Poster Board Number:

FRIDAY-001

Publishing Title:

Comparative Genomics of *Vitreoscilla* sp. Strain C1 and Other Relatives from the *Neisseriaceae* Family

Author Block:

I. Veseli, B. C. Stark, D. A. Webster, J-F. Pombert; Illinois Inst. of Technology, Chicago, IL

Abstract Body:

Gram negative β-proteobacteria from the genus *Vitreoscilla* are closely related to *Neisseria* species. While the Neisseriaceae family is infamous for harboring the gonococcus *Neisseria gonorrhoeae* and meningococcus *Neisseria meningitidis*, these two human pathogens are the exception rather than the norm and species belonging to this group are usually commensal or neutral with limited pathogenic potential. Here we sequenced the complete genome of *Vitreoscilla* sp. strain C1 to help elucidate the evolution of pathogenicity in the Neisseriaceae but also to catalog this historically important bacterial strain. The *Vitreoscilla* sp. strain C1 sequenced in this report was originally obtained from R.G.E. Murray of Western Ontario University in 1962. This strain and several others furnished by him and R.Y. Stanier of the University of California at Berkeley were examined spectrally for cytochromes and strain C1 proved to be most suitable for extracting VHb, which was originally thought to be a soluble cytochrome o. It was only after its amino acid sequence was determined 20 years later that it was identified as a bacterial hemoglobin (VHb), the first prokaryotic hemoglobin to be discovered. VHb and its genes have been extensively studied as models of prokaryotic hemoglobins and used with success to improve many biotechnological processes. Despite its great historical and practical importance, there has been some confusion about its origin in *Vitreoscilla* strain C1. Specifically, it has sometimes been incorrectly assumed that VHb comes from *Vitreoscilla stercoraria*. In fact the hemoglobin from *V. stercoraria*, as well as the transcriptional promoter for its gene, are significantly different from those in strain C1. To formally establish the difference between these species the sequence of the complete genome of C1 has been determined, annotated, and compared with that of *V. stercoraria*. DNA sequencing was done with Pacific Biosciences technology, genome assembly with SMRT Portal and Geneious software, automatic annotation with Prokka software, and manual curation with NCBI BLAST and InterProScan 5 output. Other software used for data collection, comparison and mapping included PhyML, Circos and custom Perl and bash scripts. It was found that *Vitreoscilla* sp. C1 and *V. stercoraria* are genetically distinct. Further analysis of the C1 genome is ongoing.

Author Disclosure Block:

I. Veseli: None. **B.C. Stark:** None. **D.A. Webster:** None. **J. Pombert:** None.

Poster Board Number:

FRIDAY-002

Publishing Title:

First Genome Analysis of *Entamoeba marina*

Author Block:

A. K. Mukherjee, S. Izumyama, T. Nozaki; Natl. Inst. of Infectious Diseases, Tokyo, Japan

Abstract Body:

Background: In 2015, Shiratori *et al.* first described a new species named *Entamoeba marina* and was the first among the *Entamoeba* species described till date that have a marine habitat. The genome of rich and diverse group like Amoebozoa still remained unexplored and whole genome analysis of this species will greatly enhance our understanding of the adaptation of the amoebozoan group of organisms in such diverse environments and may also lead to better resolution of amoebozoan evolution from a free living to a parasitic form or vice versa. **Methods:** The genome sequencing was performed by the Illumina HiSeq sequencing platform using both 300bp short read and 8Kb long jump distance library. The processed and cleaned DNA reads were de novo assembled into contigs and potential ORFs were identified using CLC Genomics Workbench. The ORFs were BLAST search against the non-redundant database of gene and protein sequences with cutoff E-value of 0.001. Enzyme-code mapping was also performed to understand the metabolic pathways of this organism.**Results:** The genome dataset comprised around 4500 contigs with ~12600 ORFs and an overall GC contents about 25-30%. Blast search of the putative ORF s against public databases showed that most of the *E. marina* genes have maximum similarity with other *Entamoeba* species. The enzyme mapping and similarity BLAST search in KEGG database revealed both common shared genes by *Entamoeba* and *E. marina*-specific genes. Phylogenetic analysis of some *E. marina* specific ORFs that are not present in other *Entamoeba* sp. showed a possibility of lateral gene transfer from bacteria or eukaryotic organisms.**Conclusion:** *E. marina* possesses almost similar genome contents as compared to its relalted species. The presence of *E. marina*-specific unique genes by lateral gene transfer and modification of metabolic pathways as compared to other *Entamoeba* species have made this species a unique model to study diversification and evolution of the amoebazoa. Overall the complete genome analysis of this apparently free-living marine *Entamoeba* should help us to have a much deeper picture of genome evolution of the Amoebazoa group and its relation with its adaptation to parasitic or free-living habitat.

Author Disclosure Block:

A.K. Mukherjee: None. **S. Izumyama:** None. **T. Nozaki:** None.

Poster Board Number:

FRIDAY-003

Publishing Title:

Comparative Genomics of *Streptococcus suis* 90-1330 (Serotype 2; Sequence Type 28), a Low Virulence Strain from an Important Swine and Human-emerging Pathogenic Species

Author Block:

Y. Sun[1], K. Vaillancourt[2], M. Frenette[2], D. Grenier[2], J-F. Pombert[1]; [1]Illinois Inst. of Technology, Chicago, IL, [2]Université Laval, Québec, QC, Canada

Abstract Body:

The Gram-positive α-hemolytic bacterial opportunistic pathogen *Streptococcus suis* is a major cause of concern in the swine industry and an emerging zoonotic agent that can cause sepsis, meningitis, endocarditis and other systemic issues in both pigs and humans. A total of 35 distinct *S. suis* serotypes (1 to 34 and 1/2) have been identified so far based on biochemical capsular antigen antisera assays, with strains from serotype 2 being the most dangerous for zoonotic infections. However, strain virulence can vary substantially within the same serotype and each *S. suis* serotype has been further subcategorized into sequence types (ST) based on multilocus sequence typing (MLST) analyses. Within serotype 2 strains, European ST1 isolates are known as the most virulent of all *S. suis* isolates. Closer to home, North American ST25 and ST28 strains display moderate to low virulence phenotypes, respectively. We sequenced the complete genome of *S. suis* 90-1330, a Canadian serotype 2 (SS2) - ST28 isolate producing a rare lantibiotic bacteriocin with potential therapeutic and prophylactic applications, using the Illumina and PacBio platforms. We compared it to other available complete genomes, including distinct SS2-ST28 strains from Canada and China. Using maximum likelihood phylogenomic inferences derived from 1,201 shared orthologs and genome-wide single nucleotide polymorphism (SNP) analyses, we found that all three SS2-ST28 strains of *S. suis* are genetically distinct and more closely related to representatives of serotype 3 than to other sequenced serotype 2 genomes.

Author Disclosure Block:

Y. Sun: None. **K. Vaillancourt:** None. **M. Frenette:** None. **D. Grenier:** None. **J. Pombert:** None.

Poster Board Number:

FRIDAY-004

Publishing Title:

Comparative Genomics and Epigenetics of *Sporosarcina ureae*

Author Block:

A. S. Oliver, T. Kurbessoian, M. C. Kay, K. K. Cooper; California State Univ. Northridge, Northridge, CA

Abstract Body:

Sporosarcina ureae is an aerobic, motile, spore-forming Gram-positive cocci that was originally isolated in the early 20th century from soil enrichments with elevated levels of urea. The species is unique in that it is the only known spore-forming cocci, and is currently placed in a genus exclusively composed of bacilli. Current research has been focused on the biotech potential of the unique outer cell surface layer (S-layer), and the ability to efficiently convert urea into ammonia. Specifically, researchers are using organisms that hydrolyze urea in applications such as self-healing concrete, biofuel production, and more efficient means to make fertilizer. The goal of this study is to utilize Pacific Biosciences (PacBio) DNA sequencing technology to generate complete genome sequences and to investigate genetic and epigenetic variations between strains of *S. ureae* that differ in their spatial and temporal isolation. We have sequenced the first six complete genomes and methylomes of *S. ureae*. Genomes were assembled using PacBio SMRT Analysis (v2.3.0) and Geneious (Biomatters; v9.0.4) software programs, and annotated using Rapid Annotation using Subsystem Technology (RAST; rast.nmpdr.org). The average *S. ureae* genome is 3.3 Mb in size, and contains an average 3144 CDS, 67 tRNAs and 8 rRNAs, while only one of the strains contains a plasmid (64 kb). Epigenetic analysis, using SMRT Analysis and REBASE (New England Biolabs), of the strains demonstrated evidence of several novel adenine and cytosine methylases present in *S. ureae*. Examination of the species requirement of 97% sequence identity across the 16S rRNA gene was met by all six strains. However, further analysis using *in silico* DNA-DNA hybridization (DDH), average nucleotide identity (ANI), and additional core- and pan-genome analysis demonstrated a highly divergent species or possibly some of the strains were a subspecies or new species. Further genetic analysis of the entire genus is needed to determine exactly how *S. ureae*, a spore-forming cocci, relates to the other spore-forming bacillus species in the genus *Sporosarcina*. Utilizing genomics, our analysis has begun to clarify the make up of the genus, and also found that there may be additional species of spore-forming cocci other than just *S. ureae*.

Author Disclosure Block:

A.S. Oliver: None. **T. Kurbessoian:** None. **M.C. Kay:** None. **K.K. Cooper:** None.

Poster Board Number:

FRIDAY-005

Publishing Title:

Genomic Analysis of the Human Gut Microbiome Reveals Novel Enzymes in Energy Metabolism

Author Block:

D. A. Ravcheev, I. Thiele; Univ. of Luxembourg, Esch-sur-Alzette, Luxembourg

Abstract Body:

Background: Human intestine is an organ with a unique anatomy and physiology. This organ also is an environment for about 10 to 100 trillion of microbial cells. The human gut microbiome (HGM) has been intensively studied during last years; however, respiratory capacities of the gut microbiota have been investigated for only a limited number of model. Here, we present a systematic analysis of respiration genes for ATP synthases, respiratory reductases for electron acceptors, and quinone biosynthesis encoded by the genomes of the HGM.**Methods:** We applied our genomic analysis to 254 microorganisms commonly found in the human gut. The investigated genomes belonged mostly to Firmicutes, Bacteroides, Proteobacteria, Actinobacteria, and Fusobacteria phyla of Bacteria. For the annotation of gene functions, the PubSEED platform (http://pubseed.theseed.org) was used. The gene functions annotations was done using available literature data, protein sequence similarity, protein domain structure, specificity determining positions (SDPs, http://bioinf.fbb.msu.ru/SDPfoxWeb), and genome-context based approaches, including gene chromosomal clustering and phyletic patterns.**Results:** The ATP synthases of F- and/or V-type were found in all analyzed genomes, whereas in the distribution of respiratory reductases and quinone biosynthesis variations were observed. The analysis of studied genomes revealed aerobic and anaerobic reductases for tetrathionate, thiosulfate, polysulfide, sulfite, adenylyl sulfate, heterodisulfides, fumarate, trimethylamine N-oxide, dimethyl sulfoxide, nitrate, nitrate, nitrogen oxide, nitrous oxide, selenate, and arsenate, whereas no reductases for chlorate, perchlorate, or metals were found. In addition to previously known terminal reductases, two novel reductases were predicted, being a flavin- and thiol-dependent microaerobic reductase and an anaerobic thiosulfate reductase. Three pathways for a quinone biosynthesis were found in the analyzed genomes, one for the ubiquinone (Ubi) and two for the menaquinone (Men and Mqn). During the analysis of the quinone biosynthesis pathways, four alternative forms of previously known enzymes and four novel enzymes for the Mqn pathway were predicted.**Conclusions:** This work substantially expands our knowledge on both respiratory pathways in bacteria and physiology of the HGM.

Author Disclosure Block:

D.A. Ravcheev: None. **I. Thiele:** None.

Poster Board Number:

FRIDAY-007

Publishing Title:

Phylogenomic and Phenotypic Characterizations of *Tsukamurella* Species: Reclassification of *Tsukamurella pseudospumae* as a Later Synonym of *Tsukamurella sunchonensis*

Author Block:

Y. TANG, J. L. L. Teng, S. K. P. Lau, P. C. Y. Woo; The Univ. of Hong Kong, Hong Kong, Hong Kong

Abstract Body:

Tsukamurella was first proposed as a genus in 1988 and currently comprised 11 species with validly published names. Among these, 5 species are known to be associated with human infections and 6 have been found in different environmental sources.During the characterization of *Tsukamurella* species, similar phenotypic profiles were observed between *T. sunchonensis* SCNU5^T, which was isolated from extensive foam in the aeration basin of an activated sludge process in South Korea in 2003 and *T. pseudospumae* N1176^T, which was also from foam on the surface of aeration tanks in an activated-sludge-treatment plant in UK in 2004. DNA-DNA hybridization assays proved their high relatedness (95.1±4.9% DNA-DNA relatedness, mean±SD, n=3), which was above the 70% DNA-DNA hybridization value as a gold standard criterion for delineating bacterial species. We hypothesized that these two *Tsukamurella* species be united under the same name.To test this hypothesis, we performed whole genome sequencing of the two ambiguous *Tsukamurella* species with Illumina Hi-Seq platform and used a phylogenomic approach to determine their exact taxonomic positions.After *de novo* assembly, the draft genome of *T. sunchonensis* SCNU5^T and *T. pseudospumae* N1176^T were 5.2 Mb and 5.0 Mb with G+C contents of 70.5% and 70.6% respectively. Phylogenomic analysis using the draft genome sequences was concordant to the phenotypic result. Comparative genomic analyses revealed only minor differences between the two strains, with a Genome-To-Genome Distance Calculator (GGDC) and an Average Nucleotide Identity (ANI) of 82.3% and 98.1% respectively, which was well above the recommended 70% and 95% threshold respectively for species differentiation. Sequence based comparisons exhibited high protein sequence identities of each orthologous gene present between the two genomes. We therefore proposed that the species *T. pseudospumae* should be reclassified as a later synonym of *T. sunchonensis*.This study represents the first two genomes of *T. sunchonensis* SCNU5^T and N1176 (reclassified as *T. sunchonensis* in this study). The findings also illustrate the power of phylogenomic analyses to classify bacterial strains with ambiguous taxonomic positions.

Author Disclosure Block:

Y. Tang: None. **J.L.L. Teng:** None. **S.K.P. Lau:** None. **P.C.Y. Woo:** None.

Poster Board Number:

FRIDAY-009

Publishing Title:

Comparative Genomics for Inferring Bacterial Chromosome Topologies and Their Selective Value

Author Block:

S. Khedkar, A. Seshasayee; Natl. Ctr. for Biological Sci., Bangalore, India

Abstract Body:

Genomes evolve not only in base sequence, but also in terms of their genome architecture (defined by gene organization and topology). Changes at both these levels are crucial for adaptation to stress and changing environmental conditions. While extensive genome sequence data informs us about changes in base sequences, the study of genome architecture is restricted to a few model organisms. Further, the role of genome architecture in regulation of gene expression and bacterial response to antibiotics[1] and stress[2] has made it essential to study genome architecture across a wide range of bacteria. Towards this we exploit whole genome sequence data for a range of free-living, pathogenic and non-pathogenic bacteria to study link between gene organization and chromosome topology. Using comparative genomics across ~250 pairs of closely related bacteria we show (a) many organisms show a high degree of inter-replichore translocations, which are not limited to the inversion-prone terminus (*ter*) or the *oriC* regions; (b) translocation maps may reflect chromosome topologies, and (c) inter- replichore contacts equidistant from *oriC* may serve to divert recombination events towards maintaining gene dosage gradient down the *oriC-ter* axis. In summary, we suggest that translocation maps might be a first line in defining a gross chromosome topology given a pair of closely related genome sequences, and argue that chromosome topology might help maintain gene order. Finally we think our analysis could act as a useful resource to study genome architecture across a range of bacteria, just given their genome sequence.

Author Disclosure Block:

S. Khedkar: None. **A. Seshasayee:** None.

Poster Board Number:

FRIDAY-010

Publishing Title:

Comparative Genomics and Transcriptomics of *Escherichia coli* Carrying Virulence Factors of Both Epec and Etec

Author Block:

T. H. Hazen[1], S. Daugherty[1], A. C. Shetty[1], J. P. Nataro[2], D. A. Rasko[1]; [1]Inst. for Genome Sci., Baltimore, MD, [2]Univ. of Virginia, Charlottesville, VA

Abstract Body:

Escherichia coli that are capable of causing human disease are typically classified into pathovars or pathotypes based on the illness they cause and their virulence gene content. Recent comparative genomics studies have demonstrated that both EPEC and ETEC are genomically diverse pathovars consisting of isolates in many different genomic lineages of *E. coli*. Both EPEC and ETEC are characterized by the presence of virulence factors encoded by mobile genetic elements including plasmids, phage and pathogenicity islands. In the current study we used comparative genomics and transcriptomics to characterize four *E. coli* isolates that contained virulence factors of both EPEC and ETEC. Three of the isolates contained a complete locus of enterocyte effacement (LEE) region that is usually found in EPEC, as well as the heat-labile enterotoxin (LT) that is one of the canonical virulence factors of ETEC. These isolates did not contain the bundle-forming pilus that is characteristic of typical EPEC, nor did they contain the Shiga-toxin of EHEC, thus they would be classified as atypical EPEC based only on virulence factor profiling. Phylogenomic analysis demonstrated these *E. coli* isolates were most related to a recently designated lineage of EPEC that contained both typical and atypical EPEC. A fourth *E. coli* isolate that was atypical EPEC (LEE+/BFP-) contained an autotransporter, *eatA*, which is more frequently identified in ETEC. RNA sequencing (RNA-Seq) analysis of the EPEC/ETEC isolates during growth in laboratory conditions that promote virulence factor expression of EPEC and ETEC prototype isolates demonstrated that some but not all of the virulence factors were expressed, suggesting that a coordinated regulation of chromosomal and mobile elements are required for full virulence. Genes in the LEE region exhibited increased expression under these conditions in the EPEC prototype isolate and the EPEC/ETEC isolates. The autotransporter *eatA* also exhibited increased expression under these conditions; however, the genes encoding LT did not exhibit increased expression. The findings of this study provide insight into the function of horizontally-acquired virulence factors in *E. coli* isolates that blur the designation of the *E. coli* pathovars EPEC and ETEC.

Author Disclosure Block:

T.H. Hazen: None. **S. Daugherty:** None. **A.C. Shetty:** None. **J.P. Nataro:** None. **D.A. Rasko:** None.

Poster Board Number:

FRIDAY-011

Publishing Title:

Evaluation of Horizontal Gene Transfer in the Evolutionary History of Beta-Galactosidase

Author Block:

A. Wu, L. David; Duke Univ., Durham, NC

Abstract Body:

Eukaryote-to-prokaryote horizontal gene transfer (HGT) events are believed to be rare. Here, we investigate the possibility that select bacterial beta-galactosidase genes are inherited from an ancient eukaryote-to-prokaryote HGT event. We analyzed 433 beta-galactosidase eukaryotic and prokaryotic beta-galactosidase genes downloaded from the UniProt database. These sequences were then aligned and trimmed in order to build phylogenetic trees. Inspecting our phylogenies, we observe an association between select beta-galactosidase genes in animals and host-associated bacteria. This association may be explained by an ancient HGT event, or by a series of gene duplication and loss events. Independent of inheritance mechanisms, however, these findings point to a carbohydrate-driven association between ancient bacteria and eukaryotes. Experiments are underway to test the functional role of animal-associated beta-galactosidase genes in bacteria.

Author Disclosure Block:

A. Wu: None. **L. David:** None.

Poster Board Number:

FRIDAY-012

Publishing Title:

Comparative Genomics of *Salmonella enterica* Serovars Anatum and Typhimurium Isolated from Cattle and Humans

Author Block:

S. V. Nguyen, J. L. Bono, T. P. L. Smith, R. Wang, C. M. Kelley, G. P. Harhay, D. M. Brichta-Harhay; US Meat Animal Res. Ctr., Clay Center, NE

Abstract Body:

Salmonella enterica subsp. *enterica* is an important group of pathogens capable of inhabiting a range of niches and hosts with varying degrees of impact, from commensal colonization to invasive infection. Recent outbreaks of multi-drug resistant *S. enterica*, attributed to consumption of contaminated ground beef, have led to increased scrutiny of the presence of these pathogens in red meat. To date, there has been limited comparative genomics research examining the virulence determinants that exists among serotypes of *S. enterica* associated with cattle. Such comparisons are necessary to understand disease etiology in cattle and humans.For *S. enterica* genomic comparisons, we present the complete genome sequences of 24 *S. enterica* isolates of serotypes Anatum (BA, bovine n=7; HA, human n=5) and Typhimurium (BT, bovine; HT, human n=6 each), isolated from healthy cattle or clinically infected humans. For all isolates, genomes were sequenced with single molecule real time technology and subsequently closed (HGAP3 or Celera 7.3). All circularized genomes and plasmids were annotated using a local instance of Do-It-Yourself Annotator (DIYA). Using the EDGAR comparative genomics system, phylogenetic trees were constructed and core-genome and pan-genome analyses performed between the different serotypes. Core and pan-genome values for Anatum were 4302 and 4969 ORFs, while those for Typhimurium were 4362 and 5347 ORFs respectively. Comparisons of core genomes revealed that many of the non-orthologous gene sets observed are associated with the mobilome. Specifically, phages GIFSY-1 and GIFSY-2 were only found predominantly in Typhimurium, while transposon IS903 was found in several strains of Anatum only. Analysis of the BA and HT core genomes revealed that 4118 genes were shared while 213 and 304 were unique to BA and HT, respectively. Genes unique to the HT core and possibly important for virulence, include retrons, methyltransferases, fimbrial operons, secreted effectors, and phosphotransferase systems. The results of these analyses and implications for differences in virulence potential within these serovars will be discussed.

Author Disclosure Block:

S.V. Nguyen: None. **J.L. Bono:** None. **T.P.L. Smith:** None. **R. Wang:** None. **C.M. Kelley:** None. **G.P. Harhay:** None. **D.M. Brichta-Harhay:** None.

Poster Board Number:

FRIDAY-013

Publishing Title:

Comparative Whole Genome Sequence Analysis of Long-term Surviving *Vibrio cholerae* in Nutrient-poor Lake Water Microcosms

Author Block:

S. Sinha Ray; Univ. of Florida, Gainesville, FL

Abstract Body:

Vibrio cholerae, the causative agent of the water-borne disease cholera, can shift to Growth-advantage stationary phage (GASP) phenotype in a stationary growth condition. We have previously reported a "GASP" phenotype that survived in nutrient-poor "filter sterilized" lake water (FSLW) microcosms for up to 700-days. Furthermore, we observed that the GASP persisting cells in 700-day old microcosms produced biofilms unique to FSLW. Identifying the sustained mutations in *Vibrio cholerae* GASP *phenotype will enhance our understanding of how this phenotype is selected under stressful conditions that promote its environmental fitness and adaption*. Here we report whole genome comparative analysis of wild-type *V. cholerae* N16961 El tor strain N16961, together with the same strain from a 24-hour microcosm,190 day-old microcosm and a 700-day old microcosm. Single nucleotide polymorphisms (SNPs) and INDELs were identified by using both reference mapping and *de novo* assembly approaches, including a bioinformatic pipeline optimized for cholera genomic analysis implemented in Galaxy. In particular, Freebayes was used to detect the variants and the SnpEff tool for annotating the variants and predicting their effect, while de novo assembly of the sequence reads was obtained with multiple alignments of whole genomes using MAUVE. Our analysis shows that compared to the wild type *V.cholerae* N16961 El tor strain, all three GASP phenotypes exhibited a total of 191 high quality mutations, with 50 mutations identified in the coding regions in both N16961-24 and190 days-old strain and 51mutations in 700-day-old GASP genome, respectively; out of which 28 (55%), 24 (48%) and 28 (52%) were due to INDELs, causing frameshift in several open reading frames. Point mutations consisted of 20 non-synonymous and 3 synonymous changes in all three GASP phenotypes. Among the genes exhibiting these changes, important regulatory proteins are included such as the CsrD, Ribosome GTPase, GGDEF and (p) ppGpp synthetase. In conclusion, our comparative analysis sheds light on significant genomic changes occurring in the long-time surviving GASP phenotypes, some of which may be responsible for the adaptation of the bacteria to stressful and stationary growth conditions.

Author Disclosure Block:

S. Sinha Ray: None.

Poster Board Number:

FRIDAY-014

Publishing Title:

Isolation and Genomic Diversity of Bifidobacteria Occupying the Gastrointestinal Tract of Mammals

Author Block:

K. Albert, A. C. Kundmann, R. M. Levantovsky, D. A. Sela; Univ. of Massachusetts, Amherst, MA

Abstract Body:

The genus *Bifidobacterium* contains 39 species of anaerobic rod-shaped bacteria that exhibits a characteristic bifid morphology under certain conditions. Bifidobacteria are an early colonizer of the infant gastrointestinal tract (GIT) and are considered a beneficial member of the gut microbiome in adults, albeit at lower concentrations. The goal of this study was to isolate gut-colonizing bifidobacteria from mammals and infer the molecular evolution of these strains using comparative genomics. Our overarching hypothesis is that the sequence divergence and gene complement encoded within bifidobacterial genomes reflects mammalian host divergence and may enable colonization of the specific ecological niche (e.g. non-human primate) from which it was isolated. Accordingly, bifidobacteria were isolated from various mammals (i.e. humans and non-human animals) using selective media and confirmed by genotypic and phenotypic assays. Whole genomes of bifidobacterial isolates were sequenced, with resulting reads assembled, annotated, and subjected to various comparative analyses. As a result, bifidobacterial strains were isolated and identified as several taxa and further characterized. This includes subspecies belonging to *Bifidobacterium longum*, *Bifidobacterium pseudolongum*, among other taxa typically associated with gut microbiota. In addition, strains of *Alloscardovia* spp. were isolated from non-human primates. This genus belongs to the family *Bifidobacteriacea* and is phosphoketolase positive, which was once posited as the characteristic enzymatic activity of the genus *Bifidobacterium*. In addition to comparative genomics, several metabolic and nutritional phenotypes were characterized including carbohydrate utilization profiles and fermentative end product yields.

Author Disclosure Block:

K. Albert: None. **A.C. Kundmann:** None. **R.M. Levantovsky:** None. **D.A. Sela:** None.

Poster Board Number:

FRIDAY-015

Publishing Title:

Population Genomics Traces the Evolution History of the Fungal Pathogen *Cryptococcus neoformans*

Author Block:

C. A. Desjardins[1], S. M. Sykes[1], J. Rhodes[2], Y. Chen[3], J. Perfect[3], A. Litvintseva[4], M. Fisher[2], C. A. Cuomo[1]; [1]Broad Inst., Cambridge, MA, [2]Imperial Coll. London, London, United Kingdom, [3]Duke Univ. Med. Ctr., Durham, NC, [4]CDC, Atlanta, GA

Abstract Body:

Cryptococcus neoformans causes approximately 1 million new infections resulting in 625,000 deaths per year worldwide. While global populations of *C. neoformans* are highly clonal, a unique subpopulation in sub-Saharan Africa is characterized by high genetic diversity, the presence of both mating types, and evidence of recombination. We have sequenced the genomes of roughly 400 isolates of *C. neoformans*, representing each of the three major population subdivisions: the global lineages VNI and VNII and the sub-Saharan African VNB. These include mainly clinical strains for VNI and the less commonly observed VNII, and a mix of clinical and environmental strains for VNB. Phylogenetic and linkage disequilbrium analysis revealed a deep split in the VNB group and a lack of recombination between the major lineages. Population genomic analysis highlighted patterns of recent hybridization and more ancient genetic exchange between and within subpopulations. The sub-Saharan VNB clade had substantially more diversity than the global VNI lineage. However, their genomes are also characterized by extensive regions of loss of diversity, suggesting recent selective sweeps through these populations, which could be linked to transitions between the environment and human host. These new insights highlight the evolutionary dynamics of this important fungal pathogen and a better understanding of the fundamental differences between the global and African subpopulations.

Author Disclosure Block:

C.A. Desjardins: None. **S.M. Sykes:** D. Employee; Self; Seres Therapeutics. **J. Rhodes:** None. **Y. Chen:** None. **J. Perfect:** None. **A. Litvintseva:** None. **M. Fisher:** None. **C.A. Cuomo:** None.

Poster Board Number:

FRIDAY-016

Publishing Title:

Reinstatement of the Genus *Kaistella* Within the *Flavobacteriaceae*

Author Block:

J. Newman, N. Pisani, D. Ciccarelli; Lycoming Coll., Williamsport, PA

Abstract Body:

The identification of a novel strain belonging to the *Flavobacteriaceae* led to comparative phenotypic and genomic studies with closely related reference strains that had recently undergone reclassification. The genus *Kaistella* was established in 2004 with the description of *K. koreensis*. The genus *Sejongia* was established in 2005 with the description of *S. antarctica* and *S. jeonii*. These species were moved into the genus *Chryseobacterium* in 2009 based on their similarity to *C. haifense* which itself is only distantly related to *C. gleum*, the type species of the genus. Many other species that cluster with this group have also been assigned to the genus *Chryseobacterium*. We sequenced the genomes of six members of the cluster as well as eight other members of the genus *Chryseobacterium.* Here we show that this cluster forms a monophyletic group with characteristics such as small genome size, fatty acid composition, antibiotic sensitivity and a lack of flexirubin pigment production that distinguish the group from "true" *Chryseobacterium* species. We propose that the genus *Kaistella* be reinstated with *K. koreensis* as the type species, and that the former members of the genus *Sejongia*, as well as nine *Chryseobacterium* species, including *C. solincola, C. haifense*, and *C. palustre* should be moved into the genus *Kaistella*.

Author Disclosure Block:

J. Newman: None. **N. Pisani:** None. **D. Ciccarelli:** None.

Poster Board Number:

FRIDAY-017

Publishing Title:

Complete Genome Sequence of a *Streptococcus pyogenes emm3* Strain, M3-b, Isolated from a Japanese Patient with Streptococcal Toxic Shock Syndrome Case, and Comparative Genomics of *S. pyogenes emm3* Strains

Author Block:

K. Ogura[1], S. Watanabe[2], T. Kirikae[1], **T. Miyoshi-Akiyama**[1]; [1]Natl. Ctr. for Global Hlth.and Med., Tokyo, Japan, [2]Jichi Med. Univ., Tochigi, Japan

Abstract Body:

Streptococcus pyogenes (GAS) can be subtyped according to serotype or the serotypes or genotype of the M proteins encoded by the *emm* genes. The GAS isolates harboring *emm1* or *emm3* have been found to predominate in patients with streptococcal toxic shock syndrome (STSS). To analyze the genomic background of *emm3* isolates, we have determined the complete genome sequence of an *emm3* GAS M3-b strain isolated from a patient with STSS case. The sequence showed 99% of genome coverage compared to those with the complete sequences of three previously completely sequenced *emm3* GAS isolates, MGAS 315, SSI-1, and STAB902 based on BLAST algorithm. None of these strains did not harbor functionally clustered, regularly interspaced short palindromic repeat. The sequence of the M3-b strain was compared with whole the entire genome sequences of the three strains, other MGAS 315, SSI-1, STAB902, 11 *emm3* strains isolated in the USA, and five additional 5 isolates sequenced in this study. Bayesian phylogeny using SNP concatemers showed that the most recent common ancestor of these 19 *emm3* strains appeared 84 years ago. Phylogenetic comparison and analyses of the distribution of virulence factors suggestedthat *emm3* isolates from the patients with and without STSS cases are not distinguishable from those of non-STSS cases were indistinguishable.

Author Disclosure Block:

K. Ogura: None. **S. Watanabe:** None. **T. Kirikae:** None. **T. Miyoshi-Akiyama:** None.

Poster Board Number:

FRIDAY-018

Publishing Title:

Population and Comparative Genomics of the Dimorphic Human Pathogenic Fungi

Author Block:

J. F. Muñoz[1], R. A. Farrer[2], C. A. Desjardins[2], J. E. Gallo[1], E. Misas[1], J. G. McEwen[3], O. K. Clay[4], C. A. Cuomo[2]; [1]Cellular and Molecular Biology Unit, Corporación para Investigaciones Biológicas, Medellín, Colombia, [2]Broad Inst. of MIT and Harvard, Cambridge, MA, [3]Sch. of Med., Univ. de Antioquia, Medellín, Colombia, [4]Sch. of Med. and Hlth.Sci., Univ. del Rosario, Bogota, Colombia

Abstract Body:

The Ajellomycetaceae family includes several of the dimorphic human pathogenic fungi such as *Paracoccidioides*, *Blastomyces* and *Histoplasma*. Related genera rarely cause disease in humans or are not pathogenic, such as *Emmonsia*, *Polytolypa* and *Spiromastix*. The *Paracoccidioides* genus includes two species that cause paracoccidioidomycosis (*P. brasiliensis*, *P. lutzii*). To better understand the evolution of pathogenicity of *Paracoccidioides* and other dimorphs, we performed population and comparative genome analyses of these fungi. We sequenced the genomes of 31 different isolates of *Paracoccidioides* belonging to all lineages. We completed the first annotated genomes for the PS3 and PS4 lineages, and examined gene content across each of the four lineages of *P. brasiliensis* and compared to *P. lutzii*. Comparing these reference genomes of *Paracoccidioides* and with SNPs identified in 26 additional isolates, we established the relationships of the *Paracoccidioides* lineages; we found good support from both phylogenetic analysis and principal component analysis for a separation of S1 into two subgroups (S1A, S1B). In addition we found evidence of higher exchange between the S1B group and all other lineages, and established patterns of population structure. Additionally, we sequenced, assembled and annotated the genomes of *Emmonsia* species, *P. hystricis* and *S. grisea*. Comparative analyses of these fungi with the dimorphic human pathogens identified patterns of gene conservation, protein family evolution and genomic features correlated with virulence. This population analysis provides a better understanding of the diversity and genetic exchange of *Paracoccidioides*, and the comparative analysis provided insight into the pathogenesis and life cycles of the dimorphic human pathogenic fungi.

Author Disclosure Block:

J.F. Muñoz: None. **R.A. Farrer:** None. **C.A. Desjardins:** None. **J.E. Gallo:** None. **E. Misas:** None. **J.G. McEwen:** None. **O.K. Clay:** None. **C.A. Cuomo:** None.

Poster Board Number:

FRIDAY-019

Publishing Title:

Genomic Analysis and Classification of Novel Species *Flavobacterium douthatii* Abg sp. Nov

Author Block:

K. Fischer, J. Newman; Lycoming Coll., Williamsport, PA

Abstract Body:

Strain ABG was identified in the Lycoming College Microbiology class as a potentially novel species of *Flavobacterium*, a highly diverse and well-studied genus that is particularly important in freshwater biology and fish health. 16S rRNA pairwise similarity suggested that *F. succinicans* and *F. glaciei* were most closely related to ABG. However, 16S rRNA comparisons alone are insufficient when describing a new species. Instead, global orthology scores, phylogenomic metrics, and phenotypic data should all be taken into account. *Flavobacterium* sp. ABG's genome was sequenced at 90x coverage and was assembled into 89 contigs totaling 5,270,010 base pairs. The Rapid Annotation using Subsystems Technology (RAST) website was used to annotate the genome and compare it to other Flavobacteria. Genome-wide comparisons indicated *F. hibernum* and *F. chilense* were the most closely related species. Estimated DNA-DNA Hybridization showed sufficient differences from *F. hibernum* (24.8%) and *F. chilense* (24.6%) to confirm ABG's novel species status. Average Amino Acid Identity (AAI) and Average Nucleotide Identity (ANI) were also below the species threshold of 95% further supporting that ABG should be a novel taxon. Comparative genomics identified 1272 unique genes including a paralog of lantibiotic synthesis protein LanB synthetase not yet found in other *Flavobacterium* species. The presence of a large number of unique genes and the low genome similarity of strain ABG to its closest relatives are sufficient evidence to establish this organism as a novel species within the genus *Flavobacterium* with the proposed name *Flavobacterium douthatii*. The results also support the need to compare genomes instead of relying on single gene comparisons when classifying organisms.

Author Disclosure Block:

K. Fischer: None. **J. Newman:** None.

Poster Board Number:

FRIDAY-020

Publishing Title:

Assessing Genome Wide Diversity Among Strains of *Streptococcus salivarius* Using Single Nucleotide Polymorphisms

Author Block:

R. R. Butler, III[1], J. T. A. Soomer-James[1], M. Frenette[2], J-F. Pombert[1]; [1]Illinois Inst. of Technology, Chicago, IL, [2]Université Laval, Québec, QC, Canada

Abstract Body:

Streptococcus salivarius is a ubiquitous primary colonizer of the human oral mucosa. However, little is known about the genetic diversity of this species as most of the current knowledge is restricted to limited MLST analyses. To better assess the genetic variability in this Gram-positive commensal species we sequenced two strains available from the American Tissue Culture Collection, ATCC 25975 and ATCC 27945, which are Lancefield K- and K+ respectively, the former possessing fimbriated appendages for mucosal adherence. Whole genomes of each strain were assembled using PacBio and Illumina sequencing and annotated. Genome wide pairwise SNP comparisons of these two strains plus those of four complete *S. salivarius* genomes available in GenBank were contrasted with the pairwise comparisons of ten strains of *Streptococcus thermophilus*. *S. thermophilus* is well characterized in the fermentation of dairy products, and is one of the most closely related species to *S. salivarius*. Synthetic read data for SNP comparison was generated for all strains using a portable Perl script, SSRG.pl (available via our lab's GitHub page). Pairwise SNPs for the *S. salivarius* strains ranged from 18.6-32.0 SNPs/kb, reflecting a sizeable variability in genomic content. The *S. thermophilus* strains showed markedly less variability, ranging from 0.0-8.6 SNPs/kb. The difference in polymorphism in each of the two species suggests that different environmental pressures have selected for increased divergence in *S. salivarius*, a species living in a complex oral microbiome.

Author Disclosure Block:

R.R. Butler: None. **J.T.A. Soomer-James:** None. **M. Frenette:** None. **J. Pombert:** None.

Poster Board Number:

FRIDAY-021

Publishing Title:

Functional Importance of Inteins in Microbial World

Author Block:

O. Novikova, M. Belfort; Univ. at Albany, Albany, NY

Abstract Body:

Inteins, also called protein introns, are self-splicing mobile elements found in all domains of life. A bioinformatic survey of genomic data highlights a biased distribution of inteins among functional categories of proteins in both bacteria and archaea, with a strong preference for a single network of functions containing replisome proteins. Many non-orthologous, functionally equivalent replicative proteins in bacteria and archaea carry inteins, suggesting a selective retention of inteins in proteins of particular functions across domains of life. Inteins cluster not only in proteins with related roles, but also in specific functional units of those proteins, like ATPase domains. This peculiar bias does not fully fit the models describing inteins exclusively as parasitic elements. In such models, evolutionary dynamics of inteins is viewed primarily through their mobility with the intein homing endonuclease (HEN) as the major factor of intein acquisition and loss. Although the HEN is essential for intein invasion and spread in populations, HEN dynamics does not explain the observed biased distribution of inteins among proteins in specific functional categories.We propose that the protein splicing domain of the intein can act as an environmental sensor that adapts to a particular niche and could potentially increase the chance of the intein becoming fixed in a population. There are inteins modulated in a stimulus-dependent manner which points to the possibility that some inteins may adapt to their intracellular niche and become post-translational regulatory elements. Indeed some inteins can act as sensors, and through intein chemistry can inhibit splicing under stressful environmental conditions, such as redox modulation (Callahan et al. 2011), oxidative and nitrosative stress (Topilina et al. 2015a) or cold shock (Topilina et al. 2015b). Thus, we argue that selective retention of some inteins might be beneficial under certain environmental stresses, to act as panic buttons that reversibly inhibit specific networks, consistent with the observed intein distribution.

Author Disclosure Block:

O. Novikova: None. **M. Belfort:** None.

Poster Board Number:

FRIDAY-022

Publishing Title:

Phylogenomic Analysis of *Streptococcus sinensis* Hku4^{t} Reveals a Novel Phylogenetic Group in the Genus *Streptococcus*

Author Block:

Y. TANG, Y. Huang, S. K. P. Lau, P. C. Y. Woo, J. L. L. Teng; The Univ. of Hong Kong, Hong Kong, Hong Kong

Abstract Body:

Currently, the genus *Streptococcus* comprised more than 90 species and was divided into 7 major groups, namely anginosus, mitis, salivarius, mutans, bovis, pyogenic and sanguinis. *Streptococcus sinensis* has been discovered by our group from patients with infective endocarditis in Hong Kong in 2002. Subsequently, additional cases reported from worldwide suggested that the bacterium is an emerging pathogen of global importance. Phenotypic profile of *S. sinensis* was similar to members of anginosus, mitis and sanguinis groups. Phylogenetic analyses based on *groEL* gene sequences showed that this species clustered with members of anginosus and sanguinis groups, but 16S rRNA gene sequences revealed that this species branched out from members of anginosus, mitis and sanguinis groups. Thus, the phylogenetic position of this species remained unclear.In this study, a polyphasic approach has been used to determine the taxonomic position of *S. sinensis.*The whole genome sequencing of *S. sinenesis* HKU4^{T} was performed by Illumina Hi-Seq 2500. The draft genome of *S. sinenesis* HKU4^{T} was 2.06 Mb with G+C content of 42.2%. Phylogenomic analyses using the genome sequence of *S. sinensis* HKU4^{T} and those of 69 *Streptococcus* genomes and 50 ribosomal protein gene sequences from 87 *Streptococococcus* genomes showed that *S. sinenesis*, together with *S. oligofermentans* and *S. cristatus*, formed a distinct phylogenetic clade. Dendrogram generated from hierarchical cluster analysis of matrix-assisted laser desorption ionization time-of-flight mass spectrometry also formed the same clade.On the basis of these genomic evidences, it is proposed a novel phylogenetic group, “sinensis group”, to include species of *S. sinenesis*, *S. oligofermentans* and *S. cristatus*. This study presents the first draft genome of *S. sinenesis* and reveals a novel phylogenetic clade in the genus of *Streptococcus*. The findings demonstrate the superior power of phylogenomic analyses to resolve the ambiguities in bacterial taxonomy. Detailed comparative genomic analysis on members belonging to the “sinensis group” and those of the mitis group may illustrate not only the ecology and biology of *S. sinensis* but also pathogenesis of infective endocarditis caused by members of this novel phylogenetic group.

Author Disclosure Block:

Y. Tang: None. **Y. Huang:** None. **S.K.P. Lau:** None. **P.C.Y. Woo:** None. **J.L.L. Teng:** None.

Poster Board Number:

FRIDAY-023

Publishing Title:

New Perspective on Cleanrooms and Human Pathogens

Author Block:

m. bashir[1], m. ahmed[2], t. Weinmaier[3], n. ivanova[4], t. pieber[5], **p. vaishampayan**[2]; [1]Med. Univ. of Graz, graz, Austria, [2]California Inst. of Technology, Jet Propulsion Lab., Pasadena, CA, [3]Univ. of Vienna, vienna, Austria, [4]Joint Genome Inst., walnut creek, CA, [5]Med. Univ. of Graz, Graz, Austria, graz, Austria

Abstract Body:

Strict planetary protection practices are implemented during spacecraft assembly to prevent inadvertent transfer of earth microorganisms to other planetary bodies. We wanted to evaluate if these practices selectively favors survival and growth of hardy microrganisms, such as pathogens. Three geographically distinct clean rooms were sampled during the assembly of three NASA spacecraft: Dawn, Phoenix, and Mars Science Laboratory mission. During Phoenix mission, sample sets were collected from clean room at three time points: before arrival of the Phoenix spacecraft, during the assembly and testing of the Phoenix spacecraft, and after removal of the spacecraft from the PHSF facility. All samples were subjected to whole genome shotgun metagenome sequencing on an Illumina HiSeq 2500 platform. We screened for pathogens and other virulence factors, which determine pathogenicity. Potential pathogens and their corresponding virulence factors were present in all the samples. Though the relative abundance of pathogens was lowest during the Phoenix assembly, virulence factors increased from before to during to after assembly, potentially offering a survival advantage. Decreased microbial and pathogenic diversity indicates that decontamination and preventative measures were effective and well implemented. This is the first metagenome study describing presence of pathogens in controlled enclose environments and their corresponding virulence factors. The results of this study should be considered for build environments, International space station and future manned mission to Mars.

Author Disclosure Block:

M. bashir: None. **M. ahmed:** None. **T. Weinmaier:** None. **N. ivanova:** None. **T. pieber:** None. **P. vaishampayan:** None.

Poster Board Number:

FRIDAY-024

Publishing Title:

Comparative Phylogenetic Analysis of the Microbial Communities in a Spacecraft Assembly Facility and in Epsomite Lakes

Author Block:

F. Chen[1], P. Vaishampayan[1], A. Probst[2], M. La Duc[1], B. Clark[3], M. Schneegurt[4]; [1]Jet Propulsion Lab., Pasadena, CA, [2]UC Berkeley, Berkely, CA, [3]Space Sci. Inst., Boulder, CO, [4]Wichita State Univ., Wichita, KS

Abstract Body:

The Martian surface presents numerous challenges for microbial survival. Initially discovered by the Viking lander, the high-sulfates, especially $MgSO_4$-rich nature of Martian regolith might play a role in precluding the survival and proliferation of any inadvertently introduced contaminant microorganisms. Such a notion sparks concerns relating to the unlikely event of an off-nominal landing of a spacecraft equipped with a perennial heat source. Under these conditions meltwater stemming from the subterranean ice-rich regolith might persist for an extended period of time, providing refuge for contaminant microbes to propagate, sheltered from the environmental pressures and stresses above. With this in mind, $MgSO_4$-tolerant microbes were screened from two environments relevant to the robotic exploration of Mars: a spacecraft assembly cleanroom (SAC) and natural $MgSO_4$-rich epsomite lakes. The microbial population observed in the former environment suggests the types of microbes most likely to gain access to, and contaminate, outbound spacecraft. Microbes populating the latter environment represent phylotypes whose fitness renders them capable of surviving $MgSO_4$-rich conditions. Tag-encoded 454 FLX amplicon pyrosequencing (TEFAP) was carried out on direct metagenomic extracts of environmental samples collected from a spacecraft assembly cleanroom (JPL, Pasadena, CA), Hot Lake (Oroville, WA), and Basque Lake (Kamloops, BC) using primers targeting hypervariable regions in the bacterial and archaeal 16S rRNA gene. Pyrosequencing protocols were also designed to assay the presence of certain functional genes, such as *nifH* in diazotrophs, *mcrA* in methanogens, and *dsrAB* in sulfate-reducing microorganisms. The results of this investigation, presented here, empower more knowledgeable hypotheses pertaining to the types of microbes most likely to gain access to SAC environments and thus pose a potential threat of forward contamination, and might persist for extended periods of time given an off-nominal landing or transfer to wet salty environments.

Author Disclosure Block:

F. Chen: None. **P. Vaishampayan:** None. **A. Probst:** None. **M. La Duc:** None. **B. Clark:** None. **M. Schneegurt:** None.

Poster Board Number:

FRIDAY-025

Publishing Title:

Methanogen Growth and Adaptability to Ammonium Chloride

Author Block:

C. McFerrin, R. L. Mickol, T. A. Kral; Univ. of Arkansas, Fayetteville, AR

Abstract Body:

The internal environment of Enceladus appears potentially habitable, containing mostly water, along with a few percent of carbon dioxide, methane, ammonia, and some organics. Due to a lack of oxygen and extreme subsurface environmental conditions, life there would likely be restricted to extremophilic, anaerobic organisms. On earth, methanogens, microorganisms from the domain Archaea, can thrive in anaerobic and extreme terrestrial ecosystems, suggesting methanogens could be a model for life on Enceladus. While the presence of water and nutrients, as well as the detection of methane, provide hope for life on Enceladus, the concentration of ammonia is concerning. Four methanogen species (Methanothermobacter wolfeii, Methanobacterium formicicum, Methanosarcina barkeri, Methanococcus maripaludis) were tested for their ability to survive and adapt in the presence of ammonium chloride (NH4Cl). Fifty-milliliter aliquots of four types of anaerobic methanogen growth media (MM, MSH, MSF, MS) were prepared. Ten milliliters of each medium were anaerobically transferred to each of five test tubes (n = 5). Ammonium chloride was added to each test tube, resulting in concentrations up to 6% NH4Cl. Following sterilization via autoclave, each tube was inoculated with 0.5 mL of the corresponding medium and methanogen species (MM: M. wolfeii; MSH: M. maripaludis; MSF: M. formicicum; MS: M. barkeri). The tubes were pressurized with 200 kPa H2, incubated at the organisms' ideal growth temperatures and monitored for methane production. M. wolfeii has shown methane production in 5% NH4Cl and is in the process of being adapted to higher concentrations. M. formicicum has produced significant methane in 3% NH4Cl. M. barkeri and M. maripaludis have continued to show limited success up to 3% NH4Cl. The data indicate that less methane is produced as NH4Cl concentration increases. However, the data suggest that each of the species studied could survive and produce methane in at least 3% NH4Cl concentrations. Future studies will aim to replicate findings and test additional concentrations of NH4Cl in order to determine the survival threshold for each of species.

Author Disclosure Block:

C. McFerrin: None. **R.L. Mickol:** None. **T.A. Kral:** None.

Poster Board Number:

FRIDAY-026

Publishing Title:

Methanogen Growth in the Presence of Nontronite

Author Block:

R. L. Mickol, M. Williams, J. Wray, T. A. Kral; Univ. of Arkansas, Fayetteville, AR

Abstract Body:

Spectral data indicate the presence of various clay minerals across the martian surface. Clay minerals are often created through hydrothermal alteration, suggesting long-term water-rock reactions on the planet. In certain locations, spectral signatures are consistent with those of nontronite, an Fe- and Mg-rich smectite clay. Nontronite is a hydrous phyllosilicate and, thus, can contain variable amounts of adsorbed water within its layers. Over Mars' history, these clay mineral-water assemblages may have supported microbial life. Four methanogen species (*Methanothermobacter wolfeii*, *Methanobacterium formicicum*, *Methanosarcina barkeri*, *Methanococcus maripaludis*) were tested for their ability to grow in the presence of nontronite. Methanogen growth media (MM, MSH, MSF, MS) were prepared and distributed into test tubes (10 mL per tube) in an anaerobic chamber. One, two or three grams of nontronite were placed into each type of medium. Media without nontronite served as a control. There were three tubes for each combination. Following sterilization via autoclave, each tube was inoculated with 0.5 mL of the corresponding methanogen species (MM: *M. wolfeii*; MSH: *M. maripaludis*; MSF: *M. formicicum*; MS: *M. barkeri*). The tubes were pressurized with 200 kPa H_2, incubated at the organisms' ideal growth temperatures and monitored for methane production. *M. wolfeii* and *M. formicicum* demonstrated decreased methane production with increased nontronite. Methane production was generally similar for the control, 1 g and 2 g nontronite tubes for both *M. maripaludis* and *M. barkeri*. In a second experiment, *M. barkeri* and *M. formicicum* cells were washed (centrifuged at 4200 rpm, rinsed with sterile buffer) to remove residual media, suspended in sterile buffer, and added anaerobically to tubes containing 2 g nontronite (sterile) and only sterile buffer. Tubes were pressurized with 200 kPa H_2, incubated at 37 °C and monitored for methane production. After 31 days, no methane was produced by either *M. barkeri* or *M. formicicum* in the presence of nontronite in buffer alone. This suggests that nontronite, by itself, does not provide all essential nutrients to warrant growth of methanogens. Future studies will test additional clays and clay mixtures detected on Mars for the ability to support methanogen growth.

Author Disclosure Block:

R.L. Mickol: None. **M. Williams:** None. **J. Wray:** None. **T.A. Kral:** None.

Poster Board Number:

FRIDAY-027

Publishing Title:

Bacterial Growth Tolerance to Chlorate and Perchlorate Salts Relevant to Mars

Author Block:

A. Al Soudi[1], O. Farhat[1], F. Chen[2], B. C. Clark[3], **M. A. Schneegurt**[1]; [1]Wichita State Univ., Wichita, KS, [2]NASA Jet Propulsion Lab., Pasadena, CA, [3]Space Sci. Inst., Boulder, CO

Abstract Body:

Near-surface environments on Mars are hyperarid with limited liquid water to support microbial growth. Potential sources of liquid water include deliquescent brines, liquids that form when hygroscopic salts absorb atmospheric moisture. (Per)chlorate salts form brines with low eutectic points that would remain liquid well below 0 °C on frigid Mars. Phoenix lander detected (per)chlorate salts at ~1% in polar regolith. Humidity in Mars' atmosphere appears sufficient to form deliquescent brines of (per)chlorate salts with the counterions calcium, iron, magnesium, and sodium. (Per)chlorate salts seem too oxidizing to be compatible with life, however these salts are stable and not reduced in aqueous solution. Previous studies on perchlorate-respiring anaerobes were done at concentrations far below those that significantly affect water activity or freezing point. We have examined growth tolerances to high concentrations of (per)chlorate salts using bacterial isolates from the Great Salt Plains of Oklahoma (NaCl-rich) and Hot Lake in Washington ($MgSO_4$-rich). Microbial growth tolerance to the 1% (~0.1 M) (per)chlorate level observed on Mars appears widespread among salinotolerant isolates of *Bacillus, Halomonas, Marinococcus, Nesterenkonia*, and *Planococcus*. Remarkably, growth was observed for certain isolates at 1 M (~10%; Aw ~0.96) concentrations of Na or Mg perchlorate. Tolerances to chlorate salts were higher, with growth observed at 2.75 M Na chlorate (Aw 0.89). Media with higher salt concentrations form precipitates unless acidified and iron and calcium salts were not soluble enough to substantially affect water activity. The bacteria do not appear to detoxify, reduce, or respire the anions, based on chromatographic measurements of (per)chlorate concentrations before and after cultivation. Most probable number analysis was used to measure the abundance of tolerant microbes in common oligohaline soils. Tolerance to 1% Mg perchlorate is prevalent and at 5%, 7 to 70 x 10^2 cells g^{-1} soil were observed, although no growth was detected at 10% Mg perchlorate. Our findings on microbial tolerance to (per)chlorate salts has relevance to the refinement of forward planetary protection protocols for missions to Mars and helps to set boundaries for potentially habitable regions on Mars.

Author Disclosure Block:

A. Al Soudi: None. **O. Farhat:** None. **F. Chen:** None. **B.C. Clark:** None. **M.A. Schneegurt:** None.

Poster Board Number:

FRIDAY-028

Publishing Title:

Perchlorate-Coupled Carbon Monoxide Oxidation by Extremely Halophilic *Halobacteriaceae*

Author Block:

M. R. Myers, G. King; Louisiana State Univ., Baton Rouge, LA

Abstract Body:

Recent studies have documented (per)chlorate reduction coupled to heterotrophic metabolism by extremely halophilic Euryarchaeota (*Halobacteriaceae*). (Per)chlorate reduction appeared to be a consequence of non-specific activity by the particulate dissimilatory nitrate reductase, pNar. The discovery of denitrifying CO-oxidizing *Halobacteriaceae* suggested that CO-based lithotrophic metabolism might also be coupled to (per)chlorate. This process is of particular interest, as it could support microbial activity in perchlorate-rich brines on Mars, the atmosphere of which contains substantial amounts of CO. We report here the first documented results of perchlorate-linked CO oxidation in extremely halophilic Euryarchaeota.Enrichments of salt crusts from the Bonneville Salt Flats and surrounding saline soils have yielded a library of novel CO-oxidizing isolates, including members of *Halobacterium, Haloarcula, Halorubrum, Haloterrigena, Natrinema, Halobaculum,* and *Halovenus.* Of these, isolates, *Halorubrum, Halobacterium, Haloarcula,* and *Halovenus* have been observed to denitrify using pyruvate as an electron donor. Isolates were screened for perchlorate-linked CO oxidation using a saline medium containing 10 mM perchlorate and 2.5 mM pyruvate, with or without 0.25 mM nitrate added to induce nitrate reductase. Anaerobic headspaces contained nitrogen with 10 ppm CO. Perchlorate and chlorate concentrations were quantified at the beginning and end of incubations with an ion-selective electrode or spectrophotometric analyses, respectively. Chlorite was also quantified spectrophotometrically. CO oxidation by *Halorubrum* sp. *WSN3* occurred in the presence of perchlorate and nitrate (0.25 mM), but not with perchlorate alone. Nitrate concentrations at 0.25 mM were not coupled to CO oxidation, but CO was oxidized in the presence of 25 mM nitrate. No CO uptake occurred with autoclaved controls or treatments lacking electron acceptors. Perchlorate was reduced when CO and pyruvate were present, but in incubations with CO alone, 10 ppm concentrations were too low to drive a detectable decrease. These results established CO as an electron donor for perchlorate reduction in denitrifying extreme halophiles for which nitrate reductase acts as a non-specific perchlorate reductase.

Author Disclosure Block:

M.R. Myers: None. **G. King:** None.

Poster Board Number:

FRIDAY-029

Publishing Title:

Community Analysis and Mercury Speciation in Flue Gas Desulfurization Units

Author Block:

G. Martin, J. M. Senko; The Univ. of Akron, Akron, OH

Abstract Body:

Although wet flue gas desulfurization (wFGD) systems have been largely successful at reducing SO_2 and NO_x emissions from coal combustion, they have only partially reduced Hg emissions. Hg found in forced-oxidation flue gas primary exists as elemental (Hg^0), oxidized (Hg^{2+}) and particle bound (Hg^p) mercury. Studies have shown a complex range of factors affect Hg speciation in wFGD, including physiochemical characteristics of the slurry such as ash/unburned carbon, chloride concentration, pH, and oxygen concentrations. Additionally, microbiological activities may modulate the speciation of Hg within wFGD units. On one hand, Microbiological activities may lead to reduction Hg^{2+} to Hg^0 removing the Hg by making it more volatile, thus enhancing "reemission." On the other hand, Hg^0 may be biologically oxidized to Hg^{2+}, thus minimizing Hg emission, although this Hg^{2+} may be methylated by microorgnisms, transforming the element to a species that is quite toxic and readily bioaccumulated. While microorganisms are present in wFGDs, little work has been conducted into the impacts microbial activities in wFGD units have on Hg re-emission or retention, despite the vital role of microbes in cycling Hg in the environment. wFGD slurry is a physicochemically extreme environment, operating at temperatures around 60^0C. They also contain high concentrations of dissolved solids, notably calcium, magnesium, sulfate, and chloride. Although these microorganisms have been found in wFGDs, the types and extents of their activities are not clear. The goal of this project is to characterize the wFGD slurry system and identify the microbial communities and activities within them. FGD slurry was collected from three power plants, designated A, B, and C. All systems operate at approximately 57^0C with pH 5-6, and high dissolved chloride and/or sulfate concentrations (as high as 130 mM and 118 mM, respectively). Broad microbiological activity was evaluated based on respirometry and Hg metabolism incubations. Preliminary results indicate that O2 consumption in non-sterile slurry exceeds that of biologically deactivated slurry, suggesting that at least a portion of the microorganisms associated with wFGD slurries are active. DNA was recovered from slurries (1.0-1.5 ng/.8 mL slurry) and a survey of the microbial community based on 16S rRNA gene sequences will be conducted.

Author Disclosure Block:

G. Martin: None. **J.M. Senko:** None.

Poster Board Number:

FRIDAY-030

Publishing Title:

Heterogeneous Distribution of Microbial Life Within Bonneville Salt Flats

Author Block:

S. Tahan, H. Zweifel, J. Shaver, B. KLEBA; Westminster Coll., Salt Lake City, UT

Abstract Body:

The Bonneville Salt Flats (BSF) of northwestern Utah is characterized by a perennial salt crust that spans ~ 75 square kilometers. Below the crust is a shallow brine aquifer and in the winter the aquifer increases in volume to form a shallow pond that covers the crust until temperatures warm again. Though the hydrology of the basin has been studied extensively with interest in potash mining the region has historically been viewed as void of life as no macro flora are present. Nonetheless, work from our lab has shown that viable cells can be found on the surface of the crust suggesting that BSF is inhabited after-all. Because the cultivated organisms came from surface halite we reasoned that the viable cells could take up residence on BSF via two possible, mechanisms: 1) halophiles were deposited on the surface of BSF via wind from neighboring Great Salt Lake or 2) BSF harbors its own community of halophiles that thrive in underlying strata of the crust. To begin to discern between these two possibilities we set out to determine whether viable cells could also be found in brines and subsurface strata of the crust using both culture-dependent and culture independent techniques. Fluorescence microscopy and cultivation on modified R2A plates containing 23% salt demonstrate that viable cells can be found within subsurface compartments. Because the crust is comprised of a number of chemically defined strata, the mineralogical composition of which may impede growth, we then hypothesized that different strata might harbor different concentrations of cells. To test this idea, we measure CFUs obtained from each sample and live-dead staining to calculate the density of viable cells in each compartment. The greatest concentration of living cells was found within a green layer below the surface halite layer suggesting that BSF harbors its own resident community of microbes and that specific strata within the crust are more conducive to life than others. Because salt is known to preserve ancient life-forms and salt deposits have been identified on planetary bodies other than Earth BSF has the potential to serve as a model system to address questions about the relationship between the mineralogy and chemistry of evaporite deposits and habitability as well as the preservation of life in extreme environments on Earth and beyond.

Author Disclosure Block:

S. Tahan: None. **H. Zweifel:** None. **J. Shaver:** None. **B. Kleba:** None.

Poster Board Number:

FRIDAY-031

Publishing Title:

Bacterial Biodiversity from the Eroded Surface Soils of the Island of Mayotte

Author Block:

J. R. Osman Naoum, C. Regeard, G. R. Fernandes, M. DuBow; Université Paris-Saclay, Orsay, France

Abstract Body:

"What microbes are where, and how do they live there" is now an essential question to understand life on Earth, even when comparing seemingly similar ecosystems in different locations. Metagenomic approaches, using high-throughput DNA sequencing, have become indispensable for studying populations of environmental microbes, as the vast majority of these microbes have been found to be non-cultivable under laboratory conditions. We examined the "Padza de Dapani" on the island of Mayotte off the east coast of Africa as this region is not a true (hot) desert, but resembles one due to extensive soil erosion. We collected surface soil samples from five different sites of the Padza de Dapani in Mayotte. We examined bacterial biodiversity using pyrosequencing of PCR-amplified 16S V3-V4 rDNA sequences from total extracted DNA. Our results show that in the acidic (pH 4.6 - 6), oligotrophic (organic carbon: 0.1-0.7 g/kg of soil) and mineralized (Fe: 18g/100g; Al: 12g/100g) soil samples from Mayotte, members of the *Actinobacteria*, *Proteobacteria* and *Acidobacteria* phyla dominated the bacterial populations. Interestingly, members of the genera *Acinetobacter*, *Arthrobacter* and *Bacillus* were found to be predominant in our samples, as is also observed in hot Asian deserts such as the Gobi (northern China) and Taklamaken (northwest China) deserts. The number of species (OTUs) and richness (Chao1) is higher in samples with pH >5.5, whereas the diversity (Shannon index) was found to be higher in samples with lower pH (<5) and containing organic carbon higher than 0.32 g/kg of soil. The bacterial populations were predicted based on the normalized copy number of the 16S rRNA gene using the program Tax4fun. Amino acid metabolism (12%), carbohydrate metabolism (14%) energy metabolism (7%) and membrane transport (10%) are the principal KEGG pathways in the functional diversity of bacteria present in our samples. Ongoing analyses will permit us to compare the bacterial diversity of selected worldwide soil samples with that we observe here to better understand microbial populations in oligotrophic soils and perhaps lead to ideas to prevent, stop and even reverse soil erosion.

Author Disclosure Block:

J.R. Osman Naoum: None. **C. Regeard:** None. **G.R. Fernandes:** None. **M. DuBow:** None.

Poster Board Number:

FRIDAY-032

Publishing Title:

Melainabacteria Found in Antarctic Ice-Covered Lake Vanda Pinnacles

Author Block:

K. Wall[1], T. Mackey[1], A. Jungblut[2], I. Hawes[3], D. Sumner[1]; [1]UC Davis, Davis, CA, [2]The Natural HIstory Museum, London, United Kingdom, [3]Univ. of Canterbury, Christchurch, New Zealand

Abstract Body:

Lake Vanda is a perennially ice-covered lake in Wright Valley of the McMurdo Dry Valley region of Antarctica. The lake is a microbially dominated ecosystem, with few macroscopic metazoans. Within the photic zone, the lake floor is covered with thick microbial mats, with decimeter-tall pinnacles. Previously, the biota of the the pinnacles was only known from limited morphology studies. In 2013, Lake Vanda pinnacles of a range of sizes and from two different depths were collected, and dissected into subsamples based on mat stratification. DNA was extracted and used as templates for 16s rRNA PCR. The barcoded PCR products were then sequenced on the Illumina MiSeq. DNA analysis of the mats demonstrated the presence of Melainabacteria, a deeply branching clade of bacteria which share ancestry with Cyanobacteria. Known Melainabacteria lack photosynthesis genes and have thus far been found to generally be fermenting organisms, with a reduced genome. They are typically found in the gut, and other anaerobic habitats such as aquifers and bioreactors. In contrast, Lake Vanda pinnacles are supersaturated with respect to O_2, at least in summer when the mats were sampled.The Lake Vanda pinnacles showed an unusually high percentages of Melainabacteria for environmental samples, with some samples having abundances as high as 2.9% of reads. Representatives of three groups of Melainabacteria were present, the Gastranaerophilales (YS2), Obscuribacteriales (MLE1-12), and Vampirovibrionales (SM1D11). Out of 161 samples analyzed, 157 contained Melainabacteria, and of those, 31 had abundances of greater than 1%. Of the groups of deeply branching Cyanobacteria present, the Obscuribacteriales were the most abundant, making up 94% of the Melainabacteria, and 0.68% overall abundance. The Vampirovibrionales made up most the remainder, and were present at 0.03% abundances overall. Gastaranaerophilales were present but at neglible amounts. The Melainabacteria showed distinct preferences towards certain pinnacle regions, but no distribution trends with lake depth or size of pinnacle could be determined.

Author Disclosure Block:

K. Wall: None. **T. Mackey:** None. **A. Jungblut:** None. **I. Hawes:** None. **D. Sumner:** None.

Poster Board Number:

FRIDAY-033

Publishing Title:

Fumaroles Support Oases of Microbial Life in the Highest Elevation Fumaroles on Earth

Author Block:

A. J. Solon, J. L. Darcy, L. Vimercati, S. K. Schmidt; Univ. of Colorado- Boulder, Boulder, CO

Abstract Body:

In a previous study in the dry Andes of mountains above 6000 meters above sea level (m.a.s.l.), a fumarole (geothermal vent) was discovered that supports an oasis of life in an otherwise mostly barren landscape. The initial study focused on only one fumarole at an elevation of 5825 m.a.s.l., whereas the current study re-sampled that fumarole and an additional fumarole discovered near the summit at an elevation of 6050 m.a.s.l.; the highest elevation fumarole ever investigated. For comparative purposes, three non-fumarole sites were also sampled, two at 5825 m.a.s.l. and one at 6050 m.a.s.l. Biogeochemical parameters (enzyme activity, percent carbon and nitrogen, and gas fluxes) were measured on most of the samples in addition to microbial community structure. Following DNA extraction of the soils, the QIIME pipeline was used to compare each community and statistical tests (e.g. Adonis) were done to determine the effects of fumarolic activity, elevation, and biogeochemical parameters on Alpha and Beta diversity of SSU rRNA identified18S and 16S communities. Fumarole samples had much higher Alpha diversity than all non-fumarole samples, e.g. >575 16S OTUs per 5000 sequences for fumarole soils compared to <175 per 5000 sequences for non-fumarole sites. The same pattern was seen with eukaryotes with 275 18S OTUs per 7800 sequences for fumarole soils compared to 75 per 7800 sequences for non-fumarole sites. Furthermore, Beta diversity using weighted unifrac metrics grouped fumarolic soils closely together to the exclusion of non-fumarolic soils revealing a similarity of community composition for all three domains of life. Statistical significance was confirmed using Adonis (R vegan package) to compare fumaroles to non-fumaroles; elevation and biogeochemistry had no significant effect on diversity. These findings indicate that fumarolic activity allows the proliferation of complex microbial communities even in environments that are devoid of much life due to dryness and cold associated with extreme elevation.

Author Disclosure Block:

A.J. Solon: None. **J.L. Darcy:** None. **L. Vimercati:** None. **S.K. Schmidt:** None.

Poster Board Number:

FRIDAY-035

Publishing Title:

Physiological Study of Purple Non-Sulfur Bacteria from Aquatic Environments in Puerto Rico

Author Block:

J. Rullan-Cardec, C. Rios-Velazquez; Univ. of Puerto Rico at Mayaguez, Mayaguez, PR

Abstract Body:

Purple non sulfur bacteria (PNSB) have been attracting the scientific community due to its metabolic diversity, physiology and biotechnological potential. Physiologically, some members of PNSB can growth in a wide range of temperature, salt and pH for what they are ubiquitous in nature, being isolated from different environments including those with extreme conditions. Despite of its wide distribution, it is estimated that only 3.7% of members of this group have been fully identified. The goal of this research is to study the physiology for future identification purposes of PNSB members, isolated from Heliconia and Bromeliad phytotelmata as well as water reservoirs of Puerto Rico. The isolates were partially identified by PCR amplification of 16SrDNA, followed by further sequencing and in silico analysis. To address the physiology of the isolates, growth in NaCl and pH ranging from 0 to 1% and 4 to 11 respectively were studied for each isolate. These tests were performed in 2mL-96 wells plates in Sistrom Media under phototrophic conditions for 5 days, measuring cell grow at 600nm. The data showed that 50 and 38% of the PNSB isolates from Heliconia phytotelmata could growth in a pH range from 6 to 11, and NaCl up to 3% respectively. One of these isolates was able to grow as high as 5% NaCl in the media with an optimum of 4%. In the Bromeliad phytotelmata, 79% and 60% of the isolates were able to growth in a pH range from 6 to 9 and NaCl up to 2% respectively. Two of the isolates of Bromeliad phytotelmata grew in a pH range from 5 to 11 and one of these isolates also grew at 4% of NaCl. From water reservoirs, 90% of the isolates grew in a pH range from 6 to 9, and 40% in up to 2% of NaCl in the media. Isolates belonging to the genus of *Rhodobacter* predominated in the Heliconia phytotelmata while the genus *Rhodopseudomonas* was predominant in Bromeliad phytotelmata and water reservoirs. This study confirms the diversity and versatile physiological traits of the PNSB in the studied environments, and suggests the need of a more extensive phylogenetic analysis for novel groups' determination. To our understanding this research represents the first attempt to study the physiology of PNSB from Bromeliad and Heliconia phytotelmata and water reservoirs in Puerto Rico.

Author Disclosure Block:

J. Rullan-Cardec: None. **C. Rios-Velazquez:** None.

Poster Board Number:

FRIDAY-036

Publishing Title:

Comparison of Halophyte (*Salsola stocksii*) Microbiome with Non-Halophyte (*Triticum aestivum*) Microbiome

Author Block:

S. Mukhtar; Forman christian Coll., Lahore, Pakistan

Abstract Body:

The distribution of saline soils on more than half a billion hectare worldwide, warrants attention for their efficient, economical and environmentally acceptable management practices. Halophytes are progressively utilized for human benefits. Halophyte microbiome contributes significantly to plant performance and can provide information regarding complex ecological processes involved in osmoregulation of halophytes. The basic aim of this study is to investigate the microbiomes associated with aboveground (phyllosphere), below-ground (rhizosphere) and internal (endosphere) tissues of halophytes. Culturable bacteria were characterized morphologically, physiologically, biochemically and identified by PCR amplification of specific 16S rRNA gene sequences. Sixty two strains were selected after screening of salt tolerance. It has been earlier reported that some of the osmoregulatory genes are present on the plasmid. In order to demonstrate it, plasmid curing of isolates was done by using SDS and sodium benzoate to study the effect of plasmid conferring salt tolerance. These plasmids were isolated and transformed into *E. coli* and growth response of original strains. Culture independent bacterial diversity was assessed by sequence analysis of 16S rRNA gene. Almost all strains showed optimum growth at 2-3.5M NaCl. These strains were related to *Bacillus spp.*, *Marinococcus* spp., *Micrococcus* spp., *Planococcus* spp., *Nesterenkonia* spp. and *Kocuria* spp. Mostly transformed *E. coli* were able to grow upto 3.5M NaCl concentrations. Metagenomic analysis from phyllosphere, rhizosphere and endosphere showed that approximately 40% bacteria were uncultured and unclassified. Actinobacteria, Firmicutes, Proteobacteria, Acidobacteria, Bacteriodetes, Plantomycetes, Cyanobacteria, Thermotogae and Choroflexi were predominant groups from halophyte plants. Results for cultured as well as for uncultured bacteria has revealed a wide diversity of halophilic bacterial population present in phyllosphere, rhizosphere and endosphere of halophytes.

Author Disclosure Block:

S. Mukhtar: None.

Poster Board Number:

FRIDAY-038

Publishing Title:

Functional Metagenomics, A Novel Qiagen Bioinformatics Pipeline Enables Fast High-Quality Analysis of the Functional Content of Microbial Communities

Author Block:

A. Sand, F. Strino, A. S. Christensen, M. Bundgaard, A. C. Materna; QIAGEN Aarhus, Aarhus C, Denmark

Abstract Body:

BACKGROUND: Next generation sequencing based studies have revolutionized the field of microbial ecology, revealing unprecedented insight into environmental as well as host-associated microbiomes. However, still very few tools offer efficient and user-friendly analysis of the functional content of microbiomes.**METHODS:** We have implemented a pipeline of tools for functional and comparative analysis of microbiomes in the CLC Microbial Genomics Module, a component of QIAGEN's new bioinformatics solution for microbial genomics. The first step in the pipeline produces high-quality metagenome contigs using a novel high-performance metagenome de novo assembler. The resulting contigs are subsequently CDS-annotated using MetaGeneMark, and the CDSs are annotated with PFAM protein families, GO terms and top BLAST hits (using e.g. UniProt). Finally, the relative abundance of the functional elements in each dataset is computed and presented to the user.The functional contents of individual samples can be studied in a tabular format or visualized as pie charts, and visual comparison of multiple samples is done using stacked bar charts, layered area charts and principal coordinate plots. Finally, statistical comparison of multiple microbiome samples is performed to back-up our observations.We present benchmarks of this pipeline performed on both a previously published mock community dataset and on several previously published simulated microbiome datasets designed for benchmarking of microbiome analysis solutions.**RESULTS:** We demonstrate that the new metagenome de novo assembler runs as fast as the fastest alternative but uses only a fraction of the memory required by any other benchmarked de novo assembler. At the same time it produces contigs that are both longer than the contigs produced by other popular metagenome assemblers and are of significantly higher quality than the contigs produced by any other benchmarked de novo assembler.We furthermore demonstrate that the high-quality contigs produced by the new de novo assembler enables taxonomic and functional profiling of very high quality.Finally, we demonstrate that the complete analysis pipeline, producing functional profiles from raw microbiome read datasets, is able to reliably detect changes in the functional content of microbiomes.

Author Disclosure Block:

A. Sand: D. Employee; Self; QIAGEN Aarhus. **F. Strino:** D. Employee; Self; QIAGEN Aarhus. **A.S. Christensen:** D. Employee; Self; QIAGEN Aarhus. **M. Bundgaard:** D. Employee; Self; QIAGEN Aarhus. **A.C. Materna:** D. Employee; Self; QIAGEN Aarhus.

Poster Board Number:

FRIDAY-039

Publishing Title:

Evaluation of Computational Methods for Human Microbiome Analysis Using Simulated Data

Author Block:

E. Castro-Nallar[1], S. L. Valenzuela[1], D. Simone[2], M. Pérez-Losada[2], W. E. Johnson[3], K. A. Crandall[2]; [1]Univ. Andres Bello, Santiago, Chile, [2]The George Washington Univ., Ashburn, VA, [3]Boston Univ., Boston, MA

Abstract Body:

Our understanding of the composition, function, and health implications of human microbiota has been advanced by high-throughput sequencing and the development of new genomic analyses. However, tradeoffs among alternative strategies for the acquisition and analysis of sequence data remain understudied. How do sequencing layout, sample complexity, and analysis pipeline affect taxonomic profiles? In order to approach this, we simulated metagenomic datasets reflecting different read lengths (75-1000 bp), sequencing depths (100 k-10 M), number of species (10-1000), presence of highly related strains, and various combinations of viruses/bacteria. Likewise, we simulated different database composition scenarios including presence/absence of dominant microbes in the database. The resulting simulation design yielded ~1152 datasets analyzed using five pipelines (MetaPhlan2; metaMix, PathoScope2, Sigma, and ConStrains). We evaluated pipeline performance based on ROC analysis (specificity/sensitivity), relative root mean square error, and average relative error.For instance, when one microbial species is dominant (= 50% of simulated reads), marker-based methods tend to decrease in sensitivity. Conversely, genome-based methods do not exhibit this trend, rather sensitivity decreases when simulated reads are more distributed towards the 10-50 percentiles. When simulated reads are homogenously distributed among species, we observed that both marker- and genome-based methods perform relatively better. In addition, both marker- and genome based methods exhibit larger relative errors when dealing with 1000 bp reads, presumably as a consequence of the read mapper not being optimized for such read lengths. We consistently found smaller error estimates for the 75-300 bp read length range irrespective of the method, although genome-based methods show slightly larger relative error at 75 bp.Our study enables researchers to make informed decisions relative to strengths and weaknesses of current taxonomic profiling methods, and adjust their sequencing experiments accordingly. All datasets and parameter values used in the study are freely available to ensure reproducibility and future pipeline benchmarking.

Author Disclosure Block:

E. Castro-Nallar: A. Board Member; Self; Aperiomics Inc.. **S.L. Valenzuela:** None. **D. Simone:** None. **M. Pérez-Losada:** None. **W.E. Johnson:** A. Board Member; Self; Aperiomics Inc. **K.A. Crandall:** A. Board Member; Self; Aperiomics Inc..

Poster Board Number:

FRIDAY-040

Publishing Title:

Integrated Microbiome Resource (Imr): Developing an Open and Streamlined Experimental and Analysis Pipeline for Microbiome Research

Author Block:

A. M. Comeau, **M. G. I. Langille**; Dalhousie Univ., Halifax, NS, Canada

Abstract Body:

Microbiome studies have revolutionized the microbiology field and are becoming increasingly popular. In recent years, advances in sequencing technologies and in bioinformatic methods have led to faster and more robust methods for generating and analyzing data. The Comparative Genomics and Evolutionary Bioinformatics - Integrated Microbiome Resource (CGEB-IMR: http://cgeb-imr.ca/) has streamlined and connected each essential step of a microbiome study starting with samples and ending with various plots and tables ready for interpretation within a single workweek. Our pipeline can handle up to 380 samples per sequencing run covering a variety of amplicon targets (16S, 18S, ITS, Bar-Seq, etc.). In our first year of operation we have processed 4694 samples generating 423M sequences and 236G bases from a variety of host-associated (e.g. humans, mice, rats, fish, insects, birds, reptiles) and environmental (e.g. soil, waste water, marine) biomes. These samples encompass 52 projects from 26 principal investigators from several countries. We openly present each step of this resource including primer validation, library preparation, sequencing, quality control, paired-end assembly, taxonomic annotation, functional annotation (metagenomes), predictive functional annotation using PICRUSt (16S data), statistical evaluation, and visualization. This pipeline, Microbiome Helper (https://github.com/mlangill/microbiome_helper), is continuously updated based on evolving best practices and can be replicated in other locations with only standard molecular and computational equipment, minimum personnel, and access to a bench top next-generation sequencer (e.g. Illumina MiSeq). Our results illustrate that microbiome studies can be easily conducted in various scientific settings, including for time-sensitive applications, and provide a complete experimental and analysis package that can be replicated by other microbiome researchers.

Author Disclosure Block:

A.M. Comeau: None. **M.G.I. Langille:** None.

Poster Board Number:

FRIDAY-041

Publishing Title:

Analysis of Expanded Human Mircobiome Project Data by the Hmp Data Analysis and Coordination Center

Author Block:

C. McCracken, H. Creasy, J. Crabtree, J. Orvis, A. Brady, V. Felix, **M. Giglio**, A. Mahurkar, O. White; Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract Body:

Background: The NIH Common Fund initiated the Human Microbiome Project (HMP) to explore the microbial communities of the human host and characterize their role in human health and disease. An initial set of publications in 2012 (HMP Consortium, 2012) reported analysis of the first 681 whole-metagenome-shotgun (WMS) datasets. Since then considerably more sequencing has resulted in a total of ~2400 WMS datasets from the healthy HMP cohort sampled from 18 different body sites, many from multiple visits over time. As the Data Analysis and Coordination Center for the HMP, we have carried out extensive analysis of this updated dataset.**Methods:** We developed a new assembly pipeline for this data utilizing the IDBA-UD (Peng, 2012) and DigiNorm (Brown, 2012) tools. In addition, we have done combined assemblies for all datasets that are derived from samples taken at multiple visits from the same person at the same body site. For functional annotation, we have developed a comprehensive pipeline and applied it to all of the WMS gene sets derived from the assemblies. The pipeline performs HMM, pairwise, and multiple motif searches. We have optimized the pipeline for speed by employing RAPSearch2 (Zhao, 2012) as an alternative to BLAST. Functional annotations are assigned to each predicted protein with Attributor, a tool that we developed which employs an evidence hierarchy to determine the best annotations for each protein based on the highest quality evidence. Finally, we have carried out taxonomic profiling by mapping reads to the MetaRef (Huang, 2014) collection of reference genomes to determine the phylogenetic composition of the samples.**Results:** These analysis results constitute a huge increase in the available set of HMP assemblies, gene sets, annotations and phylogenetic profiles. All results are available on our HMP Data Analysis and Coordination Center web resource at http://ihmpdcc.org/. An advanced query builder allows users to select primary sequence and derived analysis datasets using a variety of metadata filters.**Conclusions:** This work provides the microbiome research community with a significant set of analysis products and resources. These can be directly mined to answer biological questions or used as inputs for additional microbiome analyses.

Author Disclosure Block:

C. McCracken: None. **H. Creasy:** None. **J. Crabtree:** None. **J. Orvis:** None. **A. Brady:** None. **V. Felix:** None. **M. Giglio:** None. **A. Mahurkar:** None. **O. White:** None.

Poster Board Number:

FRIDAY-042

Publishing Title:

Bugbase Predicts Community-wide Microbiome Phenotypes

Author Block:

T. Ward[1], J. Lynch[1], D. Sidiropoulos[1], R. Blekhman[1], R. Fink[2], D. Knights[1]; [1]Univ. of Minnesota, St Paul, MN, [2]St. Cloud State Univ., St Cloud, MN

Abstract Body:

With the rapid expansion of microbiome studies, the complexity of microbial communities and the intricacy of host-microbiome interactions have become apparent. Many microbiome studies rely on amplicon sequencing of marker genes to determine the taxonomic composition of a microbiome. Although useful tools such as PICRUSt now allow us to estimate the total genomic content of microbiomes using marker gene surveys, functional characterizations at the level of metabolic pathways can still be challenging to interpret. We hypothesized that we could predict several biologically relevant microbiome-wide traits like Gram staining, oxygen tolerance, the ability to form biofilms, pathogenicity, stress tolerance and mobile element content at the microbiome-wide level based on existing annotations and the presence of open reading frames within the genomes of community members. By combining a curated annotation database with the PICRUSt framework we have developed BugBase, a user-friendly tool that characterizes microbiome phenotypes using marker gene survey data. BugBase, which is available as a web-application or open-source package, requires users to input an OTU table and mapping file, which are used to generate phenotype predictions in the form of publication quality plots with corresponding statistical analyses. Using published data we have predicted microbiome-wide traits to vary significantly across a variety of host and environmental parameters, including bodysite, antibiotic exposure, disease status, geographical location and pH. We believe BugBase will provide researchers with useful, broad phenotypic descriptions of microbiomes that will simplify the interpretation of complex microbial biomarkers and serve as a jumping off point for further hypothesis driven in-depth analysis.

Author Disclosure Block:

T. Ward: None. **J. Lynch:** None. **D. Sidiropoulos:** None. **R. Blekhman:** None. **R. Fink:** None. **D. Knights:** None.

Poster Board Number:

FRIDAY-043

Publishing Title:

Contact-dependent Signaling (Cds) Facilitates Mobilization of a Genomic Island in *B. Thailandensis*

Author Block:

A. M. Barrero-Tobon, P. A. Cotter; Univ. of North Carolina at Chapel Hill, Chapel Hill, NC

Abstract Body:

In their natural environment, bacteria are typically surrounded by other microorganisms and for this reason they have developed complex mechanisms to allow communication, cooperation, or competition with neighboring cells. One such mechanism utilized by bacteria to kill neighboring cells and control the surrounding population is contact-dependent growth inhibition (CDI). A major component of the CDI system is the large exoprotein BcpA, which contains a toxic C-terminus (CT) and is responsible for the induction of cell death upon delivery to a target bacterial cell. However, recent findings suggest that a delivered BcpA-CT also serves as a molecule that mediates intercellular signaling. During contact-dependent signaling (CDS) in *Burkholderia thailandensis*, the incoming BcpA-CT induces changes in gene expression in recipient cells resulting in phenotypes such as production of a yellow/brown pigment and aggregation of cells cultured in minimal media. Absence of the *bcpAIOB* locus or production of a chimeric BcpA abolishes CDS. We isolated a series of spontaneous mutants that arose from chimeric CDS-deficient strains that regained the ability to produce pigment and display other BcpA-dependent signaling phenotypes. We used transposon mutagenesis and RNAseq analyses to study these mutants and identified several pathways, such as flagellar biosynthesis and other metabolic processes, that are affected during CDS. Furthermore, we performed whole-genome sequencing to determine the genetic basis of these pseudo-revertant mutants and found that the *bcpAIOB* locus is located on a large genomic island and mobilization of this genomic element is dependent on CDS. Based on these findings we propose a mechanism in which the BcpAIOB proteins identify a neighboring target cell as "self" or "non-self" based on the *bcpAIOB* allele present. Furthermore, once a bacterial cell encounters a neighboring "non-self" cell, the BcpAIOB proteins then mediate inhibition of the target cell or perhaps transfer of the *bcpAIOB* genes to transform the cell into "self." Our work describes how, within a microbial community, the BcpAIOB proteins have the ability to mediate diverse types of interactions with different outcomes: elimination of neighboring cells to shape the population, intercellular communication, or propagation of the "self" *bcpAIOB* allele.

Author Disclosure Block:

A.M. Barrero-Tobon: None. **P.A. Cotter:** None.

Poster Board Number:

FRIDAY-044

Publishing Title:

Toxin Gene Regulation of *Clostridium perfringens* by Signal Substance Produced by *Clostridium butyricum*

Author Block:

K. Ohtani[1], K. Oka[1], M. Takahashi[1], J. Nakayama[2], T. Shimizu[3]; [1]Miyarisan pharmaceutical co.,LTD, Tokyo, Japan, [2]Faculty of Agriculture, Graduate Sch., Kyushhu Univ., Fukuoka, Japan, [3]Graduate Sch. of Med. Sci., Kanazawa Univ., Kanazawa, Japan

Abstract Body:

Background: *Clostridium perfringens* is a Gram-positive anaerobic bacteria responsible for the clostridial myonecrosis (or gas gangrane), enteritis and enterotoxemia in humans and animals by producing numerous extracellular toxins and enzymes. It is well known that the toxin gene expression of *C. perfringens* is regulated by at least two-types of cell- cell signalling systems and VirR/VirS-VR-RNA cascade which controlled under the quorum-sensing. *C. butyricum* is a normally found in soil and animal intestine and strain of MIYAIRI588 has been used as probiotics. There was an old report that *C. butyricum* has inhibitory effect on the *C. perfringens* infection but the detailed mechanisms are still unclear. Therefore, we examined the effect of *C. butyricum* on the growth and toxin gene expression of *C. perfringens.* **Methods:** *C. perfringens* and *C. butyricum* were co-cultured by using cell culture plate and 0.4um pore size ThinCert™ membrane to separate them. Then total RNA was isolated from *C. perfringens* and transcription of toxin genes was analyzed by Northern hybridization. To check the effect of the supernatant, the supernatant was collected at various growth phase and added to the cells and total RNA was isolated after 15min incubation at 37°C.**Results:** The result showed that the growth of *C. perfringens* did not change when it was cultured with *C. butyricum*, but toxin genes expression was strongly repressed. A candidate gene related production of signal substance was cloned and transformed into *C. perfringens*. The result showed the candidate gene had a negative effect on toxin gene expression. Furthermore the supernatant collected from the candidate gene overexpressed strain repressed the toxin gene expression.**Conclusions:** These data indicated that inter-species signaling of toxin gene expression of *C. perfringens* was repressed by *C. butyricum* and reduces toxin production. Now we are trying to analyze the mechanism and components of the toxin genes regulation by C. butyricum in detail.

Author Disclosure Block:

K. Ohtani: None. **K. Oka:** None. **M. Takahashi:** None. **J. Nakayama:** None. **T. Shimizu:** None.

Poster Board Number:

FRIDAY-045

Publishing Title:

Evolution in Action: *In Vivo E*xperimental Evolution of the Pathogenic Bacterium *Citrobacter rodentium*

Author Block:

S. Wiles; Univ. of Auckland, Auckland, New Zealand

Abstract Body:

Bacteria are masters at adapting to their environment, rearranging, losing or mutating their genetic material or gaining new genes from their surroundings. Indeed, studies of bacterial adaptation in simple laboratory environments have provided direct insights into important evolutionary processes. We have moved microbial evolution experiments out of the flask and into a highly complex environment which can be manipulated with ease, the laboratory mouse, to experimentally investigate the factors that influence the adaptation of pathogenic microbes. We are using the bacterium *Citrobacter rodentium* which naturally infects mice using the same 'modus operandi' as some life-threatening human *Escherichia coli* strains. C57BL/6 mice were orally inoculated with bioluminescently-tagged *C. rodentium* and individually housed animals allowed to infect naïve animals through tightly controlled mouse-to-mouse exposure, a process which was repeated weekly over a period of 6 months. 'Evolved' *C. rodentium* have been isolated following 20 transmission steps, and we are currently assessing the strains for phenotypic and genotypic changes. The strains have shown varying improvements in fitness and transmissibility compared to their ancestor, with the evolution of a hypertransmissible isolate identified. This work extends existing flask-based experimental evolution with an *in vivo* model which focuses on complex, medically applicable real-world environments.

Author Disclosure Block:

S. Wiles: None.

Poster Board Number:

FRIDAY-046

Publishing Title:

Statistical Mechanics and Thermodynamics of Viral Evolution

Author Block:

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Abstract Body:

Background: In this work we use methods drawn from physics to study the life cycle of viruses. **Methods:** We will present and analyze a model of viral infection and evolution using the "grand canonical ensemble" and formalisms from statistical mechanics and thermodynamics. This approach is interesting because it make it possible to enumerate all possible genetic states of a model virus and host as a function of two independent pressures-immune response and system temperature. **Results:** Our results prove the system has a real thermodynamic temperature, and we discover a new phase transition between a positive temperature regime of normal replication and a negative temperature “disordered” phase of the virus. From an evolutionary biology point of view, at steady state the viruses naturally evolve to distinct quasispecies. The analysis also reveals a *universal* relationship that relates the order parameter (as a measure of mutational robustness) to evolvability in agreement with recent experimental and theoretical work. **Conclusions:**Given that real viruses have finite length RNA segments that encode proteins which determine virus fitness, the approach used here could be refined to apply to real biological systems, perhaps providing insight into immune escape, the emergence of novel pathogens and other results of viral evolution.

Author Disclosure Block:

J.H. Kaufman: D. Employee; Self; IBM Research. **B. Jones:** D. Employee; Self; IBM Research. **S. Bianco:** D. Employee; Self; IBM Research. **J. Lessler:** None.

Poster Board Number:

FRIDAY-047

Publishing Title:

MDSINE: Microbial Dynamical Systems Inference Engine for Microbiome Dynamics Forecasting

Author Block:

V. Bucci[1], B. Tzen[2], N. Li[2], M. Simmons[1], T. Tanoue[3], E. Bogart[2], L. Deng[2], V. Yeliseyev[2], M. L. Delaney[2], Q. Liu[2], B. Olle[4], R. R. Stein[5], K. Honda[3], L. Bry[2], G. G. Gerber[2]; [1]Univ. of Massachusetts Dartmouth, N. Dartmouth, MA, [2]Brigham and Women's Hosp., Harvard Med. Sch., Boston, MA, [3]RIKEN Ctr. for Integrative Med. Sci., Yokohama, Japan, [4]Vedanta BioSci.s Inc., Boston, MA, [5]Sloan Kettering Inst., New York, NY

Abstract Body:

Background: The microbes that colonize our bodies are important in maintaining health. Efforts are intensifying to manipulate the microbiota to treat a variety of human diseases. Predicting temporal dynamics of host-microbial ecosystems is crucial for rational development of these treatments, to forecast how interventions will affect the microbiota over time. However, standard computational methods for analyzing microbial community structures cannot be used to predict dynamic behaviors of these ecosystems. Dynamical systems models provide an alternative and powerful analysis framework, but existing algorithms for inferring these models from microbiome time-series data suffer from major shortcomings; moreover, complete software packages have not been made available to the community. **Results:** We present the Microbial Dynamical Systems INference Engine (MDSINE), an open-source software package for inferring dynamical systems models from microbiome time-series datasets, predicting future behaviors of the microbiota, and visualizing results. Using data simulated to mimic key properties of real microbiome studies, we demonstrate that MDSINE significantly outperforms the existing method for microbial dynamical systems inference on multiple metrics. We then demonstrate the utility of our method on two new gnotobiotic mice experimental datasets, investigating infection with an enteric pathogen, *Clostridium difficile*, and stability of an immunemodulatory probiotic cocktail. For the *C. difficile* dataset, we use MDSINE to accurately forecast dynamics of the pathogen and to predict minimal sub-communities of commensal bacteria that stably coexist and inhibit *C. difficile* colonization. In the second example application, we use a new quantitative "keystoneness" measure implemented in MDSINE to determine which bacteria in the probiotic cocktail are predicted to be most crucial to maintaining integrity of the community structure in response to a dietary perturbation.

Author Disclosure Block:

V. Bucci: B. Collaborator; Self; Vedanta Biosciences. **B. Tzen:** None. **N. Li:** None. **M. Simmons:** None. **T. Tanoue:** B. Collaborator; Self; Vedanta Biosciences. **E. Bogart:** None. **L.**

Deng: None. **V. Yeliseyev:** None. **M.L. Delaney:** None. **Q. Liu:** None. **B. Olle:** A. Board Member; Self; Vedanta Biosciences. **R.R. Stein:** None. **K. Honda:** A. Board Member; Self; Vedanta Biosciences. **L. Bry:** None. **G.G. Gerber:** None.

Poster Board Number:

FRIDAY-049

Publishing Title:

Diverse Modes of Eco-Evolutionary Dynamics in Communities of Niche-Constructing Bacteria

Author Block:

K. Vetsigian; Univ. of Wisconsin-Madison, Madison, WI

Abstract Body:

Background: A recent theoretical work demonstrated that the interplay between antibiotic production and degradation can lead to robust coexistence of many microbes with different antibiotic production and resistance capabilities. The question however remains whether evolution can naturally arrive at such stable communities. **Methods:** We performed long-term, spatially-explicit eco-evolutionary simulations in which bacteria competed for a single resource in a homogeneous two-dimensional environment that was subjected to periodic mixing. The bacteria could mutate their antibiotic production and degradation capabilities with respect to multiple antibiotics, and we recorded how the diversity of phenotypes evolved over time and how it depended on parameters such as the costs of production and resistance.**Results:** We discovered that the eco-evolutionary dynamics can spontaneously arrive at diverse evolutionary-stable states, demonstrating that the interplay between production and degradation is a viable mechanism for generating diversity in one-niche environments. Moreover, evolutionary stable states were just one of the possible outcomes of the eco-evolutionary dynamics. As we varied parameters, we observed regimes with Red-Queen dynamics with no long-lived phenotypes, sudden transitions between long-persisting ecologically stable states, and intermittency in which chaotic evolutionary dynamics was interrupted by long periods of quasi-stability.**Conclusions:** We found that simple models of evolving niche-constructing bacteria can already exhibit complex and diverse modes of eco-evolutionary dynamics. These findings greatly enhance our understanding of the different possible manifestations of eco-evolutionary dynamics and the factors that control the transitions between different regimes. Our work can also provide a platform for interpreting the diverse outcomes in evolving natural microbial communities and experimental microcosms.

Author Disclosure Block:

K. Vetsigian: None.

Poster Board Number:

FRIDAY-050

Publishing Title:

Is Specialization a Typical Outcome of 1000 Generations of Evolution in Mutualism

Author Block:

D. Henderson; Univ. of Washington Bothell, Bothell, WA

Abstract Body:

It is thought that species diversification sometimes occurs when isolated subpopulations adapt to the particular features of the predators, prey, or cooperative partners that are specific to their local environment. To what extent might this hypothesis explain the diversification of microbial species, which cooperate or compete with each other in complex communities? We tested whether specialization was a common outcome of evolution in conditions requiring cooperation with another species for survival. The bacterium *Desulfovibrio vulgaris* was propagated for 1000 generations with the archaeon *Methanococcus maripaludis* in conditions that required them to cooperate through the exchange of hydrogen. To test whether *D. vulgaris* populations tended to become specialized for the *M. maripaludis* populations they evolved with (and vice versa), we revived 12 independently-evolved communities, separated the *D. vulgaris* and *M. maripaludis* populations from other another, and then paired each population back up with their sympatric partner or with 5 allopatrically-evolved partners. We then tested whether sympatric pairings were more efficient than allopatric pairings by comparing their growth rate and yield. While some *D. vulgaris* and *M. maripaludis* populations consistently caused faster community growth or productivity, there was not consistent relationship between sympatric and allopatric pairings. We used microscopy to quantify the density of *D. vulgaris* and *M. maripaludis* to test whether the absolute fitness of either species was higher in sympatric versus allopatric pairings. These tests gave similar results, showing that some *D. vulgaris* or *M. maripaludis* populations had higher absolute fitness on average across all 6 partners, but not specifically with sympatric partners. These results suggest that the first 1000 generations of adaptation to a new mutualism are not characterized by specialization.

Author Disclosure Block:

D. Henderson: None.

Poster Board Number:

FRIDAY-051

Publishing Title:

Disentangling Ecological Processes And Drivers In Subsurface Microbial Community Assembly In A Nuclear Waste Site

Author Block:

D. Ning[1], J. Wang[1], J. D. Van Nostrand[1], L. Wu[1], P. Zhang[1], Z. He[1], M. B. Smith[2], A. M. Rocha[3], S. W. Olesen[2], C. Paradis[4], J. H. Campbell[5], J. L. Fortney[4], T. L. Mehlhorn[3], K. A. Lowe[3], J. E. Earles[3], S. M. Techtmann[4], D. C. Joyner[4], D. Elias[3], K. L. Bailey[3], R. A. Hurt[3], S. P. Preheim[2], M. C. Sanders[2], M. A. Mueller[3], D. B. Watson[3], E. A. Dubinsky[6], P. D. Adams[6], A. P. Arkin[6], M. W. Fields[7], E. J. Alm[2], T. C. Hazen[4], A. Lancaster[8], B. J. Vaccaro[8], F. L. Poole[8], M. W. Adams[8], J. Zhou[1]; [1]Univ. of Oklahoma, Norman, OK, [2]MIT, Cambridge, MA, [3]ORNL, Oak Ridge, TN, [4]UTK, Knoxville, TN, [5]NWMSU, Maryville, MO, [6]LBNL, Berkeley, CA, [7]MSU, Bozeman, MT, [8]UGA, Athens, GA

Abstract Body:

Background: A central issue in ecology is understanding the processes shaping biodiversity. The groundwater in Oak Ridge Integrated Field Research Challenge site provides a rare opportunity to examine ecological processes and drivers shaping subsurface microbial diversity.**Methods:** Groundwater samples from 98 wells were analyzed for 205 environmental variables, as well as 16S rRNA genes by an Illumina MiSeq sequencer. After the phylogenetic signal was determined, the influence of selection was estimated based on beta nearest taxon index (βNTI). Then, turnovers not dominated by selection were analyzed using the Raup-Crick metric (RC) based on Bray-Curtis index to estimate the influence of dispersal limitation (RC>0.95) and homogenizing dispersal (RC<-0.95).**Results:** Across the entire site, both variable selection (44.6%) and dispersal limitation (46.3%) were important in shaping microbial diversity. Environment variables were used to fit the models with βNTI and RC using distance-based redundancy analysis. The model of βNTI identified 25 significant principal coordinates (PCs) imposing selection, with high loading of heavy metals, pH, nitrate, and DO. The model of RC identified 10 significant PCs imposing dispersal limitation, with high loading of spatial eigenvectors or metals in pellets. The spatial distribution of ecological processes showed the areas with little dispersal limitation for the microbial communities were those areas having low elevation, good diffusion of contaminants or a high level of dissolved oxygen.**Conclusions:** while contaminants and oxygen availability imposed obvious selection on subsurface microbial communities, the microbial spatial turnover was also largely affected by dispersal limitation, which may reflect actual underground dispersal conditions.

Author Disclosure Block:

D. Ning: None. **J. Wang:** None. **J.D. Van Nostrand:** None. **L. Wu:** None. **P. Zhang:** None. **Z. He:** None. **M.B. Smith:** None. **A.M. Rocha:** None. **S.W. Olesen:** None. **C. Paradis:** None. **J.H. Campbell:** None. **J.L. Fortney:** None. **T.L. Mehlhorn:** None. **K.A. Lowe:** None. **J.E. Earles:** None. **S.M. Techtmann:** None. **D.C. Joyner:** None. **D. Elias:** None. **K.L. Bailey:** None. **R.A. Hurt:** None. **S.P. Preheim:** None. **M.C. Sanders:** None. **M.A. Mueller:** None. **D.B. Watson:** None. **E.A. Dubinsky:** None. **P.D. Adams:** None. **A.P. Arkin:** None. **M.W. Fields:** None. **E.J. Alm:** None. **T.C. Hazen:** None. **A. Lancaster:** None. **B.J. Vaccaro:** None. **F.L. Poole:** None. **M.W. Adams:** None. **J. Zhou:** None.

Poster Board Number:

FRIDAY-052

Publishing Title:

Nitrate Tolerance Mechanisms In *desulfovibrio Vulgaris* Hildenborough By A Long-Term Evolution

Author Block:

B. Wu[1], A. Zhou[1], J. Li[2], J. D. Wall[3], A. Arkin[4], P. P. Adams[4], J. Zhou[1], Z. He[1], S. Chen[5]; [1]Univerisity of Oklahoma, Norman, OK, [2]Hunan Agricultural Univ., Changsha, China, [3]Univ. of Missouri-Columbia, Columbia, MO, [4]Lawrence Berkeley Natl. Lab., Berkeley, CA, [5]Hubei Univ., Wuhan, China

Abstract Body:

Background: Nitrate is considered an inhibitor of sulfate reduction in sulfate reducing bacteria (SRB). It has been suggested that the response of *Desulfovibrio vulgaris* Hildenborough (DvH) to nitrate is linked to the level of osmotic, ionic, nitrite and other less defined stressors. However, it has been reported that nitrate inhibition of DvH may be independent of nitrite production.**Methods:** In order to understand the adaptation and evolutionary mechanisms of *D. vulgaris* responses to elevated $NaNO_3$, we propagated 12 DvH populations under $NaNO_3$ stress (concentration) for 1000 generations and obtained nitrate-evolved populations (EN1-12).**Results:** The EN populations significantly increased nitrate tolerance and cell motility. Whole genome resequencing data indicated that the nitrate-evolved populations had a higher mutation frequency in genes involved in energy, amino acid, carbohydrate, nucleotide and lipid metabolism, cellular process and signal, and information storage and processing compared to the ancestral populations. Through predicting the interaction between mutated genes using String 10, we found that two gene clusters (DVU0251-DVU0245 and DVU2543-DVU2548) with a high mutation frequency may be responsible for nitrate tolerance. Further study with a deletion mutant of *DVU2547*, encoding a Crp/Fnr global transcriptional regulator in the latter gene cluster, confirmed the sequencing results.**Conclusion:** This study provides new insights into our understanding of nitrate tolerance mechanisms in *D. vulgaris.*

Author Disclosure Block:

B. Wu: None. **A. Zhou:** None. **J. Li:** None. **J.D. Wall:** None. **A. Arkin:** None. **P.P. Adams:** None. **J. Zhou:** None. **Z. He:** None. **S. Chen:** None.

Poster Board Number:

FRIDAY-053

Publishing Title:

Lymphoproliferative Disease Virus in Maine Wild Turkeys

Author Block:

J. Markham, P. Milligan; Univ. of Maine at Augusta, Augusta, ME

Abstract Body:

Repopulation of extirpated wild turkeys in Maine has been successful with an estimated fifty to sixty thousand birds currently living in the state. Assessment of the microbial health of Maine's wild turkey population is important for sustainability of agricultural livestock and the hunting industry. In 2009, Lymphoproliferative Disease Virus (LPDV) was detected by the Southeastern Cooperative Wildlife Disease Study in eastern wild turkeys, exhibiting lymphoid tumors. During the spring and summer of 2012, 15 Maine wild turkeys from Maine, 13 with overt disease, were euthanized for diagnostic examination. Skin and bone samples from the 15 birds were tested for LPDV by PCR. After confirmation of LPDV retroviral DNA in Maine wild turkeys, an additional 142 samples were assayed (4 birds with visible lesions and 138 apparently healthy birds). PCR testing of skin, bone marrow, and / or blood samples showed that 125 of 157 wild turkeys tested were positive for LPDV proviral DNA. Comparison of the estimated LPDV confidence interval in the total 2012 Maine turkey population, and our sample results showed that the margin of error at 95% confidence level (74-84%) encompasses the 80% found in our samples. Twenty-one domestic turkeys sampled in 2013 were negative for LPDV, as were 90 domestic turkeys sampled in 2014 from seven farms in central Maine. Historically, LPDV was only known to occur in domestic turkeys in Europe and Israel; however, since 2009, LPDV has been identified in wild turkeys from multiple sites throughout the Eastern United States. The implications of LPDV for wild turkey populations in Maine, as well as the risks for wild Galliformes (grouse and pheasant) and domestic fowl, are not clearly understood.

Author Disclosure Block:

J. Markham: None. **P. Milligan:** None.

Poster Board Number:

FRIDAY-054

Publishing Title:

Surrogate Host and *In Vivo* Animal Infection Assays for *Riemerella anatipestifer*

Author Block:

H-Y. Wang, C-L. Chen, G-L. Huang, **S-H. Wang**; Natl. Chiayi Univ., Chiayi city, Taiwan

Abstract Body:

Background: As the pathogen causing septicaemiae and infectious serositis in waterfowl, *Riemerella anatipestifer* is a Gram-negative facultative anaerobic rod bacterium. Twenty-one serotypes of *R. anatipestifer* had been determined, but immunizations with these serotypes are not cross-protective. Cohemolysin, OmpA, virulence plasmids and biofilm formation were suggested as the virulence factors of *R. anatipestifer*, but their roles in pathogenesis are not well characterized. **Methods:** Crystal staining was used to measure the biofilm formation. *Caenorhabditis elegans* was used as a surrogate host for screening microbial virulence factors. Japanese quail was infected by *R. anatipestifer* via nasal or subcutaneous routes.**Results:** We demonstrated that the biofilm formation ability varied in field-isolated isolates with various serotypes. Meanwhile, the infections of various biofilm-forming isolates differently shortened the life-spans of surrogate host. RA03, a serotype 2 isolate, was found much virulent to *C. elegans*. Moreover, Japanese quail (*Coturnix coturnix*) was tested as a host for *R. anatipestifer*. Riemerellosis was found in RA03 infected quails.**Conclusions:** The nematode and quail infection models may be used as good tools for studying the pathogenesis of *R. anatipestifer*.

Author Disclosure Block:

H. Wang: None. **C. Chen:** None. **G. Huang:** None. **S. Wang:** None.

Poster Board Number:

FRIDAY-055

Publishing Title:

Qualitative Assessment Of Salmonella Specie In Poultry And Poultry Facilities

Author Block:

R. Ohenhen[1], E. M. ODOYA[2]; [1]Univ., Ekpoma, Nigeria, [2]Univ., Benin, Nigeria

Abstract Body:

Background: In developing world, many commercial poultry farm are associated with poor personal hygiene of poultry workers, poor sanitation of poultry environment, infestation of the environment with rodents and insects, all of which could aid the transmission of bacteria. Bacteria such as Salmonella species can develop resistance to routine elimination practice, chemical treatment and antibacterial drugs. The Objective of this study was to determine the distribution of Salmonella species in poultry and poultry facilities and to carry out drug susceptibility test on the bacterial isolates. **Materials**:Ten commercial poultry farms were used for this study. Samples were collected from Poultry and Poultry facilities. Selenite F medium was used for the culturing and identification was done using standard microbiological procedures. Using Agar culture plates, drugs susceptibility test was conducted with the following antibiotics: Sulphamethaxozole, Chloramphenicol, Gentamicin, Tetracycline, Ciprofloxacine, Cefotaxime and Doxycycline. **Results**: Salmonella species were isolated from all samples collected except from the floor of one farm. Susceptibility test was positive for Gentamicin only with 17mm zone of inhibition (standard diameter for sensitivity ≥ 15mm). The zone of inhibition diameter in Sulphamethaxozole (9mm/≤10mm standard), Tetracycline (11mm/≤14mm standard) and Deoxycycline (10mm/≤10mm standard). Intermediate diameter of zone of inhibition was recorded in Chloramphenicol at 15mm/ 13-17mm standard, Ciprofloxacine at 16mm/16-20mm standard and Cefotaxime 18mm/15-22mm standard. **Conclusion**: Poultry feaces of various thickness was observed adhered on surfaces in the farms. This could impact negatively on the efficacy of cleaning and disinfection procedure. Salmonella is known tosurvive for weeks in dust in poultry environment and in dried feaces of poultry and poultry feed . Infection would always occur with recontamination.The fact that Gentamicine was the only drug susceptible to isolate prove indiscriminate and abuse in application of antibiotics in the commercial poultry farms studied. We recommend establishment of strict Hygiene Protocols for farm circumstances alongside flexible management of "all in" and " all out" principle.

Author Disclosure Block:

R. Ohenhen: None. **E.M. Odoya:** None.

Poster Board Number:

FRIDAY-056

Publishing Title:

Unraveling Myxozoan Parasite Diversity In Catfish Aquaculture In The Southeastern United States

Author Block:

T. G. Rosser[1], L. M. Pote[1], L. H. Khoo[2], D. J. Wise[2], N. R. Alberson[1], E. T. Woodyard[1], S. M. Quiniou[3], W. A. Baumgartner[1], T. E. Greenway[2], C. C. Mischke[2], **M. J. Griffin**[2]; [1]Coll. of Vet. Med., Mississippi State Univ., Mississippi State, MS, [2]Thad Cochran Natl. Warmwater Aquaculture Ctr., Mississippi State Univ., Stoneville, MS, [3]Warmwater Aquaculture Res. Unit, Agricultural Res. Service, United States Dept. of Agriculture, Mississippi State, MS

Abstract Body:

Myxozoan parasites are a persistent threat to the health and production of cultured and wild fish species throughout the world. The Myxozoa represent a diverse group of >2,000 described species from fish of both fresh and marine environments. The two-host life cycle usually involves a myxospore stage in the fish intermediate host and a pelagic, waterborne actinospore stage released from aquatic annelids. Of the numerous myxozoan species from economically important fish, <10% have experimentally or molecularly confirmed life cycles. In the southeastern United States, catfish producers experience significant losses annually attributed to *Henneguya ictaluri*, the causative agent of proliferative gill disease (PGD) in channel catfish (*Ictalurus punctatus*) and blue catfish (*Ictalurus furcatus*) x channel catfish hybrids. In addition to *H. ictaluri*, our team has identified at least 10 distinct species of *Henneguya* endemic to catfish aquaculture as well as a new species of *Unicauda*. Moreover, two previously undescribed *Myxobolus* spp. have been identified from smallmouth buffalo (*Ictiobus bubalus*) raised in polyculture with catfish in a failed effort to biologically control PGD. Species descriptions supplemented with 18S rRNA gene sequence data can be linked to corresponding actinospore stages through direct sequence comparisons allowing for molecular elucidation of unknown life cycles. At present, three of the four known *Henneguya* life cycles are from catfish aquaculture in Mississippi, U.S.A. and have been described by our research group. Herein we discuss our most recent findings regarding the diversity of myxozoans indigenous to catfish aquaculture and future studies concerning myxozoans associated with farm-raised catfish in the southeastern United States.

Author Disclosure Block:

T.G. Rosser: None. **L.M. Pote:** None. **L.H. Khoo:** None. **D.J. Wise:** None. **N.R. Alberson:** None. **E.T. Woodyard:** None. **S.M. Quiniou:** None. **W.A. Baumgartner:** None. **T.E. Greenway:** None. **C.C. Mischke:** None. **M.J. Griffin:** None.

Poster Board Number:

FRIDAY-057

Publishing Title:

Genome Sequencing, Metabolic and Antibiotic Resistance Phenotyping of Diverse Nasopharyngeal Bacteria Isolated from Cattle in an Epidemiological Study of Bovine Respiratory Disease Complex

Author Block:

G. P. Harhay[1], D. M. Harhay[1], J. L. Bono[1], T. P. L. Smith[1], M. P. Heaton[1], M. L. Clawson[1], C. G. Chitko-Mckown[1], S. F. Capik[2], K. D. DeDonder[3], M. D. Apley[2], B. V. Lubbers[2], B. J. White[2], R. L. Larson[2]; [1]USDA-ARS Meat Animal Res. Ctr. (USMARC), Clay Center, NE, [2]Kansas State Univ., Manhattan, KS, [3]Vet. and BioMed. Res. Ctr., Inc, Manhattan, KS

Abstract Body:

Background: Despite over 100 years of research to reduce the incidence and impact of bovine respiratory disease complex (BRDC) in North American feed yard cattle, outbreaks still occur accounting for up to 75% of feed yard cattle morbidity. BRDC is the primary driver of health-related antibiotic treatment and is the most costly disease of feed yard cattle. Pathogenicity and virulence determinants of bacteria associated with BRDC remain incompletely understood and constitute a barrier to effective and precise treatment. **Goal**: Integrate genomic and phenotyping technologies to characterize molecular entities, pathways, and biological processes in nasopharyngeal bacteria to identify pathogenicity and virulence determinants associated with BRDC. **Approach**: A multi-institutional team with expertise in veterinary medicine, microbiology, genomics, and bioinformatics collaborated in a 28-day study to isolate diverse nasopharyngeal bacteria from 180 calves sampled at both sale barn and feed yard. Two thousand bacterial isolates from 28 case and 28 matched control animals were identified to species level. We have initially identified over 40 species that are not represented by closed genomes in GenBank. Closed genomes from these and other bacteria will seed downstream transcriptomic, epigenomic, and biochemical analyses to identify promoters, genes, and pathways from *in vitro* experiments. Antibiotic resistance and metabolic profiles are being characterized. **Results**: The newly closed genomes of over ten nasopharyngeal bacterial species and their antibiotic resistance profiles will be presented and available at GenBank. Genera include *Achromobacter*, *Comamonas*, and *Kurthia*. We will discuss our approaches to integrating genomic information with metabolic phenotypes to identify molecular entities, pathways, and biological processes that are enriched or depleted in genes that distinguish bacteria from case and control animals.

Author Disclosure Block:

G.P. Harhay: None. **D.M. Harhay:** None. **J.L. Bono:** None. **T.P.L. Smith:** None. **M.P. Heaton:** None. **M.L. Clawson:** None. **C.G. Chitko-Mckown:** None. **S.F. Capik:** None. **K.D.**

DeDonder: None. **M.D. Apley:** None. **B.V. Lubbers:** None. **B.J. White:** None. **R.L. Larson:** None.

Poster Board Number:

FRIDAY-058

Publishing Title:

Identifying *Bordetella* Species Cultured from Turkeys with Upper Respiratory Infections

Author Block:

A. Reveille, S. Johnson, H. Madison, A. Brouillette, L. Harb, **L. M. Temple**; James Madison Univ., Harrisonburg, VA

Abstract Body:

Bordetella avium is the causative agent of bordetellosis, a contagious upper-respiratory tract disease that poses a problem to commercially raised turkeys. In recent years clinical manifestations similar to bordetellosis have been appearing in turkeys that are vaccinated against *B. avium*. Tracheal swab cultures of these turkeys revealed the presence of *Bordetella hinzii*, a commensal organism of the respiratory tracts of poultry that has been regarded as non-pathogenic. Reports of humans becoming infected with certain strains of *B. hinzii* have been recorded, creating a health hazard if turkeys are found to be reservoir hosts for these strains. In this study microbiological techniques were used to differentiate *B. hinzii* from *B. avium* in samples of bacterial isolates from various locations including North Carolina, Utah, Missouri, and Iowa. Identification methods included hemagglutination, which differentiates *B. hinzii* (negative) from *B. avium* (positive). Previous identification of these samples was based largely on colony morphology on MacConkey agar and hemagglutination results, with the majority of the isolates identified as *B. avium*. In this study hemagglutination was repeated on these samples, and PCR was run using specific PCR primers for either *B. avium* or *B. hinzii*. Of twenty isolates collecting over 30 years, ten were positive and ten were negative. Gel electrophoresis of the species-specific PCR products from the 20 isolates showed 12 positive for the *B. hinzii*-specific primers and 1 positive for the *B.avium*-specific primers. The remaining 7 isolates, which appeared to be *B. avium* or *B. hinzii* by colony morphology, did not test positive for either species-specific primer set. A similar trend is emerging from some isolates collected within the last year. We conclude that the infectious agent of bordetellosis may include other than either *B. avium* or *B. hinzii*, which is a novel and unexpected finding. Further work will include 16srDNA sequencing and biochemical tests to identify the unknown agents.

Author Disclosure Block:

A. Reveille: None. **S. Johnson:** None. **H. Madison:** None. **A. Brouillette:** None. **L. Harb:** None. **L.M. Temple:** None.

Poster Board Number:

FRIDAY-059

Publishing Title:

Intestinal Colonization of Healthy Brazilian Cattle with Multidrug Resistant Extended-spectrum Beta-lactamase Producing *Escherichia coli*

Author Block:

J. Palmeira, H. Ferreira; Univ. of Porto, Porto, Portugal

Abstract Body:

Background: Intestinal colonization of food-producing animals with extended-spectrum beta-lactamase (ESBL) producers represents a public health concern. Brazil is one of the biggest bovine producers for meat and milk consumption and a big exporter. This study aimed to detect multidrug resistant (MDR) ESBL producing *Enterobacteriaceae* in feces of healthy bovine from the North of Brazil.**Methods:** Isolates were obtained from bovine fecal samples of 22 productions in the North of Brazil. Selection of the isolates was performed on MacConkey agar with oxiimino-beta-lactams, tetracycline, and ciprofloxacin. Samples were previously incubated in TSB. Susceptibility testing was achieved by disk diffusion method according to the CLSI. Bacterial presumptive identification was performed with CHROMagar Orientation. ESBL producers were screened by the double disk synergy test. ESBL coding genes were screened by PCR with specific primers $_{bla}$TEM, $_{bla}$OXA and $_{bla}$SHV; and $_{bla}$CTX-M.**Results:** From 188 cattle fecal samples, 267 *Enterobacteriaceae* isolates were obtained, showing resistance to tetracycline (73%), amoxicillin (59,9%), sulfamethoxazole + trimethoprim (44,9%), ciprofloxacin (33,7%), cefotaxime (30,7%), chloramphenicol (14,9%), amoxicillin + clavulanic acid (14,2%), cefoxitin (5,6%) and gentamicin (4,4%). A total of 176 isolates were MDR. Seventy of the selected *Enterobacteriaceae* isolates (26,2%) were ESBL-producing *Escherichia coli*. Thirty five of the total fecal samples (18,6%) showed ESBL producers with the following beta-lactamase gene profile: $_{bla}$SHV (11,4%); $_{bla}$TEM (11,4%); $_{bla}$CTX-M Group 2 (G2) (7,1%); $_{bla}$CTX-M Group 8 (G8) (7,1%); $_{bla}$CTX-M Group 1 (G1) and $_{bla}$OXA (2,8%); $_{bla}$SHV and $_{bla}$TEM (2,8%); $_{bla}$CTX-M G1 (1,4%); $_{bla}$CTX-M G1 and $_{bla}$TEM (1,4%); $_{bla}$CTX-M G2 and $_{bla}$TEM (1,4%); $_{bla}$CTX-M G2 and $_{bla}$OXA (1,4%); $_{bla}$CTX-M G8 and $_{bla}$SHV (1,4%); and $_{bla}$OXA (1,4%). Results show the prevalence of $_{bla}$CTX-M, present in 24,3% of ESBL-producing *E. coli*. ESBL producing isolates showed different associated resistance with other antibiotic families, showing a polyclonal spread.**Conclusions:** Results show a high prevalence of ESBL-producing *E.coli*, presenting $_{bla}$CTX-M and MDR phenotype, colonizing these animals. Farm animals may act as reservoirs of MDR bacteria, highlighting the relevance of the One Health approach of this reality.

Author Disclosure Block:

J. Palmeira: None. **H. Ferreira:** None.

Poster Board Number:

FRIDAY-060

Publishing Title:

Identification of the Pan and Core Genomes for *Mannheimia haemolytica* Genotypes 1 and 2

Author Block:

M. L. Clawson[1], G. Schuller[1], A. M. Dickey[1], J. L. Bono[1], R. W. Murray[2], M. T. Sweeney[2], M. D. Apley[3], K. D. DeDonder[3], S. F. Capik[3], R. L. Larson[3], B. V. Lubbers[4], B. J. White[3], G. P. Harhay[1], M. P. Heaton[1], C. G. Chitko-McKown[1], D. M. Harhay[1], T. P. L. Smith[1]; [1]USDA/ARS/ US Meat Animal Res. Ctr., Clay Center, NE, [2]Zoetis, Kalamazoo, MI, [3]Kansas State Univ., Manhattan, KS, [4]Kansas State Univ., Olathe, KS

Abstract Body:

Mannheimia haemolytica normally populates the upper respiratory tract of cattle and is recognized as a major cause of bovine respiratory disease. Recently, two major genotypes (1 and 2) of *M. haemolytica* were identified from whole genome sequencing and analyses of 1,145 North American isolates. Only genotype 2 associated with bovine respiratory disease, although genotype 1 isolates were detected in diseased cattle. The genotypes were defined by the mapping of short sequence reads from each isolate to a reference genome followed by nucleotide polymorphism identification. Thus, closed circular genomes for these isolates were not assembled, and gene regions unique to the sequenced isolates in comparison to the reference genome were not identified. The goal of this study was to characterize the *M. haemolytica* pan genome, as well as the core genome for genotypes 1 and 2 using closed circular genomes. This could lead to the identification of effective intervention strategies for controlling genotype 2 *M. haemolytica* in cattle. Accordingly, we went back to the original collection of 1,145 isolates and identified sets of genotype 1 and 2 isolates that efficiently characterized genetic variation within and between the genotypes. The isolates from each set were all re-sequenced and assembled *de novo* into closed circular genomes. Analyses of these genomes revealed the pan genome of North American *M. haemolytica* as well as distinct core genomes for genotypes 1 and 2. These core genomes can be mined for proteins or other biological determinants that are specific to genotype 2, and suitable for potential intervention strategies like vaccine development.

Author Disclosure Block:

M.L. Clawson: None. **G. Schuller:** None. **A.M. Dickey:** None. **J.L. Bono:** None. **R.W. Murray:** None. **M.T. Sweeney:** None. **M.D. Apley:** None. **K.D. DeDonder:** None. **S.F. Capik:** None. **R.L. Larson:** None. **B.V. Lubbers:** None. **B.J. White:** None. **G.P. Harhay:** None. **M.P. Heaton:** None. **C.G. Chitko-McKown:** None. **D.M. Harhay:** None. **T.P.L. Smith:** None.

Poster Board Number:

FRIDAY-061

Publishing Title:

Genetic Characterization of *Listeria monocytogenes* Isolated from Cases of Listeriosis in Ruminants in the Upper-Midwest

Author Block:

A. J. Steckler, M. Cardenas Alvarez, N. Dyer, T. M. Bergholz; North Dakota State Univ., Fargo, ND

Abstract Body:

Encephalitis associated with *Listeria monocytogenes* infections is an important disease of ruminants worldwide. Listeriosis can lead to significant economic losses at the farm level due to morbidity and the high mortality it causes in animals. In addition to these concerns for farmers and ranchers, veterinary listeriosis can also pose a potential food safety risk due to the potential for *L. monocytogenes* to enter the food supply via the farm environment. Our goal was to assess the genetic diversity of clinical listeriosis isolates from ruminants in the upper Midwest states, a population not well-studied. To determine the frequency and distribution of *L. monocytogenes* serotypes, we used a multiplex PCR to assign each isolate to a serogroup. The majority of isolates (7/11) belong to serotype 1/2a, and the remainder belong to serogroup 4bde (2 isolates) and 4ac (2 isolates). Multi-locus sequence typing (MLST) using 7 loci was implemented to investigate the genetic diversity of the isolates from the region. Loci sequences were compared to all known sequence types using the Pasteur Institute *L. monocytogenes* MLST database. One isolate of serogroup 4ac was found to have two loci with novel alleles, leading to a new sequence type, ST 897, which belongs to lineage 3. The seven isolates of serotype 1/2a all belong to lineage 2, with two isolates each assigned to ST 7, ST 91, and ST 230, despite being isolated from geographically distinct areas and at different times over the past year. Isolate information from the MLST database indicates that ST 7 isolates are widespread and have been isolated from food, the environment, and human clinical as well as animal clinical cases worldwide. Our results indicate that the isolates causing listeriosis in ruminants in the upper Midwest are genetically diverse, with new sequence types of *L. monocytogenes* still being discovered. Our results also demonstrate that isolates from ruminants in this region belong to the same genetic subtypes as isolates found in the food supply and human cases of listeriosis, and further studies are warranted to understand the ecology of this pathogen in the farm environment.

Author Disclosure Block:

A.J. Steckler: None. **M. Cardenas Alvarez:** None. **N. Dyer:** None. **T.M. Bergholz:** None.

Poster Board Number:

FRIDAY-062

Publishing Title:

Effect of Temperature and Organic Acids on the Viability of *Campylobacter* spp. in Dairy Cattle Manure

Author Block:

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Abstract Body:

Background: Livestock manure is stored in concrete tanks and represents a reservoir of human-associated pathogens. With the application of manure as an organic fertilizer, these pathogens transfer to humans via water, food and air and cause potential public health risks. Among several bacterial pathogens, *Campylobacter* spp. exists as a commensal organism in the gastrointestinal tracts of domestic animals which is one of the most common causes of human gastroenteritis worldwide. The study was designed to determine the viability of *Campylobacter* spp. in dairy cattle manure samples from various dairy cattle manure storage tanks. **Methods:** Samples were anaerobically incubated for 28 weeks under two temperature regimens simulating the winter to summer (eight weeks at 4ºC, followed by a gradual four-week increase at 25ºC) or the summer to winter (sixteen weeks at 25ºC, followed by a gradual four-week decrease at 4ºC) seasonal periods. Moreover, another series of *in vitro* manure incubations were performed to study the effect of organic acids (addition of acetate at 8.5 g/L or propionate at 2 g/L; final conc.) on *Campylobacter* survival. Viability was determined using a culture-based viable count plating method. **Results:** Overall, the cells of *Campylobacter* spp. survived at both temperature conditions for 28 weeks with no significant difference, and a maximum of one log reduction was observed. In contrary, cells of *Campylobacter* spp. survived up to two weeks in diluted (three-fold with autoclaved water) manure treated with acetate and in undiluted manure containing acetate and propionate at the above-mentioned concentrations. However, viability of *Campylobacter* cells was observed for at least four weeks in diluted manure suggest that the dilution might influence *Campylobacter* survival. **Conclusions:** The temperature-based assay results show that temperature did not have an impact on the viability compared to the organic acids. This suggests that acetate and propionate could be used for reducing the rate of survival of *Campylobacter* spp. in manure. The results would help in improving guidelines on manure management to minimize the risks of pathogen transfer from animal manures to the human and food chain.

Author Disclosure Block:

I. Khan: None. **M. Cloutier:** None. **G. Talbot:** None. **E. Topp:** None. **M.G. Miltenburg:** None. **M. Libby:** None.

Poster Board Number:

FRIDAY-063

Publishing Title:

Investigation of Sulfate Reducing Bacteria Populations and Their Activity in Anaerobic Manure Digesters

Author Block:

B. St-Pierre[1], A-D. G. Wright[2]; [1]South Dakota State Univ., Brookings, SD, [2]Univ. of Arizona, Tucson, AZ

Abstract Body:

Background: Biogas produced from anaerobic digestion of manure not only consists of methane and carbon dioxide, but also contains other minor gases, such as hydrogen sulfide (H_2S). Mitigation of H_2S emissions from anaerobic manure digesters is important to minimize health risks and reduce financial losses. In many anaerobic environments, H_2S is produced by sulfate reducing bacteria (SRBs) as a result of their anaerobic respiration. In manure digesters, the phylogenetic affiliation and activity of SRBs remain poorly characterized, and their investigation could yield valuable insight that would improve current mitigation strategies.**Methods:** SRB populations were analyzed in untreated manure and anaerobic digestate samples collected from three dairy farms that operate manure digesters in the state of Vermont (USA). Farms were selected based on the H_2S emissions reported by their respective operators: inherently high (GVS), inherently low (MPH), or moderate as a result of management practices (MXW). SRB diversity was investigated by high-throughput sequencing of PCR-generated amplicons of the SRB-specific *dsrAB* gene.**Results:** Manure samples from all farms showed a similar composition with a high prevalence of SRBs related to species of the genus *Desulfovibrio* (84.2 - 97.6% of sequence reads). In contrast, *Desulfosporosinus*-related SRBs were by far the most dominant in all digestate samples (96.9 - 98.3% of sequence reads). Further analysis revealed two major Operational Taxonomic Units (OTUs) for *Desulfovibrio*-related SRBs, compared to one OTU for *Desulfosporosinus*-related SRBs. We also report that, while digestate from the GVS farm was populated with a higher abundance of SRBs and contained higher levels of sulfate, it produced low (< 300 ppm) to non detectable amounts of H_2S when maintained without supplementation as batch cultures *in vitro*. We were only able to reproducibly observe production of high levels of H_2S (>1000 ppm) from digestate when it was mixed with untreated manure as a co-culture.**Conclusions:** Our results suggest that differences in H_2S production amongst different dairy manure digesters may not be caused by differences in SRB composition, but rather by other factors (chemical and/or biological) that remain to be determined.

Author Disclosure Block:

B. St-Pierre: None. **A.G. Wright:** None.

Poster Board Number:

FRIDAY-064

Publishing Title:

Comparison of *Edwardsiella ictaluri* Isolates from Different Hosts and Geographic Origins

Author Block:

M. J. Griffin[1], S. R. Reichley[1], T. E. Greenway[1], S. M. Quiniou[2], C. Ware[1], D. X. Gao[1], P. S. Gaunt[1], R. P. E. Yanong[3], D. B. Pouder[3], J. P. Hawke[4], E. Soto[5]; [1]Mississippi State Univ., Stoneville, MS, [2]USDA, Stoneville, MS, [3]Univ. of Florida, Ruskin, FL, [4]Louisiana State Univ., Baton Rouge, LA, [5]Univ. of California - Davis, Davis, CA

Abstract Body:

The intraspecific variability of *Edwardesiella ictaluri* isolates from different origins was investigated. Isolates were recovered from farm-raised catfish (*Ictalurus punctatus*) in Mississippi, USA, tilapia (*Oreochromis niloticus*) cultured in the Western hemisphere, and zebrafish (*Danio rerio*) propagated in Florida, USA. These isolates were phenotypically homologous and antimicrobial profiles were largely similar. Genetically, isolates possessed differences that could be exploited by repetitive-sequence mediated PCR and *gyrB* sequence, which identified three distinct *E. ictaluri* genotypes: one associated with catfish, one from tilapia, and a third from zebrafish. Plasmid profiles were also group specific, and correlated with rep-PCR and *gyrB* sequences. The catfish isolates possessed profiles typical of those described for *E. ictaluri* isolates, however, plasmids from the zebrafish and tilapia isolates differed in both composition and arrangement. Furthermore, some zebrafish and tilapia isolates were PCR negative for several *E. ictaluri* virulence factors. Isolates were serologically heterogenous, as serum from a channel catfish exposed to a catfish isolate had reduced antibody activity to tilapia and zebrafish isolates. This work identifies three genetically and serologically distinct strains of *E. ictaluri* from different origins using rep-PCR, 16S, *gyrB* and plasmid sequencing, in addition to antimicrobial and serological profiling.

Author Disclosure Block:

M.J. Griffin: None. **S.R. Reichley:** None. **T.E. Greenway:** None. **S.M. Quiniou:** None. **C. Ware:** None. **D.X. Gao:** None. **P.S. Gaunt:** None. **R.P.E. Yanong:** None. **D.B. Pouder:** None. **J.P. Hawke:** None. **E. Soto:** None.

Poster Board Number:

FRIDAY-065

Publishing Title:

Genotypification and Virulence Genes Detection in *Pasteurella multocida* Isolates from Alpacas

Author Block:

R. Rimac, R. Hurtado, R. Rosadio & L. Maturrano; Univ. Natl. Mayor de San Marcos (UNMSM), Lima, Peru

Abstract Body:

Background: The South Americans camelids (SAC) production is the main economic resource for the Andean communities but the high mortality rates caused by infectious diseases such as acute pneumonia induced by *Pasteurella multocida* is the main restrain in its production. *P. multocida* is frequently isolated from fatal cases alpaca and it appears to be the responsible for the fatality. There is few studies of this bacterium in SAC animals and we lack detail information on type of LPS (serovar) and identification of virulence genes that could lead to develop control strategies including vaccines. For this reason, the objective of this study was genotypified *P. multocida* (n=25) strains isolated from acute pneumonia as well as to identify some virulence genes present in this pathogen. **Materials and Methods:** The 25 strains isolated were evaluated by multiplex PCR following the protocol described by Townsend et al (1998) to test the *P. multocida* specific *kmt* gen, to the serogroup we used the capsular genes as described by Townsend et al (2001) and the LPS genes to genotypified (Harper et al, 2015). Then, the virulence genes *toxA*, *tbpA*, *pfhA* and *hgbB* were tested according to Lichtensteiger et al (1996), Ewers et al (2006) and Atashpaz et al (2009). **Results:** All the *P. multocida* strains tested were classified as serogroup A and genotyped LPS 6 indicating that these serovars could be classified as 10, 11,12 or 15. The virulence genes *toxA* and *tbpA* were detected in all isolates. **Conclusion:** All strains tested were serogroup A, similarly to those detected in cattle and sheep and the presence of *toxA* and *tbpA* genes to those already reported in sheep where the *toxA* in being implicated and main responsible of fatality outcomes. The isolates were positive to genotype LPS 6 similar to those detected in cattle (serovar 10) and goat (serovar 12). The serogroup A and genotype LPS 6 are the most frequently detected in pneumonia from alpacas and the presence of *toxA* and *tpbA* may well be responsible for the pathology of acute pneumonia in Peruvian alpacas.

Author Disclosure Block:

R. Rimac: None.

Poster Board Number:

FRIDAY-066

Publishing Title:

Expanding Our Knowledge Of The Edwardsiella

Author Block:

S. R. Reichley[1], M. J. Griffin[1], C. Ware[1], G. C. Waldbieser[2], H. C. Tekedar[1], T. E. Greenway[3], L. Khoo[1], M. L. Lawrence[1], P. S. Gaunt[1], D. J. Wise[4]; [1]Mississippi State Univ. Coll. of Vet. Med., Mississippi State, MS, [2]USDA-ARS Warmwater Aquaculture Res. Unit, Stoneville, MS, [3]Thad Cochran Natl. Warmwater Aquaculture Ctr., Stonevill, MS, [4]Thad Cochran Natl. Warmwater Aquaculture Ctr., Stoneville, MS

Abstract Body:

Edwardsiella spp. have been linked to disease outbreaks in cultured and wild freshwater and marine fish species across the globe. Recent investigations of the genotypic and phenotypic variability of *Edwardsiella tarda* resulted in the division of *E. tarda* into three genetically distinct taxa. This led to the adoption of *E. piscicida* and the proposal of *E. anguillarum* as new species within the *Edwardsiella* genus. Phylogenomic investigations have demonstrated significant genetic differences between these phenotypically ambiguous taxa. To this end, real-time polymerase chain reaction (qPCR) assays were developed for the rapid differentiation and quantification of *E. piscicida*, *E. tarda*, and *E. anguillarum*. *In vivo* studies performed using intracoelomic injections in catfish fingerlings (mean weight: 17.3g; range 7.3 - 40.9g) identified a median lethal dose (LD_{50}) of 3.9×10^5 CFU for *Edwardsiella piscicida* and 5.9×10^7 CFU for *Edwardsiella tarda*. Negligible mortality was observed in catfish injected with *E. anguillarum d*oses as high as 5.0×10^8 CFU, suggesting significant variation in pathogenicity of these isolates in channel catfish. This work supported a previous survey of archived bacterial isolates that suggested *E. piscicida* is more common in North American catfish aquaculture than *E. tarda.* These assays, coupled with a previously established qPCR assay for *E. ictaluri*, were employed in a multiplex fashion for the purpose of rapidly discriminating between fish pathogenic *Edwardsiella* spp. (*E. ictaluri*, *E. piscicida*, *E. tarda*, and *E. anguillarum*). In addition to this work, forty-seven *Edwardsiella* spp. isolates from different hosts and geographic origins were characterized by two commercial phenotypic identification kits, plasmid profiling, PCR-mediated DNA fingerprinting and sequencing of 16S rDNA, *gyrB*, and *sodB* genes in efforts to consolidate the current dissonance associated with previous reports of *Edwardsiella* spp. infection in fish.

Author Disclosure Block:

S.R. Reichley: None. **M.J. Griffin:** None. **C. Ware:** None. **G.C. Waldbieser:** None. **H.C. Tekedar:** None. **T.E. Greenway:** None. **L. Khoo:** None. **M.L. Lawrence:** None. **P.S. Gaunt:** None. **D.J. Wise:** None.

Poster Board Number:

FRIDAY-067

Publishing Title:

Evaluation Of Exposure To Common Tick-Borne Bacteria In Select Northeastern Oklahoma Equine Population

Author Block:

T. L. Orme, D. Kirk, **S. Ruskoski**; Northeastern State Univ., Broken Arrow, OK

Abstract Body:

Background: *Anaplasm*a *phagocytophilium, Ehrlichia canis, Borrellia burgdorferi,* and *Rickettsia rickettsii* are common tick -borne bacteriapathogens that infect both humans and animals. via tick bites. There is a paucity of information regarding exposure to these organisms in horses. The purpose of this study is to evaluate and document equine exposure to these tick-borne pathogensbacteria in Northeast Oklahoma during the 2014 and 2015 tick seasons.**Materials:** Blood was collected form from 41 horses in March/April and again in November/December of 2014 and 2015. Sera were tested for antibodies to *A. phagocytophilium, E. canis, B. burgdorferi,* and *R. rickettsii* using an indirect immunoflourescent antibody (IgG) assay.**Results:** While none of the horses presented with fever or lethargy at the time of collection, the largest exposure was to *R. rickettsia* with 9(22%) showing a 4-fold or greater increase in antibody titers. Only 1 (3%) and 2 (5%) horses had a 4-fold or greater exposure to *A.phagocytophilium* and *E. canis,* respectively. However, 21 (51%) of the horses tested positive to antibodies against *B. burgdorferi,* while 10 (24%), 8 (20%), 8 (20%) had antibodies present against *A.phagocytophilium, E. canis,* and *R. rickettsiia,* respectively.**Conclusion:** The results of this study indicate that these horses were exposed to *R. rickettsiia* and *B. burgdorferi* to a greater extent than to *A. phagocytophilium* and *E. canis* during the 2014 and 2015 summers.

Author Disclosure Block:

T.L. Orme: None. **D. Kirk:** None. **S. Ruskoski:** None.

Poster Board Number:

FRIDAY-068

Publishing Title:

Phenotypic Diversity in an Emerging Infectious Disease

Author Block:

K. Perez[1], N. Mullen[1], J. Canter[2], D. H. Ley[3], M. May[1]; [1]Univ. of New England, Biddeford, ME, [2]Univ. of Connecticut, Storrs, CT, [3]North Carolina State Univ., Raleigh, NC

Abstract Body:

Parameters of disease ecology are affected by contact between individuals in the population, transmission rates, clinical presentation, and virulence capacity of the infectious agent. The avian pathogen *Mycoplasma gallisepticum* (MG) is a known pathogen of poultry, and newly emerging pathogen of house finches wherein it is associated with lethal conjunctivitis. Factors known to mediate virulence of MG in poultry include cytadherence, sialidase activity, peroxide production, and possibly biofilm formation. We have quantitatively assessed these factors for over twenty MG isolates from house finches from a wide geographic distribution across the continental United States. Statistically significant ($P<0.05$) differences were observed across strains for sialidase activity, cytoadherence, and hydrogen peroxide production. No significant differences were observed for biofilm formation. Analysis of variance in adherence to distinct sialic acid linkages representing diverse cellular host ligands is ongoing. Quantitative variance in virulence-associated traits is consistent with within-host evolutionary adaptation in response to a change in ecological niche by a parasitic pathogen.

Author Disclosure Block:

K. Perez: None. **N. Mullen:** None. **J. Canter:** None. **D.H. Ley:** None. **M. May:** None.

Poster Board Number:

FRIDAY-069

Publishing Title:

Proline as a Formic Acid Stress Protectant on the Fermentation of Glucose to Ethanol by *Saccharomyces* spp.

Author Block:

C. E. Oshoma[1], C. Powell[2], C. Du[3]; [1]Univ. of Benin, Benin City, Nigeria, [2]Univ. of Nottingham, Loughborough, United Kingdom, [3]Univ. of Huddersfield, Huddersfield, United Kingdom

Abstract Body:

Background: During bioethanol production from lignocellulosic hydrolysate yeasts are frequently exposed to various forms of fermentation stress such as nutritional starvation, metabolites production and fermentation inhibiting compounds produced during pretreatment and hydrolysis of the lignocellulosic material. These inhibitors hamper efficient ethanol production as they are toxic to the microbes conducting the fermentation. Formic acid is one of these inhibitors released into the hydrolysates at a concentration of 10 -30 mM. Previously found that proline act as a compatible solute that enhances yeast tolerance to stress by improving fermentative ability. Proline functions, in vitro, maybe protein and membrane stabilization, lowering T_m of DNA and scavenging of reactive oxygen species.**Methods:** The strains used in this study were *Saccharomyces cerevisiae* NCYC2592 and *Saccharomyces arboricolus* 2.3319. Phenotypic microarrays (PM) was carried out to study the effect of different concentrations of proline (0 - 50 mM) on metabolic profiles of *S. cerevisiae* NCYC2592 and *S. arboricolus* 2.3319 strains in the presence of 40 mM formic acid. Experimental procedure was carried out in 150 ml (mini) fermentation vessels using Yeast nitrogen base (YNB) medium.**Results:** In this study, addition of proline to the media to improve tolerance of yeast to formic acid during fermentation was investigated. The observation was that proline at 30 mM in the medium had higher cell count than the control medium as the fermentation progresses, and cell viability of proline medium was 94 and 91% after 24 hours for strain *S. cerevisiae* NCYC2592 and *S. arboricolus* 2.3319 respectively. The proline medium revealed a higher accumulation of ethanol than the control medium in both strains. The results showed that the modification of growth medium with little concentration of proline increased formic acid tolerance and fermentative ability of yeast cells.**Conclusions:** Overall results suggest that lower concentrations of proline in the media may improve yeast tolerance to formic acid and increase bioethanol production, although the mechanism of leading to this increase remains to be elucidated.

Author Disclosure Block:

C.E. Oshoma: None. **C. Powell:** None. **C. Du:** None.

Poster Board Number:

FRIDAY-070

Publishing Title:

Glycerol Fermentation and Hydrogen Metabolism by *Escherichia coli*: New Approaches to Enhance Hydrogen Production

Author Block:

A. POLADYAN, K. Trchounian, A. Trchounian; Yerevan State Univ., Yerevan, Armenia

Abstract Body:

Hydrogen (H_2) metabolism is a basic process upon bacterial fermentation. H_2 is produced from formate, the end product of glycerol or glucose fermentation of *Escherichia coli*, by formate dehydrogenase H and hydrogenase (Hyd) enzymes. *E. coli* possesses four [Ni-Fe]-Hyd enzymes for activity of which heavy metals can be required. Besides, H_2 is efficient energy source for the future and organic waste glycerol can be used as a cheap carbon source. Glycerol and glucose fermentation redox routes by *E. coli* and their regulation by oxidizing and reducing reagents were investigated at different pHs: cell growth was followed by decrease of pH and redox potential. H_2 production and redox potential (ORP) kinetics from positive to negative level were investigated with two redox Pt (sensitive to H_2) and Ti-Si electrodes: simultaneously using both electrodes during bacterial growth provides information not only on general redox processes but also H_2 production rate and yield. Depending on carbon (glycerol and glucose) source different ORP kinetics was observed. Reducers (DL-dithiothreitol or dithionite) stimulating effect on H_2 production was shown. The influence of formate alone or its mixture with glycerol on growth of *E. coli* wild type and mutants with deletions of key subunits of different Hyd enzymes and H_2 production in batch culture was analyzed at different pHs. The results obtained identify the conditions when formate alone or with glycerol had stimulatory effects on bacterial growth and H_2 production. Furthermore, the importance of some heavy metals and their mixtures at low concentrations (<0.1 mM) for H_2 production was shown: H_2 yield was markedly stimulated 1.7- to 3-fold in the presence of Ni^{2+}+Fe^{3+}, Ni^{2+}+Fe^{3+}+Mo^{6+} and Fe^{3+}+Mo^{6+} mixtures. Discrimination between Fe^{2+} and Fe^{3+} was established. Some interaction of Ni^{2+} with Fe^{2+} was suggested to be effective increasing bacterial biomass and with Fe^{3+} determining activity of H_2 producing Hyds. The findings reveal the ORP level and reductive conditions essential role for H_2 production in *E. coli* upon glycerol fermentation. Mixed carbon sources or addition of some heavy metal ions enhance H_2 production. Co-fermentation with organic acids and oxidizing and reducing reagents effects and heavy metals ions can be considered as novel approaches to regulate and enhance H_2 production.

Author Disclosure Block:

A. Poladyan: None. **K. Trchounian:** None. **A. Trchounian:** None.

Poster Board Number:

FRIDAY-071

Publishing Title:

Comparative Effects of Cu(Ii) and Ni(Ii) Ions Low Concentrations on *Rhodobacter sphaeroides* Growth Characteristics and Hydrogen Production

Author Block:

L. Gabrielyan, A. Trchounian; Yerevan State Univ., Yerevan, Armenia

Abstract Body:

Different heavy metals are involved as "essential" elements in metabolism and hydrogen (H_2) production by bacteria through stimulating the responsible enzymes and related metabolic pathways. Copper (Cu) and nickel (Ni) ions in high concentration (>0.1 mM) are toxic, disturbing the membrane permeability and inhibiting enzymes activity. The inhibitory effect of these ions was reported for various bacteria, but the effect of Cu and Ni ions low concentrations on photosynthetic bacteria *Rhodobacter sphaeroides* has not been investigated yet. Many chemical reagents and biologically active compounds show bioeffects at low and ultra-low concentrations. It is interesting, how the effects of compounds at low concentrations differ from those of relatively high concentrations. The aim of the present work was the investigation of comparative effects of Cu(II) and Ni(II) low concentrations on growth properties and H_2 yield in *R. sphaeroides* MDC6522 from Armenian mineral springs. The bacterial growth characteristics were studied during phototrophic growth of *R. sphaeroides* in 1-5 μM Cu^{2+} and Ni^{2+} containing media. Specific growth rate by addition of Cu^{2+} and Ni^{2+} changed in differed manner: in the presence of 5 μM Cu^{2+} growth rate was ~3.5-fold lower in comparison with control (no addition), and has increased ~1.5-fold in medium with 5 μM Ni^{2+}. These changes may be resulted by action of the ions on redox potential (E_h) or by direct effect of these ions on bacterial membrane. Low concentrations of Cu and Ni ions were discovered to affect the E_h drop of the *R. sphaeroides* growth medium. Cu^{2+} and Ni^{2+} were shown to have an enhancing effect on the E_h drop after 72 h of bacterial growth. The increase of concentration from 1 to 5 μM enhanced the stimulatory effect of Ni^{2+}. The effect of Cu and Ni ions on H_2 yield in *R. sphaeroides* presented a different picture. H_2 yield in R. sphaeroides after 72 h of growth was enhanced ~1.5- and ~2.5-folds with 1 μM $Cu^{2+}$and Ni^{2+}, respectively. 5 μM Cu^{2+} inhibited H_2 production ~1.3-fold, whereas in the presence of 5 μM Ni^{2+} H_2 yield was ~3 fold higher in comparison with control. Thus, the results point out discrimination between Cu^{2+} and Ni^{2+} low concentrations effects and reveal new regulatory pathways to enhance H_2 yield in *R. sphaeroides*.

Author Disclosure Block:

L. Gabrielyan: None. **A. Trchounian:** None.

Poster Board Number:

FRIDAY-072

Publishing Title:

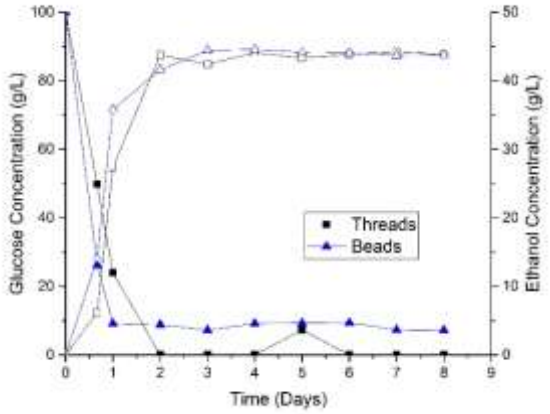
Enhanced Ethanol Productivity Through The Use Of Alginate Gel Threads

Author Block:

A. Nordmeier, D. Chidambaram; Univ. of Nevada Reno, Reno, NV

Abstract Body:

Background: The increasing rate at which our known fossil fuel reserves are being depleted coupled with the increasing production of greenhouse gases (GHG) is of serious concern. Switching to fuels produced through renewable sources will lower net carbon output to the atmosphere and reduce effects caused by GHG. There has been significant focus on the production of ethanol using microbes, as so-produced ethanol is considered biofuel and helps meet the Renewable Fuel Standards *(1)*. Current methods of ethanol production is done through a batch based system, which have long processing times and large infrastructure costs (2). Continuous flow reactors (CFR) are known to reduce cost, size, and time but are not industrially conducted at large production rates (3). CFRs with enhanced rates of productivity are needed. Alginate threads have been shown to have higher diffusion rates over that of the typically used beads(4). **Methods:** *Zymomonas mobilis* strain ZM4 (ATCC 31821) immobilized in Ca-alginate was used for fermentation in a CFR. Ethanol production was studied using two different types of immobilization; beads and threads. The production of ethanol and the consumption of glucose were monitored using a high performance liquid chromatograph.**Results:** Although both methods of immobilization are able to obtain similar ethanol concentrations, the use of threads as an immobilization technique could offer the advantage of increased flow rates for the feedstock. As shown in Figure 1, the threads in the CFR consumed all the glucose present, while the beads were unable to fully ferment all the glucose present at the given flow rate.**Conclusions:** Faster flow rates of the feedstock into CFRs with threads could significantly enhance the ethanol yield as well as the rate of production. Further analysis on the use of threads for ethanol production will be discussed.



Author Disclosure Block:

A. Nordmeier: None. **D. Chidambaram:** None.

Poster Board Number:

FRIDAY-073

Publishing Title:

Improved Acetic Acid Resistance in *Saccharomyces cerevisiae b*y Overexpression of the *Whi2* gene Identified Through Inverse Metabolic Engineering

Author Block:

Y. Chen, Female, Na Wei; Univ. of Notre Dame, South Bend, IN

Abstract Body:

Developing acetic acid resistant *Saccharomyces cerevisiae* is important for economical production of biofuels from lignocellulosic biomass, but the goal remains a critical challenge due to insufficient knowledge on effective genetic perturbation targets for improving acetic acid resistance in the yeast. This study employed a genome-wide inverse metabolic engineering approach to successfully identify a novel gene target *WHI2* (encoding a cytoplasmatic globular scaffold protein) which elicited improved acetic acid resistance in *S. cerevisiae.* Overexpression of *WHI2* significantly improved glucose and/or xylose fermentation under acetic acid stress in engineered yeast. Analysis of expression of *WHI2* gene products determined that acetic acid induced endogenous expression of Whi2 in *S. cerevisiae*. Meanwhile, the *whi2*Δ mutant strain had substantially higher susceptibility to acetic acid than the wild type, suggesting the important role of Whi2 in acetic acid response in *S. cerevisiae*. Additionally, overexpressing *WHI2* and a cognate phosphatase gene *PSR1* had a synergistic effect in improving acetic acid resistance, suggesting that Whi2 could function in combination with Psr1 to elicit acetic acid resistance. These results enriched our understanding of yeast response to acetic acid stress and provided a new strategy to construct acetic acid resistant yeast strains for renewable biofuel production.

Author Disclosure Block:

Y. Chen: None.

Poster Board Number:

FRIDAY-074

Publishing Title:

Simultaneous Saccharification and Fermentation of L-Lactic Acid Production from Starch by Genetically Engineered Aspergillus Oryzae

Author Block:

S. Wakai[1], N. Asai-Nakashima[1], H. Tsutsumi[2], Y. Hata[2], C. Ogino[1], A. Kondo[1]; [1]Kobe Univ., Kobe, Japan, [2]Gekkeikan Sake Co Ltd, Kyoto, Japan

Abstract Body:

Background: Lactic acid is a commodity chemical that is widely used in the food, pharmaceutical, textile, leather, and chemical industries. It can be produced biologically, and it is a source of polylactate. Polylactate has been a focus of recent studies for its use as a biodegradable alternative to petroleum-based plastic products. Nowadays, bio-based lactic acid is produced from starchy biomass, while bioproduction by lactic acid bacteria require for utilization of expensive enzymes. Therefore, we attempted to examine bioproduction of lactic acid from starch by a filamentous fungus *Aspergillus oryzae* which has a strong advantage over other organisms because it produces various amylolytic enzymes.**Methods:** Lactic acid-producing *A. oryzae* strains were constructed by genetic engineering. The bovine (*Bos taurus*) *LDH-A* gene sequence was modified based on the codon usage of *A. oryzae* and synthesized. The synthesized artificial gene fragment was cloned into pISI, which contains the *sodM* promoter and *glaB* terminator from *A. oryzae*. Using the resulting plasmid, the *A. oryzae* strain (*A. oryzae* LDH) with the bovine l-lactate dehydrogenase gene was constructed. Furthermore, an authentic lactate dehydrogenase gene on *A. oryzae* chromosome was disrupted by replacing with *pyrG* fragment. The resulting strain named as *A. oryzae* LDHΔ871. Using these strains, lactic acid fermentation from glucose and starch was examined.**Results:** The *A. oryzae* LDH produced 38 g/L of lactate from 100 g/L of glucose. Disruption of the wild-type lactate dehydrogenase gene in *A. oryzae* LDH improved lactate production. The resulting strain *A. oryzae* LDHΔ871 produced 49 g/L of lactate from 100 g/L of glucose. So *A. oryzae* strains innately secrete amylases, *A. oryzae* LDHΔ871 produced approximately 30 g/L of lactate from various starches, dextrin, or maltose (all at 100 g/L). Finally, *A. oryzae* LDHΔ871 produced 45 g/L of lactic acid from a mixture of starch (50 g/L) and glucose (50 g/L) as the substrate.**Conclusions:** This is the first report describing the simultaneous saccharification and fermentation of lactate from starch using a pure culture of transgenic *A. oryzae*. Our results indicate that *A. oryzae* could be a promising host for the bioproduction of useful compounds such as lactic acid.

Author Disclosure Block:

S. Wakai: None. **N. Asai-Nakashima:** None. **H. Tsutsumi:** None. **Y. Hata:** None. **C. Ogino:** None. **A. Kondo:** None.

Poster Board Number:

FRIDAY-075

Publishing Title:

Substantial Improvement in Methyl Ketone Production in *E. coli* Using Metabolic Engineering and Metabolic Modeling

Author Block:

E-B. Goh, E. E. K. Baidoo, J. D. Keasling, H. G. Martin, H. R. Beller; Joint BioEnergy Inst., Emeryville, CA

Abstract Body:

We have engineered *Escherichia coli* to overproduce aliphatic methyl ketones (MK) in the C_{11} to C_{15} (diesel) range; this group of MK includes 2-undecanone and 2-tridecanone, which have favorable cetane numbers and are also of importance to the flavor and fragrance industry. Overall, we have made specific improvements that resulted in more than 10,000-fold enhancement in MK titer relative to that of a fatty acid-overproducing *E. coli* strain. The first generation of engineered *E. coli* (Goh et al. 2012) produced ~380 mg/L of MK in rich medium and had modifications that included: (a) overproduction of β-ketoacyl-coenzyme A (CoA) thioesters achieved by modification of the β-oxidation pathway (specifically, overexpression of a heterologous acyl-CoA oxidase and native FadB, and chromosomal deletion of *fadA*) and (b) overexpression of a native thioesterase (FadM). We have subsequently made additional genetic modifications that included balancing overexpression of *fadR* and *fadD* to increase fatty acid flux into the pathway, consolidation of the pathway from two plasmids into one, codon optimization, and knocking out key acetate production pathways. These latest modifications have led to a MK titer of 1.4 g/L with 1% glucose in shake flask experiments, which represents 40% of the maximum theoretical yield (the best values reported to date). *In vitro* assays with purified pathway enzymes suggested that flux towards β-ketoacyl-CoAs may be diminished by the promiscuous thioesterase activity of FadM, providing insight on how to fine-tune expression of pathway genes for further optimization of MK production. Using another approach for improving MK production, metabolic modeling was used to identify gene deletions that could improve flux through the MK pathway. One of the specified knockouts, Δ*scgE*, which was annotated as a homolog of Rpe (ribulose-5-phosphate epimerase), improved MK production by >50% relative to the base strain (EGS1710). ^{13}C-glucose experiments have recently been performed on the knockout strain along with the DH1 wild type and base strain (EGS1710) to obtain more comprehensive metabolic flux profiles that will refine the genome-scale model and enable additional improvements in MK production.

Author Disclosure Block:

E. Goh: None. **E.E.K. Baidoo:** None. **J.D. Keasling:** K. Shareholder (excluding diversitied mutual funds); Self; Amyris, Lygos. **H.G. Martin:** None. **H.R. Beller:** None.

Poster Board Number:

FRIDAY-076

Publishing Title:

Phenotypic Evidence That the Iron Transport Gene *fec*a Can Influence Ethanol Tolerance in *Escherichia coli*

Author Block:

K. Lupino, M. J. Hickman, G. A. Caputo, G. B. Hecht; Rowan Univ., Glassboro, NJ

Abstract Body:

Background: Biofuel research with the long term goal of industrial-scale production of alcohols has focused on fermentations by various microbial biocatalysts. To make such fermentations economical, the biocatalyst must have significant alcohol tolerance and the ability to utilize five carbon sugars that are abundant in lignocellulosic feedstocks. The *Escherichia coli* K12 derivative strain FBR5 was developed for its ability to metabolize five carbon sugars and ferment to ethanol efficiently. Our lab subsequently isolated ethanol tolerant mutants of FBR5. Several of these strains were found to have just a single silent mutation in the iron transport gene *fec*A. It has been reported that upregulation of multiple genes involved in iron transport is associated with increased ethanol tolerance. Thus, it was hypothesized that *fec*A specifically may play a role in the ethanol tolerance of *E. coli*.**Methods:** *E. coli* strains differing in *fec*A expression were tested for ethanol tolerance using two methods: a minimal inhibitory concentration (MIC) assay and cell survival experiments with various ethanol amounts.**Results:** MIC assays were performed with a *fecA* deletion strain and another strain capable of overexpressing *fecA* in the presence of IPTG. MIC assays performed with these strains were inconclusive. However, survival curve experiments demonstrated overexpression of *fecA* conferred 10 fold greater survival compared to control strains after as little as a 10 minute exposure to 16.5% (v/v) ethanol. This difference generally increased with time. Surprisingly, the *fec*A deletion strain also maintained 10 fold greater survival compared to its parental control throughout a 60 minute exposure to 16.5% ethanol. Deletion of *fec*A was additionally associated with increased cell survival in experiments using 17% ethanol.**Conclusions:** These data suggest that mutations resultant in increased or decreased expression of *fec*A are associated with a marked increase in the ethanol tolerance of *Escherichia coli*. Further characterization of the correlation between ethanol tolerance and genes associated with iron transport, including *fec*A, is pertinent to the use of *E. coli* as a microbial biocatalyst in industrial scale production of alcohols.

Author Disclosure Block:

K. Lupino: None. **M.J. Hickman:** None. **G.A. Caputo:** None. **G.B. Hecht:** None.

Poster Board Number:

FRIDAY-077

Publishing Title:

Production of Oleaginous Biodiesel Precursors in *Acinetobacter baylyi* Adp1

Author Block:

A. Chistoserdov, R. Ukey, R. Bajpai; UL Lafayette, Lafayette, LA

Abstract Body:

Due to a possible threat of the global climate change and dwindling supplies of fossil fuel, the humanity is actively seeking alternative sources for transportation fuel. Heterotrophic microorganisms offer an interesting alternative (or a complement) to the algal biodiesel production. *A. baylyi* produces large quantities of triacylgycerides and wax esters (TAGs and WEs) due to a unique bifunctional enzyme, wax synthase/diacylglycerol acyltransferase (WS/DGAT). To improve the production of biodiesel precursors in *A. baylyi*, a sequential inactivation of several genes (two lipases, acyl-CoA reductase and acyl-CoA dehydrogenase) was carried out using the *loxP*/Cre system. This would allow us to streamline the flow carbon towards fatty acids and then to the final products, TAGs and WEs. A four-fold increase in the TAG production was observed when three mutations (in two lipases and acyl-CoA dehydrogenase) were combined in the same strain of *A. baylyi*. Interestingly, an addition of a mutation in acyl-CoA reductase decreased production of oleaginous compounds. In order to further enhance the flow of carbon towards TAGs and WEs, several genes involved in both TAG and WE were cloned. However, their expression in *A. baylyi* did not markedly improved production of oleaginous compounds. It appears that thioesterases, which hydrolyze both acyl-CoA and acyl-ACP derivatives, plays the key role in production or removal of TAG and WE precursors. Therefore, thioesterases from several sources (*Escherichia coli*, *Enterococcus faecalis*, *A. baylyi*) were cloned and overexpressed and their contribution to production of free fatty acids, TAGs and WEs was investigated. It was shown that a truncated form of the AcTesA thioestratse from *A. baylyi* leads to the highest accumulation of fatty acid intermediates, preferentially producing palmitoleic and myristic acids. An unoptimized expression of AcTesA' in combination with WS/DGAT, led to further twofold increase in accumulation of TAGs in *A. baylyi*.

Author Disclosure Block:

A. Chistoserdov: None. **R. Ukey:** None. **R. Bajpai:** None.

Poster Board Number:

FRIDAY-078

Publishing Title:

Engineering *Pseudomonas putida* to Produce Adipic Acid from Lignocellulosic Biomass

Author Block:

J. A. Meadows, J. M. Gladden; Joint BioEnergy Inst., Emeryville, CA

Abstract Body:

Adipic acid is an important industrial dicarboxylic acid that is made worldwide. Annually, about 2.5 billion kg is produced and is primarily used as a precursor in the manufacturing of nylon, as well as plasticizers, food additives, and polyurethane resins, to name a few. With there being no significant occurrence in nature, it is manufactured from chemicals derived from petroleum, an environmentally harmful process. By using plant components as starting material and engineering an industrially relevant bacterial host, we have fashioned an approach that is ecofriendly for making adipic acid. *Pseudomonas putida* KT2440 was metabolically engineered to produce adipic acid from rich and a chemically defined media containing sugar and aromatic compounds derived from lignocellulose. Using a multi-omic approach, we were able to assess the metabolic, transcript, and protein profiles to identify bottlenecks in the engineered pathways. This allowed for targeted modifications to the pathways to increase adipic acid titers. This work demonstrates that *P. putida* is a viable bacterial host for producing adipic acid from biomass and is a potential alternative to manufacturing adipic acid that does not rely on petroleum-based feedstocks.

Author Disclosure Block:

J.A. Meadows: None. **J.M. Gladden:** None.

Poster Board Number:

FRIDAY-079

Publishing Title:

Investigation of Relationship between 2,3-Butanediol Toxicity and Production during Growth of *Paenibacillus polymyxa*

Author Block:

C. C. Okonkwo, V. Ujor, T. C. Ezeji; The Ohio State Univ./OARDC, Wooster, OH

Abstract Body:

Understanding 2,3-butanediol (2,3-BD) tolerance in *Paenibacillus polymyxa* DSM 365 is critical to its engineering to produce economically viable quantities of 2,3-BD. While *P. polymyxa* is unable to generate more than 3% 2,3-BD in batch fermentation systems in our laboratory, different concentrations of 2,3-BD ranging from 15 to 111 g/L have been reported by various research groups. Hence, we investigated the response of *P. polymyxa* to high 2,3-BD concentrations.To determine the 2,3-BD production capacity of *P. polymyxa*, fed-batch and batch fermentations during which *P. polymyxa* was challenged with 2,3-BD (20, 40 and 60 g/L) at 0 h was conducted. Furthermore, *P. polymyxa* was challenged with incremental 2,3-BD concentrations (20, 40 and 60 g/L at 12, 24 and 36 h, respectively) to mimic 2,3-BD accumulation during batch and fed-batch fermentations. Using serial dilution, gas chromatography, spectrophotometric and HPLC techniques, and assays, cell growth, residual glucose, 2,3-BD and acetoin were analyzed.In fed-batch cultures (6-L bioreactor) 2,3-BD accumulated to a maximum concentration of 47 g/L despite the presence of residual 13 g/L glucose in the medium. Concomitantly, accumulation of acetoin, the precursor of 2,3-BD, in the bioreactor increased after maximum 2,3-BD concentration was reached, suggesting that 2,3-BD was reconverted to acetoin after the concentration threshold for 2,3-BD tolerance was exceeded. Batch cultures of *P. polymyxa* challenged with 20, 40 and 60 g/L 2,3-BD at 0 h showed a concentration dependent growth inhibition response to 2,3-BD, inhibiting the growth by. 18, 33 and 100 %, respectively, relative to the control. Interestingly, 2,3-BD was reconverted to acetoin when its concentration reached 60 g/L in *P. polymyxa* cultures challenged with incremental 2,3-BD at 12, 24, and 36 h, possibly to alleviate 2,3-BD toxicity.Collectively, our findings indicate that 2,3-BD-mediated toxicity is a major metabolic impediment to 2,3-BD overproduction, thus, making it an important metabolic engineering target for rational design of a 2,3-BD-overproducing strain.

Author Disclosure Block:

C.C. Okonkwo: None. **V. Ujor:** None. **T.C. Ezeji:** None.

Poster Board Number:

FRIDAY-081

Publishing Title:

Screening for Biofuel Producing *Clostridium* Species

Author Block:

S. Riedel, C. Olumba, K. Lee, G. Barding, W-J. Lin; Cal Poly Pomona, Pomona, CA

Abstract Body:

The search for sustainable renewable energy has been one of the primary areas of research. The solventogenic *Clostridium beijerinckii* is known for its production of biofuels through the acetone-butanol-ethanol (ABE) fermentation pathway. Among the three fermentation products, butanol is considered a direct replacement of gasoline due to its compatibility with the gasoline engine. In our previous study, 92 *C. beijerinckii* strains were isolated from horse fecal samples collected from three horse farms, Cal Poly Pomona, Mt. SAC, and a private farm in Glendora. To screen for strains that possess the ABE fermentation pathway, 3 sets of PCR primers were designed to detect the presence of the genes encoding for acetoacetate decarboxylase, butanol dehydrogenase and alcohol dehydrogenase, which are essential for the production of acetone, butanol, and ethanol, respectively. The screening of the 92 *C. beijerinckii* isolates yielded a total of 5 isolates that are positive for the three genes tested. To set up the quantitative analysis of butanol production, the ABE positive isolates, as well as the positive control (*C. beijerinckii)* and a negative control (*C. perfringens)* were grown in the synthetic P2 medium for 48 hours. The organic compounds were extracted and subjected to gas chromatographic analysis with a flame ionization detector (GC-FID). The presence of butanol peak at 7.4 minutes is confirmed in the positive control, but not the negative control strains. The ABE-gene positive isolates showed variable butanol yields based on our GC-FID analysis. The conditions for optimal butanol production are also studied.

Author Disclosure Block:

S. Riedel: None. **C. Olumba:** None. **K. Lee:** None. **G. Barding:** None. **W. Lin:** None.

Poster Board Number:

FRIDAY-082

Publishing Title:

Metabolic Engineering to Enhance Furfural Tolerance during Cellulosic Butanol Fermentation by Glycerol-Supplemented *Clostridium beijerinckii*

Author Block:

C. Agu, V. C. Ujor, T. C. Ezeji; The Ohio State Univ. and Ohio Agricultural research and Dev. Ctr., Wooster, OH

Abstract Body:

The inability of *Clostridium beijerinckii* to efficiently utilize glycerol is a major impediment to adopting glycerol metabolism as strategy to increase NAD(P)H regeneration, mitigate inhibitor (e.g. furfural) toxicity, and improve butanol titer during fermentation of lignocellulosic biomass hydrolysates (LBH). Therefore, it was reasoned that metabolic engineering to enhance glycerol utilization in *C. beijerinckii* will increase NAD(P)H regeneration and improve butanol production from furfural-replete LBHTowards this goal, we sought to systematically excise glycerol catabolic arsenal from a hyper-glycerol utilizing bacterium (*Clostridium pasteurianum*) and clone into *C. beijerinckii*. Glycerol dehydrogenase (gldh), the first enzyme in glycerol catabolism, catalyzes an NAD(P)H yielding reaction, dehydrogenation glycerol to dihydroxyacetone. As a preliminary step, *C. pasterianum* gldh genes - F502 12753 and 6017 were overexpressed as fusion construct in an *E. coli-Clostridium* shuttle vector - pWUR460 under the control of constitutive thiolase promoter, followed by electroporation into *C. beijerinckii*. The generated strain, *C. beijerinckii*-gldh was used to conduct batch acetone-butanol-ethanol (ABE) fermentation with glucose medium supplemented with glycerol and 0, 4, 5, or 6 g/L furfural. Using gas chromatography, high performance liquid chromatography and spectrophotometric assays, cell growth, acetone-butanol-ethanol (ABE) production, glycerol utilization, and furfural detoxifying capacity of *C. beijerinckii*-gldh were analyzed and compared with the wildtype *C. beijerinckii*At high furfural concentration (6 g/L), the growth of and ABE production by *C. beijerinckii*-gldh were 20% and 87%, respectively, higher than the wildtype. Similarly, the ABE productivity was 2.4-folds higher than the *C. beijerinckii* wildtype and glycerol utilization was significantly ($p<0.05$) increased by 15%.Taken together, gldh overexpression in *C. beijerinckii* improved its tolerance to furfural and glycerol utilization, suggesting that overexpression of the entire *C. pasteurianum* glycerol catabolic arsenal and optimization may significantly improve fermentation of dilute acid pretreated LBH to butanol.

Author Disclosure Block:

C. Agu: None. **V.C. Ujor:** None. **T.C. Ezeji:** None.

Poster Board Number:

FRIDAY-083

Publishing Title:

Identification of Butanol Tolerant Genes in *Lactobacillus mucosae*

Author Block:

S. Liu, N. Qureshi; USDA, ARS, NCAUR, Peoria, IL

Abstract Body:

Background: Butanol, though in low concentrations, is produced biologically through fermentation of lignocellulosic biomass-derived substrates by Gram-positive *Clostridium* species. However, naturally available butanol fermenting microbes are sensitive to stress caused by increased production of butanol and the presence of various inhibitors from biomass hydrolyzates. Thus for commercial production, new strains with better tolerance to butanol and inhibitors are desired. One strategy is to genetically modify available species of *Clostridium* by introducing stress tolerance genes. The rationale of this study is to seek butanol tolerance genes from other Gram-positive species which might be better suited than those from the Gram-negative *Escherichia coli* or eukaryotic *Saccharomyces cerevisiae*. Several butanol tolerant *Lactobacilli* were found capable of growth in 3-4% butanol after long term adaptation. In this study, *Lactobacillus mucosae* BR0605-3was used to identify new butanol tolerance genes, since this strain showed most robust growth in 4% butanol and the genome sequence of *L. mucosae* is publically available. **Methods:**Total cellular proteins from duplicate cultures of *L. mucosae* BR0605-3 grown in MRS with 0 and 4% butanol were extracted and subjected to 2D-gel electrophoresis. Comparisons of 2D gel images between 0% and 4% butanol grown cultures indicated expression levels changed in response to growth in 4% butanol. Both increases and decreases of individual spots were measured and calculated from the intensity on the gel images. Based on the significance and multitude of changes, 50 spots were chosen and analyzed using LC-MS/MS**Results:** A total of 31 protein spots were identified either from the L. mucosae database through ProteinLynx Global Server (PLGS) or from the Mascot database through NCBI and SwissProt. The identification of butanol stress related proteins will be presented and the results will help us to understand the molecular mechanisms governing tolerance to high butanol concentrations.**Conclusions:** This study will lead to cloning of specific genes related to butanol tolerance, which may be used for improving biocatalysts for efficient conversion of biomass to biofuel butanol.

Author Disclosure Block:

S. Liu: None. **N. Qureshi:** None.

Poster Board Number:

FRIDAY-084

Publishing Title:

Engineering Thiolase Enzyme Specificty

Author Block:

Y. Tarasova, B. Bonk, B. Tidor, K. L. J. Prather; Massachusetts Inst. of Technology, Cambridge, MA

Abstract Body:

Inspired by polyhydroxyalkanoate (PHA) biosynthesis, a heterologous pathway for the production of a variety of 3-hydroxyalkanoic acids (3HAs) has been developed in *Escherichia coli*. 3HAs produced by this pathway are chiral and can serve as precursors for many useful biopolymers, pharmaceuticals and solvents. The pathway can also be extended by overexpression of additional enzymes that act on the 3HA-CoAs to produce many other classes of compounds, such as aldehydes and alkanes, of variable chain length. However, this pathway suffers from 3-hydroxybutyrate (3HB) byproduct formation, due to the high catalytic activities of the pathway enzymes towards their native substrates. In this work, we focus on the thiolase enzyme as a selectivity control point for the pathway. A structure-guided computational approach was used to predict mutants with increased activity towards the condensation of longer chain acyl-CoA thioesters and/or with decreased activity towards the condensation of two acetyl-CoA molecules. Candidate mutant thiolases were then screened *in vivo* using formation of PHAs as a readout of enzyme selectivity. The best mutant resulted in over a ten-fold increase in selectivity for formation of the longer chain product, 3-hydroxyhexanoate, as compared to the wild type, while maintaining high overall conversion. This mutant was then characterized *in vitro*, and confirmed to have lower activity towards the condensation of two acetyl-CoAs. Results on the application of these highly specific mutant thiolases towards the synthesis of longer chain products, such as 4-methyl pentanol will be presented. Further *in vitro* characterization of thiolase mutants is underway and will serve to expand our understanding of the sequence-structure-function relationships for this important class of enzymes.

Author Disclosure Block:

Y. Tarasova: None. **B. Bonk:** None. **B. Tidor:** None. **K.L.J. Prather:** None.

Poster Board Number:

FRIDAY-085

Publishing Title:

Bioprocessed Polysaccharide Powders from the Liquid Culture of Lentinus Edodes Fungal Mycelia with Curcuma Longa (Turmeric) Alter the Chicken Macrophage Responses to *Salmonella* Enteric Species

Author Block:

D. Han[1], H. T. Lee[1], **J. B. Lee**[1], N. Y. Kim[2], Y. B. Kim[2], J. W. Yoon[1]; [1]Coll. of Vet. Med. & Inst. of Vet. Sci., Kangwon Natl. Univ., Chuncheon, Korea, Republic of, [2]Res. Inst. for Vet. Sci., & BK21 PLUS Program for Creative Vet. Sci. Res., Coll. of Vet. Med., Seoul Natl. Univ., Seoul, Korea, Republic of

Abstract Body:

Various pharmacological activities of *Curcuma longa* (turmeric) have been reported, including anti-inflammatory and antioxidant effects. In this study, bioprocessed polysaccharide powders (BPPs) from the liquid culture of *Lentinus edodes* fungal mycelia containing *Curcuma longa* (referred to as BPP-Curcuma) were extracted in water at 37°C and examined their biological effects on the avian-specific or zoonotic *Salmonella enterica* species as well as their responses to the chicken-derived macrophage cells, HD-11, during the salmonella infection. Our experimental analyses revealed that the water extracts of BPP-Curcuma (i) did not affect the growth kinetics of *Salmonella* spp. evaluated, (ii) induced the alteration of the bacterial secretome profiles compared to the untreated control groups, (iii) showed increased phagocytic activities in the HD-11 cells when infected by *Salmonella* spp., but did not affect their bacterial killing activities, and (iv) greatly up-regulated the transcription expression of TNF-α IL-1β, iNOS, and IL-10. Interestingly, such cytokine expression appeared to be modulated when the HD-11 cells were pre-exposed with *Salmonella spp*. Taken together, these results imply the biological activity of BPP-Curcuma on both *Salmonella spp*. and macrophages during an avian infection.

Author Disclosure Block:

D. Han: None. **H.T. Lee:** None. **J.B. Lee:** None. **N.Y. Kim:** None. **Y.B. Kim:** None. **J.W. Yoon:** None.

Poster Board Number:

FRIDAY-086

Publishing Title:

Tailor-Made Construction of Yeast Adsorbing Rare Earth Elements through Screening from Yeast Library Displaying Random Peptides

Author Block:

K. Kuroda, R. Ito, H. Hashimoto, M. Ueda; Kyoto Univ., Kyoto, Japan

Abstract Body:

Rare earth elements (REEs) consist of 17 kinds of metal elements and belong to rare metals. REEs are commonly used in high-tech products such as liquid crystal displays, IT devices, and hybrid vehicles. Thus, demands for REEs are increasingly growing with the development of high-tech industries, leading to rising prices of REEs. For stable supply and procurement of REEs, the efficient recovery of REEs from nature, wastewater, and discarded high-tech products is one of the most important technology to be developed. We have established yeast display system and applied it into metal-ion adsorption on yeast cell surface by displaying metal-binding proteins or peptides [1, 2]. However, there is no natural metalloprotein that binds REEs and is few examples of artificial REEs-binding peptides. In this study, we attempted to construct REEs-binding yeast by screening from yeast library displaying random peptides.For the preparation of yeast library, peptides with random sequences based on $(NNK)_{25}$ codon was displayed by fusion with cell wall-anchoring domain of α-agglutinin. After the incubation of the prepared yeast library with Tb^{3+}-charged magnetic beads, yeast cells that were bound to beads were recovered and isolated on agar medium. The isolated clones were examined for adsorption ability in Tb^{3+} solution. Several peptide-displaying yeasts showed the enhanced adsorption of Tb^{3+} compared to wild-type yeast. Furthermore, this enhanced adsorption was specific for Tb^{3+}. Therefore, the combination of yeast cell surface engineering and screening would be an efficient strategy to create a protein or peptide for specific adsorption of target REEs.[1] K. Kuroda and M. Ueda (2010) *Appl. Microbiol. Biotechnol.* **87**, 53-60[2] K. Kuroda and M. Ueda (2011) *Curr. Opin. Biotechnol.* **22**, 427-433

Author Disclosure Block:

K. Kuroda: None. **R. Ito:** None. **H. Hashimoto:** None. **M. Ueda:** None.

Poster Board Number:

FRIDAY-088

Publishing Title:

Indigenous Hydrocarbon-Contaminated Niger Delta Soil Bacteria Possess Biosurfactant Production Potential for a More Sustainable Environmental Bioremediation

Author Block:

S. B. Akinde, A. A. Adebunmi, I. O. Awoniyi, A. E. Ibilibor; Osun State Univ., Osogbo, Nigeria

Abstract Body:

The work is a preliminary investigation of indigenous biosurfactant production by bacterial flora obtained from chronically hydrocarbon-contaminated Niger Delta soil for microbially enhanced oil recovery (MEOR) and sustainable environmental bioremediation in the region. A total of 99 bacterial strains were isolated from 13 hydrocarbon-contaminated soil samples and presumptively identified by their morphological and biochemical characteristics. Blood haemolysis, oil displacement and emulsification index screening were used to select hyper-active biosurfactant-producing isolates which were identified by 16S rRNA sequencing. The bacterial strains belonged to the genera: *Aeromonas* (38), *Bacillus* (14), *Citrobacter* (1), *Clostridium* (5), *Corynebacterium* (14), *Enterobacter* (9), *Klebsiella* (7), *Providencia* (1) and *Pseudomonas* (4). 16S rRNA sequencing however authenticated the identity of 8 selected hyper-active strains and further characterized them to species level as *Clostridium sporogenes* (NGS-BH1), *Citrobacter freundii* (NGS-BH2), *Clostridium sp* (NGS-BH3), *Aeromonas hydrophila* (NGS-BH4), *Enterobacter cancerogenus* (NGS-M7), *Aeromonas caviae* (NGS-M17), *Aeromonas caviae* (NGS-M18), *Enterobacter hormaechei* (NGS-M19). The dry weight ± standard deviation of biosurfactant produced by the 8 hyper-active strains ranged between 0.39 ± 0.06 g (NGS-M17) and 1.72 ± 0.07 g (NGS-M7). Local biosurfactant production by indigenous microflora can facilitate cost-effective and rapid environmental bioremediation as well as other industrial applications in Niger Delta Nigeria.

Author Disclosure Block:

S.B. Akinde: None. **A.A. Adebunmi:** None. **I.O. Awoniyi:** None. **A.E. Ibilibor:** None.

Poster Board Number:

FRIDAY-089

Publishing Title:

Evaluation of Native Bacterial Strains to Formulate an Oil Degradative Consortium

Author Block:

P. Teran Morales, F. Merino Rafael, S. Gutierrez Moreno; Natl. Univ. of San Marcos, Lima, Peru

Abstract Body:

Nowadays the oil industry is the main component of the world´s energetic matrix. The oil extraction and handling processes have the inherent risks of contamination, causing a progressive deterioration of the environment. In spite of this, there are ecofriendly alternatives like bioremediation process that is gaining world recognition. For this process, the selection and characterization of microorganisms is the main key to success. In Peru this process is being applicated, recently. The objective of this research was the evaluation of bacterial strains isolated from oil contaminated soil to formulate a consortium with high oil degradation ability. We evaluated 44 bacterial strains, isolated from Talara oil contaminated soil, provided by SAVIA Peru Company. The selection was based on the degradative activity on crude oil (Mills et al., 1978), emulsifying activity, growth capacity on petroleum derivates: gasoline, kerosene, paraffin and vacuum waste; and the inexistence of antagonism between them. At first, the strains were identified by API20NE and APICH50 system and then by 16S rRNA gene. The results were statistically analyzed with Principal Component Analysis and ANOVA. Results revealed that on crude oil, the 16% of the strains showed a growth comparable with 1-3 McFarland scale. The 7% of strain showed an emulsifying activity higher than 1 UAE/ml. Growth evaluation on different substrates showed that 100% (44) of the strains grew on paraffin and gasoline, whereas only 15% (7) was able to grow on vacuum waste and the 84% (37) on kerosene. These results indicated a great variability of oil degradative ability of the strains. Then, 9 strains were selected to check the antagonism between them: *Pseudomonas aeruginosa* 68P, 202P, 24, 210P, 165, *Bacillus subtilis* 202G, 68G, 106, 210G. After the antagonism test, the consortium was formulated with *Pseudomonas aeruginosa* 68P, 202P and *Bacillus subtilis* 202G. In conclusion, the formulated consortium could be used in the bioremediation process of the oil soil where were isolated.

Author Disclosure Block:

P. Teran Morales: None. **F. Merino Rafael:** None. **S. Gutierrez Moreno:** None.

Poster Board Number:

FRIDAY-090

Publishing Title:

Biodegradation of Crude Oil by Pure Bacterial Culture in Liquid Medium

Author Block:

H. Kim[1], J. Kim[1], H. Kim[2], S-S. Lee[1]; [1]Kyonggi Univ., Suwon, Korea, Republic of, [2]Hyundai Construction & Engineering, Yongin, Korea, Republic of

Abstract Body:

Byproducts from crude oil are the important energy source for everyday life. However, their accidental and untreated release of the petroleum product into the environment contaminates the soil and water which creates several health and environmental problems. Such long term contamination of crude oil products into the environment has enriched several microorganisms which have potential to degrade such toxic compounds. These microorganisms generally degrades oil components in the order of *n*-alkane, branched-chain alkane, branched alkane, low molecular weight *n*- alkyl aromatic, mono-aromatics, cyclic alkane, polycyclic aromatic hydrocarbon to asphaltene. In this study we selected thirty one bacteria which were previously isolated bacteria from different oil contamination sites. The degradation efficiency by pure cultures were performed using liquid extraction and liquid analysis method via GC-FID. Decrease in the low molecular *n*-alkanes peak was analyzed through GC-FID after 7 and 14 days of interval. In addition, molecular analysis was also performed to analyze the genes involved in the crude oil biodegradation. Among all the tested bacteria, strain *Ochrobactrum pecoris* and strain SBR17 showed highest oil degradation ability. The crude oil degradation efficiency of strain *Ochrobactrum pecoris* and SBR17 was 63.3 % and 69.0 % after 7 days of incubation at their optimal growth conditions. Crude oil degradation ability from strain *Gordonia malaquae*, *Pseudomonas monteilli*, *Massilia niastensis* was also recorded up to 39.5 % after 14 days of incubations. In addition, molecular analysis was also confirmed the presence of *CYP153, alkB* and *ladA* in these bacteria which are involved in the crude oil biodegradation. Our findings from this study indicated the presence of several potential bacteria which have highly efficient crude oil degradation ability. Further studies have to be performed to optimize and enhance the removal efficiency and it gives a gateway for the bioremediation of crude oil contamination. We will do through the GC-MS, figure out product degradation pass way by pure culture bacteria.

Author Disclosure Block:

H. Kim: None. **J. Kim:** None. **H. Kim:** None. **S. Lee:** None.

Poster Board Number:

FRIDAY-093

Publishing Title:

Biotransformation of PAHs by *Sphingobium barthaii* and Investigation of DNA Damage by LC/ESI-MS/MS

Author Block:

K. Fukuoka, **R. A. Kanaly**; Yokohama City Univ., Yokohama, Japan

Abstract Body:

During the biodegradation of aromatic environmental pollutants by bacteria, catechols are produced that undergo redox cycling with aromatic quinones. Quinones are a group of highly reactive compounds that react with DNA and cellular macromolecules or produce reactive oxygen species in cells. Not much is known about the effects of these compounds on bacteria during polycyclic aromatic hydrocarbon (PAH) biodegradation. Towards the development of methods to investigate such phenomena, biodegradation and exposure experiments were conducted with the soil bacterium *Sphingobium barthaii* with analyses by liquid chromatography electrospray ionization tandem mass spectrometry (LC/ESI-MS/MS). LC/ESI-MS/MS product ion scan analyses of a 160 Da product detected in extracts from *S. barthaii* during growth on PAHs identified it as the PAH-catechol, 1,2-dihydroxynaphthalene. Thereafter, *S. barthaii* cells were exposed to 1,2-naphthoquinone and cellular DNA was extracted, digested and purified to their corresponding 2'-deoxynucleosides. DNA adductomics methods were applied to detect unidentified DNA modifications by analyzing the 2'-deoxynucleosides by LC/ESI-MS/MS. Results showed that at least one high molar mass DNA modification was detected with a molar mass of 425 Da. Product ion scan analyses revealed fragments that pointed towards an identity of a dihydroxynaphthalene adduct. LC/ESI-MS/MS analysis of products from a reaction of 1,2-naphthoquinone with 2'-deoxyguanosine yielded a 425 Da compound with a retention time that matched the product detected from bacterial cells and supported an identity of 1,2-dihydroxynaphthalene-4-N7-deoxyguanosine. At the same time, other DNA adducts were unambiguously identified from exposed and unexposed cells by LC/ESI-MS/MS techniques including N^6-methyl-2'-deoxyadenosine, and the oxidative adduct, 8-oxo-deoxyguanosine. This work contributes to our understanding of the effects of PAH biodegradation products on cellular functions during exposure to PAHs. Future work shall involve investigation of the production of depurinating adducts.

Author Disclosure Block:

K. Fukuoka: None. **R.A. Kanaly:** None.

Poster Board Number:

FRIDAY-094

Publishing Title:

A New Approach for Phenotype-based Identification of Key Enzymes in Polycyclic Aromatic Hydrocarbon Metabolism from Mycobacteria Using Transposon Mutagenesis and a Pah Spray Plate

Author Block:

S-J. Kim, O. Kweon, C. E. Cerniglia; FDA/NCTR, Jefferson, AR

Abstract Body:

Background: Despite considerable knowledge of bacterial PAH metabolism, key enzymes and their pleiotropic and epistatic behaviors responsible for high-molecular-weight (HMW) PAH biodegradation remain poorly understood. HMW PAHs, such as benzo[*a*]pyrene, are genotoxic compounds that tend to persist in the environment. A number of studies have shown that mycobacteria and other gram positive bacteria have the ability to degrade the HMW PAHs. However, to improve rates of HMW PAH biodegradation, new research approaches and tools are necessary to obtain knowledge on the enzymatic mechanisms involved in the degradation of these environmental contaminants. In this study, we describe, using HMW PAH-degrading *Mycobacterium vanbaalenii* PYR-1 as a model organism, a rapid, low-cost, and high-throughput screening for function-dependent selection of mutants with metabolic discrepancy or defect in the metabolism of HMW PAHs. **Methods:** The approach has four steps: i) preparation of mycobacterial electrocompetent cells, ii) construction of a transposition mutant library using EZ-Tn5 <R6Kγori/KAN-2> Tnp transposome, iii) phenotypic screening, using a PAH spray plate method, and iv) rescue cloning of transposed genomic DNA. **Results:** Using this new method, we identified more than 20 mutants with HMW PAH metabolic discrepancy or defect from ~4,000 transposon mutants of *M. vanbaalenii* PYR-1. For example, a mutant 8F7 lost its ability to degrade pyrene, while still being able to degrade fluoranthene and the mutant was shown to be defective in the gene *nidA*, encoding a ring-hydroxylating oxygenase enzyme, which is essential for the metabolism of pyrene in PAH-degrading mycobacteria. By sequencing analysis, we confirmed the position of Tn5-based genetic perturbation of selected mutants mostly in the genes responsible for PAH degradation. **Conclusion:** Combination of the approaches such as transposon mutagenesis and phenotypic assays has provided insights into HMW PAH biodegradation for bioremediation applications. In addition, this new phenotype-based approach may be applicable to other PAH-degrading bacterial strains and could also be modified to screen a mutant(s) able to degrade only a certain group of PAHs.

Author Disclosure Block:

S. Kim: None. **O. Kweon:** None. **C.E. Cerniglia:** None.

Poster Board Number:

FRIDAY-096

Publishing Title:

Diesel Degradation in Presence of Copper and Microaerobiosis by *Pseudomonas extremaustrali*s

Author Block:

L. Rossi, N. I. Lopez, L. J. Raiger Iustman; FCEyN, Univ. de Buenos Aires, Buenos Aires, Argentina

Abstract Body:

Microorganisms adapted to contaminated environments represent an alternative to bioremediation of pollutants. Diesel is a widely distributed one, being its composition a mixture of alkanes that could be degraded by some microorganisms. Cooper is an essential metal for most organisms, but at high concentrations commonly detected in diesel-polluted waters can be lethal. Previous work show that *P. extremaustralis* was able to degrade diesel in biofilm growth conditions but not in shaken flasks, and tolerate up to 4mM $CuSO_4$ in aerobiosis. It was also described that co-substrate addition could enhance xenobiotic degradation. The aim of this work was to determine if *P.extremaustralis* was able to degrade diesel in microaerobiosis without biofilm formation and to evaluate the effect of glucose as co-substrate and Cu in diesel degradation. *P. extremaustralis* 14-3b strain was used for the assays. Cultures of 20 mL were incubated for 7 days at 30 °C in 100 mL capped bottles with E_2 minimal medium, supplemented with 8 mM KNO_3 and 1% v/v Diesel. To determine the growth variables, different Cu and glucose concentrations were evaluated: $CuSO_4$ 0.1 to 1.5 mM, and glucose 0.5 to 20 g/L. Diesel degradation was evaluated by extracting the remnant hydrocarbon with 10% v/v n-hexane, and analyzing by GC. A residual hydrocarbon percentage was calculated using a control without bacteria as 100%. The growth conditions were finally set at $CuSO_4$ 0.1 mM for it was the highest full soluble concentration in this media, and 0.5 g/L glucose. *P. extremaustralis* was able to degrade 16% of the total diesel when it was added as sole carbon source, and no differences were observed when glucose was added as co-substrate or Cu was present. When the culture media was supplemented with both glucose and Cu, however, 30% degradation was achieved. Analyzing each alkane in particular, there was a clear degradation in the ones with 14 to 21 Carbon atoms, consistent with the results found in biofilm. This suggests that the addition of a co-substrate and a stress promoter as Cu in a sub lethal concentration could enhance diesel biodegradation in *P. extremaustralis*.

Author Disclosure Block:

L. Rossi: None. **N.I. Lopez:** None. **L.J. Raiger Iustman:** None.

Poster Board Number:

FRIDAY-097

Publishing Title:

Isolation and Characterization of Anaerobic Microbial Communities from Hydraulic Fracturing Fluids

Author Block:

S. Brewer[1], **M. F. Campa**[2], A. G. M. Amaral[1], J. L. Fortney[1], S. M. Techtmann[3], K. S. Fitzgerald[1], T. C. Hazen[1]; [1]Univ. of Tennessee, Knoxville, TN, [2]Bredsen Ctr. for Interdisciplinary Res. and Graduate Ed., Knoxville, TN, [3]Michigan Technological Univ., Houghton, MI

Abstract Body:

Hydrocarbon production from hydraulic fracturing of gas shale in the US has skyrocketed and is projected to keep growing. This water intensive drilling process creates toxic wastewater without an efficient disposal method. Because this method involves projecting fluid 1-3 km deep into the Earth, it is likely that microbial communities adapted to the extreme conditions of the subsurface have accumulated in the produced water. The goal of this study is to identify microorganisms that might have bioremediation capabilities for the toxic flowback water and characterize microbes isolated from fracking water samples in anaerobic conditions. Water samples were obtained from hydraulic fracturing locations in the Marcellus shale of Pennsylvania. These water samples include six different collections of flowback water, a flowback mix tank, and three different treatment tanks. Inoculations from the water samples were grown in anaerobic conditions in high salinity marine media and halotolerant hydrocarbon degradation dependent media. Samples were also grown at ambient temperature and at 37°C. DNA was extracted, and 16S rRNA gene Sanger sequencing was used to identify individually isolated microbes. Illumina sequencing was used to yield genetic information about the overall microbial communities. The Biolog Omnilog, a high-throughput phenotype microarray, was used to determine the genotype-phenotype characteristics of some of the most significant isolates. Early results show presence of numerous anaerobic microbes with metabolic variability and bioremediation potential including sulfate reducers and hydrocarbon degraders. There also have been a considerable number of human pathogens identified with the capability for antibiotic resistance from biocide exposure.

Author Disclosure Block:

S. Brewer: None. **M.F. Campa:** None. **A.G.M. Amaral:** None. **J.L. Fortney:** None. **S.M. Techtmann:** None. **K.S. Fitzgerald:** None. **T.C. Hazen:** None.

Poster Board Number:

FRIDAY-098

Publishing Title:

Oil Sludge Degradation by *Lysinibacillus sphaericus* and *Geobacillus sp.*

Author Block:

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Abstract Body:

Bioremediation is an environmentally friendly and affordable technology for the removal of pollutants from petrochemical industry which does not introduce any additional chemicals to the environment. *Bacillus* species have been identified as being involved in crude oil degradation, however, little is known about the degradative potential of other related genera as *Lysinibacillus* and *Geobacillus*. We aim to evaluate the oil sludge degradative capacity of a consortium made up of *L. sphaericus* and *Geobacillus* sp. native strains of Colombia in landfarming processes carried out in greenhouse and field.Twelve impermeable membrane coated containers with oil sludge (API gravity 39.3°) and soil were placed (0.05:0.95) in greenhouse and field. Two kinds of experiments were set, the first one was open to the atmosphere and the second was totally coated. Bacterial consortium composed of *L. sphaericus* and *Geobacillus* sp. strains was added to the soil; negative controls had no inoculum. All of the experiments were replicated three times. Samples were taken after 8, 15 and 50 days to evaluate hydrocarbon degradation by gas chromatography and bacterial growth by plate count method. Samples of volatile organic compounds (VOCs) were taken from the closed containers and analyzed by high performance liquid chromatography. Statistical significance was evaluated by ANOVA and Tukey-Kramer test. Bacterial titer remained almost constant in all of the greenhouse experiments. A significant decrease in the number of bacteria was seen in experiments carried out in field, however a bacterial titer greater than 10^6 was still present on day 50. A significant difference is observed in total petroleum hydrocarbons (TPHs) concentration in open and closed inoculated containers as well as in non-inoculated and inoculated containers. The net TPHs degradation percentages due to biodegradation in greenhouse experiments were 68.7% and 19.4% for open and closed containers, respectively, whereas in field experiments were 39.7% and 33.9%. VOCs concentrations in the headspace were comparable to that of TPHs in soil, however VOCs biodegradation percentages were more than 50% both in greenhouse and field.Both inoculated open and closed containers presented a TPH removal percentage that was higher than the one for non-inoculated containers, which make the oil sludge degradative capacity of the strains under study evident.

Author Disclosure Block:

M. Hernández: None. **J. Dussán:** None.

Poster Board Number:

FRIDAY-099

Publishing Title:

Anaerobic Degradation of Alkanes (*n*-Hexane) by the Denitrifying Betaproteobacterium Strain Hxn1

Author Block:

K. Trautwein, A. Strijkstra, L. Wöhlbrand, R. Rabus; Carl von Ossietzky Univ. Oldenburg, Oldenburg, Germany

Abstract Body:

The main source of alkanes in the environment is crude oil and its refined products. Alkanes are the least reactive hydrocarbons due to absence of polar bonds. Nevertheless, aquatic and soil microorganisms are able to completely mineralize these energy-rich compounds under oxic and anoxic conditions. Aerobic degradation employs oxygenases and reactive oxygen species (O_2-derived) to activate the alkane, followed by oxidation of the primary or secondary alcohol to a fatty acid and its further degradation by conventional β-oxidation. In contrast, O_2-independent activation under anoxic conditions proceeds by radical addition of the subterminal carbon atom of the alkane to fumarate by (1-methylalkyl)succinate synthase (MasCDE; with the large subunit MasD bearing the glycyl radical). The enzymes catalyzing the subsequent degradation of the resulting (1-methylalkyl)succinate and regenerate the co-substrate fumarate are still unknown.This study aimed to investigate the proposed anaerobic degradation pathway for *n*-hexane in the betaproteobacterium strain HxN1 (Wilkes *et al.*, 2002), degrading C_6-C_8 alkanes with nitrate as terminal electron acceptor. The availability of a whole-genome shotgun database combined with comprehensive proteome analyses (2D DIGE, shotgun, membrane) allowed the identification of *n*-hexane-specific proteins (soluble and membrane) in strain HxN1. Differential proteome analysis by 2D DIGE revealed proteins specifically formed in cells grown with *n*-hexane, as compared to cells grown with hexanoate (caproate), propionate or acetate. The majority of *n*-hexane-specific identified proteins localize in a confined region (130 kb) of the genomic database, which include two identical copies of MasCDE (encoded in close proximity to each other), substrate:CoA ligases, a methylmalonyl-CoA mutase, and β-oxidation-related enzymes possibly involved in downstream degradation of *n*-hexane-derived, CoA-activated fatty acids. The presence of many genes related to transposases and mobile elements as well as the lower (by 6%) GC content in this region (as compared to the GC content of the whole-genome shotgun database) may suggest that the ability to degrade alkanes was horizontally acquired by the ancestors of strain HxN1.

Author Disclosure Block:

K. Trautwein: None. **A. Strijkstra:** None. **L. Wöhlbrand:** None. **R. Rabus:** None.

Poster Board Number:

FRIDAY-100

Publishing Title:

Hexadecane-Degrading Bacteria: Inoculums For Efficient Soil Rhizoremediation

Author Block:

Y. Bernier-Casillas, K. F. Álamo-Rodríguez, J. R. Pérez-Jiménez; Univ. del Turabo, Gurabo, PR

Abstract Body:

Rhizoremediation has been improved by efficient combination of plants and microbes to reduce harm of pollutants in nature. Bacterial contribution to alkane degradation has been documented for polluted and temperate sites. Alkanes, as hexadecane, reach the environment by anthropogenic activities and natural processes, including plant exudates. We hypothesized that if we stimulate microbes naturally selected in soil then plant development will be enhanced and remediation will proceed. Our objective is to evaluate which hexadecane-degrading bacteria (HDB) promote plant development. Soil samples were collected along Caño Martín Peña (San Juan, PR). HDB were isolated by aerobic cultivation on mineral media supplemented with hexadecane as sole carbon source and subjected to 16S rDNA sequencing. Isolates were tested in vitro for colonization factors. A selection of them was inoculated in soil microcosms, with *Phaseolus vulgaris* seeds, to evaluate their contribution to plant growth. All prospects were able to grow in hexadecane (1% v/v). Colonization factors revealed diverse capabilities among fifty-eight strains: phosphate solubilization (7%), production of indole acetic acid (3%), ammonia (17%), protease (34%), and siderophore (4%). Most of the factors were harbored in twenty strains, including members of *Klebsiella* and *Pseudomonas*. Plant microcosms have shown efficient plant growth, mostly by *Klebsiella* sp. HDB prevailed across Neotropical ecosystems, despite pollution level, are phylogenetically diverse, and harbors novel genetic variants. In the elucidation of colonization factors, the most specialized ones occur in a minimal fraction of the isolates. These hexadecane-degrading bacteria provide novel isolates to deal with pollution and disclose processes in nature.

Author Disclosure Block:

Y. Bernier-Casillas: None. **K.F. Álamo-Rodríguez:** None. **J.R. Pérez-Jiménez:** None.

Poster Board Number:

FRIDAY-102

Publishing Title:

Novel Taxa Are Dominant In Mangrove Swamp Of Niger Delta, Nigeria

Author Block:

C. J. OGUGBUE, C. C. NWANKWO, G. C. OKPOKWASILI; Univ. OF PORT HARCOURT, Port harcourt, Nigeria

Abstract Body:

Taxonomic study has greatly progressed with the use of metagenomics which provides detailed insights into the microbial diversity of ecosystems that hitherto, was not possible using traditional technique. Three crude oil contaminated soils and three pristine soils in the Niger Delta mangrove swamps were studied. Roche 454 Metagenomic sequencer was used to produce all data sets while bioinformatics were used to analyze data sets. DNA sequencing was performed using the Next Generation Sequencing technique to determine the nucleotide sequences of all microorganisms present in the soil sample while the microbial compositions were identified using CLO bio software after BLASTX Analysis. A total of 20 phyla and 36 classes were identified in both polluted and unpolluted swamp soils. Some of the nucleotide sequences showed no significant similarity and were classified as potential No hits; most of which belonged to the unknown phylum, This unknown phylum was the most dominant phylum in the polluted soils investigated with an abundance of 49.86 % and the second most dominant in the unpolluted soils with an abundance of 27.5 %. They also had a higher relative abundance when compared to *Proteobacteria* phylum and their frequency of occurrence was 91.7%. PCR amplification and sequencing revealed isolates of base pair lengths, 784, 732 and 627 which had no significant similarity with other isolates at the NCBI website. Functional gene analysis indicated a 100% amplification of hydrocarbon degrading genes (alkM, Ppalk B and ndoB genes) of these novel isolates and they were more abundant in the polluted soil when compared to the unpolluted soil. The Agglomerative Hierarchical Clustering method, Pearsons correlation, Principal Component Analysis and Canonical Correspondence Analysis were employed to reveal the similarities among phyla, the ecological interactions at polluted and unpolluted sites, as well as the influence of environmental variables on potential novel isolates.. Results of this study suggest an abundance of potential novel bacteria in the Niger Delta mangrove swamp with potentials for hydrocarbon degradation and bioremediation of crude oil polluted soil.

Author Disclosure Block:

C.J. Ogugbue: None. **C.C. Nwankwo:** None. **G.C. Okpokwasili:** None.

Poster Board Number:

FRIDAY-103

Publishing Title:

Identification and Characterization of Oxidized Fatty Acids Biotransformed by Novel Marine Sediment Derived Bacterium

Author Block:

S. Goldberg, B. Haltli, R. Kerr; Univ. of Prince Edward Island, Charlottetown, PE, Canada

Abstract Body:

Background: Marine sediments are known as a rich source of diverse and potentially novel microbes, yet these habitats remain largely underexplored. New species of bacteria have the potential for biosynthesizing novel natural products with pharmaceutical or industrial applications. Here, a bacterial library was derived from marine sediment collected in the Caribbean Sea off San Salvador, Bahamas. We hypothesized that cultivating bacteria from marine sediment using a variety of media would yield novel species with potential for discovery of novel natural products. **Methods:** A bacterial library of 57 isolates was cultivated from marine sediment. Small-subunit rDNA sequencing identified 43 unique OTUs using a similarity cutoff of 99%. A novel gram-negative bacterium, RKSG952, was identified. Based on 16S rDNA gene sequence of 96.9% identity to known species, the bacterium has been phylogenetically described as a new species related to *Labrenzia*. Interestingly, isolate RKSG952 produced some bioactive compounds only when fermented in the presence of oils. This allowed identification of metabolites for further investigation. **Results:** The strain RKSG952 produces large amounts of oxidized fatty acids only when fermented in the presence of oils by bio-transforming the major components of the oils. The purified hydroxy-fatty acids exhibit antibacterial activity against MRSA and VRE. There is precedence in the literature where *Pseudomonas aeruginosa* and *Micrococcus leuteus* are, among others, bacterial species that have been reported to convert fatty acids into a variety of oxidized fatty acids. Additionally, this bacterium also exhibits degradation capabilities of long-chain hydrocarbons. The structure elucidation of the oxidized fatty acid products and the characterization of the biotransformation will be reported here. **Conclusions:**Hydroxy-fatty acids can be used as substrates for secondary metabolites with bioactivity and signaling molecules. They can also be useful for production of industrial fine chemicals. This strain may be producing oxidized fatty acids as a surfactant, to aid in degradation of hydrocarbons. The capabilities of this novel bacterium are interesting when considering environmental applications, such as oil spill bioremediation.

Author Disclosure Block:

S. Goldberg: None. **B. Haltli:** None. **R. Kerr:** I. Research Relationship; Self; Nautilus Biosciences.

Poster Board Number:

FRIDAY-104

Publishing Title:

Biodegradation of Engine Oil by Fungi from Mangrove Habitat

Author Block:

F. Ameen Hasan; King Saud Univ., Riyadh, Saudi Arabia

Abstract Body:

Pollution of land and water by petroleum compounds is a matter of growing concern necessitating development of methodologies including microbial biodegradation to minimize the impending impacts. It has been extensively reported that fungi from polluted habitats have the potential to degrade pollutants including petroleum compounds. Red Sea being treaded extensively for transport of oil is substantially polluted due to leaks, spills and occasional accidents. Tidal water, floating debris and soil sediment was collected from mangrove stands on three polluted sites along the Red Sea coast of Saudi Arabia and forty five fungal isolates belonging to 13 genera were recovered from these samples. The isolates were identified on the basis of sequence analysis of 18S rRNA gene fragment. Nine of these isolates were found to be able to grow in association with engine oil as sole carbon source under *in vitro* conditions. These selected isolates and their consortium accumulated greater biomass, liberated more CO_2 and produced higher levels of extracellular enzymes during cultivation with engine oil as compared to the controls. These observations were authenticated by gas chromatography-mass spectrophotometry (GC-MS) analysis, which elucidated that many high mass compounds present in the oil before treatment either disappeared or showed diminished levels.

Author Disclosure Block:

F. Ameen Hasan: None.

Poster Board Number:

FRIDAY-105

Publishing Title:

An Evaluation of the Suitability of Legumes in Phytoremediation of Crude Oil Polluted Soil

Author Block:

F. I. Esumeh, **A. R. AKPE**, O. Agholor, G. I. Okwu; Ambrose Alli Univ., Edo State, Nigeria

Abstract Body:

The remediation of crude oil polluted soils has been a major problem in oil producing countries and the use of plants to clean up such soils has been on investigation. Standard procedures were used in this study to evaluate the suitability of *Glycine max* and *Arachis hypogea* in the remediation of crude oil polluted soil. Results revealed that crude oil pollution had significant ($P>0.05$) negative effect on plant growth parameters such as plants height, leaf area, number of root nodules, leaf number, root length and number, chlorophyll content, shoot fresh and dry weights. Physicochemical analysis showed general increase in pH, organic matter and moisture content in planted soils while nitrogen increased in fertilized planted soils only. Calcium, phosphorus and potassium increased in fertilized soils. A total of 69 bacterial isolates from 13 genera (9 Gram negatives and 4 Gram positives) were encountered in this study while the fungal isolates were 42. *Pseudomonas aeruginosa* and *Aspergillus niger* were the most predominant crude oil degrading bacteria and fungi respectively. The total heterotrophic and crude oil degrading bacterial counts ranged from $1.0\pm0.58 \times 10^7$ to $30.0\pm0.58 \times 10^7$ $cfug^{-1}$and $0.67\pm0.33 \times 10^7$ to $15.0\pm0.00 \times 10^7$ $cfug^{-1}$ respectively. The total heterotrophic and crude oil degrading fungal counts ranged from $1.0 \pm0.00 \times 10^7$ to $15.0 \pm0.58 \times 10^7$ $cfug^{-1}$ and $1.0\pm0.00 \times 10^7$ to $6.0\pm0.58 \times 107cfug^{-1}$ respectively. The total petroleum hydrocarbon (TPH) was significantly ($P<0.05$) lower in planted than unplanted soils with those planted with *Glycine max* having the lowest value. The highest percentage reductions in TPH were 84.59% and 82.35% for 2% crude oil polluted soil sample planted with *Glycine max* and *Arachis hypogea* respectively. The reduction of total petroleum hydrocarbons in unplanted soil (control) was 31.87%. This study has shown that legumes are promising candidates for phytoremediation of crude oil polluted soils. It also revealed that *Glycine max* is a better phytoremediants than *A. hypogea* and that the total petroleum hydrocarbons are degraded better in planted soils than in unplanted soil.

Author Disclosure Block:

F.I. Esumeh: None. **A.R. Akpe:** None. **O. Agholor:** None. **G.I. Okwu:** None.

Poster Board Number:

FRIDAY-106

Publishing Title:

Inventory and Metabolic Potential of Molecular Fossils Degrading Bacteria from Petroleum Hydrocarbon Contaminated Sites

Author Block:

S. Yadav, S-S. Lee; Kyonggi Univ., Suwon, Korea, Republic of

Abstract Body:

Molecular fossils are a group of compounds, primarily hydrocarbons, found in oils, rocks, soil and sediment extracts. They are the most recalcitrant part of the petroleum hydrocarbon which has been persisted from different geological scale of time. There is no available report regarding the degradation of molecular fossils degrading bacteria. This research is focused on the discovery of novel molecular fossils degrading bacteria from desert and coastal samples collected from Kuwait. Desert and coastal habitats of Kuwait are being discharged by high load of petroleum hydrocarbon from more than 20 years of duration. Such contamination of hydrocarbons have enriched highly tolerant and rarer bacterial members which could be potential degrader of the molecular fossils. To analyze the bacterial diversity analysis from selected soil samples from Kuwait an improved cultivation based approach was adopted. Cultivated bacterial strains were identified based on small subunit ribosomal (16S rRNA) gene sequencing. 16S rRNA gene sequence analysis grouped the cultivated bacterial isolates into the genus *Massilia*, *Pseudomonas*, *Noviherbaspirillum*, *Alkanindiges* and *Skermanella*. Morphological, physiological and genetic analysis indicated that long term crude oil contamination have enriched several novel bacterial members with potential metabolic pathway. Their metabolic pathways are involved in the degradation of highly recalcitrant compounds i.e. molecular fossils. Further research focuses on GC-MS metabolite foot-printing and genome sequencing to reveal the metabolic pathway and their potential to degrade the pure compounds of steranes, hopanes, tetracyclic polyprenoids, C^{31} homohopanes, gammacerane and isorenieratane group of molecular fossils

Author Disclosure Block:

S. Yadav: None. **S. Lee:** None.

Poster Board Number:

FRIDAY-107

Publishing Title:

Biodegradation of Petroleum-Based Plastics by Insects: Mealworms vs. Polystyrene

Author Block:

J. YANG[1], Y. YANG[1], W-M. WU[2], J. ZHAO[3], L. JIANG[1]; [1]Beihang Univ., Beijing, China, [2]Stanford Univ., Stanford, CA, [3]BGI-Shenzhen, Shenzhen, China

Abstract Body:

Polystyrene (PS), commonly known as Styrofoam, accounts for about 7.4% (20 Mt/y) of the global petroleum-based plastic consumption. To date, PS is generally considered to be the plastic most resistant to biodegradation and the accumulation of PS wastes in the environment has been a global concern. Here we demonstrate that mealworms, common insect larvae of *Tenebrio molitor* Linnaeus, can eat Styrofoam and degrade it as sole diet for living over one month. We tested mealworms obtained at Beijing, China and found that they consumed Styrofoam up to 30-40 mg per 100 worms per day at ambient temperature. The fecula were collected and the chemical and physical properties were analyzed by gel permeation chromatography (GPC), solid-state ^{13}C cross-polarization/magic angle spinning nuclear magnetic resonance (CP/MAS NMR) spectroscopy, and thermogravimetric Fourier transform infrared spectroscopy (TG−FTIR). Analyses of fecula showed that cleavage/ depolymerization of long-chain PS molecules and the formation of depolymerized metabolites occurred. With a short retention time less than 24 hrs in mealworm digestion system, the carbon of ingested PS was mainly mineralized to CO_2 up to 47.7% during a 16 day test period. ^{13}C-tracer tests confirmed that the ^{13}C-PS was converted to $^{13}CO_2$ and partially incorporated into lipid biomass. Suppression of the gut bacteria of mealworms with antibiotic gentamicin (60 μg/g food) impaired their ability to depolymerize PS and mineralize PS into CO_2. One bacterial strain *Exiguobacterium* sp. YT2, capable of degrading PS, was isolated from the mealworm gut, based on its growth on PS film with obvious damage, change in hydrophobicity and shift of average molecular weight of residual PS film, formation of water-soluble daughter products and PS film weight loss of 7.4% over 60 days. These results indicate that PS can be mineralized rapidly within mealworm gut, and the gut bacteria play an essential role in PS biodegradation. The discovery reveals a new fate for plastic in the environment and suggests a new promising area of research on a technical approach to biodegradation of plastic wastes.

Author Disclosure Block:

J. Yang: None. **Y. Yang:** None. **W. Wu:** None. **J. Zhao:** None. **L. Jiang:** None.

Poster Board Number:

FRIDAY-108

Publishing Title:

Biodegradation Potentials of Indigenous Bacteria in a Nigerian Institute Palm Oil Mill Effluent

Author Block:

C. C. Isitua[1], I. Ahaotu[2], E. I. Atuanya[3]; [1]Afe Babalola Univ. Ado-Ekiti (ABUAD), Ado-Ekiti, Nigeria, [2]Univ. of PortHarcourt, PortHarcourt, Nigeria, [3]Univ. of Benin, Benin City, Nigeria

Abstract Body:

Nigeria produces large quantities of palm oil effluent arising from the processing of palm fruits obtained from various small holdings and big oil palm plantationsscattered all over the country. These effluents ultimately enter the environment, thereby constituting sources of environmental pollution. The biodegradation of palm oil mill effluent by indigenous bacteria was studied by observation of changes in some physico-chemical characteristics of the effluent. Based on cultural, morphological and biochemical characterization, bacteria isolated from the effluent were identified as *Flavobacterium breve*, *Bacillus sphaericus*, *Bacillus brevis*, *Corynebacterium diphtheriae*, *Bacillus licheniformis* and *Pseudomonas aeruginosa*. Incubation of the bacteria at different temperatures and hydrogen ion concentrations for 48 hours, showed that 50% grew at 4^{o}C, 100% grew at 28^{o}C, 83.34% grew at 37^{o}C, while 66.67% grew at 50^{o}C and all the isolates survived only the neutral (7.4) hydrogen ion concentration. *F. breve* and *C. diphtheriae* isolates gave the highest turbidity of screen test for the utilization of palm oil effluent as sole carbon sources. The physico-chemical characteristics of the raw effluent samples were studied and the pH values ranged from 4.10 - 7.30, biochemical oxygen demand (BOD) ranged from 532.40 – 665.50 mg/l while chemical oxygen demand (COD) ranged from 797.25 – 997.50 mg/l. The effects of *F. breve* and *C. diptheriae* isolates on the physico-chemical characteristics of the effluent (supplemented and unsupplemented) were carefully noted and compared using shake flask degradation experiment. There was an observed reduction in pH, from 7.40 – 6.50, BOD, from 440.14 – 45.01 mg/l and COD, from 659.63 – 86.96 mg/l of the carbon supplemented effluent sample with consortium of *F. breve* and *C. diptheriae* isolates. There was a high positive correlation between mean population counts of palm oil mill effluent utilizing bacteria with BOD and COD. T-test values also showed that there were significant differences ($P< 0.05$) in all the parameters analyzed. The study revealed the high potency of *F. breve* and *C. diphtheriae* isolates and the possibility of using them in biodegradation of palm oil mill effluent.

Author Disclosure Block:

C.C. Isitua: None. **I. Ahaotu:** None. **E.I. Atuanya:** None.

Poster Board Number:

FRIDAY-110

Publishing Title:

Iron Related Bacteria Near Shipwrecks in the Gulf of Mexico

Author Block:

L. A. FITZGERALD[1], D. Haridas[2], A. L. Cockrell-Zugell[3], J. C. Biffinger[1], P. A. Fulmer[1], L. J. Hamdan[4]; [1]Naval Res. Lab., Washington, DC, [2]American Society for Engineering Ed., Washington, DC, [3]Janssen Pharmaceutical Companies of Johnson & Johnson, Malvern, PA, [4]George Mason Univ., Manassas, VA

Abstract Body:

Background: Water samples were collected near six shipwrecks located in the Gulf of Mexico during April-May 2015 field activities. Two control sites (Ewing Bank and Halo), two heavily impacted sites (Mica and U-166), and two moderately impacted sites (Anona and Viosca Knoll) were chosen in relation to the 2010 Deepwater Horizon oil spill. These samples were examined for iron-related bacterial consortia. **Methods:** Water samples were collected close to the sediment, analyzed by inductively coupled plasma mass spectrometry (ICP-MS), and used as the inoculum in an Iron Related Bacteria Biological Activity Reaction Test (IRB-BART™) assay. All IRB-BART™ assays were performed according to the manufacturer's protocol and were analyzed 4, 8, and 16 days after inoculation. At each of these time points the assay cultures were analyzed to detect changes in the microbiome (metagenomic analysis), estimate Fe^{2+} and total Fe concentrations (bathophenanthrolinedisulfonate (BPS) assay), and observe morphological changes (SEM). **Results:** ICP-MS analysis identified trace levels of iron (<1.5 μM) at the control sites but significantly higher concentrations (220-795 μM) at the heavily impacted sites. One of the control sites (Halo) and one site in the moderately impacted area (Viosca Knoll) had the greatest number of culturable iron related bacterial populations, and culture growth was not correlated with environmental Fe concentrations (0.3 and 30.8 μM, respectively). The BPS assay showed that after 16 days of growth one of the heavily impacted sites (U-166) contained lower concentrations of soluble Fe (~3mM) when compared to the other samples, which could indicate cellular Fe uptake or Fe precipitation. In most samples (except Anona), the concentrations of Fe^{2+} increased until 8 days of growth, then declined between 8 and 16 days. **Conclusion:** Water samples were cultivated in an iron rich environment to examine iron-related bacterial consortia. Mica and U-166 samples took longer to cultivate in an IRB-BART™ assay, indicating lower bacterial numbers, even though the inoculum contained the highest iron concentrations. However, all samples showed changes in Fe^{2+} concentrations over time.

Author Disclosure Block:

L.A. Fitzgerald: None. **D. Haridas:** None. **A.L. Cockrell-Zugell:** None. **J.C. Biffinger:** None. **P.A. Fulmer:** None. **L.J. Hamdan:** None.

Poster Board Number:

FRIDAY-111

Publishing Title:

Novel Denitrifying Microbial Consortiums Identified from Shipwreck Sites in the Gulf of Mexico

Author Block:

D. Haridas[1], J. Biffinger[2], T. Boyd[2], P. Fulmer[2], L. Hamdan[2], L. Fitzgerald[2]; [1]American Society for Engineering Ed., Washington, DC, [2]Naval Res. Lab., Washington, DC

Abstract Body:

Background: Shipwreck sites serve as a rich source of unique adaptable microbial populations that have largely remained undiscovered. The low temperature, lack of sunlight and decreased oxygen levels enable microbes to adopt unique metabolic adaptations. Therefore, the marine dark biosphere was sampled **t**o identify cultivable novel microbes that exhibit energy efficient metabolic pathways which include denitrification and carbon fixation.**Methods:** Water samples collected near two shipwreck sites, Halo and U-166, were enriched for denitrifying bacteria using the denitrifying Biological Activity Reaction Test assay. Sterile Nunc tubes containing one of three modified Indole Nitrite (IN) medium (nitrate only (N), carbonate only (C) and without nitrate and carbonate (ONC)) were used. Nitrogen gas was bubbled through the Nunc tubes for 15 mins prior to inoculation. Growth kinetics and variation in elemental composition using Ion chromatography (IC) for the consortia over a period of 24h were analyzed. All analyzes were performed in triplicate.**Results:** Growth kinetic studies of the Halo microbial consortium showed that the microbial population multiplied only in the N medium while no growth was observed in the C and the ONC medium. With the U-166 microbial consortium, growth was observed in all three different medium compositions but optimum growth was observed in the N medium. IC studies showed a steady decline in nitrate levels for the denitrifying microbial supernatants cultured from the water samples obtained from Halo (734mg/L to 0.7mg/L) and U-166 (727mg/L to 2.5mg/L) which was followed with an increase in nitrite levels before leveling off (Halo- 1.4mg/L to 133.59mg/L to 4.25mg/L and U-166- 1.1mg/L to 239.4 mg/L to 2.2mg/L).**Conclusions:** The above study shows that the denitrifying microbial consortia isolated from both the shipwreck sites are cultivable under laboratory conditions and are mutually exclusive as they exhibit varied growth profiles and rates of nitrate utilization. This resulted in different levels of nitrate utilization thus supporting the objective that novel microbial populations exist at various ship wreck sites. This enables the discovery of unique microbes that can pave way for identifying novel microbial processes.

Author Disclosure Block:

D. Haridas: None. **J. Biffinger:** None. **T. Boyd:** None. **P. Fulmer:** None. **L. Hamdan:** None. **L. Fitzgerald:** None.

Poster Board Number:

FRIDAY-112

Publishing Title:

Efficiency of Cholera Smart II Water Test in Detecting *Vibrio cholerae* in Ballast Water

Author Block:

A. Chen, S.M. Rashed, A. Huq, R.R. Colwell; Univ. of Maryland, College Park, MD

Abstract Body:

Ballast water is essential to stabilize and operate ocean-bound shipping vessel safely, but can pose significant ecological, public health, and economic problems. Because ballast water can serve as a vehicle for transmission of pathogenic microorganisms to new environments, ballast water can be treated by filtration, chemical, and/or UV irradiation to remove or inactivate pathogens. Current regulations address acceptable levels of human pathogenic fecal coliforms, such as *Escherichia coli* and *Enterococcus spp.,* and including *Vibrio cholerae,* to control their spread via ballast water discharge. Accurate detection of pathogens that persist after treatment is critical for determining the efficiency of treatment systems. Although there are several EPA approved standard operating protocols for detection of *E. coli* and *Enterococci*, there is none for detection of *V. cholerae* in ballast water. Current regulations allow <1 CFU toxigenic *V. cholerae* per 100 mL and is typically determined using colony blot hybridization, DFA or PCR. However, these methods can be time consuming and difficult to perform. In this study, Cholera SMART II water test was employed to determine its efficacy and potential in the SOP for *V. cholerae* detection. Cholera SMART II is a rapid, lateral flow, colorimetric immunoassay for detection of *V. cholerae* O1 in water samples. Briefly, membrane filtered natural water collected from Baltimore Harbor, MD, was resuspended in 1x APW and amended by addition of 10^1, 10^2, 10^3,10^4 environmental or clinical *V. cholerae* O1 cells and incubated for 4 hours at 35°C and tested using SMART II. Samples were stored at room temperature for 4 days and retested with SMART II. Preliminary results show that SMART II is effective in detecting as few as 10^3 cells after 4 hours and 10^2 after 4 days at room temperature. Use of Cholera SMART II is a simple method for rapid and accurate detection of *V. cholerae* and is beneficial for validation of the safety of ballast water treatment systems.

Author Disclosure Block:

A. Chen: None.

Poster Board Number:

FRIDAY-113

Publishing Title:

Visualization of Rhodopsin-utilizing Cells in the Chesapeake Bay

Author Block:

K. Miller[1], J. Keffer[1], C. Sabanayagam[2], J. Maresca[1]; [1]Univ. of Delaware, Newark, DE, [2]Delaware Biotechnology Inst., Newark, DE

Abstract Body:

Microbial rhodopsins are a family of transmembrane proteins, with photosensitive retinal cofactors, found in every domain of life. Rhodopsins respond to light by transporting ions across the cell membrane or by initiating a signaling cascade that leads to altered gene expression. Rhodopsins are abundant in nature, and recent estimates indicate that up to 70 percent of microbial cells in some aquatic environments encode rhodopsin genes, suggesting that more bacteria utilize sunlight than previously thought. However, these estimates are based on gene abundances, not direct observation. In order to determine the abundance of functional rhodopsins, visualization of these low-fluorescing proteins is essential. We recently developed a method that uses total internal reflection fluorescence (TIRF) microscopy to identify rhodopsin-containing cells in environmental samples. Here, we use TIRF microscopy to quantify the total number of rhodopsin-containing cells in water samples collected along the Chesapeake Bay, demonstrating that rhodopsin production is correlated with daylight and salinity. As up to ~65 percent of cells produce rhodopsins, microbial capture and utilization of sunlight by rhodopsin-type photosystems is thus common throughout an estuary.

Author Disclosure Block:

K. Miller: None. **J. Keffer:** None. **C. Sabanayagam:** None. **J. Maresca:** None.

Poster Board Number:

FRIDAY-114

Publishing Title:

Xylan-degrading Bacteria Isolated and Characterized from Eastern Mediterranean Sea

Author Block:

K. R. McBride, Anthony Rossi, Hannah Woo, Jing Wang, Nicole Labbe, Terry Hazen; Univ. of Tennessee, Knoxville, Knoxville, TN

Abstract Body:

Hemicellulose xylan is an important structural component within plant cell walls that is difficult to degrade. There is relatively little information on the diversity of bacteria that produce xylan-degrading enzymes, or the necessary conditions to maximize enzyme production. Additional knowledge about xylanase producing bacteria could lead to more efficient ways to break down plant material for biofuels. Marine environments were sampled because they are rich in hemicellulosic biomass. This study aims to identify xylan-degrading bacteria by sequencing isolates from Eastern Mediterranean Sea, and measuring their growth rates on xylan media. Isolates were cultivated from a laboratory incubation of xylan-amended seawater. A set of ninety isolates were sub-cultured several times on synthetic seawater agar with xylan as the sole carbon source. All strains were then identified by 16S rRNA gene sequencing. Isolates were closely related to *Halomonas*, *Pseudomonas*, *Joostella*, *Glaciecola* and *Janibacter*. None of the species found are well-known xylan degraders. Growth curve data and preliminary metabolic tests with xylan show that several isolates have high xylanase producing ability. Understanding more about xylan degradation could find more cost effective ways to produce biofuels.

Author Disclosure Block:

K.R. McBride: None.

Poster Board Number:

FRIDAY-115

Publishing Title:

Challenging Actinomycetes: A Non-Related New Strain M11-108 Isolated from Marine Sediments Off Peru with Unknown Antimicrobial Activity

Author Block:

U. TARAZONA[1], W. serrano[2], K. RODRIGUEZ[2], R. OLAECHEA[2], e. marguet[3], M. VALLEJO[3], L. Liza[4], N. Galindo[4], j. león[1]; [1]Univ. Natl. Mayor de San Marcos, LIMA, Peru, [2]Univ. CIENTÍFICA DEL SUR, LIMA, Peru, [3]Univ. Natl. de la Patagonia SJB, trelew, Argentina, [4]Univ. Natl. Mayor San Marcos, Lima, Peru

Abstract Body:

The emergency of bacterial resistance to antibiotics nowadays represents a huge problem in human health. Actinobacteria are very well known organisms that are able to produce secondary metabolites. As part of a big oceanographic campaign in the central coast off Peru organized between September to Decemeber 2005, we collected marine sediments from two locations. Our main objective was to isolate actinomycetes from unexplored hábitats.Sediments samples were collected from two locations in front of the central coast off Peru: Ancon Bay (11°81S, 77°39W) and Independence Bay (14°34S, 76°18W). Sediments were obtained with a Van-Been grab at depths of 35 to 100 m and surface alliquotes were placed in sterile 50-ml Falcon tube and kept frozen at 4°C. Before culturing, samples were dry and wet warm-treated to eliminate other undesirable microflora. Then 20 gr of wet sediments were diluted in sterile marine sea-water to 10-5 dilutions and 0.1 ml of ech were inoculated in marine agar and Casein-Starch agar plates. Pigmented and non-pigmented forming a concentric ring pattern colonies were selected for further purification in agar slants.Based on colony morphologies, microscopic evaluation and biochemical testing 25 suspected actynomicetes were isolated in pure culture. The most conspicuous strain M11-108 has shown antagonistic activity against pathogenic vibrios and to other drug-resistant pathogens e.g. vancomycin-resistant *Enterococcus faecium*. Comparison of the nearly complete 16S rRNA gene sequences against sequences in the GenBank database indicates that almost all 25 strains belong to the familiy of Actinomicetales. However, this molecular probe also revealed that the strain M11-108 is far related to this group. The close relative of this new isolate were members of the familiy Alcanivoracaceae of the Proteobacteria showing a nucleotide similarity of 83%.This finding may indicate that unexplored habitats in the world still harbor unknown organisms that are able to produce novel antibiotics and other biomolecule therapeutic agents. Based on our study strain M11-108 may represents a novel species in a new genus within the family Alcanivoracaceae.

Author Disclosure Block:

U. Tarazona: None. **W. serrano:** None. **K. Rodriguez:** None. **R. Olaechea:** None. **E. marguet:** None. **M. Vallejo:** None. **L. Liza:** None. **N. Galindo:** None. **J. león:** None.

Poster Board Number:

FRIDAY-116

Publishing Title:

Preliminary Report on Microbial Flora Isolated from Pollutedand Non-polluted Coastal Waters of Karachi, Pakistan

Author Block:

A. Shaheen[1], H. S. Baig[2], **S. U. Kazmi**[1]; [1]Immunology and Infectious Disease Res. Lab. - Dadabhoy Inst. of Higher Ed., Karachi, Pakistan, [2]Insti1Natl. Inst. of Oceanography, ST-47, Block-1, Clifton, Karachi-75600, Pakistan, Karachi, Pakistan

Abstract Body:

Background: Marine pollution has now become worldwide environmental concern. Continuous discharge of untreated industrial effluent, municipal and power plant's contaminated wastewater has been a serious threat to marine habitat, marine life, aesthetic values and interest of visitors to coastal areas. Karachi, the largest city of Pakistan and industrial hub is located at latitude 24° 48´ N and longitude 66° 59´ E on coast of the Arabian Sea. It suffers serious environmental problems because of population pressure and increasing industrial activities. **Methods:** In this investigation, samples of sediment, water, flora and fauna were taken from nine selected stations on Karachi coast included three stations represented major creeks i.e. Korangi, Gizri and Chinna Creek. These samples were taken in north-east and south-west monsoonal period during 2014. **Results:** Bacterial flora isolated and identified from samples collected from these sites by conventional method. Among isolated and identified bacteria e.g. Vibrio alginolyticus, E.coli and Streptococuss anginosus were the most dominant species contributing 21.43, 19.64 and 15.18 percent of total assemblage respectively. Among selected sample stations, Korangi creek station was found to be most polluted with coliform and other pathogenic bacteria. **Conclusions:** These results clearly indicate that threats from these pathogens are not only to marine life but also to the large number of visitors coming to beaches and residents of surrounding area. Moreover; immediate action should be taken to restrict the growth of these pathogens by taking measures to treat the municipal and industrial effluent to avoid outbreak of any disease in future.

Author Disclosure Block:

A. Shaheen: None. **H.S. Baig:** None. **S.U. Kazmi:** None.

Poster Board Number:

FRIDAY-117

Publishing Title:

Phylogenetic Analysis of Microbial Sediment Communities from Two Salt Marshes in the South Shore Estuary Reserve

Author Block:

J. M. Coombs, A. Espinosa; Adelphi Univ., Garden City, NY

Abstract Body:

Estuarine ecosystems are becoming increasingly impacted by anthropogenic contaminants from terrestrial sources. In particular, heavy metals can become trapped in estuary sediments after binding to particulate matter in the water column or through precipitation due to gradients in water chemistry. This results in increasing the concentrations of heavy metals in estuary sediment, which can negatively impact sediment microbial communities. In this study, a vibracoring technique was used to obtain an 8 foot sediment core from a salt marsh in the South Shore Estuary Reserve (SSER) of Long Island, NY. Metals analysis was performed on 8 core sub-samples, and 16S rRNA gene clone libraries were constructed from metagenomic DNA extracted at three different core depths. Surface core samples were also obtained from a second salt marsh in the SSER, one located close to two potential point sources of anthropogenic pollution. Although there was some variation in metal concentration with respect to depth in the vibracore-extracted sediment, differences were not significant except for the deepest core sub-sample, which had much lower values for all 6 metals examined in this study. This layer of the core differed from others in that it was comprised mainly of sand. Most environmental clone sequences from the vibracore subsamples clustered phylogenetically with bacterial taxa such as the Deltaproteobacteria or Chloroflexi, which have previously been detected in salt marsh sediments. While some operational taxonomic units (OTUs) appeared to have a cosmopolitan distribution across core subsamples, others such as the Spirochetes appeared to be depth-limited. High throughput sequencing of metagenomic DNA from the second salt marsh site detected some of the same phylogenetic groups although the data set exhibited more phylogenetic diversity due to the depth of sequence coverage. Neither of the two salt marsh sites examined in this study was considered to be impacted by heavy metals. However, the data collected on microbial sediment community structure in these two marshes provides baseline measurements that will inform future work on marshes impacted by point source contamination in the SSER.

Author Disclosure Block:

J.M. Coombs: None. **A. Espinosa:** None.

Poster Board Number:

FRIDAY-118

Publishing Title:

Diversity and Distribution of Microbial Communities in Subtropical Coastal Wetland Sediments

Author Block:

M. K. Cheung, C. K. Wong, K. H. Chu, H. S. Kwan; The Chinese Univ. of Hong Kong, Hong Kong, Hong Kong

Abstract Body:

Wetland sediments are inhabited by an abundant amount of ecologically important microbes. However, since most molecular studies to date have only focused on the bacterial communities, information on the diversity and distribution of wetland archaea and fungi is still very limited. Here, we elucidated the genetic diversity and composition of bacterial, archaeal and fungal communities in sediments of a coastal Ramsar wetland in subtropical Hong Kong by using Ion Torrent next-generation sequencing and examined their spatial distribution between mudflat and mangrove regions in the wetland.Bulk surface sediment samples were collected in triplicate from seven locations in mudflat and three locations in mangrove during the wet season in 2015. The V1-V2 hypervariable regions of the bacterial and archaeal 16S rRNA genes and the internal transcribed spacer regions of fungi were amplified and sequenced together on an Ion Torrent PGM system. Raw sequence reads were demultiplexed, quality-filtered and analyzed using the QIIME pipeline. Reads from the same microbial group were clustered into operational taxonomic units (OTUs) at 97% similarity. Taxonomic information was acquired by aligning representative OTUs to the Greengenes and UNITE reference datasets for prokaryotes and fungi, respectively.Bacterial communities in the mudflat and mangrove sediments were both dominated by the phyla *Proteobacteria* and *Chloroflexi*. Archaeal communities in the mudflat and mangrove sediments were dominated by, respectively, members in the deep-sea hydrothermal vent euryarchaeotic group I and the ammonia-oxidizing genus *Nitrosopumilus*. Ascomycetes dominated the fungal communities in mangrove whereas unidentified members overwhelmed the mudflat sediments. There were > 2700, >1200 and >180 observed OTUs of bacteria, archaea and fungi in the sediment samples, respectively. For all three microbial groups, mudflat and mangrove samples formed distinct clusters in principal coordinates analysis plots.In conclusion, our study has revealed a high bacterial and archaeal diversity, moderate fungal diversity, and spatial differentiation of all these groups in subtropical coastal wetland sediments.

Author Disclosure Block:

M.K. Cheung: None. **C.K. Wong:** None. **K.H. Chu:** None. **H.S. Kwan:** None.

Poster Board Number:

FRIDAY-119

Publishing Title:

Identification of Polycyclic Aromatic Hydrocarbon and the Microbial Flora from an Oil Spill Contaminated River in Nigeria

Author Block:

U. Elijah, **S. Machunga-Mambula**; Univ. of Abuja, Abuja, Nigeria

Abstract Body:

Polycyclic Aromatic Hydrocarbons (PAHs) are an important group of obstinate ubiquitous chemicals that occur naturally as organic pollutants in the environment which originate from the incomplete combustion of coal (liquefaction), volcanic eruptions, crude oil and/or accidental leakage of petroleum products (asphalts/creosote) into our environment. Water and sediment samples from Qua Iboe River near an oil pollution site and Akpa Atak Eka River an unpolluted site both in Akwa Ibom State, Nigeria were analysed for microbial and PAH content. The PAHs present in the water and sediment samples analysed through Gas Chromatography Mass Spectrometry (GC-MS) were anthracene and naphthalene with the concentration of 2.4%. The bacterial load of the contaminated and uncontaminated river in CFU were 6.7×10^{6} cfu/ml to $4..8\times10^{8}$cfu/ml and 3.1×10^{6} cfu/ml to 2.0×10^{8}cfu/ml while the total coliform (TC) count ranged from 6.8×10^{6}cfu/ml to 5.6×10^{8}cfu/ml and 3.1×10^{6}cfu/ml to 2.2×10^{8}cfu/ml respectively. The organisms isolated were *Bacillus spp, Rhodococcus, Serratia spp, Escherichia coli, Staphylococcus spp, Micrococcus spp.* The bacterial strains isolated from this environment metabolized Anthracene and Napththalene. The degradability of this polycyclic aromatic hydrocarbon by these indigenous bacterial isolates shows a promise towards its application in bioremediation technique.

Author Disclosure Block:

U. Elijah: None. **S. Machunga-Mambula:** None.

Poster Board Number:

FRIDAY-120

Publishing Title:

Detection of Free-Living Amoebae and Amoeba Resisting Bacteria in Beach Waters in Southeastern Georgia

Author Block:

M. Mears, C. Karcs, D. Capps, S. Warang, A. Aslan, M. E. Eremeeva; Georgia Southern Univ., Statesboro, GA

Abstract Body:

Background: Kings Ferry Park in Savannah, Georgia, has been under a permanent health advisory since 2004 due to high levels of Enterococci; however, other information about microorganisms circulating at this site is lacking. We examined the occurrence and distribution of free-living amoebae (FLA), and FLA associated bacteria in water and sediment samples in the park and the surrounding area along the Ogeechee River.**Methods:** Water and sediment samples were collected monthly from 14 sites along the beach and river around Kings Ferry Park. Sample filtrates were used for isolation of FLA and bacteria. Amoebae were isolated using the walk-out method. Bacterial isolates were established using co-culturing with *Acanthamoeba castellanii* followed by colony isolation on charcoal yeast extract agar plates with and without BCYE supplement. DNA was extracted using Qiagen QIAmp protocol. Identification of amoeba isolates was completed by sequencing 18S rRNA gene; bacteria were identified by sequencing 16S rRNA gene and *Legionella* 23S-5S rRNA intergenic region.**Results:** 155 isolates of amoebae were obtained from water and sediment samples collected from the beach and surrounding sites. *Vermamoeba vermiformis* was a predominant species; *Naegleria clarki* and *Acanthamoeba spp.* were also found. 17 species of bacteria which are obligate or opportunistic pathogens of human and animals were recovered. Bacteria of *Bacillus cereus* complex were the most frequently isolated followed by *Stenotrophomonas maltophilia and Acinetobacter baumanii. Legionella anisa* and *Mycobacterium sp.* were also detected. **Conclusions:** All three species of amoebae isolated as a part of this study can harbor diverse amoeba-resistant bacteria; many bacteria isolated are known to carry a variety of antibiotic resistance genes. The origin, sources, and spatial and temporal distribution of these pathogens in the area need further study. In depth characterization of these microorganisms and their interactions is critical to assess the risk of human and companion animal exposure to these common groups of organisms as a result of various aquatic recreational activities.

Author Disclosure Block:

M. Mears: None. **C. Karcs:** None. **D. Capps:** None. **S. Warang:** None. **A. Aslan:** None. **M.E. Eremeeva:** None.

Poster Board Number:

FRIDAY-121

Publishing Title:

Microbial Community Succession after Press Disturbance of a Sulfidic, Phototrophic Habitat

Author Block:

E. S. Cowley[1], S. Bhatnagar[2], S. H. Kopf[3], K. Hanselmann[4], S. C. Dawson[2], J. R. Leadbetter[1], D. K. Newman[1], S. E. Ruff[5]; [1]California Inst. of Technology, Pasadena, CA, [2]Univ. of California, Davis, Davis, CA, [3]Univ. of Colorado, Boulder, CO, [4]ETH-Z, Zurich, Switzerland, [5]Univ. of Calgary, Calgary, AB, Canada

Abstract Body:

Experimental environmental disturbance is a means of analyzing successional processes in natural ecosystems. We investigated several sites in a small area of Trunk River, an organic-rich, sulfidic pool near Woods Hole, MA. In its undisturbed steady state this ecosystem contains thick layers of decaying seagrass in a shallow water column with steep gradients of sulfide, light, and temperature. Some areas exhibit a bright yellow microbial suspension, termed “Lemonade”, that transiently forms above the organic matter and disappears within a few days. We hypothesized that the microbial succession within this “bloom” is linked to a physical disturbance and follows distinct spatiotemporal patterns.We removed the organic matter layer, mixed the water column and studied the reestablishment of habitats and microbial communities using next-generation sequencing and a comprehensive set of physicochemical parameters. After the press disturbance the ecosystem was homogeneous comprising the taxonomically diverse communities, which were characteristic for Trunk River. With time the communities underwent substantial change, evolved striking sulfide and oxygen gradients while distinct niches emerged. After around 7 days the ecosystem seemed to be mature and was largely dominated by a single species of Green Sulfur Bacteria, *Prosthecochloris vibrioformis*, a member of the phylum *Chlorobi*, while other layers in the floating mats included *Chlorobi* that consisted mainly of organisms belonging to the genus *Chlorobaculum*.

Author Disclosure Block:

E.S. Cowley: None. **S. Bhatnagar:** None. **S.H. Kopf:** None. **K. Hanselmann:** None. **S.C. Dawson:** None. **J.R. Leadbetter:** None. **D.K. Newman:** None. **S.E. Ruff:** None.

Poster Board Number:

FRIDAY-122

Publishing Title:

Putative Glucosyltransferase Gene (*ycj*M) of *Escherichia coli* as a Biomarker to Unravel Contamination Sources and History of Water

Author Block:

A. Shen, D. Deng, F. Liu, M. Reed, M. Dolan-Timpe, **G. ZHENG**; Lincoln Univ., Jefferson City, MO

Abstract Body:

Background: Information on contaminant sources and history of surface water and groundwater is important for water resource management. The putative glucosyltransferase gene (*ycj*M) of *Escherichia coli* (*E. coli*) was reported to be highly associated with enteric *E. coli.* The objective of this study was to determine if gene *ycj*M could be used as a biomarker in assessing the sources and history of surface water and groundwater. The underlying hypothesis was that surface water and shallow groundwater (mostly young water) were highly linked with enteric *E. coli* (*ycj*M positive), while deeper groundwater (mostly old water) with environmental *E. coli* (*ycj*M negative).**Methods:** A total of over 200 strains of *E. coli* were isolated from surface water and groundwater with different depths at eight different locations within the Goodwater Creek Experimental Watershed in northern central Missouri. Presence or absence of gene *ycj*M for each *E. coli* isolate was determined by the polymerase chain reaction (PCR) assay. The association of *ycj*M with the relative age of water was statistically analyzed (Student's t-test) using both the electric conductivities of the water samples collected at different depth, which was considered a chemical indicator of the relative age for water, and the history of fertilizer uses. **Results:** The ycjM positive rates in the E. coli population were relatively high and similar for surface water and shallow groundwater, suggesting recent contamination from animal wastes. The rates for groundwater were relatively low and negatively correlated with electric conductivities and the depths of groundwater well, demonstrating increasing domination of environmental E. coli (ycjM negative) in deeper groundwater (older). **Conclusions:** Gene *ycj*M of *E. coli* may be used as a biomarker in determining the sources and history of surface water and groundwater for effective water resource management.

Author Disclosure Block:

A. Shen: None. **D. Deng:** None. **F. Liu:** None. **M. Reed:** None. **M. Dolan-Timpe:** None. **G. Zheng:** None.

Poster Board Number:

FRIDAY-123

Publishing Title:

Detection of Enteric Viruses and Index Indicators for Evaluating Sachet Water Quality

Author Block:

O. T. Adekunle[1], O. B. Shittu[1], F. O. Olufemi[1], O. O. Odedara[1], T. O. C. Faleye[2], M. O. Adewumi[2], J. A. Adeniji[2]; [1]Federal Univ. of Agriculture, Abeokuta, Nigeria, [2]Univ. of Ibadan, Ibadan, Nigeria

Abstract Body:

Background: Microbiological safety of sachet water remains a public health problem in Nigeria as most brands on quality assessment do not conform to drinking water standards. There is a need to assess sachet drinking water for possible enteric viruses and some index indicators.**Methods**: A total of sixty samples of sachet water were obtained from 5 different brands between February (peak of dry season) and April (rainy season) 2013. Water samples of the same brands were pooled together and concentrated with Polyethylene glycol (PEG) 6000. Viral detection was conducted using Polymerase Chain Reaction techniques targeting specific genes in adenovirus, rotavirus and norovirus. *Cryptosporodium parvum, Giardia lamblia* and *Escherichia coli* as index indicators along with other organisms were detected using standard methods.**Results**: Viral analyses revealed that only one sample which was collected at the peak of dry season tested positive for adenovirus while rotavirus and norovirus were absent in all samples. Adenovirus had a prevalence rate of 20% (1/5) in February and 6.7% (1/15) over three months of collection. No oocyst of *Cryptosporodium parvum* or ova of *Giardia lamblia* or any form of parasite was found in all batches of water collected. However, *Salmonella enterica* serovar *Typhi*, *Pseudomonas aeruginosa*, *Shigella dysentariae*, and *Escherichia coli* were detected.**Conclusions**: Adenovirus was detected by PCR in a sachet water sample that tested negative for *Escherichia coli*, oocyst of *Cryptosporodium parvum* and ova of *Giardia lamblia.* There is the need to screen sachet water periodically for enteric viruses even when they meet bacteriological standards.

Author Disclosure Block:

O.T. Adekunle: None. **O.B. Shittu:** None. **F.O. Olufemi:** None. **O.O. Odedara:** None. **T.O.C. Faleye:** None. **M.O. Adewumi:** None. **J.A. Adeniji:** None.

Poster Board Number:

FRIDAY-124

Publishing Title:

Lapse in Municipal Water Corrosion Control Linked to Elevated *Legionella* Levels in Building Plumbing

Author Block:

D. O. Schwake, E. Garner, O. Strom, A. Pruden, M. Edwards; Virgnia Tech, Blacksburg, VA

Abstract Body:

A recent crisis occurred in Flint, MI after the city switched to the corrosive Flint River as its drinking water source and discontinued corrosion control. Though the city has since returned to their former supply, the distribution system may have been irreversibly damaged. During a period of rampant corrosion, water problems were widespread, chorine residuals were low, and microbial growth in the distribution system was high. As low chlorine residual and conditions conducive to microbial growth are known factors associated with *Legionella pneumophila* proliferation, we hypothesized that the lack of corrosion control could elevate the levels of these bacteria in buildings. In a broad field sampling of water from the municipal distribution system just before the switch back to the former water source, we detected low levels of *Legionella* in residences, but a more in-depth sampling at two local hospitals revealed high levels of *L. pneumophila* gene markers. Furthermore, publically available data suggest a legionellosis outbreak occurred concurrently with the use of the corrosive water within Flint. This unfortunate situation presented a unique opportunity to examine how deteriorating infrastructure and shifts in water management practices may influence waterborne disease. As this study is ongoing, the main objectives are to: 1) monitor *Legionella* within high-risk premise plumbing systems for comparison to data gathered while the city was sourcing drinking water from the Flint River; 2) measure water quality parameters associated with the previously utilized water source and examine correlations with occurrence of *Legionella*; and 3) explore associations between these results with reported legionellosis infection data in Flint to identify factors that may trigger increased incidence. Though Flint is an extreme case, the findings regarding the relationship between corrosion related infrastructure damage and opportunistic pathogen proliferation will have broad relevance. As water utilities continue to grapple with aging water infrastructure, reduced water demand due to water conservation, dwindling water resources, and poorer water quality, water distribution will become more conducive for the growth and survival of *Legionella* and other opportunistic pathogens, putting communities at increased risk of contracting disease.

Author Disclosure Block:

D.O. Schwake: None. **E. Garner:** None. **O. Strom:** None. **A. Pruden:** None. **M. Edwards:** None.

Poster Board Number:

FRIDAY-125

Publishing Title:

Reduced Infectivity Of Viable But Non-Culturable *helicobacter Pylori* in Water

Author Block:

K. F. Boehnke[1], K. A. Eaton[2], C. Fontaine[2], M. Valdivieso[2], L. H. Baker[2], C. Xi[1]; [1]Univ. of Michigan, Sch. of Publ. Hlth., Ann Arbor, MI, [2]Univ. of Michigan, Med. Sch., Ann Arbor, MI

Abstract Body:

Background: In epidemiologic studies, *Helicobacter pylori* infection has been consistently associated with lack of access to clean water and proper sanitation. We previously showed that exposure to culturable *H. pylori* in drinking water can be infectious in mice (estimated ingested doses of $5.26*10^2$-$5.26*10^6$ CFU). However, *H. pylori* exists in a viable but non-culturable (VBNC) state in water, and the infectivity of this form is not well characterized. In this set of studies, we examined the infectivity of VBNC *H. pylori* in mice.Materials and Methods: *H. pylori* strain SS1 was used in all studies. The VBNC state was induced by adding freshly grown SS1 to sterilized tap water and waiting until culturability was lost. Viability of VBNC *H. pylori* was examined using Live/Dead staining microscopy. Four studies were performed, with *Helicobacter*-free C57/Bl6 mice exposed to different doses of VBNC *H. pylori* via gavage or in drinking water (Table 1). After exposure, *H. pylori* infection in the stomach was evaluated using quantitative culture, PCR for the VacA gene, and urease test.Results: See table 1. Live/Dead staining showed that all inocula contained viable cells. Quantitative culture results were negative for all mice. In DNA extracted from stomachs of the gavaged mice, PCR was positive in 8 of 16 mice.Table 1. Overview of experiments

Exposure groups	***Exposure and duration***	***Estimated dose of VBNC cells***	***Quantitative culture outcome (positive/total)***	***PCR outcome (positive/total)***
40 C57/BL6 immunocompetent mice (20 male, 20 female)	Exposure to one day of 10^9 cells/L of VBNC *H. pylori* in drinking water. Sacrificed 4 weeks after final exposure.	~$3.4*10^6$ cells	0/40	0/40
40 C57/BL6 immunocompetent mice (20 male, 20 female)	Exposure to six days of 10^9 cells/L of VBNC *H. pylori* in drinking water.	~$5.3*10^7$ cells	0/40	0/40

	Sacrificed 2 weeks after final exposure.			
10 C57/BL6 Severe Combined Immunodeficient mice (4 male, 6 female)	Exposure to one day of 10^9 cells/L of VBNC *H. pylori* in drinking water. Sacrificed 1 week after final exposure.	~$5.4*10^6$ cells	0/10	0/10
16 C57/BL6 immunocompetent mice (16 male)	Gavaged with 4 doses of ~10^8 cells of VBNC *H. pylori* over 2 weeks. Sacrificed 4 weeks after final dose.	~$6*10^8$ cells	0/16	8/16

Conclusions: In this study, doses of VBNC failed to cause infection, despite being substantially higher than infectious doses of culturable *H. pylori*. This suggests that VBNC *H. pylori* is less infectious in mice than the culturable form. Future studies will evaluate the VBNC infectivity of other *H. pylori* strains. This study provides a new approach to evaluate risks of waterborne *H. pylori*.

Author Disclosure Block:

K.F. Boehnke: None. **K.A. Eaton:** None. **C. Fontaine:** None. **M. Valdivieso:** None. **L.H. Baker:** None. **C. Xi:** None.

Poster Board Number:

FRIDAY-126

Publishing Title:

Inevitability of an Enhanced Monitoring Strategy to Reduce Water Borne Illness Combining Indicators of Sanitary Protection and Measuring Water Quality

Author Block:

N. T. Bukhari[1], S. U. Kazmi[2]; [1]RRRL, Karachi, Pakistan, [2]DIHE, Karachi, Pakistan

Abstract Body:

Background: World Health Organization estimated after 2000 assessments that there are 4 billion cases of diarrhea burst each year in addition to millions other cases of illness associated with lack of access to clean water. It is now well established that infectious diseases are transmitted primarily through water supplies contaminated with human and animal excreta and inappropriate storage procedures. Out breaks of water borne diseases continue to occur throughout the world but such out breaks in developing counties like Pakistan lead to millions deaths among children.**Objective:** The objective of the study was to identify pathogens in contaminated drinking water as a major contributing factor in infantile diarrhea. **Methods:** Total 57 drinking water samples from each source of low socio economic areas of Karachi Pakistan were collected according to WHO guidelines for drinking water quality assessment. Water Sample of about 200 ml each was collected from Government filter plants, cooling containers, storage tank and Municipal tap water, labeled and transported to IIDRL lab for bacteriological analysis. Detailed examination was done by direct microscopy of centrifuged water sample deposit. Water Samples were analyzed and indicator organisms i.e. total and fecal coliform (*E.coli*) were detected by most probable number (MPN) method. Rotavirus antigen was detected by PCR method.**Results:** Out of 57 samples collected, 32 (56%) found positive after bacteriological analysis by MPN technique. Further found that samples were carrying E.coli in 9 (16%), salmonella in 3 (5%), Klebsiella in 2 (4%), Pseudomonas in 6 (11%), mix infection in 5 (8.78% including Rota virus presence, and parasites in 3 & EPEC in 2) samples. Rota virus and EPEC were confirmed by PCR method. Presence of pathogens in every sample confirmed that the water transportation and storage method are not hygienic.**Conclusions:** Enhanced Monitoring system is required in Karachi Pakistan and it should be placed at different disposal points like Water Storage, Water Delivery Channels, Municipality Storage Areas, different point within water supply system and End User Storage Point. This monitoring system will help to identify the area where water is being contaminated leading towards preventive measures. which may reduce the epidemics as well as the financial burden.

Author Disclosure Block:

N.T. Bukhari: None. **S.U. Kazmi:** None.

Poster Board Number:

FRIDAY-127

Publishing Title:

High Prevalence of Virulent *Escherichia coli* Belonging B1 Phylogroup in Municipal Water Supply in Dhaka, Bangladesh

Author Block:

J. Ferdous[1], R. B. Rashid[1], S. Saima[1], S. Tulsiani[2], P. K. M. Jensen[2], **A. Begum**[1]; [1]Univ. of Dhaka, Dhaka, Bangladesh, [2]Univ. of Copenhagen, Copenhagen, Denmark

Abstract Body:

Escherichia coli is a commensal organism of the digestive tracts of many vertebrates, including humans. Contamination of drinking water with pathogenic *E. coli* is a serious public health concern.This study focused on the distribution of phylogenetic groups and virulence gene profile of *E. coli* isolated from drinking water in Arichpur, a low income area of Dhaka, Bangladesh.The distribution of the phylogroups and virulence genes were investigated in 200 isolates among them 110 isolates were from municipal water supply system and 90 were from household drinking water. Gene profile of virulence factors was done based on the presence of *eltB, estA, vt1, vt2, eaeA,* pCVD432, *bfpA, ial, ipaH* by PCR. The classification of the isolates into 4/5 major groups (A, B1, B2, D2, D3) was done based on the distribution of *chuA, yjaA* and DNA fragment tspE4.C2 genes.Results demonstrated predominance of phylogroup B1 78.5 % (157/200) followed by B2 phylogroup 13% (26/200) and phylogroup D 8.5% (17/200).The genes *eltB, estA* and *eaeA* was present in 27.39% (43/157), 37% (74/157), and 10.5% (21/157) of B1 phylogroup isolates, respectively. The calculated chi-square value and P-value were 10.23 and 0.001. Therefore, it can be inferred municipal water supply was a greater contributor of pathogenic *E. coli* from the B1 phylogroup.Usually commensals fall in the Phylogroups A and B1. The presence of greater number of virulent B1 phylogroup isolates originating from municipal water supply indicates that the supply system might be contaminated with virulent *E. coli* such as enterotoxigenic *E. coli* carrying mobile genetic elements such as plasmids which might be transferred to the commensal strains. Greater proportion of commensal strains in household water demonstrates low level of contamination of virulent *E.coli* from asymptomatic shedders.

Author Disclosure Block:

J. Ferdous: None. **R.B. Rashid:** None. **S. Saima:** None. **S. Tulsiani:** None. **P.K.M. Jensen:** None. **A. Begum:** None.

Poster Board Number:

FRIDAY-128

Publishing Title:

Evaluation of Ultrafiltration and Elution Solutions as a Means to Concentrate Indicators of Fecal Contamination in Water Bodies

Author Block:

J. W. Conrad, V. J. Harwood; Univ. of South Florida, Tampa, FL

Abstract Body:

Fecal contamination of recreational and drinking water is a major concern for public health as it can lead to infections from waterborne pathogens. Usually these pathogens are in low densities and are difficult to detect. Microorganisms that are abundant in feces, e.g. enterococci, *Escherichia coli* and members of *Bacteroidales* (GenBac) are used to detect fecal contamination and as a proxy for pathogens; however, pathogens generally require more extensive concentration strategies such as ultrafiltration via a semipermeable membrane and techniques to recover organisms from filters. Here, the efficiency of ultrafiltration, with hollow fiber filtration (HF) (with a Rexeed-25S filter cartridge) and HF with foam elution (HF-FE) (Innovaprep high volume elution fluid 0.75% tween/25mM Tris), were tested for enterococci, GenBac and human polyomaviruses (HPyVs) in Florida fresh (HF n=2, HF-FE n=2) and salt (HF n=2, HF-FE n=2) surface waters, with enumeration by quantitative PCR (qPCR). Microorganisms in 10L of water were concentrated ~50 fold (to ~200ml) by HF, resulting in no loss of enterococci or GenBac, compared to the control membrane filtration; however, 90% of HPyVs were lost. The HF concentrate was further reduced in volume 5-10000-fold by centrifugal ultrafiltration (CUF) which resulted in up to a 99% loss of enterococci and GenBac by qPCR. Inhibition of qPCR and DNA recovery from extraction procedures were tested using salmon sperm DNA. Inhibition was not detected in the HF concentrate of any sample (n=4) but minor inhibition (change of < 2 C_T) and DNA loss was noted in some CUF samples. CUF was deemed unsatisfactory for concentrating the targets due to loss and inhibition. HF-FE may enhance recovery of bacterial and viral targets from large-volume samples compared to HF alone for analysis of waterborne pathogens and microbial source tracking (MST) targets.

Author Disclosure Block:

J.W. Conrad: None. **V.J. Harwood:** None.

Poster Board Number:

FRIDAY-129

Publishing Title:

Efficacy of Inactivation of Human Enteroviruses by Multiple-wavelength Uv Leds

Author Block:

H. Ryu[1], S. Beck[2], L. Boczek[1], N. Brinkman[1], K. Linden[2], O. Lawal[3], S. Hayes[1]; [1]Environmental Protection Agency, Cincinnati, OH, [2]Univ. of Colorado, Boulder, CO, [3]AquiSense Technologies, Florence, KY

Abstract Body:

Background: Ultraviolet (UV) light has been successfully used for treating a broad suite of pathogens without the concomitant formation of carcinogenic DBPs. However, conventional mercury UV lamps have some practical limitations in water treatment applications, such as the inefficiency of energy consumption and more importantly potential mercury contamination upon disposal of the lamps. The recent invention of a novel light-emitting-diodes (LED) device generating germicidal UV wavelengths could eliminate the aforementioned limitations. In this study, we investigated the efficacy of multiple-wavelength UV LEDs for inactivating USEPA contaminant candidate list (CCL) RNA enteroviruses.**Methods:** Serotype representatives of the four human enteric species of enteroviruses such as coxsackievirus A10 (CVA10), echovirus 30 (Echo30), poliovirus 1 (PV1), and enterovirus 70 (EV70) respectively were selected as testing RNA viruses. Bench-scale performance evaluation was conducted using a collimated beam (CB) apparatus with LEDs emitting at 260 nm, 280 nm, and the combination of 260|280 nm together, as well as a monochromatic low-pressure (LP) UV lamp at 254 nm for comparison. The CB tests were performed with mixed stocks of four viruses. Infectious virus concentrations were determined using an ICC-RTqPCR.**Results:** The 260 nm LED was most effective at inactivating all enteroviruses tested, followed by the 260|280 nm LED, LP UV and lastly the 280 nm LED. These results are favorably comparable to our companion study with MS2 coliphage (RNA virus), suggesting no synergistic inactivation of RNA viruses by the 260|280 nm combination. Specifically, for a 2-log inactivation credit by the most effective 260 nm LED, UV doses averaged approximately 8 mJ/cm^2 for CVA10 and PV1, 10 mJ/cm^2 for EV70, and 13 mJ/cm^2 for Echo30. These UV doses for the viruses tested yielded much greater inactivation rate constants than MS2 coliphage and the most UV resistant adenoviruses.**Conclusions:** Overall, UV LEDs showed the capability to effectively inactivate the CCL enteroviruses tested. Superior performance of the 260 nm LED to conventional LP UV encourages further studies on its applicability for sustainable water treatment and other CCL pathogens.

Author Disclosure Block:

H. Ryu: None. **S. Beck:** None. **L. Boczek:** None. **N. Brinkman:** None. **K. Linden:** None. **O. Lawal:** None. **S. Hayes:** None.

Poster Board Number:

FRIDAY-130

Publishing Title:

Microbiological Quality and Microbial Diversity of Packaged Ice in Southern California

Author Block:

K. Lee, P. Lwin, L. Ab Samad, S. Riedel, A. Magin, W-J. Lin; Cal Poly Pomona, Pomona, CA

Abstract Body:

Background: Microbial contamination in food and water may post a threat to public health. Ice is defined as a food by the U.S. Food and Drug Administration (FDA). According to the International Packaged Ice Association (IPIA), approximately 2 billion bags of ice are sold from retail, wholesale, and vending producers each year in the U.S. Out of 700 commercial ice-making companies, 200 of the aforementioned are not presented by the IPIA and do not comply to specific packaged ice processing standards.**Methods:** Non-IPIA complied samples were collected from gas stations, liquor stores, or convenient stores in Los Angeles, Orange, San Diego/Imperial, and San Bernardino/Riverside counties. The microbiological quality of non-IPIA complied ice samples were compared with the IPIA-complied packaged ice samples using microbiological, molecular, and sequencing analyses.**Results:** Among 132 non-IPIA complied packaged ice samples analyzed, 15 samples contained unsatisfactory level of heterotrophs, (≥ 500 Most Probable Number [MPN]/100 ml), 41 samples contained unsatisfactory level of coliforms (≥1 MPN/100 ml), 19 samples had staphylococci, and 70 samples had yeast/mold. None of the 24 IPIA-complied samples had unacceptable microbial levels. None of the samples analyzed showed the presence of the pathogens, *Salmonella*. Next generation sequencing (NGS) results revealed that there are more diverse microbial populations in non-IPIA than IPIA samples, including opportunistic pathogens.**Conclusions:** Our results revealed the microbiological quality of non-IPIA and IPIA complied ice samples in southern California. These findings may lead to a better enforcement of processing standard on packaged ice.

Author Disclosure Block:

K. Lee: None. **P. Lwin:** None. **L. Ab Samad:** None. **S. Riedel:** None. **A. Magin:** None. **W. Lin:** None.

Poster Board Number:

FRIDAY-131

Publishing Title:

Occurrence of Opportunistic Pathogens *Legionella pneumophila* and Non-Tuberculous Mycobacteria in Hospital Plumbing Systems

Author Block:

J. Hoelle[1], M. Coughlin[2], E. Sotkiewicz[2], J. Lu[1], S. Pfaller[1], M. Rodgers[1], H. Ryu[1];
[1]Environmental Protection Agency, Cincinnati, OH, [2]Weas Engineering, Westfield, IN

Abstract Body:

Background: Opportunistic premise plumbing pathogens (OPPPs) such as *Legionella pneumophila*, *Mycobacterium avium*, and *Pseudomonas aeruginosa* are frequently detected in the plumbing systems of large buildings. The ability of these organisms to form biofilms and to grow in phagocytic amoeba are thought to provide some insulation from chlorine and other conventional water disinfectants. Copper-silver (Cu-Ag) ionization is one water treatment technology used in many hospital water systems as an additional barrier to the growth of waterborne pathogens. In this study, we investigated the occurrence of three OPPPs and phagocytic amoebae and the efficacy of Cu-Ag ionization in hospital plumbing systems.**Methods:** A total of 197 water and 31 swab samples were collected from 14 buildings in one city, including two installed with Cu-Ag ionization. The samples were analyzed for *Legionella* by culture and *L. pneumophila*, nontuberculous mycobacteria (NTM) (*M. avium* and *M. intracellulare*), *P. aeruginosa*, and two amoeba (*Acanthamoeba* and *Vermamoeba vermiformis*) by qPCR.**Results:** Of the 166 water samples from facilities without Cu-Ag ionization, *L. pneumophila* were the most prevalent pathogen detected by qPCR (44%, 73/166), followed by *V. vermiformis* (13%), NTM (11%), and *P. aeruginosa* (6%). Of *Legionella* positive samples, 35 and 25 were confirmed as *L. pneumophila* serotype 1 by culture and qPCR, respectively. Overall, OPPPs were more prevalent in warm water with low chlorine residuals than in cold water with higher chlorine residuals. In the two facilities using Cu-Ag ionization, the prevalence of *Legionella* in the water was low (3%, 1/31), whereas *M. avium* was detected at a high frequency (61%, 19/31) by qPCR. In addition to water, six of 26 swab samples were positive by qPCR for *L. pneumophila* including four serotype 1 positives, whereas *M. avium* was detected in one of five swabs from Cu-Ag ionization facilities. The other target microorganisms were not detected in any swab samples.**Conclusions:** Our observations that Cu-Ag ionization may control *Legionella* but enhance colonization of NTM in building plumbing systems should encourage further studies on the reduction in risks from waterborne pathogens afforded by new treatment technologies.

Author Disclosure Block:

J. Hoelle: None. **M. Coughlin:** None. **E. Sotkiewicz:** None. **J. Lu:** None. **S. Pfaller:** None. **M. Rodgers:** None. **H. Ryu:** None.

Poster Board Number:

FRIDAY-132

Publishing Title:

Assessment of Next Generation Sequencing as a Tool for Microbial Community Signature Analysis in Reused Water

Author Block:

M. Leddy[1], N. Hasan[2], C. Foster[1], R. Colwell[3]; [1]Orange County Water District, Fountain Valley, CA, [2]CosmosID, Rockville, MD, [3]Univ. of Maryland, College Park, MD

Abstract Body:

Background: Recycled water can be used for almost any purpose when treated from a health and environmental perspective. The Groundwater Replenishment System (GWRS), California, is the world's largest water purification system (100 million gallons per day). GWRS takes highly treated wastewater and disinfects it using a three step advanced treatment process: microfiltration (MF), reverse osmosis (RO), and ultraviolet light with hydrogen peroxide, producing high quality reclaimed water for injection into the groundwater basin that is the principal water supply for over two million people. Despite rapid growth of use and reuse practices for reclaimed water, the identification of microbial community at different levels of treatment remains largely unknown. The lack of rapid and accurate identification methods for total communities is one important reason for this knowledge gap. Culture-based methods are too limiting since they can only identify the organisms that can be grown. PCR based methods are faster, but targeted only to a limited number of organisms and static markers, which erode over time. Moreover, these approaches frequently underestimate pathogen removal through the advanced treatment process that can lead to overdesign and unnecessary costs. Next Generation Sequencing (NGS) combined with the GENIUS algorithm is used to identity microbial community signatures and their transformations through GWRS treatment. **Methods:** Total DNA is extracted from water and biofilm samples collected from plant influent (Q1) and biofilm scrapings from MF and RO membranes. Samples are sequenced by Illumina HiSeq and analyzed by GENIUS Bioinformatics Package. **Results:** Diverse communities are identified in Q1 and from biofilm scrapings and enables detection of fecal indicators, pathogens, antibiotic resistance and virulence factors, as well as genes encoding biogeochemical processes such as pollutant biodegradation. Fewer organisms are expected through MF and RO filtration, with the least number of organisms in the RO. Unique signatures of microbial community are identified in the MF and RO. **Conclusion:** The results will be used to assess the application of NGS for recycled water, highlighting both the benefits and limitation of this technology for water treatment processes.

Author Disclosure Block:

M. Leddy: None. **N. Hasan:** None. **C. Foster:** None. **R. Colwell:** None.

Poster Board Number:

FRIDAY-133

Publishing Title:

Assessment of Microbial Diversity in Water Samples Using Next Generation Sequencing

Author Block:

N. Jothikumar, A. K. Kahler, V. R. Hill; CDC, Atlanta, GA

Abstract Body:

Next-generation genomic and metagenomic sequencing represents a powerful tool for environmental testing , including improving methods for detecting pathogens in environmental samples, characterizing the microbiological community associated with environmental pathogens, improving understanding of environmental mechanisms of disease transmission, and determining the effectiveness and impacts of water and waste treatment systems. We developed multiple sets of primers targeting different regions of the 16S gene for metagenomic analysis to identify a broad range of bacteria in environmental samples. The primers targeted divergent taxa likely to be present in diverse water types. We performed 16S metagenomics sequencing using the Illumina MiSeq platform and a 600 cycle reagent kit for environmental water samples for a comprehensive assessment and to compare taxonomic diversity. Out of the five hyper variable V regions studied, V3 yielded the highest number of identified species, followed by the V1-V3 region, V5-V6 region, V4-V5 region, and V4-V6 region. The V3 assay, with amplicon lengths of 193 bp, showed the greatest species diversity. The V3 primer set enabled identification of more bacterial species from water samples than a commercially available primer set targeting the V1-V3 region. Based on these study results, the 16S rRNA gene V3 region provided more information to identify a greater number and diversity of bacterial species, and should be a useful tool for bacterial community studies of water systems.

Author Disclosure Block:

N. Jothikumar: None. **A.K. Kahler:** None. **V.R. Hill:** None.

Poster Board Number:

FRIDAY-134

Publishing Title:

Unique Environmental *E. coli* Strains Isolated from Aquatic Environments

Author Block:

T. Seale[1], E. T. Steenkamp[1], V. S. Brözel[2], S. N. Venter[1]; [1]Univ. of Pretoria, Pretoria, South Africa, [2]South Dakota State Univ., Brookings, SD

Abstract Body:

The gut of warm-blooded animals is widely considered to be the primary habitat of *E. coli*, but some strains can persist and possibly multiply in environments outside of the primary host. Several studies have indicated that *E. coli* isolates from the environment primarily belong to phylogroups A and B1. These environments are also thought to be the primary habitat for the closely related species currently designated as Clades III - V. The aim of this study was to determine whether the *E. coli* populations associated with freshwater reservoirs in subtropical South Africa belonged to the typical phylogroups and clades associated with this type of environment. For this purpose, *E. coli* was initially isolated from water plants, sediment and water from two peri-urban reservoirs as well as from sewage before treatment and release into these reservoirs. *E. coli* was also isolated from aquatic plants collected from six additional reservoirs in the area. Phylogroups were assigned using the Clermont method and isolates were clustered using phylogenetic analysis of the concatenated *uidA, rpoS, mutS* and *fadD* gene fragments. As expected, most of the isolates belonged to phylogroup B1 (35%), but numerous isolates belonging to phylogroups B2 (22%) and D (25%) were also obtained. Amongst the B2 and D isolates obtained from water hyacinths and plant debris many formed part of unique clusters that were not closely related to any of the sewage isolates. The genome sequences for four isolates from these unique clusters were determined to compare their gene content with those of typical phylogroup B2 or D isolates. The isolates associated with hyacinths and plant debris encoded a number of unique genes but also lacked several of the genes typically encoded by isolates of phylogroups B2 or D. These findings indicate that phylogroup B2 and D isolates exist that have undergone niche adaptation for persistence in aquatic environments.

Author Disclosure Block:

T. Seale: None. **E.T. Steenkamp:** None. **V.S. Brözel:** None. **S.N. Venter:** None.

Poster Board Number:

FRIDAY-135

Publishing Title:

Miniature Pulsed-Electric Field Device for On-Site Disinfection of Water

Author Block:

O. W. LEE, P. S. HUNG, K. W. LAM, **H. ZHANG**, K. L. YEUNG; The Hong Kong Univ. of Sci. and Technology, Hong Kong, Hong Kong

Abstract Body:

Open water features such as water ponds and fountains are common in modern architecture and interior design. Improper maintained, they could become reservoir for microbes and pests. Chemical disinfectants (i.e., chlorination) are often used, but can lead to malodor and material corrosion. This work examines an alternate water disinfection technology based on pulsed-electric field (PEF). Microbes subjected to pulsed-electric field often experienced a change in cell permeability and on occasion irreversible poration that lead to cell death. A miniature PEF device using a low voltage circuit and micro-engineered electrodes were designed and fabricated. The narrow gap between electrodes means intense electric field can be generated, whereas using a low voltage, low powered circuit allows the use of battery to power the device. Laboratory studies show the miniature PEF is effective against common Gram- bacteria (i.e., *E. coli*) commonly found in water with 2 log reduction in less than 1 min. The device was used in a water feature located at HKUST to measure its effectiveness under practical use condition. An unpowered device was used for control. Study showed that better than 70 % reduction in heterotropic bacteria can be achieved by the powered device under a 1 L per min flow. The device can be operated without battery recharging for up to a month and is effective in controlling the bacteria contamination in the water.

Author Disclosure Block:

O.W. Lee: None. **P.S. Hung:** None. **K.W. Lam:** None. **H. Zhang:** None. **K.L. Yeung:** None.

Poster Board Number:

FRIDAY-136

Publishing Title:

Multiple-Year Investigation of Bacterial Contamination in a Midwest Agricultural Watershed

Author Block:

E. Badger, A. Johnson, J. Denney, J. Enos-Berlage; Luther Coll., Decorah, IA

Abstract Body:

Background: Surface waters in the midwestern United States face serious water quality challenges, in part due to the intensity of agricultural activity in this region. In Iowa, where over 90% of the land is used for agriculture, approximately 75% of assessed waterways are impaired (1). The 20,000 acre Dry Run Creek Watershed in Northeast Iowa is impaired for bacteria, a particular concern because this stream flows through major recreational areas. To increase our understanding of contributing factors, as well as explore complexities of bacterial water quality analysis, we assessed distinct watershed sites under varying environmental conditions over a multi-year period. **Methods:** Between 2010-2015**,** ten sites were evaluated for land use and water quality under both dry and wet weather conditions. Levels of *Escherichia coli*, and physical and chemical field parameters, were measured from May-October during 2010, 2011, 2013, and 2015. In addition, phosphorus and nitrogen compounds were measured during 2010 and 2011. AN(C)OVA tests, 99% bootstrapped CIs, and Spearman rank order correlations were performed as appropriate. **Results:** Watershed sites were distinguished by adjacent land use, including relative levels of row crop agriculture, direct livestock access, perennial grass cover, and natural woodlands. Average levels of *E. coli* varied between sites and increased dramatically in response to rain events. Interestingly, different patterns were observed during dry and wet weather conditions in terms of site-specific average *E. coli* concentrations. Positive correlations were found between *E. coli* levels and some nutrients ($p < 0.01$). In addition, *E. coli* levels were positively correlated with amount of rainfall but negatively correlated with time elapsed between rainfall and sampling ($p < 0.05$). Finally, our data exhibited seasonal patterns, as well as substantial changes in some parameters between years having dramatic precipitation differences. **Conclusions:** The condition-dependent shifts in the highest bacteria-contributing sites suggest different types of sources and mitigation strategies. Collectively, these data underscore the importance of sampling during diverse conditions, and taking site-specific characteristics, rainfall amount, timing of sampling, and seasonal and annual variation into consideration when interpreting bacterial water quality data.

Author Disclosure Block:

E. Badger: None. **A. Johnson:** None. **J. Denney:** None. **J. Enos-Berlage:** None.

Poster Board Number:

FRIDAY-137

Publishing Title:

Performance of a Pepper Mild Mottle Virus Rt-Qpcr Method in Coastal Waters Exposed to Untreated Wastewater Pollution and Its Simulated Risk of Gastrointestinal Illness

Author Block:

E. M. SYMONDS[1], C. Sinigalliano[2], M. Gidley[2], W. Ahmed[3], S. M. McQuaig-Ulrich[4], M. Breitbart[1]; [1]Univ. of South Florida, Saint Petersburg, FL, [2]U.S. Natl. Oceanographic and Atmospheric Admin., Miami, FL, [3]Commonwealth Scientific and Industrial Res. Organisation, BRISBANE, Australia, [4]St. Petersburg Coll., Clearwater, FL

Abstract Body:

Pepper mild mottle virus (PMMoV) is a sensitive indicator of human fecal pollution. Its elevated concentrations in untreated domestic wastewater, as well as persistence throughout the wastewater treatment process, create the possibility that PMMoV detection in surface waters may overestimate the extent of fecal pollution. In order to better understand the significance of PMMoV detection in surface waters, this study determined the potential health risks associated with detecting PMMoV using a previously published RT-qPCR assay. First, the limits of detection (LOD) and quantification (LOQ) of this assay were determined for coastal waters seeded with untreated wastewater. The analytical LOQ for the RT-qPCR PMMoV was between 10 and 100 gene copies and the assay's standard curves average regression coefficient of 0.990 and an efficiency of 99.38%. In Florida coastal waters exposed to known sources of fecal pollution, PMMoV was detected in greater concentrations and/or more frequently at all sites compared to the following markers: human polyomavirus (HPyV), animal-specific *Bacteroidales* markers, and *Enterococcus* spp. Following the assumption that viruses within untreated wastewater would be diluted to the same extent upon discharge, Quantitative Microbial Risk Assessment (QMRA), with human norovirus (NoV) as a reference pathogen, was then used to calculate the health risks associated with untreated wastewater pollution. Simulated QMRA suggests that PMMoV detection in coastal waters exposed to untreated wastewater exceeds the 2012 U.S. Environmental Protection Agency illness benchmark (1986 equivalent, 36 illnesses/1,000 swimmers). Future work, including epidemiological studies, is needed to further investigate the use of PMMoV as an indicator as well as to confirm the relationship between PMMoV presence and gastrointestinal illness in coastal waters.

Author Disclosure Block:

E.M. Symonds: None. **C. Sinigalliano:** None. **M. Gidley:** None. **W. Ahmed:** None. **S.M. McQuaig-Ulrich:** None. **M. Breitbart:** None.

Poster Board Number:

FRIDAY-138

Publishing Title:

Necrotizing Fasciitis Due to *Vibrio cholerae* Non-O1/Non-O139 after Exposure to Austrian Bathing Sites

Author Block:

F. J. Allerberger[1], S. Hirk[1], S. Huhulescu[1], E. Gschwandtner[2], M. Hermann[2], S. Neuhold[2], A. Zoufaly[2], A. Indra[1]; [1]Austrian Agency for Hlth.and Food Safety (AGES), Wien, Austria, [2]Wiener Krankenanstaltenverbund, Wien, Austria

Abstract Body:

We report on two cases of necrotizing fasciitis of the lower leg due to nontoxigenic *Vibrio cholerae*. A 73 year old woman (case 1) and an 80 year old man (case 2) were hospitalized with symptoms of necrotizing fasciitis on July 18 and August 15, 2015, respectively. In both cases, symptoms started the day after swimming in local ponds. Swabs gained intraoperatively and a blood culture from the male patient, yielded *Vibrio cholerae* non-O1/non-O139, negative for cholera toxin gene *ctx* and positive for hemolysin genes *hly*A and *hly*B. Water samples taken from pond A on August 17, 2015 (32 days after exposure of case 1) and from pond B on August 20, 2015 (7 days after exposure of case 2) yielded non-O1/non-O139 *Vibrio cholerae* in most-probable numbers of >11,000 per 100 ml each. The occurrence of two cases of necrotizing fasciitis within a one month period related to two Austrian non-saline bathing waters, previously not known to harbor *Vibrio cholerae*, is probably linked to the prevailing extreme weather conditions (heat wave, drought) this summer in Austria. While case 1 was discharged in good clinical condition after 73 days, case 2 died after four months of hospitalization. Public health authorities are challenged to assess the effects of long-term climate change on pathogen growth and survival in continental bodies of fresh water.

Author Disclosure Block:

F.J. Allerberger: None. **S. Hirk:** None. **S. Huhulescu:** None. **E. Gschwandtner:** None. **M. Hermann:** None. **S. Neuhold:** None. **A. Zoufaly:** None. **A. Indra:** None.

Poster Board Number:

FRIDAY-139

Publishing Title:

Survival of Various Pathogenic Microorganisms in Surface Water and Sediments in Western New York

Author Block:

L. Farovitch, J. Lodge; Rochester Inst. of Technology, Rochester, NY

Abstract Body:

Western New York (WNY) is well known for its vacation and recreational water spots. In 2005-2006, of the 4,412 reported cases of disease outbreak due to recreational water use, 91% were associated with acute gastrointestinal (GI) illness. Several recreational waters in WNY include: Durand Eastman Beach, Charlotte Beach, Genesee River, Black Creek, Irondequoit Bay and Oatka Creek. To improve the safety of swimming in recreational water, adequate monitoring of water-quality is necessary. In addition to *Escherichia coli* strains (e.g., O157:H7), other enteric pathogens such as *Salmonella typhimurium, Shigella sonnei*, and *Yersinia enterocolitica*, may be responsible for contaminated recreational waters. Because these enteric pathogens are not normally found in surface waters and sediments, it is important to determine the survival rates of these enteric bacteria in recreational waters in WNY. Also, the survival of *Pseudomonas aeruginosa* (pathogenic strain) was determined in some of the local water and sediment samples. All bacterial strains used in this study were isolated from various patients presenting GI disease symptoms. The data from late Fall samples indicated that ~90% of *P. aeruginosa* and *Y. enterocolitica* survive longer (6 and 4 days, respectively) in both sediment and water samples from Black Creek and Durand Eastman Beach than other samples. On average, the survival of organisms in surface waters and sediments ranked from greatest to least as follows: *P. aeruginosa* > *Y. enterocolitica* > *S. sonnei* > *S. typhimurium* > *E. coli*. However, the preliminary data from late Summer samples showed that *E. coli*, *S. typhimurium* and *Y. enterocolitica* but not *S. sonnei* survived longer in water from Durand Eastman Beach (*P. aeruginosa* was not tested in late Summer samples) where it suggests that changes in survival of these pathogenic organisms in recreational waters in WNY depend on seasonal variation. The pH of surface waters and sediments differed by ~1 pH unit (8.1 and 7.4, respectively), indicating that pH does not appreciably affect survivability of these pathogenic organisms. Future studies will examine the effects of climate changes and nutrients on the survival of pathogens by collecting the samples in winter and spring.

Author Disclosure Block:

L. Farovitch: None. **J. Lodge:** None.

Poster Board Number:

FRIDAY-140

Publishing Title:

Ultraviolet A Induced Phenotype Heterogeneity of *Pseudomonas aeruginosa* and *Enterococcus faecalis* in Synthetic Stormwater

Author Block:

D. Ng, A. Kumar, B. Cao; Nanyang Technological Univ., Singapore, Singapore

Abstract Body:

Background: Stormwater is an important resource. In surface reservoirs, stormwater is subjected to natural self-purification activities such as UV disinfection by sunlight. UVA radiation in sunlight plays a key role in inactivation of bacterial pathogens [1]. However, there is limited knowledge on the fate of bacterial pathogens in stormwater during solar disinfection, especially on how some bacteria survive solar disinfection, which is critical for stormwater management. In this study, the effects of UVA radiation in sunlight on the fate of an opportunistic pathogen *Pseudomonas aeruginosa* and a fecal indicator bacterium *Enterococcus faecalis* in synthetic stormwater were investigated. **Methods:** Bacteria were exposed to a UVA radiation intensity similar to that in sunlight for a period of 2 weeks and culturability was determined every 2 days by CFU counts. qPCR analysis was used to determine the viable but not culturable (VBNC) state of *E. faecalis*. The decrease in culturability of *E. faecalis* and *P. aeruginosa* was described by the single-phase decay model. Small colony variants of *P. aeruginosa* were characterized by biofilm, motility and reactive oxygen species (ROS) assays.**Results:** In the presence of UVA, the culturability of *E. faecalis* decreased drastically while culturability of unexposed *E. faecalis* persisted. Exposure to UVA induced *E. faecalis* to enter the viable but not culturable (VBNC) state. In contrast, there was only approximately a 2-log reduction in the CFU counts of *P. aerguinosa* exposed to UVA radiation. Instead, *P. aeruginosa* formed small colony variants (SCV) in response to UVA radiation treatment. Compared to typical colonies, SCV had reduced biofilm formation ability and motility as well as faster adaptation to reactive oxygen species stress. Culturability of unexposed *P. aeruginosa* persisted throughout the duration of the experiment.**Conclusions:** UVA induces phenotype heterogeneity in *P. aeruginosa* and *E. faecalis* which may enable their survival when exposed to sunlight. Hence, phenotype heterogeneity may play a key role in the survival of bacteria during solar disinfection. The findings of this study will contribute towards effective stormwater management strategies.

Author Disclosure Block:

D. Ng: None. **A. Kumar:** None. **B. Cao:** None.

Poster Board Number:

FRIDAY-141

Publishing Title:

Interaction between *Legionella pneumophila* and *Pedobacter glucosidilyticus*

Author Block:

K. Paranjape, H. Trigui, N. Mendis, D. Rodriguez-Mendez, S. P. Faucher; McGill Univ., St-Anne de Bellevue, QC, Canada

Abstract Body:

Background: *Legionnella pneumophila* (*Lp*), a Gram-negative bacterium, is the causative agent of Legionnaires' disease and Pontiac Fever. The primary source of Legionnaire's disease outbreaks is cooling towers, which get contaminated by microorganisms. The contamination within a cooling tower can create an ecosystem that is favourable for the establishment of *Lp* strains. *Lp* is a known facultative intracellular parasite of 13 different amoebal species and thus problematic cooling towers usually contain some of these species. Chemical and thermal control methods are implemented, in order to decrease outbreaks. However, these methods are usually expensive, damaging and harmful. Moreover, different microorganisms present within a cooling tower may either impede or promote the establishment of different *Lp* strains.The research presented here examines the interaction between the bacterial species *Pedobacter glucosidilyticus* (*Pg*), a Gram-negative environmental species isolated from an artificial water system, and *Lp*.**Methods & Results:** *Pg* was isolated from a man-made water system. This species was examined for its capacity to survive in water for extended period of time, its effect on *Lp* survival in water and its effect on *Lp* when co-cultured in the presence of *Acanthamoeba castellanii (Ac)*, an amoebal host cell for *Lp*. *Pg* and *Lp* can both survive in water for extend periods of time. When co-cultured together in water, no significant decrease in CFU count could be observed. However, when these two strains were co-cultured in the presence of *Ac*, *Lp* showed no signs of growth whereas *Pg* grew rapidly over the incubation period. Additionally, *Pg* grew with *Ac* exclusively but not in the infection buffer alone.**Conclusion:** In conclusion, our results suggest that *Lp* and *Pg* can both survive in water for extended periods of time. Furthermore, both these strains do not affect one another's survivability in water. However, both strains are capable of growing in the presence of *Ac*, but *Pg* seems to outcompete *Lp* for intracellular growth. This would indicate that *Pg* is probably using the amoebal species for its own growth and thus may be a potential parasite of *Ac.* The lack of growth of *Lp*, when co-cultured with Amoeba and *Pg*, may either be due to an active inhibition by *Pg* or could be caused by *Pg* higher growth efficiency in *Ac*.

Author Disclosure Block:

K. Paranjape: None. **H. Trigui:** None. **N. Mendis:** None. **D. Rodriguez-Mendez:** None. **S.P. Faucher:** None.

Poster Board Number:

FRIDAY-142

Publishing Title:

Development of the Smart Sample Concentration System for Microbial Monitoring of Potable Water in the International Space Station

Author Block:

A. Page[1], A. Adolphson[1], M. Hornback[1], A. Checinska[2], K. Venkateswaran[2]; [1]InnovaPrep LLC, Drexel, MO, [2]Jet Propulsion Lab., Pasadena, CA

Abstract Body:

Human habitation of space requires unique systems for providing safe potable water. The International Space Station (ISS) Water Processor Assembly treats wastewater and condensate, turning it into potable water, and as such requires routine microbial monitoring. The existing microbial detection methods are culture-based, posing a high risk of additional health hazards in the space environment, and operational challenges in micro-gravity. Molecular detection technologies have progressed significantly in the last several decades and provide the greatest potential for overcoming these challenges. However, these methods outpaced development of sample concentration techniques, which are necessary for rapid detection of low concentrations of pathogens in drinking water. At present, NASA is challenged with a system that could reliably measure 50 cells per mL of drinking water. Since most available molecular detection technologies use only a few microliters of sample per reaction, water samples must be concentrated at least 1,000 times to achieve reliable and reproducible results. In response to this need, InnovaPrep joined with NASA to develop the ISS Smart Sample Concentrator (iSSC).In a pilot study, multiple one-liter water samples were processed through a hollow fiber membrane filter concentration cell, capturing microorganisms in the fiber lumen. Following capture, the microorganisms were eluted using a novel wet foam elution process in which a viscous, wet foam was used to sweep the captured particles from the membrane surface and dispense them in a concentrated sample volume of less than 500 μL. Microbiological tests performed with one-liter water samples spiked with *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus epidermidis* and assayed by qPCR confirmed that the iSSC effectively produces samples that are over 1,000 times more concentrated than unprocessed samples.Findings from completed and future development of the iSSC will provide a foundation for improvement of future rapid concentration and microbial detection systems for space- and earth-based drinking water.

Author Disclosure Block:

A. Page: A. Board Member; Self; InnovaPrep LLC. D. Employee; Self; InnovaPrep LLC. **A. Adolphson:** D. Employee; Self; InnovaPrep LLC. **M. Hornback:** D. Employee; Self; InnovaPrep LLC. **A. Checinska:** None. **K. Venkateswaran:** None.

Poster Board Number:

FRIDAY-143

Publishing Title:

Preliminary Report on Microbial Flora Isolated from Polluted and Non-polluted Coastal Waters of Karachi, Pakistan

Author Block:

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Abstract Body:

Marine pollution has now become worldwide environmental concern. Continuous discharge of untreated industrial effluent, municipal and power plant's contaminated wastewater has been a serious threat to marine habitat, marine life, aesthetic values and interest of visitors to coastal areas. Karachi, the largest city of Pakistan and industrial hub is located at latitude 24° 48´ N and longitude 66° 59´ E on coast of the Arabian Sea. It suffers serious environmental problems because of population pressure and increasing industrial activities. In this investigation, samples of sediment, water, flora and fauna were taken from nine selected stations on Karachi coast included three stations represented major creeks i.e. Korangi, Gizri and Chinna Creek. These samples were taken in north-east and south-west monsoonal period during 2014. Bacterial flora isolated and identified from samples collected from these sites by conventional method. Among isolated and identified bacteria e.g. *Vibrio alginolyticus, E.coli* and *Streptococuss anginosus* were the most dominant species contributing 21.43, 19.64 and 15.18 percent of total assemblage respectively. Among selected sample stations, Korangi creek station was found to be most polluted with coliform and other pathogenic bacteria. These results clearly indicate that threats from these pathogens are not only to marine life but also to the large number of visitors coming to beaches and residents of surrounding area. Moreover; immediate action should be taken to restrict the growth of these pathogens by taking measures to treat the municipal and industrial effluent to avoid outbreak of any disease in future.

Author Disclosure Block:

A. shaheen: None. **H.S. baig:** None. **S.U. kazmi:** None.

Poster Board Number:

FRIDAY-144

Publishing Title:

Study of Microbial Communities across the Rio Piedras River Watershed

Author Block:

A. Lugo, E. Torres, F. Godoy-Vitorino; Inter American Univ. of Puerto Rico, San Juan, PR

Abstract Body:

Background: The Río Piedras river is located in an urban watershed fully contained within the metropolitan area of San Juan within two geographic regions of Puerto Rico: the Northern Coastal Lowlands Humid Alluvial Section and the Humid Northern Foot Hills Northeastern Cretaceous Section. Due to an increase in the urban population, it has been impacted with human activity over the years raising numerous conservation challenges for this important water resource in the San Juan area. We have evaluated the microbiome complexity at three sites of the river, over an urbanization gradient, and hypothesize that the structure and diversity of the bacterial communities change according to the anthropogenic impact levels. **Methods**: Three 1L water samples were collected in Las Curias (pristine site 1), Old aqueduct (medium human impact site 2) and University Gardens (high human impact site 3). The water was filtered using 0.2 uM membrane disk filters, followed by gDNA extraction using the MOBIO Power soil and Illumina sequencing of the V4 region of the 16S rDNA gene. Data analyses was done using QIIME with the SILVA database as taxonomic reference. **Results:** A total of 1,444,201 raw sequences underwent strict quality and size filtering resulting in 135,237 high quality sequences (>250bp, no singletons and no chloroplasts) which clustered into 1,034 OTUs based on a 97% similarity. A total of 26 phyla in 135 families were found with overall dominance of Proteobacteria, Actinobacteria, Bacteroidetes, Chlorobi, Cyanobacteria and Firmicutes. The more pristine site showed only 6 phyla with a dominance of Proteobacteria (Rhodobacteraceae) and Actinobacteria (Sporichthyaceae) while the medium impacted areas had 15 phyla and a dominance of Chlorobi (f__OPB56), Actinobacteria (Sporichthyaceae) and Bacteroidetes (Bacteroidaceae); and the most impacted site had 24 phyla with a dominance of Proteobacteria (Comamonadaceaea), Firmicutes (Ruminococcaceae) and Bacteroidetes (Bacteroidaceae). Richness was as high as 500 species in areas of high human impact and as low as 100 taxa in the low impact site. **Conclusion:** We found an unprecedented diversity of microbial communities in water samples of medium and high human impacts, indicating greater ecological complexity alerting for the risk of water contamination due to sewage discharges and posing a public health risk.

Author Disclosure Block:

A. Lugo: None. **E. Torres:** None. **F. Godoy-Vitorino:** None.

Poster Board Number:

FRIDAY-145

Publishing Title:

Chemical Transmutation of *Penicillum italicum* rlm55$_i$ for Enhanced Cellulase Hyper-production

Author Block:

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Abstract Body:

The inability of wild fungi to secrete appreciable amount of enzymes limited their potential application in enzyme production technology. This study investigated the enhancement of cellulase biosynthesis potential of *Penicillum italicum* RLM55$_i$ through chemical treatment. The fungal spores were incubated with a fixed concentration of different chemical mutagens (sodium azide, ethyl methane sulphonate, nitrous acid and hydroxylamine) over a period of 90 min. with the recovery of mutants at every 15 min. Mutant with highest cellulase-producing capability at the selection points was further screened in a submerged culture system at 28±2^{o}C and 120 rpm for 5 days, for quantitative estimation of cellulase production. The chemicals exhibited different stimuli on the isolate. Results of the study revealed that the chemicals impacted cellulase activity as follows; nitrous acid, 2.26 fold in *RLM$_{NO}$45*; sodium azide, 2.34 fold in *RLM$_{NA}$-30;* ethyl methane sulphonate, 3.51 fold in RLM$_{MS}$*75*; and hydroxylamine, 3.94 fold in *RLM$_{NH}$*45 as compared to the parent strain; and the highest achieved with the respective mutagen under the experimental conditions. Protein biosynthesis was highly enhanced in the following mutants by the corresponding chemical treatment: *RLM$_{NA}$-90* (2.08 fold), *RLM$_{MS}$60* (4.34 fold), *RLM$_{NO}$60* (5.60 fold), and *RLM$_{NH}$*30 (5.13 fold). Of all the agents, hydroxylamine exhibited the most suitable mutagenic potential, thus could be further exploited in the improvement of the strain for overproduction of cellulases.

Author Disclosure Block:

T.C. Ekundayo: None.

Poster Board Number:

FRIDAY-146

Publishing Title:

Plant Cell Wall Degrading Enzymes From *A. niger* Using Novel Natural Substrates

Author Block:

D. Kumari, S. A. Khan; Univ. of Karachi, Karachi, Pakistan

Abstract Body:

Background: Fungi elaborate a wide variety of plant cell-wall degrading enzymes, including xylanases, pectinases, amylases and cellulases. Although these enzymes have applications in a number of industries, but their production at the commercial scale is limited because of higher costs of the purified substrates. Hence, natural substrates can be considered as an alternate source for their production. The present study was aimed to explore the novel and unconventional substrates for production of plant cell-wall degrading enzymes.**Methods:** Enzyme production was studied using indigenously isolated fungal strain of *Aspegillus niger* by growing it on peels of banana, grapefruit and pomegranate, sugarcane bagasse, leaves of *Eucalyptus camaldulensis*-and stem of two halophytic plants i.e. *Halopyrum mucronatum*, *Desmostachya bipinnata* under solid-state fermentation (SSF) and submerged fermentation (Smf) conditions. The crude enzyme preparation was screened for xylanase, pectinase, amylase and cellulase (β-glucosidase, endoglucanase and filter-paperase) production**Results:** The results revealed the highest levels of xylanase production as compared to other enzymes; higher titres were obtained under SSF when strain was grown on stems of *D. bipinnata* and *H. mucronatum*. Xylanase production was followed by pectinase levels in *E. camaldulensis*-leaves. Considerable levels of amylase were produced from sugarcane baggasse and *E. camaldulensis*-leaves. Additionally, appreciable amounts of endoglucanase and β-glucosidase were noted using grapefruit-peels and *H. mucronatum*-stem respectively.**Conclusions:** Hence, significant level of xylanase production was observed from stems of *D. bipinnata* and *H. mucronatum*, and could be used for large scale xylanase production at the commercial level.

Author Disclosure Block:

D. Kumari: None. **S.A. Khan:** None.

Poster Board Number:

FRIDAY-147

Publishing Title:

Microbiological Status of Cocoa Beans Fermentation for Optimal Utility

Author Block:

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Abstract Body:

Raw cocoa, *Theobroma cacao,* with its astringent taste and flavor needs fermentation to obtain the usual characteristic cocoa flavor and taste. A number of microflora are involved in spontaneous cocoa beans fermentation but not all are desirable. The quest for food security for the now and future, one of the primary objectives of the 2000 Millennium Development Goals, demands improvement in processes. This research examined the microbiological status of cocoa bean fermentation for optimal utility.A five-day fermentation of fresh cocoa bean of variety S/7, F3 Amazon hybrid was carried out in 50kg wooden boxes. A mixed microbial population of yeast and bacteria were isolated as the microflora. The physio-chemical were determined during the fermentation period. Morphological and biochemical characteristics for the identification of the isolates were carried out. Qualities of fermented beans were established using the Cut Test Method. The Nutritional Status of the final product (cocoa powder) was determined by standard chemical method of food analysis.Results gave gradual increase in the Temperature, with corresponding decrease in P^H while Titretable acidity increased due to the microbial activities. The Total Viable Count of the fermenting isolates in CFU/g, gave a growth format of progressive increase in microbial population throughout the fermentation period, though these were highest between day 1 and day 3 and gradually slowed down in day 4 and 5 which could have resulted from the different physiological roles of the mixed microbial population. Twenty five (25) isolates of the essential flora were obtained with a distribution that showed that Yeasts dominated the fermentation the first 24 hours followed by Lactic acid bacteria and then Acetic acid bacteria. The proximate analysis showed that Protein content of the fermented samples increased with the microbial fermentation in compliance with the recommended level for normal child growth. Crude fibre, Crude ash and Crude fat contents all decreased after fermentation.A mixed microbial controlled fermentation with *Saccharomyces cerevisiae*, *Lactobacillus plantarum* and *Acetobacter acetic* played vital roles in enhancing cocoa beans quality for optimal utilization, and therefore recommended.

Author Disclosure Block:

M.I. Araoye: None.

Poster Board Number:

FRIDAY-148

Publishing Title:

Biosynthesis of Ethylene Gas by Various Microorganisms from Different Sources

Author Block:

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Abstract Body:

Background: To quantify and compare the Ethylene gas production by a range of selected microorganisms.**Methodology & Results:** Ethylene is a plant hormone that triggers the ripening of fruit which results in the transformation of complex sugars to the simpler forms, fruit softening, and degreening of the skin of the fruit. This plant hormone is involved in processes like plant growth, fruit ripening, senescence and development of plant. Both endogenous and exogenous ethylene can induce the ripening of fruits. Ethylene coordinates in the expression of such genes, which increase the rate of respiration, autocatalytic ethylene production, degradation of chlorophyll, synthesis of carotene, conversion of starch or complex sugars into their simpler forms and increased enzymatic activity of cell-wall degradation.Different microorganisms were checked for the production of ethylene under controlled condition at different pH values and various temperature.Ethylene gas was detected by commercially available Ethylene specific analyzer., Listeria produced Ethylene in highest amount, i.e. 19.5ppm /100ml of TSB. **Conclusion:** This method can be adapted easily because of its easy production and cost effectiveness

Author Disclosure Block:

M. Munir: None. **M. Hamid:** None. **S.T. Hakim:** None. **S.G. Nadeem:** None.

Poster Board Number:

FRIDAY-149

Publishing Title:

Colonic Fermentation of Cranberry A-Type Proanthocyanidins: Prebiotic Potential?

Author Block:

L. A. Doherty[1], K. Racicot[1], S. Arcidiacono[1], K. Kensil[1], O. Chen[2], J. W. Soares[1]; [1]US Army NSRDEC, Natick, MA, [2]Tufts Univ., Boston, MA

Abstract Body:

From a military perspective, the gut microbiome may serve as a useful tool to not only enhance Soldier gut and immune health, but also improve survivability, protection and performance. Our works centers on elucidating the systematic processes of colonic bacterial metabolism of dietary components and the influence on bacterial community dynamics, inflammation and immunity. To date, primary focus has been related to investigating the prebiotic potential of cranberry A-type proanthocyanidins (PAC) through *in vitro* colonic fermentation. Static and dynamic batch fermentations were performed utilizing fecal inocula derived from three individuals in a nutrient-rich anaerobic media supplemented with purified cranberry PAC. Total growth assessment through protein content analyses revealed a linear increase in growth as a function of PAC dosage. Bacterial population dynamics, determined through 16S RNA sequencing, indicated an increase in *Blautia spp.*, associated with increased metabolic byproducts and nutrient assimilation, and *Bifidobacterium spp.*, a known probiotic, concomitant with a decrease in *Bacteroides spp.*, associated with gut inflammation. GC-FID analysis of metabolic products indicated a PAC-dependent increase in propionate production, which possesses putative health effects. GC/MS identified two phenolic metabolites, (4-hydroxyphenyl) acetic acid and (3-(4-hydroxyphenyl)-propionic acid that are associated with anti-inflammatory activity. The results suggest cranberry PAC may have a prebiotic effect on gut microbiota in vitro with potential effects in vivo, particularly at an elevated intake. To further elucidate knowledge related to PAC metabolism, PAC-dependent growth under conditions simulating the multiple domains of the colon was investigated, revealing domain-dependent growth behavior. Domain-specific bacterial population and metabolic byproduct assessments are ongoing. Efforts toward understanding the gut microbiota metabolism of PAC and the influence on innate immune function could impact future dietary supplementation approaches, particularly in relation to building resiliency to military-relevant stressors through nutritional strategies to enhance performance.

Author Disclosure Block:

L.A. Doherty: None. **K. Racicot:** None. **S. Arcidiacono:** None. **K. Kensil:** None. **O. Chen:** None. **J.W. Soares:** None.

Poster Board Number:

FRIDAY-150

Publishing Title:

Industrially Important Enzymes and Microbial Lipids for Biodiesel Synthesis from Organic Municipal Solid Wastes

Author Block:

A. Azad, Islam K, Ahmed J, Iqbal A, Sohag MMH, RakibuzzamanSM, Rana MM, Hakim A, Hasan M and Hossain S; Shahjalal Univ. of Sci. and Technology, Sylhet, Bangladesh

Abstract Body:

Municipal solid wastes (MSW) in the open dumping of developing countries result in environmental pollution, public health hazards and climate change. Organic MSW (OMSW) can be converted to bioresources through production of industrially and commercially important products, and thus the environmental pollution and climate change caused by MSW may be mitigated. We have isolated and identified proteolytic and cellulolytic bateria and fungi from MSW and cowdung. Based on the 16S rDNA sequence, the bacterial isolates were *Bacillus cereus*, *Serratia marcescens* and *Pseudomonas aeroginosa*, and based on the 18S rDNA sequence, the fungal isolates were *Aspergillus oryzae, A. fumigatus and A. Flavus*. Significant level of protease and cellulase were produced from bacterial and fungal isolates, respectively by using OMSW as raw materials in the shake flask and bioreactor. Physicochemical parameters such as temperature, pH, aeration, agitation, substrate concentrations etc were optimized for protease and cellulase production in the bioreactor. Protease production was scaled-up 2.5-3.0 fold in the bioreactor with reduction in fermentation period. The SDS-PAGE showed that the estimated molecular mass of the partially purified protease from *S. marcescens* was ~38 kDa and that from *P. aeroginosa* was ~25 kDa. Partially purified protease from both sources was characterized and enzyme kinetics were determined. Genome-wide analysis revealed that the genomes of *A. oryzae, A. fumigatus and A. Flavus* had 22, 25 and 23 cellulase genes, respectively. Some homologous cellulase genes were isolated from the three fungus isolates. Physicochemical parameters for microbial lipids production from oliagenous yeast *Lipomyces starkeyi* for biodiesel synthesis by using OMSW were optimized. Amount of accumulated lipids of *L. starkeyi* grown in OMSW hydrolysate media under optimal conditions was ~45% (v/w) of dry biomass of the yeast cells. Gas chromatographic analysis revealed that the fatty acid composition of this microbial lipid is similar to that of vegetable oils. Our study indicates that OMSW might be a valuable alternative feedstock for production of microbial lipids and industrially important enzymes.

Author Disclosure Block:

A. Azad: None.

Poster Board Number:

FRIDAY-151

Publishing Title:

Transcriptional Profiling Reveals Molecular Basis and Novel Gene Targets for Improved Resistance to Multiple Fermentation Inhibitors in *Saccharomyces cerevisiae*

Author Block:

Y. Chen, Jiayuan Sheng, Tao Jiang, Joseph Stevens, XueyangFeng, Na Wei; Univ. of Notre Dame, South Bend, IN

Abstract Body:

Lignocellulosic biomass is a promising source of renewable biofuels and bioproducts. However, pretreatment of lignocellulosic biomass generates fermentation inhibitors that are toxic to industrial microorganisms such as *Saccharomyces cerevisiae*. A critical challenge on developing *S. cerevisiae* with improved inhibitor resistance lies in incomplete understanding of molecular basis for inhibitor stress response and insufficient knowledge on effective genetic targets for increasing yeast resistance to disparate fermentation inhibitors. We applied comparative transcriptomic analysis to determine the molecular basis for acetic acid and/or furfural resistance in *S. cerevisiae*. The genes associated with stress responses of *S. cerevisiae* to single and mixed inhibitors were revealed. Specifically, we identified 184 consensus genes that were differentially regulated in response to the distinct inhibitor resistance between wide type and mutant *S. cerevisiae*. Bioinformatic analysis next revealed key transcription factors (TFs) that regulate these consensus genes. Two transcription factors, Sfp1p and Ace2p, were uncovered as novel overexpression gene targets for the first time for their functions in improving yeast resistance to mixed fermentation inhibitors. The study demonstrated an omics-guided metabolic engineering framework and also helped advance the fundamental understanding of the mechanisms of inhibitor resistance in yeast.

Author Disclosure Block:

Y. Chen: None.

Poster Board Number:

FRIDAY-152

Publishing Title:

Could Sucrose Be a Better Alternative Carbon Source for Methionine Production by *Bacillus cereus* S8?

Author Block:

V. N. Anakwenze[1], C. C. Ezemba[2], C. M. Ogbukagu[1], A. B. Ilodinso[1], C. C. Ekwealor[1], O. G. Anakwenze[1], I. A. Ekwealor[1]; [1]Nnamdi Azikiwe Univ., Awka, Anambra, Nigeria, Awka, Nigeria, [2]Renaissance Univ., Ugbawka, Enugu, Nigeria, Enugu, Nigeria

Abstract Body:

Methionine is one of the essential amino acid secreted by various microorganisms. It can be supplemented in food and feed for proper growth and body functions of mammals. There is an increased need for methionine production by fermentation as a result of the ban on the use of synthetic methionine in organic farming in many countries. *Bacillus cereus* S8 recovered from Nigerian soil and characterized by 16S rRNA sequencing was used for methionine production in submerged medium employing sucrose as the carbon source. The effects of medium/fermenter volume ratio, inoculum size, nitrogen, growth factors, bivalent metals, surfactants, vitamins and amino acids were studied. In shake flask experiments, a 20% medium/fermenter volume ratio and a 5% inoculum size increased methionine yield. Sucrose at 40g/l stimulated methionine production in *B. cereus* S8. Ammonium sulphate was the best nitrogen source for methionine production and at 10g/l improved methionine yield. All growth promoting substances except the mixture of yeast extract and casein enhanced methionine yield by *B. cereus* S8. Tween 80 at 0.2 (%v/v) gave the highest methionine yield of 1.53mg/ml. All the vitamins studied improved methionine production by *B. cereus* S8 with the highest concentration being produced when pyridoxine at 10.0µg/ml was used. Ba^{2+} at 0.1µg/ml improved methionine production in *B. cereus* S8. DL-leucine stimulated the highest methionine production of 1.54mg/ml in *B. cereus* S8 . Time course experiments for methionine production by *B. cereus* showed that maximum methionine production was obtained after 96h. This study has shown that methionine producing bacteria can be recovered from Nigerian soil. When compared with previous study, sucrose was found to stimulate less of methionine than other carbon sources studied.

Author Disclosure Block:

V.N. Anakwenze: None. **C.C. Ezemba:** None. **C.M. Ogbukagu:** None. **A.B. Ilodinso:** None. **C.C. Ekwealor:** None. **O.G. Anakwenze:** None. **I.A. Ekwealor:** None.

Poster Board Number:

FRIDAY-153

Publishing Title:

Production of Galacto-Oligosaccharides Using Chitosan Based Immobilized Whole Cells of *Lactococcus lactis* Containing a Hyperthermostable Beta-Galactosidase Enzyme

Author Block:

L. Yu, D. J. O'Sullivan; Univ. of Minnesota, St. Paul, MN

Abstract Body:

Galacto-oligosaccharides (GOS) are desirable prebiotics that are non-digestible and are proposed to have a 'bifidogenic' effect. Recently, we developed an efficient whole-cell production system for GOS using a hyperthermostable beta-galactosidase enzyme from *Sulfolobus solfataricus* that was overexpressed in *Lactococcus lactis*. In order to facilitate the food-grade use of this whole-cell GOS production system, we developed an immobilization process for the whole-cells that could function at very high temperatures. Permeabilized whole-cells of *L. lactis* containing high levels of this beta-galactosidase enzyme were encapsulated in chitosan beads. Prior to immobilizing the whole cells, a UV treatment methodology was developed to degrade DNA from the permeabilized cells such that the recombinant DNA in the cells was below the level of detection by PCR. The efficiency of GOS production was analyzed by incorporating the immobilized beads in a reaction mixture containing lactose at various concentrations and conditions. This revealed that the optimum pH for GOS synthesis using these immobilized cells was 5.5, which is slightly lower than the free whole-cells (pH6). Also, the optimum temperature for GOS synthesis using the immobilized whole-cells increased to 90^{o}C, which is 5 degrees higher than the free whole-cells (85^{o}C). The highest GOS production was obtained using 40% lactose. Approximately 60% of lactose was converted into 3-OS and 4-OS starting with 5% of initial lactose using immobilized enzyme. The enzyme beads retained ~ 50% activity after 2 cycles of GOS production. In conclusion, these immobilized whole-cells can be used for efficient GOS production in a food-grade manner.

Author Disclosure Block:

L. Yu: None. **D.J. O'Sullivan:** None.

Poster Board Number:

FRIDAY-154

Publishing Title:

Sequence-Based Mining and Characterisation of a Novel Acetyl Xylan Esterase from a Hot Desert Hypolith Metagenome

Author Block:

F. A. Adesioye, T. Makhalanyane, S. Vikram, D. A. Cowan; Univ. of Pretoria, Pretoria, South Africa

Abstract Body:

Acetyl xylan esterases (AcXEs) are Carbohydrate-Active Enzymes (CAZymes) that hydrolyse ester bonds to liberate acetic acid in acetylated polymeric xylan and xylooligosaccharides during enzymatic saccharification of hemicellulose, a major bottle-neck during bioconversion of lignocellulosic biomass for sustainable biofuel production. While most AcXEs have been identified from a range of lignocellulose-degrading microorganisms via culture-based methods, metagenomic screening methods allow access to novel genes of the ≥90% uncultured microorganisms within any given environmental sample.In this study, NaMet2, a novel AcXE-encoding gene was identified via *in silico* bio-mining of a Namib Desert (a hot coastal desert possessing unique climatic conditions) soil hypolith metagenomic sequence dataset. Sequence homology data showed that NaMet2 possessed highest similarity (69%) to AcXE from *Arthrobacter* sp. 35W (WP 02655436.1) and 53.4% similarity to the AcXE gene from *Bacillus pumilus* (a well characterized model CE7 AcXE). NaMet2 shared the GXSXG conserved motifs, characteristic of the CE7 CAZy family, and was found to possess the Ser-His-Asp catalytic triad with active site residues located at Ser187, His307 and Asp273. Gene synthesis, restriction cloning and expression of NaMet2 yielded a recombinant functional AcXE, NaM2, with a molecular weight of 35.9kDa and estimated isoelectric point of 5.47 evidenced by SDS-PAGE analysis. A >95% pure fraction of NaM2 was obtained as a his-tagged protein via cobalt-affinity purification and fast-pressure liquid chromatography. Agar well diffusion assays indicated its activity on tributyrin and acetylated xylan. Acetylesterase activity and specific activity of NaM2 on p-nitrophenol acetate as determined via spectrophotometric assays were $0.55Uml^{-1}$ and $27.5Umg^{-1}$, respectively. The V_{max} and K_M values of the enzyme were $0.46Uml^{-1}$ and 0.1mM, respectively, with a turnover rate (k_{cat}) of $0.26s^{-1}$. Crystallization studies using sitting drop method yielded x-ray diffracting protein crystals suitable for structural analysis. These results suggest that novel functional AcXEs may be discovered via sequence-based metagenomics.

Author Disclosure Block:

F.A. Adesioye: None. **T. Makhalanyane:** None. **S. Vikram:** None. **D.A. Cowan:** None.

Poster Board Number:

FRIDAY-155

Publishing Title:

Bioreactor Production Optimization of the Family of Fungal Antibiotics Mdn-0057 & Mdn-0058

Author Block:

V. Gonzalez-Menendez, J. Martin, N. el Aouad, J. Tormo, F. Reyes, **O. Genilloud**; Fundacion MEDINA, Granada, Spain

Abstract Body:

The novel family of broad spectrum Gran negative antibiotics MDN-0057 and MDN-0058 was previously reported as isolated from the liquid cultures of a grass endophytic fungus. In this study we describe the optimization of the *antibiotics* production and scale-up fermentation in 7L bioreactors as part of the development studies performed to ensure the large scale production of these novel compounds. The Biolog FF MicroPlate system was applied for evaluating the use of 95 carbon and nitrogen sources in fermentation media by the producing fungal strain. The substrate utilization patterns guided the selection of nutritional components for media optimization of MDN-57 production. Scaled-up fermentations were performed in 7L Applikon BioBundle bioreactors combining both 6 bladed Rushton impellers (100 mm) and marine 3 bladed impellers (60 mm, vortex). Cultivations were carried out for 10 days at 22 ^{o}C, air flow rate was adjusted to 2L/min, with an agitation speed of 500 r.p.m and a pH initially adjusted to 5.5. In fungal pre-optimization studies, fungal culture growth was monitored by absorbance in each well of the FF MicroPlate and D-cellobiose resulted to be the best substrate for our strain. The the optimized medium YEC containing D-cellobiose as principal carbon source ensured the best yield for antibiotic production in the 7 liter bioreactor, with a 2-fold yield in comparison with YES medium (sucrose as carbon source). The FF MicroPlate was shown to be a useful tool for the prior selection of the best nutritional conditions for large scale fungal fermentations in large scale. A strong correlation was found between substrate utilization, growth, and MDN-57 production.

Author Disclosure Block:

V. Gonzalez-Menendez: None. **J. Martin:** None. **N. el Aouad:** None. **J. Tormo:** None. **F. Reyes:** None. **O. Genilloud:** None.

Poster Board Number:

FRIDAY-156

Publishing Title:

Fermentation Optimization of an Engineered *Streptomyces* Strain for Production of Wdb-002, A Novel Fk506-Like Compound

Author Block:

R. A. Giacobbe, D. Nguyen, D. Vo, K. Kenyon, U. Swaminathan, L. Yang, H. Huang, J-Q. Gu, B. R. Bowman, K. Nguyen; WarpDrive Bio, Inc., Cambridge, MA

Abstract Body:

Background: An engineered strain of *Streptomyces* S583 was found to produce the novel FK506-like compound WDB-002, which comprises two isomeric forms WDB-002a and WDB-002b. S583 fermentation was optimized to improve production yield for isolation of gram quantities of WDB-002 to support *in vitro* and *in vivo* studies. In this work, we evaluated the effect of various carbon and nitrogen sources, pH and fermentation time on WDB-002 yield. **Methods:** Components at various concentrations in 8430, a complex medium containing mannitol, cottonseed meal, yeast extract, minerals and oil were examined in shake flasks. Selected media were also tested in 5 L bioreactors. Medium pH values, packed cell volume and WDB-002 yield determined by HPLC were monitored over a time course.**Results:** A sequential drop-out experiment identified critical and inhibitory components in the complex, mannitol-containing medium 8430. Yeast extract and phosphate were found to be inhibitory for WDB-002 production. The culture experienced an initial drop in pH (from pH 6.5 to 4.5) followed by a pH increase to pH >8 at day 6, concomitant with ceased WDB-002 production. The pH increase may be an indicator of culture lysis, consistent with the decreased packed cell volume. Mannose, maltose and fructose were found to be superior carbon sources to mannitol. In a 5 L bioreactor, the production rates with the tested carbon sources did not change significantly, but fructose supported a prolonged production phase compared to that in a mannitol medium. In the best medium tested, WDB-002 production reached 700 mg/L in shake flasks and 300 mg/L in 5 L bioreactors versus the original yields of 200 and 140 mg/L, respectively. In addition, WDB-002a and WDB-002b were preferentially produced in pH <7 and pH >8, respectively.**Conclusions:** *Streptomyces* S583 fermentation for WDB-002 production in a complex medium was significantly enhanced by >3-fold in shake flasks and >2-fold in 5 L bioreactors using fructose compared to mannitol. The medium pH profile could serve as an indicator for the completion of WDB-002 production phase.**Key words:** *Streptomyces* S583, WDB-002, fermentation, flask, bioreactor, FK506-like

Author Disclosure Block:

R.A. Giacobbe: None.

Poster Board Number:

FRIDAY-157

Publishing Title:

Flavodoxin Reduction by Electron Bifurcation and Na^+-Dependent Oxidation by Nad^+ in *Acidaminococcous fermentans*

Author Block:

N. Pal Chowdhury, W. Buckel; Max Planck Inst. for Terrestrial Microbiol., Marburg, Germany

Abstract Body:

Electron bifurcation with electron transferring flavoprotein (Etf) and butyryl-CoA dehydrogenase (Bcd) in *Acidaminococcous fermentans* enables the endergonic reduction of ferredoxin by NADH driven by the exergonic reduction of crotonyl-CoA to butyryl-CoA also by NADH [1]. Here we show that recombinant flavodoxin from *A. fermentans* produced in *Escherichia coli* can replace ferredoxin. Under limiting concentrations of crotonyl-CoA, flavodoxin is reduced to the semiquinone, whereas with an excess of this oxidant the fully reduced quinol state of flavodoxin is obtained. Kinetic data show that the reduction of the quinone to the semiquinone ($E°'_1$ = -60 mV) is about 3-times slower than that of the semiquinone to the quinol ($E°'_2$ = -430 mV), though the opposite is expected when considering the redox potentials. Re-oxidation of the quinole is achieved by hydrogenase resulting in the equation: Crotonyl-CoA + 2 NADH + 2 H+ → Butyryl-CoA + 2 NAD^+ + H_2. Membrane preparations of *A. fermentans*, containing a very active ferredoxin-NAD reductase (Rnf), catalyze the re-oxidation of the quinol to the semiquinone by NAD^+. Due to the high redox potential of the semiquinone, further oxidation to the quinone is not possible. The combination of Etf, Bcd and Rnf recycles not only the semiquinone but also half of the NAD^+, resulting in the simple equation: Crotonyl-CoA + NADH + H^+ → Butyryl-CoA + NAD^+. In this system the apparent K_m of flavodoxin was determined as 9 µM, 45-times higher than that of ferredoxin by recycling with hydrogenase, K_m = 0.2 µM [1]. Furthermore, this assay allowed demonstrating the postulated dependence of Rnf from *A. fermentans* on Na^+ or Li^+ [2], which has been already verified for Rnf from *Acetobacterium woodii* [3]. Following values were measured: K_m = 120 µM Na^+ or 275 µM Li^+. Etf contains two FAD, one of which exhibits normal redox potentials, $E°'_1 < E°'_2$, whereas those of the other are similar to the potentials of flavodoxin, $E°'_1 > E°'_2$ (see above). This inverse behavior leads to a model of flavin-based electron bifurcation.

Author Disclosure Block:

N. Pal Chowdhury: None. **W. Buckel:** None.

Poster Board Number:

FRIDAY-158

Publishing Title:

Selection of a Potential Starter Culture for Milk Fermentation

Author Block:

S. O. Akintayo, I. F. Fadahunsi; Univ. of Ibadan, Ibadan, Nigeria

Abstract Body:

The ability of Lactic acid bacteria (LAB) to grow and survive in milk is being exploited in industrial and biotechnological applications. Although considerable studies have been reported on the fermentation of milk, however, not so much work has been documented on the selection of LAB strains from milk of the Nigerian local cattle breeds for their starter culture potentials. A total of 110 LAB were isolated from raw milk of *Sokoto gudali* cattle breed. The isolates were screened for their proteolytic activities on skimmed milk media with isolates A07, F06 and A01 showing the highest zone of clearance of 18.5mm, 18.5mm and 18.0mm respectively and were selected for the studies of their growth in different constituents of milk. A01, F06 and A07 were identified as *Pediococcus acidilactici*, *Lactococcus raffinolactis* and *Leuconostoc mesenteriodes* respectively using cultural, biochemical, physiological and molecular characterization techniques. *Leuconostoc mesenteriodes* showed the highest growth in all the milk components that were used in this study. The three LAB species selected showed a growth range of 6.46 log cfu/ml to 10.91 log cfu/ml in lactose with *Leuconostoc mesenteriodes* showing the highest growth of 10.91 log cfu/ml while *Pediococcus acidilactici* recorded the lowest growth of 9.78 log cfu/ml. In medium containing leucine as the only amino acid, the viable counts of *Pediococcus acidilactici*, *Lactococcus raffinolactis* and *Leuconostoc mesenteriodes* in log cfu/ml at zero hour were 6.39, 6.36 and 6.38 respectively which increased to 9.31 log cfu/ml, 9.21 log cfu/ml, 9.92 log cfu/ml respectively after 24 hours. Similarly in all other substrates (casein, lysine, glutamic acid, aspartic acid, stearic acid and oleic acid) tested in this study, *Leuconostoc mesenteriodes* showed the highest growth. It was observed that the highest quantity of lactic acid (15.31mg/ml) was produced by *Leuconostoc mesenteriodes*. The same trend was also observed in the production of diacetyl and hydrogen peroxide by the three tested microorganisms. Due to its ability to grow maximally in milk components, *Leuconostoc mesenteriodes* shows potential as starter culture for milk fermentation.

Author Disclosure Block:

S.O. Akintayo: None. **I.F. Fadahunsi:** None.

Poster Board Number:

FRIDAY-159

Publishing Title:

Optimization of Fermentation Conditions for Improved Biosurfactant Production by *Pseudomonas monteilii* AF064458 and *Citrobacter murliniae* AF025369

Author Block:

C. G. Anaukwu[1], C. C. Ezemba[2], V. N. Anakwenze[1], I. A. Ekwealor[1]; [1]Nnamdi Azikiwe Univ., Awka, Nigeria, Awka, Nigeria, [2]Renaissance Univ. , Enugu Nigeria, Enugu, Nigeria

Abstract Body:

Background: Biosurfactants are secondary metabolites produced by microorganisms. Owing to their unique features which includes low toxicity and biodegradability, biosurfactant have gained importance over synthetic surfactants. The composition and emulsifying activity of the biosurfactant not only depends on the producer strain but also on the fermentation conditions such as the carbon source, the nitrogen source as well as the C:N ratio and other physical parameters. The effects of fermentation conditions on biosurfactant production by *Pseudomonas monteilii* AF064458 and *Citrobacter murliniae* AF025369 were studied.**Methods:** *Pseudomonas monteilii* AF064458 and *Citrobacter murliniae* AF025369, isolated from soil contaminated with spent-engine oil were used for biosurfactant production. Shake flask fermentation experiments were conducted in mineral salt medium and the effects of medium/fermenter volume ratio, carbon and nitrogen sources, growth factors, pH and agitation speed on biosufactant production by the isolates were examined.**Results:** An increase in biosurfactant production by *P. monteilii* AF064458 was observed when 40% medium/fermenter volume ratio was used, while 50% ratio improved biosurfactant accumulation in the broth culture of *C. murliniae* AF025369. Maltose at 1% and glucose at 2% concentrations enhanced biosurfactant production by *P.monteilii* AF064458 and *C. murliniae* AF025369 respectively. Sodium nitrate was the nitrogen of choice for both isolates, and at 1% and 0.5%, biosurfactant production by *P. monteilii* AF064458 and *C. murliniae* AF025369 respectively were improved. At a pH of 7.5, *P. monteilii* AF064458 gave high biosurfactant yield, while *C. murliniae* AF025369 stimulated an increase at pH of 8.0.The effect of agitation speed revealed that biosurfactant production in *P.monteilii* AF064458 and *C. murliniae* AF025369 was optimal at 200rpm.**Conclusions:** This study has shown that optimizing the fermentation conditions in mineral salt medium improved biosurfactant yield by *Pseudomonas monteilii* AF064458 and *Citrobacter murliniae* AF025369. Optimization of nutritional and physical parameters is recommended for substantially higher biosurfactant production by microorganisms.

Author Disclosure Block:

C.G. Anaukwu: None. **C.C. Ezemba:** None. **V.N. Anakwenze:** None. **I.A. Ekwealor:** None.

Poster Board Number:

FRIDAY-160

Publishing Title:

Characterization of Phenolic Acid Decarboxylase (*padc*) from Lactic Acid Bacteria Isolated from Fermenting Mash (Moromi) of Awamori, Okinawan Traditional Distilled Beverage

Author Block:

K. Nakasone; Kindai Univ., Higashihiroshima, Japan

Abstract Body:

Awamori is traditional distilled beverage made from steamed Thai-Indica rice in Okinawa, Japan. For brewing the liquor, two microbes, local black-koji fungus *Aspergillus luchuensis* and awamori yeast *Saccharomyces cerevisiae*, are involved. After the mash fermenting (moromi) completed by the multiple parallel fermentation (simultaneous saccharification and ethanol production), the moromi is distilled under atmospheric pressure. One of unique flavors of awamori is characterized by vanillin generated by oxidation of 4-vinylguaiacol (4-VG). In the moromi fermentation, the microbial phenolic acid carboxylase (PAD) decarboxylate ferulic acid in cell wall of the rice with concomitant production of 4-VG. Thus, activity and function of the enzyme during the moromi fermentation are important for the awamori flavors. The microbial source(s) or origin(s) of the enzyme are still unclear and the gene was recently detected on the draft genome of *A. luchuensis*. Besides two microbes, importance of other microbes such as lactic acid bacteria as a contaminant in the moromi are suggested, because many awamori breweries are built as an open system from outside. In this study, several lactic acid bacteria from the awamori moromi were tried to isolate, in order to characterize biochemistry and function of PAD(s).At first, 10 milliliters of fermenting mashes (moromi) from an awamori brewery in Okinawa were collected and filtered. The concentrate on the filter was statically cultured at 30°C using liquid MRS medium containing 1% calcium carbonate. Single colony isolations on the solid MRS medium were repeated using the turbid cultures. Several genera of lactic acid bacteria such as *Lactococcus* and *Lactobacillus* were found and detected by fermentation test and 16SrDNA analyses. Then, these bacteria were cultured and the chromosomal DNA was purified. According to genome sequences of *Lactococcus* and *Lactobacillus* species, degenerate primers of the phenolic acid decarboxylase were designed and PCR was performed. After confirmation of nucleotide sequence of the gene, pColdI expression vectors for his-tagged protein, were constructed. These purified enzymes were also confirmed purity using SDS-PAGE and the biochemical analyses are being characterized.

Author Disclosure Block:

K. Nakasone: None.

Poster Board Number:

FRIDAY-161

Publishing Title:

Utilization of Lignocellulosic Waste (Plantain Peel) and Subsequent Generation of Organic Acids of Industrial Importance by *Streptomyces albus* strain DOB KF977551

Author Block:

O. M. Buraimoh, A. K. Ogunyemi, S. A. Boyejo, O. O. Amund, M. O. Ilori; Univ. of Lagos, Lagos, Nigeria

Abstract Body:

The global economy is faced with challenges of obtaining industrial raw materials in a sustainable manner. One way through which this could be addressed is the bioconversion of wastes generated in large quantities from agricultural activities into industrial raw materials. This warranted our study of the ability of a Streptomyces strain to utilize plantain peel as substrate for the production of organic acids of industrial importance. Growth was carried out in submerged fermentation for a period of 21days using Streptomyces albus strain DOB isolated from a tropical estuarine while plantain peel was the sole carbon source. The ability of the organism to grow on the peel was evaluated at intervals using a spectrophotometer (620 nm), changes in the pH of the medium was also monitored. After extraction, the analytes (1ml) including the uninoculated control was each injected into the GCMS to detect the by-products of fermentation. Increase in the optical density (up to 2.20 nm) showed that the organism utilized the plantain peel as a growth substrate. The pH dropped from 7.2 and fluctuated between 6.08 and 6.87 throughout the period of the experiment. The result of the GCMS showed that propionic, acetic, lactic, oxalic, nvaleric, isovaleric, cis -aconitic acid, nbutyric acid, trans -aconitic and iso-butyric acids were the by- products of fermentation of plantain peels by this strain. Organic acids are used as additives and preservatives in the food and pharmaceutical industries; hence, the biological production is significant because it allows the use of raw materials tagged as “wastes” and simultaneously removes wastes from the environment. Plantain peel appears to be an ideal substrate for microbial process for the production of organic acids.

Author Disclosure Block:

O.M. Buraimoh: None. **A.K. Ogunyemi:** None. **S.A. Boyejo:** None. **O.O. Amund:** None. **M.O. Ilori:** None.

Poster Board Number:

FRIDAY-162

Publishing Title:

Transformation of Acacetin during the Fermentation of Safflower Seed (*Carthamus tinctorius L.*) with *β*-Glucuronidase Producing Lactic Acid Bacteria

Author Block:

K. Lee, S-S. Lee; Natural Sci., Suwon-si, Korea, Republic of

Abstract Body:

This study was undertaken to investigate the possible application *β*-glucosidase producing lactic acid bacteria as a functional starter cultures to obtain the bioactive flavonoid, acacetin in fermented safflower seeds. Safflower seeds (*Carthamus tinctorius* L.) are used as traditional medicine for the treatment of osteoporosis and rheumatoid arthritis in Korea. In the present study, the influence of two types of lactic acid bacteria (*Lactobacillus acidophilus*, *Leuconostoc mesenteroides*) on antioxidant activities and acacetin contents of fermented safflower seeds was determined and compared with those of their unfermented safflower seeds. Safflower seeds fermented with *L. acidophilus* showed the highest antioxidant activity and the greatest increase in the contents of acacetin. The percentage of DPPH and ABTS in safflower seeds fermented with *L. acidophilus* increased from 59.48% and 48.16%, respectively, to 71.43% and 63.76 after 28 days. Results of the HPLC analysis indicated the change of phenolic compounds during the fermentation of safflower seeds with *L. acidophilus*. Acacetin which is a flavonoid compound in safflower seeds has anti-inflammatory, anti-cancer activity and were increased from 0.27 mg/g to 1.23 mg/g by *L. acidophilus* but composition of acacetin was not changed by *L. mesenteroides*. Transcriptional analysis showed that *β*-glucosidase (*gusA*) was expressed in *L. acidophilus* and was not expressed in *L. mesenteroides*. From the study, *L. mesenteroides* have less efficiency than *L. acidophilus*. These results demonstrated that *L.acidophilus* could be potentially used as functional starter to improve the antioxidant activity of safflower seeds.

Author Disclosure Block:

K. Lee: None. **S. Lee:** None.

Poster Board Number:

FRIDAY-163

Publishing Title:

New Function of Microbial Enzyme Involved in Dehydration Reaction

Author Block:

M. KOBAYASHI, M. Yamada, Y. Hashimoto, T. Kumano; Univ. of Tsukuba, Tsukuba, Japan

Abstract Body:

We have extensively studied microbial metabolism of toxic compounds with a triple bond between carbon and nitrogen, such as nitriles [R–CN]. In *Pseudomonas,* whose nitrile hydratase enzyme was previously used for the industrial acrylamide production and is now used for the production of 5-cyanovaleramide, we discovered the enzyme gene organization composed of seven genes and clarified the regulation mechanism of their gene expression.The nitrile-synthesizing enzyme is a unique enzyme, which catalyzes the dehydration of aldoxime [R-CH=N-OH] into nitrile [R-CN] even in an aqueous solution. This enzyme contains a heme as the prosthetic group. Unlike the utilization of H_2O_2 or O_2 as a mediator of the catalysis by other heme-containing enzymes, this enzyme is notable for the direct binding of a substrate to the heme ion. The ferrous enzyme, which contains a five-coordinated high-spin heme and a His residue as its proximal ligand, is the reactive form of the enzyme. Another histidine in the distal heme pocket was found to play a crucial role in the catalysis. We next clarified the crystal structure of the enzyme and its detailed reaction mechanism. We initially found catalase activity of the enzyme, when H_2O_2 was used as a substrate. We also determined kinetic parameters of this reaction.

Author Disclosure Block:

M. Kobayashi: None. **M. Yamada:** None. **Y. Hashimoto:** None. **T. Kumano:** None.

Poster Board Number:

FRIDAY-164

Publishing Title:

Optimization of Cultural Conditions of *Bacillus cereus* Rs16 for Methionine Accummulation Using Agricultural Products

Author Block:

C. C. Ezemba[1], V. N. Anakwenze[2], G. C. Anaukwu[3], C. E. Chukwujekwu[1], I. A. Ekwealor[4]; [1]Renaissance Univ., Ugbawka, Enugu. Nigeria, Enugu, Nigeria, [2]Nnamdi Azikiwe Univ., Awka Nigeria., Anambra, Nigeria, [3]Nnamdi Azikiwe Univ., Awka Nigeria, Awka, Nigeria, [4]Nnamdi Azikiwe Univ., Awka. Nigeria, Enugu, Nigeria

Abstract Body:

Background: Methionine is an α-protein dietary essential amino acid for human and other mammals for normal growth. Its deficiency has also been related to childhood rheumatic fever, muscle paralysis, hair loss, depression, schizophrenia, and Parkinson's liver deterioration. Methionine-producing bacteria characterized as *Bacillus cereus* RS16 based on 16s rRNA sequencing which was previously isolated from soil in Awka, Nigeria was screened for methionine production in submerged medium. **Method:** Corn Starch hydrolysate, and defatted groundnut meal were used as carbon and nitrogen sources respectively for methionine production. The influences of medium/fermenter volume, inoculum size, pH, agitation speed, growth stimulators, amino acid, antibiotics, bivalent metals, vitamins and fermentation time on methionine production were studied. **Result:** A 20% medium/fermenter volume ratio, 5% inoculum size, pH of 7.0 and agitation speed of 170rpm increased methionine yield. A 40g/l corn-starch hydrolysate and 20g/l defatted groundnut meal stimulated a methionine level of 2.66mg/ml. All growth stimulators at (0.1%w/v), DL-leucine (0.01%w/v), penicillin at all concentrations, Zn^{2+} (5.0mg/ml) and pyridoxine at 100.0µg/ml enhanced methionine accumulation by *Bacillus cereus* RS16. Methionine level of 3.79mg/ml was produced after 96h fermentation. **Conclusion:** The study has shown that methionine production by *B. cereus* RS16 is depended on medium composition and that agricultural products can be utilized as fermentation substrates.

Author Disclosure Block:

C.C. Ezemba: None. **V.N. Anakwenze:** None. **G.C. Anaukwu:** None. **C.E. Chukwujekwu:** None. **I.A. Ekwealor:** None.

Poster Board Number:

FRIDAY-165

Publishing Title:

Utilization of Starch Hydrolysates and Defatted Proteins by *Bacillus* Strains for Methionine

Author Block:

C. C. Ezemba[1], K. S. Dike[2], V. N. Anakwenze[3], C. M. Ogbukagu[4], C. C. Ekwealor[4], I. A. Ekwealor[3]; [1]Renaissance Univ., Ugbawka, Enugu State Nigeria, Enugu, Nigeria, [2]Nnamdi Azikiwe Univ., Awka, Nigeria, Awka, Nigeria, [3]Nnamdi Azikiwe Univ., Awka Nigeria, Awka, Nigeria, [4]Nnamdi Azikiwe Univ. Awka Nigeria, Awka, Nigeria

Abstract Body:

Background: L-methionine is an essential amino acids required by man and animal. They are used as food and feed supplement, pharmaceutical adjuvants, and are produed by fermentative process by bacteria and yeasts. Because agricultural products are rich in nutrients, cheap and economical, their use as fermentative substrates in methionine production was studied **Methods:** *Bacillus cereus* DS13, *Bacillus cereus* RS16, *Bacillus cereus* AS9 were used for methionine production. Shake flask experiments were conducted to examine the effects of 2% (w/v) starch hydrolysates of wheat, sorghum, cassava, cocoyam, yam, plantain, millet, corn, potato, rice and defatted plant proteins from pigeon pea, cowpea, bambaranut, and groundnut as carbon and nitrogen sources respectively for the production of methionine. **Results:** All *Bacillus* strains accumulated different methionine levels in their broth cultures: *Bacillus cereus* DS13, 2.32mg/ml with sorghum/groundnut: *Bacillus cereus* RS16, 2.22mg/ml with corn/groundnut and *Bacillus cereus* AS9, 1.78mg/ml with plaintain/bambaranut.**Conclusions:** This study has shown that agricultural products have great potential to be utilized in methionine production.

Author Disclosure Block:

C.C. Ezemba: None. **K.S. Dike:** None. **V.N. Anakwenze:** None. **C.M. Ogbukagu:** None. **C.C. Ekwealor:** None. **I.A. Ekwealor:** None.

Poster Board Number:

FRIDAY-166

Publishing Title:

Viability And Thermal Resistance Of *bacillus Atrophaeus* Atcc 9372 Spores Cultivated In Orange'S Bagasse

Author Block:

E. H. Lenhardt, E. E. Santo, T. C. Vessoni Penna, **M. Ishii**; Univ. of São Paulo, São Paulo, Brazil

Abstract Body:

Background: Fruit production is of paramount importance to Brazil's economy, highlighting the cultivation of orange species. Regarding orange's juice manufacturing, this industrial process generates large amounts of residue waste (50% of fruit weight), rich in nutrients that could be used as substrate for microorganism cultivation. Spores of *Bacillus atrophaeus* ATCC 9372 are known as a bacteria resistant form and are used as producers of enzymes of industrial interest and as biological indicators to monitor and to validate sterilization cycles ensuring the sterility of medical articles. The aim of this work is to evaluate the viability and thermal resistance of *Bacillus* spores obtained using orange's bagasse, to be used as bioindicator to monitor sterilization processes.**Methods:** Surface oranges were rubbed, rinsed in tap water and immersed in a 1% peracetic acid solution for 60 minutes. The fruit was processed in a juice centrifuge to separate the bagasse, (pulp, peel and seeds, which was frozen at -18°C), from the juice. Cultivation media was prepared using from 1.5 g to 18.5 g of orange´s bagasse into from 50mL up to 130 mL of water, sterilized, filtered through gauze, inoculated with 0.1 g/L of *B. atrophaeus* ATCC 9372 spores and incubated into orbital shaker at 150 rpm / 37°C for 3 and 6 days. After this period, the culture was centrifuged (4000 rpm / 4°C / 25 minutes). The pH media, spores viability and thermal resistance, expressed in terms of D-values at 102°C were determined.**Results:** After 3 and 6 days of cultivation, using 10.0g of orange bagasse, viable spores achieved 5.0 x 10^7 CFU/mL (pH 7.8 (±0.01)) and 1.0 x 10^9 CFU/mL (pH 8.8 (±0.01)), respectively. The spores developed showed appropriate thermal resistance being D value = 1.32 minutes and D = 1.79 minutes for 10.0 g of bagasse after 3 and 6 days of cultivation, correspondingly. At standard media, TSB, after 6 days of cultivation, viable spores achieved 1.0 x 10^6 spores/mL (pH 8.75 (±0.05)) and D value = 1.0 min, being less resistant compared to orange's bagasse media ones.**Conclusions:** The thermal resistance of *Bacillus* spores suggest that the use of orange bagasse corresponds to a small cost-effective media and an ecologically friendly initiative to obtain bioindicators to be applied for industrial and healthcare purposes.

Author Disclosure Block:

E.H. Lenhardt: None. **E.E. Santo:** None. **T.C. Vessoni Penna:** None. **M. Ishii:** None.

Poster Board Number:

FRIDAY-167

Publishing Title:

***n*-Acetyl-D-Glucosamine as an Advanced Feedstock for Biocatalytic Conversion to Triacylglycerol by *Rhodococcus opacus* Pd630, Towards Sustainable Biodiesel Production**

Author Block:

J. Palmer, M. Phou, C. Brigham; Univ. of Massachusetts Dartmouth, North Dartmouth, MA

Abstract Body:

Rhodococcus opacus strain PD630 has demonstrated the ability to accumulate triacylglycerol (TAG) as a carbon storage molecule when in nutrient limiting conditions. Based on broad substrate tolerability and high cell density culturing capacity, *R. opacus* PD630 has become a model oleaginous prokaryotic species for TAG accumulation. TAG may be converted into fatty acid methyl esters (FAMEs) and burned as diesel fuel. Here we demonstrate predicted relevant fuel properties of FAMEs (cetane number and lower heating value) derived from *R. opacus* PD630 grown in a defined media containing the amino sugar, *N*-acetyl-D-glucosamine (NAG), as the sole carbon source, based on previously developed models. NAG is a primary component of chitin, which has recently become a polymer of interest for industrial applications, as it is a nutrient rich waste product disposed of in mass quantities from the seafood/fishery industry. Additionally, we demonstrate the ability for *R. opacus* PD630 to achieve flask culture cell densities, when grown in defined media with NAG, more than 3X greater than when grown on other refined sugars (glucose, fructose, and sucrose) with the same starting conditions. This characteristic suggests that chitin and its monomeric subunit, NAG, hold great potential for being an ideal feedstock for industrial biodiesel production and *R. opacus* PD630 holds promising biocatalytic characteristics.

Author Disclosure Block:

J. Palmer: None. **M. Phou:** None. **C. Brigham:** None.

Poster Board Number:

FRIDAY-168

Publishing Title:

Enhanced Viability of Microencapsulated Probiotic Bacteria in Alginate and Chitosan Microbeads During Storage and Simulated Digestion

Author Block:

T. W. Yeung, I. J. Arroyo-Maya, E. F. Üçok, K. A. Tiani, D. J. McClements, D. A. Sela; Univ. of Massachusetts Amherst, Amherst, MA

Abstract Body:

Probiotic microorganisms are incorporated in foods and other applications to modulate the function of one's microbiome to impact health. However, maintaining viability of live cells during storage and gastrointestinal transit remains an obstacle for nutritional interventions. Accordingly, encapsulating lactococcal and bifidobacterial cells within food-grade hydrogel biopolymers was investigated to mitigate sensitivity to environmental stresses. Calcium alginate microbeads were formed by dripping a microbe-alginate mixture into a calcium solution, and were confirmed to envelop cells via microscopy. Temporal cell viability was evaluated by culture-based approaches. Encapsulating the facultative anaerobe *Lactococcus lactis* subsp. *cremoris* prolonged survival during aerobic storage, improving viability by 3 log CFU in comparison to free cells. Whereas, encapsulating obligate anaerobes *Bifidobacterium longum* subsp. *infantis* and *B. longum* subsp. *longum* strains showed little difference in viability over time between encapsulated and free cells. Indeed overall survival of bifidobacteria strains was low, decreasing by up to 10 log CFU in 14 days, suggesting that oxygen diffusion within the beads is limiting. An outer coating of chitosan changed the physicochemical properties as confirmed by increased zeta potential. However *B. longum* viability was not prolonged appreciably with the secondary coat. Encapsulated microbeads were subjected to simulated salivary, gastric, and intestinal fluids *in vitro*. Encapsulation maintained cellular viability in the salivary phase. In contrast, viability was significantly reduced when exposed to the low pH conditions of the gastric, as well as the modeled intestinal phase. Hydrogel beads have potential for improving the stability and efficacy of specific probiotics in various food products. Future studies optimizing encapsulated cell preparations based on inherent traits will enable colonic delivery more efficient than conventional practices.

Author Disclosure Block:

T.W. Yeung: None. **I.J. Arroyo-Maya:** None. **E.F. Üçok:** None. **K.A. Tiani:** None. **D.J. McClements:** None. **D.A. Sela:** None.

Poster Board Number:

FRIDAY-169

Publishing Title:

Genetic Instability of *Shigella flexneri* from a Foodborne Outbreak in Hawaii

Author Block:

C. GRIM[1], R. Y. Kanenaka[2], K. A. Lampel[1]; [1]Food and Drug Admin., Laurel, MD, [2]Hawaii State Dept. of Hlth., Pearl City, HI

Abstract Body:

Background: A foodborne outbreak caused by *Shigella flexneri* affected over 270 people in Hawaii in 1996. Epidemiological data indicated that poi, contaminated by an infected preparer, was the transmission vector. Pulsed-Field Gel Electrophoresis (PFGE) data showed that most of the patient isolates were indistinguishable from G106, an isolate from the poi preparer, indicating a single source outbreak. However, PFGE patterns of some isolates differed slightly from G106.**Methods:** To resolve these genetic differences, genomic DNA was extracted from 14 *Shigella* poi isolates and sequenced on a MiSeq (Illumina). Reads were trimmed and de-novo assembled with CLC Genomics Workbench. For SNP based analysis, the CFSAN SNP pipeline was used to produce a SNP matrix by mapping unprocessed reads on the reference genome of *Shigella flexneri* 2a strain 301 (GenBank NC_004337.2/NC_004851.1). Phylogeny was calculated and visualized using Mega, version 6.06. Genomes were annotated with RAST and comparative genomics was performed using the SEED viewer.**Results:** A total of 9,697 SNPs were identified between the 14 poi isolates and the reference genome. In general, most SNPs, compared to the reference genome, were shared by all poi outbreak isolates. However, closer examination of the "clonal" poi outbreak group revealed a significant number of SNP variations between isolates. Comparative genomics also revealed an unusually high number of disparate genomic regions between isolates, with the majority of these being flanked by insertion sequences (IS). Many of these genomic regions encoded putative stress response or resistance operons.**Conclusions:** The genomes of *Shigella* isolates associated with this foodborne outbreak attributed to poi exhibited a high degree of genetic instability, apparently attributable to the mechanism of IS elements. All isolates formed a tight phylogenetic cluster, compared to the reference genome used; however, this high degree of genomic instability within an outbreak cluster may have a significant impact on future epidemiological investigations involving *Shigella*. Variations in the PFGE profiles and genomic differences indicate that this outbreak was caused by a strain of *S. flexneri* that had undergone relatively rapid changes in its genome and plasmid during either the outbreak or isolation.

Author Disclosure Block:

C. Grim: None. **R.Y. Kanenaka:** None. **K.A. Lampel:** None.

Poster Board Number:

FRIDAY-170

Publishing Title:

Whole Genome Analysis of a Predominant *Escherichia coli* O157:H7 in Animals Reveals Unique Genetic Features That May Confer Persistency in Hosts

Author Block:

C. Lee[1], L. Teng[1], D. Park[2], K. C. Jeong[1]; [1]Univ. of Florida, Gainesville, FL, [2]Keymyung Univ., Daegu, Korea, Republic of

Abstract Body:

Shiga toxin-producing *Escherichia coli* (STEC) O157:H7 is an important foodborne pathogen that causes outbreaks of hemorrhagic colitis and hemolytic uremic syndrome. Cattle are a major asymptomatic reservoir of STEC O157 which primarily colonizes the terminal recto-anal junction. Persistent STEC O157 strains found in animals and on farms are presumably well adapted to hosts and environments and are responsible for a large part of O157 outbreaks. These environmentally persistent isolates can potentially convert cattle to become bacterial super-shedders, secreting O157 at a rate of more than 10^4 CFU/g of feces. Previous studies have revealed that an *E. coli* O157:H7 subtype strain (FRIK2455) was predominant on a farm while other clonal variants were rarely isolated (FRIK2069 and FRIK2533). However, genetic factors that may explain its predominance in cattle are not understood. In this study, we conducted whole genome sequencing using the PacBio sequencing technique to identify genetic factors that may explain predominance of FRIK2455 in cattle. By conducting comparative genome analysis of closed-genomes, we found that these strains share similar genetic composition and structure of chromosomes, but distinct features in plasmids. Only the predominant strain FRIK2455 carries a plasmid, p35K that encodes a type IV secretion system (T4SS) that may provide an advantage for survival in hosts and environments by enhancing colonization of this pathogen.

Author Disclosure Block:

C. Lee: None. **L. Teng:** None. **D. Park:** None. **K.C. Jeong:** None.

Poster Board Number:

FRIDAY-171

Publishing Title:

Stress-Induced Regulatory Mutations Generate Biofilm Diversity in an *Escherichia coli* O157:H7 Population

Author Block:

G. A. UHLICH, C-Y. Chen, B. J. Cottrell, L-H. Nguyen; ERRC, WYNDMOOR, PA

Abstract Body:

Background: Expression of *Escherichia coli* major biofilm components, curli fimbriae and cellulose, require the CsgD transcription factor. A complex regulatory network allows environmental control of *csgD* transcription and biofilm formation. However, most serotype O157:H7 strains contain prophage insertions in the *csgD* regulator, *mlrA*, or mutations in other regulators that restrict *csgD* expression. These barriers can be circumvented by certain compensating mutations that restore higher *csgD* expression. One mechanism is via *csgD* promoter mutations that switch sigma factor utilization. Variants utilizing either *rpoD* or mutated *rpoS*, and showing strong or weak curli/biofilm expression, respectively, have been identified in glycerol freezer stocks of a foodborne outbreak strain. In this study we used WGS and RNA-seq to study genotypic alterations accumulated during storage stress and their effect on CsgD-dependent phenotypes. **Methods:** Variants 43894OW (RpoS-dependent, curli-) and 43894OR (RpoD-dependent, curli+), isolated from a freezer stock prepared from an ATCC 43894 culture (aka CDC EDL932), were subjected to WGS and RNA-seq using an Illumina platform. Curli expression and biofilm formation were assayed using Congo red (CR) dye affinity and crystal violet dye retention, respectively. Gene deletions were constructed by Red ET recombination. Protein fusions were constructed in plasmid pSE380. **Results:** Enhanced expression ($p<0.05$) of *csgD*-dependent biofilm components and regulators accompanying stronger biofilm and CR-binding phenotypes was observed in RpoD-dependent strain 43894OR compared to 43894OW. A duplicated 13-kb chromosomal region was found in 43894OW that resulted in significantly higher *hnr* expression. Hnr can speed RpoS degradation in stationary-phase and a recombinant Hnr further repressed biofilm and CR phenotypes in a strain with normal RpoS-CsgD regulation (intact *mlrA* and WT *rpoS*). **Conclusion:** Mutations emerging during stressful conditions can have significant and opposing effects on CsgD-dependent phenotypes, creating individuals with phenotypic presentations that diverge from the parent population. Such individuals would increase population fitness for survival under extreme conditions.

Author Disclosure Block:

G.A. Uhlich: None. **C. Chen:** None. **B.J. Cottrell:** None. **L. Nguyen:** None.

Poster Board Number:

FRIDAY-172

Publishing Title:

Whole Genome Sequence Analysis Of Shiga Toxin Producing *e.Coli* O103:H2 Associated With Diarrhea In White Tailed Deer

Author Block:

D. S. S. Wijetunge[1], A. Sebastian[2], I. Albert[2], S. Kariyawasam[1]; [1]Pennsylvania State Univ., State College, PA, [2]Bioinformatics Consulting Ctr, Pennsylvania State Univ., University Park, PA

Abstract Body:

Shiga Toxin Producing *E. coli* (STEC) is one of the major causes of foodborne infections that leads to approximately 2,801,000 acute illnesses per year worldwide. Although *E. coli* O157:H7 has been the predominant cause of STEC infection, the Foodborne Diseases Active Surveillance Network (FoodNet) has reported a recent increase in non-O157 STEC illnesses since 2000. Approximately, 83% of non-O157 STEC outbreaks are associated with six serogroups, including *E. coli* O103. As with O157 STEC, common sources for non-O157 STEC infections are cattle and other ruminants. The objective of the present study was to analyze the whole genome of STEC strain PSUO103 (O103:H2 serotype), which was isolated from a white-tailed deer with severe diarrhea to study its zoonotic potential. Genome sequencing was performed using both Ion-Torrent PGM and PacBio sequencing technologies followed by Rapid Annotation using Subsystem Technology. Analysis of the genome revealed it consists of a circular chromosome of 5.449 Mb in size and a 73 kbp plasmid. Both the chromosome and plasmid of PSUO103 displayed a high degree of similarity (95% coverage with 99% similarity at the nucleotide level) to the genome of *E. coli* O103:H2 str. 12009 that was isolated from a human patient with hemorrhagic diarrhea in Japan in 2001. A total of 68 genomic islands were detected in PSUO103 chromosome, which included islands encoding the locus of enterocyte effacement and Shiga-toxin 1. The zoonotic potential of STEC PSUO103 strain was evaluated by its ability to bind two human colonic epithelial cell lines (Caco-2 and CCD841_CoN) and produce Shiga toxins as determined by Vero cell cytotoxicity assay.

Author Disclosure Block:

D.S.S. Wijetunge: None. **A. Sebastian:** None. **I. Albert:** None. **S. Kariyawasam:** None.

Poster Board Number:

FRIDAY-173

Publishing Title:

Transcriptomic Analysis of *Salmonella enterica* Forc_015, a Clinical Pathogen Isolated from Human Blood, in Contact with Cabbage

Author Block:

S. Kim[1], H. Kim[2], H. Yoon[1]; [1]Ajou Univ., Suwon-si, Korea, Republic of, [2]Dankook Univ., Cheonan-si, Korea, Republic of

Abstract Body:

FORC_015 is a rod-shaped,facultative anaerobic, Gram-negative bacterium isolated from the blood of an infected patient in Korea. The complete genome sequence is released in NCBI NZ_CP011365. Recently, an increasing number of reports have shown that *Salmonella* survives and persists inside raw vegetables as well as animal hosts. To gain insight into the systematic gene regulation of *Salmonella* in contact with raw vegetables, cabbage in a M9 medium broth was inoculated with FORC_015 and the bacterial mRNA was analyzed using RNA-Seq technology. Sorting out genes with expression changes of three-fold or more by the contact with cabbage showed 114 genes up-regulated and 106 genes down-regulated. To understand genes whose expression is controlled for the adaptation to raw vegetables, genes increased or decreased fairly as an operon were chosen. Genes of *nar* operon (nitrate reductase) and *cit* operon (citrate lysase) were up-regulated, while genes of *thi* operon (thiamine biosynthesis) and *sit* operon (iron transport) were down-regulated. *Salmonella* mutant strains lacking each operon were constructed and their growth rates were compared in the presence and absence of cabbage. Except Δ*thi* mutant, all mutant strains showed comparable fitness to that of wild type *Salmonella* regardless of the presence of cabbage. However, the Δ*thi* mutant whose growth was significantly attenuated in a M9 medium condition was complemented by the addition of cabbage in the medium, indicating that vegetables like cabbage are exploited as a good source of vitamin B1 by *Salmonella*. These results suggest that the inhibition of thiamine biosynthesis in *Salmonella* and the abolishment of thiamine uptake by *Salmonella* may be considered as promising control measures against *Salmonella* infection via raw vegetables. Key words: *Salmonella enterica*, Vegetable, Virulence

Author Disclosure Block:

S. Kim: None. **H. Kim:** None. **H. Yoon:** None.

Poster Board Number:

FRIDAY-174

Publishing Title:

Wgs Analysis of Antimicrobial-Resistant *Salmonella* from Animal Feed and Pet Food

Author Block:

K. Domesle[1], C. Li[1], Q. Yang[1], X. Li[2], B. Ge[1]; [1]U.S. Food and Drug Admin., Ctr. for Vet. Med., Office of Res., Laurel, MD, [2]U.S. Food and Drug Admin., Ctr. for Vet. Med., Office of Surveillance and Compliance, Rockville, MD

Abstract Body:

The prevalence of antimicrobial resistance genes among *Salmonella* isolates recovered from animal feed and pet food commodities is not well documented. In this study, 47 antimicrobial-resistant *Salmonella* isolates were selected from over 700 *Salmonella* recovered by the U.S. Food and Drug Administration between 2004 and 2012 from animal feed and pet food samples. The isolates were selected based on their unique phenotypic resistance, and further characterized for the presence of antimicrobial resistance genes by whole genome sequencing and queried against an in-house antimicrobial resistance database. The majority (76.6%) of isolates were recovered from pet food, primarily dog food, and comprised 29 domestic and 18 imported samples. Twenty-four *Salmonella* serovars were represented, with Typhimurium and group B monophasic representing 25.5% and 8.5% of selected isolates, respectively. Phenotypic resistance was most common to tetracycline (65.9%) and aminoglycosides (52.2%), followed by cephems (19.1%), folate pathway inhibitors (17.8%), β-lactams (15.6%), phenicols (14.9%), and quinolones (13.0%). Multidrug resistance (resistant to ≥ 3 antimicrobial classes) was observed in 16 (34.0%) isolates, all except two were recovered from pet food. One *Salmonella* Agona isolate from dog food and one *Salmonella* Uganda from canola meal, both from imported samples, were resistant to 7 and 6 classes of antimicrobials, respectively. With WGS, tetracycline resistance genes *tetA*, *tetB*, *tetC*, *tetD*, and *tetG* and aminoglycoside resistance genes *aph(6)-Ic*, *aph(6)-Id*, *aph(3'')-Ib*, *aph(3')-Ia*, *aph(3')-Id*, *aph(3')-II*, among others, were detected. The *sul1* and *sul2* genes accounted for sulfonamide resistance whereas $bla_{\text{CMY-2}}$, $bla_{\text{TEM-1}}$, $bla_{\text{carB-2}}$, bla_{OXA}, and bla_{TEM} were among the β-lactam resistance genes found. The agreements between phenotypic and genotypic determination of resistance at the antimicrobial level ranged from 53.3% for folate pathway inhibitors to 85.1% for phenicols and the agreement was 17.0% at the isolate level. In summary, diverse antimicrobial resistance genes were present in *Salmonella* serovars from animal feed and pet food and WGS effectively provided in-depth characterization of resistance genes among these isolates.

Author Disclosure Block:

K. Domesle: None. **C. Li:** None. **Q. Yang:** None. **X. Li:** None. **B. Ge:** None.

Poster Board Number:

FRIDAY-175

Publishing Title:

Genome-Wide Analysis Reveals *Salmonella enterica* Genes Involved in Proliferation within Tomatoes (An Alternative Host) with Implications for Human Health

Author Block:

M. H. de Moraes[1], P. Desai[2], D. R. Perez[1], S. Porwollik[2], W. Chu[2], R. Canals[2], M. McClelland[2], M. Teplitski[1]; [1]Univ. of Florida, Gainesville, FL, [2]Univ. of California, Irvine, Irvine, CA

Abstract Body:

The increasing number of salmonellosis outbreaks linked to tomatoes is a major concern for public health. Non-typhoidal *Salmonella* is capable of reaching high cell numbers, up to 10^7 colony forming units (CFU) per tomato fruit, making them a vehicle that allows the pathogen to re-infect the preferred animal hosts. Despite its importance for public health, the genetic mechanisms involved in the interactions of human enteric pathogens with alternate hosts, such as edible plants, remain largely unknown. In this study, a high-density transposon library of *S. enterica* Typhimurium ATCC 14028 was screened to identify loci involved in persistence within tomatoes. We identified 1245 loci disruption of which led to reduced fitness within tomatoes (FDR < 0.1). Competitive fitness assays using isogenic mutants confirmed screening results. Mutants for biosynthesis of lipopolysaccharide core and O-antigen had reduced fitness. Lipopolysaccharide is required for *Salmonella* virulence in animals, indicating that an overlap of colonization mechanisms in animals and plants may benefit *Salmonella* when using tomatoes as an alternative host. The global nitrogen regulator *glnG* had a major role in the *Salmonella*-tomato interaction; its deletion led to a severe defect in proliferation within fruits ($\log_2$[CI]= -3.54). Auxotrophic mutants for arginine, glutamine, leucine, valine, isoleucine, methionine, serine, threonine and tryptophan had decreased fitness within tomatoes, and *in vivo* reporters confirmed that these pathways are also highly expressed within tomatoes. Moreover, tomato genotype influenced amino acid requirements; *Salmonella* auxotrophic mutants for glutamine, leucine, valine, isoleucine, methionine and serine were less fit in *rin* tomato mutants, known to differentially accumulate amino acids during ripening. The nitrogen metabolism genes identified in our screening were within the core genomes of *Enterobacteriaceae,* suggesting that adaptations in this clade are related to nutritional requirements when adopting plants as hosts.

Author Disclosure Block:

M.H. de Moraes: None. **P. Desai:** None. **D.R. Perez:** None. **S. Porwollik:** None. **W. Chu:** None. **R. Canals:** None. **M. McClelland:** None. **M. Teplitski:** None.

Poster Board Number:

FRIDAY-176

Publishing Title:

Rna-Seq Data Reveals Sigma H-Dependent Regulation of Competence Genes in *Listeria monocytogenes*

Author Block:

Y. Liu, R. H. Orsi, K. J. Boor, M. Wiedmann, V. Guariglia-Oropeza; Cornell Univ., Ithaca, NY

Abstract Body:

Background: *Listeria monocytogenes*, the foodborne pathogen which causes serious infection listeriosis, not only is a public health concern but also remains a problem in food industry. Among the four alternative σ factors of *Listeria monocytogenes*, σ^B remains the best understood and it has been shown to control a large regulon and play important roles in stress response and virulence. The function of σ^H, however, has not yet been well defined.**Method:** An *L. monocytogenes* strain with deletions of all 4 alternative σ factor genes (Δ*BCHL*) was used as the background strain, which was further modified to overexpress *sigH* (Δ*BCHL::*P_{rha}*-sigH*). A new bio-informatics approach was applied to compare normalized RNA-seq coverage (NRC), between Δ*BCHL::*P_{rha}*-sigH* and a Δ*BCHL* control, using sliding windows of 51 nt along the whole genome rather than comparing NRC calculated only for annotated genes as BaySeq does.**Results:** With this novel sliding window method, we identified 6 transcription units (TUs) that are transcribed from σ^H-dependent promoters and five of which are newly identified. Interestingly, among them, three are operons encoding competence genes (*comGABCDEFG*, *comEABC*, *coiA*). While these promoters were highly conserved in *L. monocytogenes*, none of them were found in all *Listeria* spp.**Conclusion:** Our data indicate that a number of *L. monocytogenes* competence genes are regulated by σ^H. This regulation is conserved in the pathogenic strains of *L. monocytogenes*, but not in other non-pathogenic *Listeria* strains, suggesting a possible novel role of σ^H-dependent competence genes in *L. monocytogenes* virulence. Additionally, the sliding window approach described here could also give more insights into RNA-seq data, particularly for genes that are transcribed from multiple promoter elements only some of which show differential transcription.

Author Disclosure Block:

Y. Liu: None. **R.H. Orsi:** None. **K.J. Boor:** None. **M. Wiedmann:** None. **V. Guariglia-Oropeza:** None.

Poster Board Number:

FRIDAY-177

Publishing Title:

Evaluation of a 16s rRNA Amplicon Sequencing Protocol for the Identification of Bacteria in Environmental Swabs

Author Block:

A. A. Rodriguez[1], J. R. White[2], C. L. Randell[1], D. A. Sela[3]; [1]U.S. FDA, Winchester, MA, [2]Resphera BioSci.s, Baltimore, MD, [3]Univ. of Massachusetts, Amherst, MA

Abstract Body:

FDA routinely collects environmental swabs from food manufacturing facilities. These are analyzed for the presence of foodborne pathogens by cultivation methods. The analysis involves enrichment and isolation of the organism of interest. Furthermore, a separate analysis is required for each pathogen. 16SRrNA amplicon sequencing allows simultaneous identification of bacteria in a mixed community without prior enrichment or isolation. Our goal was to evaluate this technique as an alternative to cultivation methods. Two mock bacterial communities were prepared encompassing 12 genera and including foodborne pathogens *Listeria monocytogenes, Salmonella enterica, Escherichia coli* and *Staphylococcus aureus.* The first community consisted of a mixture of DNA extracted from 15 strains. The second consisted of a mixture of broth cultures from 10 strains. The later was spread onto stainless steel surfaces, allowed to dry, and swabbed, at which point the DNA was extracted. Dual-indexed DNA libraries targeting regions V3 and V4 of the 16S rRNA gene were prepared and sequenced on an Illumina MiSeq for 500 cycles. The resulting reads were analyzed using four bioinformatic pipelines 1) MiSeq Reporter, 2) Resphera Insight, 3) QIIME with UCLUST and 4) QIIME with RDP. All the pipelines tested correctly identified the genus of all bacteria present in the mock communities. Some foodborne pathogens were identified to the species level as follows: MiSeq Reporter - *S. aureus* and *S. enterica*; Resphera Insight- *S. enterica* and *E. coli*; QIIME with UCLUST- *S. aureus;* and QIIME with RDP- *S. aureus* and *E. coli*. None of the pipelines was able to speciate *L. monocytogenes*, moreover, QIIME with RDP misidentified it as *L. seeligeri*. The protocol evaluated permitted genus-level identification of mixed bacterial communities extracted from swabs without prior enrichment. We were able to accurately identify species *S. aureus, E. coli* and *S. enterica,* pathogens of relevance to the food industry. No one pipeline tested was able to speciate all bacteria. This is possibly due to limitations in the diversity of regions V3 and V4. Alternative primer sets targeting other variable regions will next be evaluated in an attempt to improve species identification.

Author Disclosure Block:

A.A. Rodriguez: None. **J.R. White:** N. Other; Self; founder of Resphera Biosciences. **C.L. Randell:** None. **D.A. Sela:** None.

Poster Board Number:

FRIDAY-178

Publishing Title:

Do Dna Extraction Methods Affect Shotgun Metagenomic Library Generation and Sequencing Output of Spice Microbiomes?

Author Block:

L. Ewing[1], G. Gopinath[1], N. Addy[2], D. Hanes[1], J. Jean-Gilles Beaubrun[1]; [1]FDA, Laurel, MD, [2]Oak Ridge Inst. for Sci. and Technology, Oak Ridge, TN

Abstract Body:

Next Generation Sequencing (NGS) is a critically useful tool for answering a variety of scientific questions. Currently we are using shotgun metagenomic sequencing to investigate foodborne pathogens in spices. Spices are difficult matrices to analyze because their essential oils contain antimicrobial properties that can inhibit detection methods. Previous studies using NGS to analyze leafy greens were successful using Qiacube (Qiagen) extracted DNA. However, spice samples did not generate quality results with the same consistency. Quality DNA is essential for generating libraries that can be sequenced and, in turn, affects NGS data. It's possible that physical and chemical properties of spices affect the purity of DNA which could interfere with the biochemistry of NGS, so we designed a study to compare the effects of three DNA extraction methods. Spices were enriched following a modification of the BAM using corn oil as an additive to the primary enrichment broth in an attempt to neutralize antimicrobial properties. Samples were collected throughout the enrichment process. DNA was extracted using the Qiacube, PowerPlant Pro DNA Isolation Kit (Mo Bio) and the MagNA Pure Compact (Roche). Libraries were prepared using Illumina's Nextera kit and run on the MiSeq; they were quantified using Qubit (Invitrogen) and the Agilent Bioanalyzer. NGS data was analyzed using CLC Bio's Genomic Workbench and Metaphlan2. Quality of the sequencing data was evaluated based on average length, total length and number of contigs and N50. The quality of the NGS data and metagenomics profiles were comparable for samples using all three DNA extraction methods. However, the MoBio samples exhibited the highest rate of samples that successfully produced quality libraries suitable for sequencing, 90%, versus 85% for MagNA Pure Compact and 81% for Qiacube. The results of this study suggest that using a MoBio DNA extraction method, which separates spice debris by pelleting and contains additional methods for removal of inhibitors, may be beneficial for metagenomics-based detection of pathogens in spice samples. An optimized laboratory workflow for pathogen detection in spices will allow for maximum quality and consistent results in samples that may be limited in number, as the case may be with food outbreak samples.

Author Disclosure Block:

L. Ewing: None. **G. Gopinath:** None. **N. Addy:** None. **D. Hanes:** None. **J. Jean-Gilles Beaubrun:** None.

Poster Board Number:

FRIDAY-179

Publishing Title:

First Detection and Identification of *Candidatus* Neoehrlichia mikurensis in Korea

Author Block:

D-M. Kim, P. Jha, N. Yun, C-M. Kim; Chosun Univ. Coll. of Med., Gwang-ju, Korea, Republic of

Abstract Body:

Background: *Candidatus* Neoehrlichia mikurensis (CNM; family Anaplasmataceae) is an emerging tick-borne pathogen causing a systemic infecton mostly in persons with underlying hematologic or autoimmune diseases. The aims of this study were to identify CNM in small mammals in Republic of Korea (ROK).**Methods:** We investigated the presence of CNM in small mammals captured in south-western part of ROK, which was screened for CNM by molecular methods. Samples were initially screened by PCR targeting the *groEL* gene, and confirmed by PCR targeting the CNM 16S rRNA gene. For positive samples, the amplified fragments were sequenced.**Results:** 28.57 % of the investigated mammals were positive for CNM-DNA. All of the captured animals were identified as striped field mouse (*Apodemus agrarius).***Conclusions:** The presence of this pathogen in the investigated areas in these rodents points towards the need for more specific investigation on its role as human pathogens.

Author Disclosure Block:

D. Kim: None. **P. Jha:** None. **N. Yun:** None. **C. Kim:** None.

Poster Board Number:

FRIDAY-180

Publishing Title:

Detection of *Salmonella spp.* in Peruvian Guinea Pigs at First Parturition by Pcr Multiplex

Author Block:

A. M. Chero, G. Marcelo, G. Diaz, R. Rosadio & L. Maturrano; Univ. Natl. Mayor de San Marcos (UNMSM), Lima, Peru

Abstract Body:

Background: The production of guinea pigs in Peru was originated in the Andes of the country. This species is traditionally consumed by Peruvian population, besides of being an economic activity. Salmonellosis is the most important bacterial disease in this production, generating high rates of morbility and mortality. There are over 2500 serotypes of *Salmonella* spp., of which *Salmonella* Typhimurium have been reported more frequently in guinea pigs in Peru in animals without clinical signs of disease (Perez, 1975; Ortega, 2009; Morales, 2012), followed by *Salmonella* Enteritidis in another countries (Fish *et al*, 1968, Bartholomew *et al*, 2014). The risk of isolating *Salmonella* spp. of asymptomatic animals consists in the introduction of these in farms without presence of this bacteria, generating the disease because of a stress event, and the possibility of transmission to humans by direct contact or foodborne. For this reason, the aim of the present study was to identify the most frequent serotypes (Typhimurium or Enteritidis), isolated through microbiological standardized protocols. **Materials and methods:** We evaluated the presence of *Salmonella* spp. in 272 rectal swabs from guinea pigs without evidence of clinical signs, after their first parturition, from a commercial farm in Lima, Peru. The microbiologic method used is based on laboratory protocols according to the ISO-6579 (2002). DNA was isolates and analyzed by PCR Multiplex, in order to detect *inv*A, *prot*6E and *fli*C genes; which are specific for *Salmonella* spp., *Salmonella* Enteritidis and *Salmonella* Typhimurium, respectively, according to Jamshidi (2009). **Results:** At microbiological methods, eight of all animals evaluated were positive a *Salmonella* spp. Strains isolated were analyzed by PCR multiplex; all of them amplified *inv*A gene, belonging to the genus *Salmonella*. Seven isolates amplified *fli*C gene, belonging Typhimurium serotype. The remaining isolated was identified only as *Salmonella* spp. and Enteritidis serovar was no detecting. **Conclusion:** In this study, 2.94% of asymptomatic carrier guinea pigs at first parturition were detected in a farm that did not present outbreak of salmonellosis four years before. The predominant serovar was Typhimurium, we did not find Enteritidis and one isolate belongs to genus Salmonella, but no Typhimurium or Enteritidis.

Author Disclosure Block:

A.M. Chero: None.

Poster Board Number:

FRIDAY-181

Publishing Title:

Fungal Infection and Mycotoxin Contamination of Vietnamese Coffee Beans

Author Block:

T. Dao[1], L. Phan[1], H. Tran[2], M. Eeckhout[3], F. Devlieghere[4]; [1]Ho Chi Minh city Univ. of Food Industry, Ho Chi Minh city, Viet Nam, [2]Cai Lan Oils & Fats Industries Co., Ltd, Ho Chi Minh city, Viet Nam, [3]Lab. for applied mycology, Univ. Ghent, Gent city, Belgium, [4]Lab. of Food Microbiol. and Food Preservation, Univ. Ghent, Gent city, Belgium

Abstract Body:

Background: The estimated loss of agricultural products due to mold contamination in Vietnam is approximately 20-25% per year including reduced productivity and product quality. Mycotoxin such as aflatoxins, ochratoxins and fumonisins produced by molds are major concerns. Nowadays, Vietnam is the second biggest coffee producer in the world. Coffee is one of the most popular drinks of the Vietnamese people and has been exported to USA and EU countries. Therefore, it is important to have knowledge on the mold contamination and identification on Vietnamese coffee beans in order to improve the quality of Vietnamese coffee. **Methods:** Samples of coffee beans were collected from different regions of Vietnam (central highlands, Northern and Southern provinces⋯). In total, 80 samples of coffee beans were analyzed. Mold isolation from coffee beans was performed by direct plating on Dichloran Rose Bengal Chloramphenicol (DRBC) agar and identification was performed by DNA sequencing. **Results:** This study showed that 80% of the coffee samples were positive for *Aspergillus* sp. such as: *Aspergillus fumigatus*, *A. flavus*, *A. niger*, *A. oryzae* and *A. terreus*. The diversity of the mold population was correlated with geographical coffee origin. *Aspergillus niger*, *A. flavus* were more frequently detected in coffee bean samples from Southern area of Vietnam. HPLC analysis revealed that *A. flavus* strains were able to produce Aflatoxin AB1. **Conclusion:** This research has confirmed the biodiversity of molds from different regions in Vietnam. *A. flavus* was the dominant species infecting Vietnamese coffee beans. *A. flavus* is capable to produce Aflatoxin AB1, this mold was detected in 70% of the coffee bean samples.1

Author Disclosure Block:

T. Dao: None. **L. Phan:** None. **H. Tran:** None. **M. Eeckhout:** None. **F. Devlieghere:** None.

Poster Board Number:

FRIDAY-182

Publishing Title:

Microbial Load in Hard Corals of Burma Nella- Andaman Islands

Author Block:

K. Revathi, Ph.D, V. Gunasekaran; Ethiraj Coll., Chennai, India

Abstract Body:

Background: Microbial communities associated with the healthy corals play a major role in production of nutrition and also maintaining the health by producing antibiotics in coral animals. All healthy corals contains large population of algae, bacteria, archea and these microbes provide benefits to their host by various mechanisms including photosynthesis, nitrogen fixation and infection protection. Coral microbiology is an emerging field, driven largely by a desire to understand, and ultimately prevent, the world destruction coral reef. **Methods:** In this study five species of hard corals (Acropora aspera, Favia matthai, Goniastrea retiformis, Porites annae, Porites solida) from Burma Nella in Andaman were studied for microbial load.**Results:** The bacterial population ranged from 12.8 x 103 to 2.80 x 103 CFU/ml. Porites solida was registered maximum microbial load and Porites annae gave minimum microbial load. Altogether, 6 genera and 7 species viz. Aeromonas hydrophila, Bacillus megaterium, Bacillus subtilis, Enterobacterium sp, Flavobacterium hibernum, Pseudomonas aeruginosa, and Vibrio communis were identified from these five corals. Among them, the Pseudomonas aeruginosa was most abundant bacteria found in all coral samples followed by Vibrio communis.**Conclusions:** The aim of this study is to known the association of microbes in healthy corals and provide a theoretical reference for better protection and maintaining the coral's biodiversity.

Author Disclosure Block:

K. Revathi: None. **V. Gunasekaran:** None.

Poster Board Number:

FRIDAY-183

Publishing Title:

Absolute Quantification of Viable *Lactobacillus acidophilus* Ncfm® and *Bifidobacterium animalis* s*ubsp*. *lactis* bl-04™ Using Strain-Specific Digital Pcr

Author Block:

S. J. Z. Hansen, W. Morovic; DuPont, Madison, WI

Abstract Body:

Total cell count (TCC) is the industry-accepted reference method for probiotic enumeration. However, it has a wide variation of 25-30% and cannot always differentiate between similar strains commonly found within blended products. Alternatively, Digital PCR (dPCR) is a technique that can quantify singular DNA targets through the detection of TaqMan® probe fluorescence. The objective of this work was to develop an absolute enumeration method utilizing strain-specific dPCR that produces results that improves the enumeration range of TCC. Optimization of the method included the use of Propidium Monoazide (PMA), a dye that inhibits the amplification of DNA in dead and damaged cells, and mechanical disruption. This creates a strategy to prevent amplification of DNA from non-viable cells as well as ensure all healthy cells are sampled. These treatments were applied to cell suspensions of freeze-dried *Lactobacillus acidophilus* NCFM® and *Bifidobacterium animalis* subsp. *lactis* BL-04™ and quantified with the chip-based Life Technologies' Quantstudio® 3D Digital PCR system. Statistical analysis of the reproducibility and repeatability between the TCC and dPCR results validated the method for quantifying probiotic cultures, giving consistent readings within 12% variation, well within the acceptable range of TCC. In all, the use of dPCR for the enumeration of probiotics not only allows results consistent with TCC, but also provides a solution for strain-specific enumeration that is not currently available for multi strain products.

Author Disclosure Block:

S.J.Z. Hansen: None. **W. Morovic:** None.

Poster Board Number:

FRIDAY-184

Publishing Title:

Characterization of the Contaminant Bacterial Communities in Fed-Batch and Continuous Fermentation Process for Ethanol Production

Author Block:

M. L. Bonatelli, M. C. Quecine, A. P. Bini, C. A. Labate; ESALQ/USP, Piracicaba, Brazil

Abstract Body:

Brazil is the largest sugarcane ethanol producer in the world and ethanol demand has increased as it is considered a sustainable alternative to fossil fuel. During the fermentation process, that may operate either in fed-batch or continuous mode, bacterial contamination is responsible for significant economic losses. Thus, we compared the profile of the bacterial communities from two distilleries in Brazil, each operating a different fermentation processes. Bacterial communities were accessed through Illumina culture-independent 16S rDNA gene sequencing and qPCR was used to quantify total bacteria. Both distilleries showed similar bacterial density 5,05 ± 0,14 Log CFU/mL; however, 16S rDNA sequencing showed differences between the two distilleries. It was reported 219 genera belonging to 12 different phyla, several of them that have never been described in this environment, but 91% up to 99% of sequences were affiliated to genus *Lactobacillus* in the samples. Alpha diversity only showed a correlation through the fermentation tanks in continuous mode. Beta diversity clearly separated distilleries and the operational taxonomic units that were differentially represented, belonged mainly to *Lactobacillus*, where several of them were unique to distillery with continuous mode, whereas others from the genera *Weissella* were more abundant in fed-batch mode. *Pediococcus*, *Acetobacter* and *Anaerosporobacter* were more abundant in continuous mode. Predictive functional gene was made using PICRUSt and reinforced clustering within the distilleries. It also revealed that several classes of genes, as carbohydrate metabolism and membrane transport, mainly related to fermentation process, were significantly different between the distilleries. Our data strongly suggests that bacterial communities are distinct between distilleries, it may be related to fermentation process operation and future works should focus on looking for specific control method to each distillery.

Author Disclosure Block:

M.L. Bonatelli: None. **M.C. Quecine:** None. **A.P. Bini:** None. **C.A. Labate:** None.

Poster Board Number:

FRIDAY-185

Publishing Title:

Combining the Use of a Novel Substrate, Sorghum Bran, with Strain Improvement to Produce High Yields of Itaconic Acid

Author Block:

A. M. Ahmed El-Imam[1], C. Du[2], P. S. Dyer[1]; [1]Univ. of Nottingham, NOTTINGHAM, United Kingdom, [2]Univ. of Huddersfield, Huddersfield, United Kingdom

Abstract Body:

Itaconic acid (IA) is a bulk chemical produced by submerged fermentation of sugars by *Aspergillus terreus*. It could potentially replace many petroleum-based monomers in a many industrial applications. However, the cost of sugars makes its use uneconomical. Several approaches are being utilised to decrease the cost of IA production and these include attempts to increase yield, improve fermentation strains and utilise cheaper raw materials; many of which are promising in improving itaconic acid production economics. The present work combines two such strategies. Firstly, IA was produced from a novel substrate, sorghum bran (SB), which is the waste accumulated from the processing of *Sorghum bicolor* grains. To this end, SB composition and suitability for IA production was investigated, and then pretreatment conditions optimised to obtain a sugar-rich fermentable liquor. Secondly, strain improvement was attempted. *Aspergillus terreus* strains (n=45) were screened on SB hydrolysate for IA production. The most promising strains were then subjected to two strain improvement techniques. Mutagenesis was carried out by UV irradiation of the spores of strains 49-5 and 49-22. Alternatively, mating types of the strains were determined and sexual crossing undertaken where possible. Novel strains obtained via both methods are being tested for IA output. The SB contained about 53% residual starch, 20% protein and varying amounts of minerals, and thus showed potential for IA production. Screening showed yields from 0.1-2.6 g/L on SB hydrolysate. Following UV irradiation, a mutant of 49-22 showed a **6-fold** increase in yields relative to parents at 16.4 g/L of IA. It was observed that 28 strains were *MAT 1-1* while 18 were of *MAT 1-2* mating types. Sexual crossing yielded large numbers of cleistothecia (sexual reproductive structures of *A. terreus*) containing mature ascospores. Work is currently underway to verify clonality of the progeny by molecular markers, and their IA yields. This is the first report of itaconic acid production from sorghum bran, and also the first reported attempt to use sexual crossing to improve yields from *A. terreus*. These results could have significant impact on the economics of renewable itaconic acid production and consequently reduce dependence on petroleum-based monomers

Author Disclosure Block:

A.M. Ahmed El-Imam: None. **C. Du:** None. **P.S. Dyer:** None.

Poster Board Number:

FRIDAY-186

Publishing Title:

Characterizing and Quantifying Bacterial Populations Associated with Nutrient Removal in Full-scale Sequencing Batch Reactors

Author Block:

J. T. Johnston, S. F. Behrens, T. M. LaPara; Univ. of Minnesota, Minneapolis, MN

Abstract Body:

The bioreactors of municipal wastewater treatment plants are designed to rapidly metabolize nutrients to prevent ecological harm when treated wastewater is released to the environment. Despite the reliance on microbes, relatively little is known regarding the types and quantities of microorganisms that reside in full-scale wastewater treatment bioreactors. The goal of the present study was to track the microbial community composition in full-scale sequencing batch reactors using deep sequencing (Illumina MiSeq) of PCR-amplified 16S rRNA gene fragments and quantitative PCR targeting, functional genes associated with specific nutrient removal activities. These results were then statistically correlated to plant performance data. Results suggest complex but dynamic bacterial communities but stable functional community for ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA). These results suggest that tracking specific bacterial populations by qPCR can become a useful tool for monitoring wastewater treatment bioreactors.

Author Disclosure Block:

J.T. Johnston: None. **S.F. Behrens:** None. **T.M. LaPara:** None.

Poster Board Number:

FRIDAY-189

Publishing Title:

Development and Validation of a Species-Specific Multiplex Pcr Protocol for the Identification of Commercial Probiotic Products

Author Block:

B. J. Zabel, W. Morovic, B. Stahl; DuPont Nutrition and Hlth., Madison, WI

Abstract Body:

The Dietary Supplements industry has made large strides in product quality and safety under regulatory entities such as FDA, EFSA, and HealthCanada, however molecular methods still lag behind requirements. Recent reports have shown that label claims do not always describe the contents of dietary supplements, including probiotic products (Mills *et al.* 2015). In order to identify multiple bacterial species in products, we developed an end-point, multiplex PCR method that identifies 20 common species and sub-species of probiotic products.We collected whole genome sequences from public and proprietary strains of common probiotic species of the genera: *Lactobacillus, Bifidobacterium, Lactococcus, Streptococcus, and Pediococcus*. Utilizing polymorphism in the glucose-6-phosphate isomerase gene, a single copy core housekeeping gene, we were able to design primers targeting various amplicon sizes leading to an assay of four reactions that detect 20 species and sub-species.To validate these results, we tested the multiplex PCR against controls and determined the optimum detection limit of each to be 1E5 CFU/mL. Then, we obtained 20 various commercial formulations to compare with the method.We processed these samples with the MoBio PowerSoil gDNA extraction kit and were able to remove the inhibitors and identify the samples, all of which amplified well.Preliminary results show that all species claimed on the labels were detected. However, six of the products have additional bacteria not noted on the label. The amplicon strengths seemed to be affected by the concentration of bacteria in the product, but this could not be confirmed because formulations were not always present on the label. This highlights the need for a quantitative method for multi-strain blends.In conclusion, the molecular method we developed can detect up to 20 different species and sub-species common in probiotic products. When used with commercial samples, we showed that the contents to do not always match the label claim. Highlighting the need for oversight of regulatory testing on probiotic dietary supplements, to ensure label accuracy and customer confidence.

Author Disclosure Block:

B.J. Zabel: None. **W. Morovic:** None. **B. Stahl:** None.

Poster Board Number:

FRIDAY-190

Publishing Title:

Direct Capture and Expression of the Didemnin Anti-Cancer Agents from the Marine Bacterium *Tistrella mobilis*

Author Block:

J. Zhang, Bradley S. Moore; Univ. of California, San Diego, La Jolla, CA

Abstract Body:

The cyclic depsipeptide didemnin B was the first marine natural product to enter clinical trials as a chemotherapeutic agent. Although the compound and several analogs were originally isolated from tunicates, it was recently discovered that two species of free-living marine bacteria, *Tistrella mobilis* from the Red Sea and *Tistrella bauzanesis* from the Pacific Ocean, also produce didemnins. To investigate the biosynthesis and unique post-synthetase activation mechanism of these compounds, the 75-kb biosynthetic gene cluster was directly captured from *Tistrella mobilis* KA081020-065 using transformation-associated recombination (TAR) in yeast. Heterologous expression and genetic manipulation of the pathway provides a mechanism for didemnin B biosynthesis, as well as a proposed route to the natural analogue aplidine (dehydrodidemnin B), a drug with orphan designation in the EU.

Author Disclosure Block:

J. Zhang: None.

Poster Board Number:

FRIDAY-191

Publishing Title:

Diversity of Novel Alkaline Protease Producing Bacteria from Chilika Lake

Author Block:

A. Narayan Panda, S. Mishra, L. Ray, T. K. Adhya, M. Suar, V. Raina; Sch. of Biotechnology, .Bhubaneswar, India

Abstract Body:

Chilika lake is the largest brackish water lake in India situated in the east coast of Odisha. The lake is a biodiversity hotspot & is a storehouse of industrially important marine microorganisms. Enzymes from marine or brackish environment have unique properties & have proven industrial applications. Alkaline proteases are known active ingredients of the commercial detergents & have wide application in leather & tanning industries. Culture-based isolation of alkaline protease producing bacteria from different stations & identification by biochemical & molecular methods. Microbial identification & characterization by using polyphasic approach. 30 different sampling sites from the lake were investigated for the isolation of alkaline proteases. 13 isolates showing highest zone in plate assay within a pH range of 9-11. By using casein as a substrate at pH 10, three bacterial isolates i.e KGS4, KGW1 & PKS7 were characterized by polyphasic taxonomy. The preliminary phylogenetic analysis based on 16SrRNA sequence & BLAST analysis showed that closest phylogenetic neighbors of KGS4, KGW1, CHW3, & PKS7 were *Bacillus stratosphericus* 41KF2a, *Halobacillus trueperi* SL-5 & *Rheinheimera aquimaris* SW-353, respectively. The 16SrRNA sequence similarities were found to be 99.5%, 99.1%, & 98.58%, respectively. The isolated strains showed alkaline protease activity at pH10 & were morphologically & biochemically distinct from their closest taxonomic neighbors which may be considered as a novel feature. The alkaline protease activities as measured by activity assay of strains KGS4, KGW1& PKS7 were 4.1U/ml, 3.8U/ml, 2.2U/ml respectively, at pH 10. PCR amplification of *subtilisin e* (EMI14709) gene for alkaline protease was detected in KGS4. The isolated strains showed alkaline protease activity at pH10 & were morphologically & biochemically distinct from their closest taxonomic neighbors which may be considered as a novel feature. On the basis of phenotypic, chemotaxonomic properties, phylogenetic analysis, strain KGW1^T KGS4^T & PKS7 should be placed in the genus Halobacillus, Bacillus & Rheinheimera as novel species, for which the *Halobacillus marinus* sp. nov. , *Bacillus kharajalensis* sp. nov. *Rheinheimera pleomorphicus sp. nov*. were proposed. The type strains were KGW1^T (= DSM 29522=KCTC 33609) , KGS4^T (=DSM 29521=KCTC 33610) & PKS7^T (=KCTC 42365)

Author Disclosure Block:

A. Narayan Panda: None. **S. Mishra:** None. **L. Ray:** None. **T.K. Adhya:** None. **M. Suar:** None. **V. Raina:** None.

Poster Board Number:

FRIDAY-192

Publishing Title:

Genome Analysis and Comparative Genomics of Two *escherichia Coli* isolates Associated with Salpingo-Peritonitis in Commercial Layer Hens

Author Block:

K. H. E. M. Karunathilake, D. S. S. Wijetunge, A. Sebastian, I. Albert, S. Kariyawasam; Pennsylvania State Univ., University Park, PA

Abstract Body:

Egg peritonitis in laying hens is a major cause of morbidity and mortality leading to significant economic losses to the table-egg industry worldwide . Unlike other forms of colibacillosis, egg peritonitis and associated salpingitis (salpingo-peritonitis) is restricted to adult hens in egg production . Objective of the present study was to identify novel virulence traits of *E. coli* associated with salpingo-peritonitis using the whole genome sequencing approach. To accomplish this objective, we have sequenced two *E. coli* strains isolated from the ovaries and oviducts of two laying hens with egg peritonitis. These two *E. coli* strains designated PSUO2 (O2 serogroup) and PSUO78 (O78 serogroup) were sequenced and assembled using PacBio sequencing technology and genomes were annotated using the NCBI prokaryotic annotation pipeline. Both sequences were analyzed and compared with the genomes of five *E. coli* strains isolated from avian colibacillosis with the orthology defined by >70% identity and >70% coverage. Around 400 accessory genes, including, putative adhesins, autotransporters, and heme utilization systems that might be involved in pathogenesis of layer salpigo-peritonitis were identified in the two sequenced genomes.

Author Disclosure Block:

K.H.E.M. Karunathilake: None. **D.S.S. Wijetunge:** None. **A. Sebastian:** None. **I. Albert:** None. **S. Kariyawasam:** None.

Poster Board Number:

FRIDAY-193

Publishing Title:

Isolation, Screening and Optimization of Medium Composition for Protease Production by *Bacillus sp*

Author Block:

C. FLORES-FERNANDEZ, L. Alejandro Paredes, A. Zavaleta Pesantes; Univ. Natl. Mayor de San Marcos, Lima, Peru

Abstract Body:

Background: *Bacillus* is isolated from different environments and has been widely reported as proteases producer, the most important enzymes in the industrial market. Nowadays, studies are aimed at finding microorganisms from extreme environments that produce proteases with peculiar properties and optimizing the production of these for subsequent application in the industry. In this study, a strain of *Bacillus sp*. with proteolytic activity was isolated and medium for protease production were optimized. **Methods:** The soil samples were collected from Pilluana Salterns in San Martin, Peru. For the isolation, samples were plated in nutrient agar plates with 5 % NaCl and incubated at 37 °C for 24 h. Screening of protease producing bacteria was carried out with crude extract produced by each strain on nutrient agar plates with gelatin, skim milk, casein, and proteins from leguminous as, Cicer arietinum, Pisum sativum and Glycine max (1 % w/v). Enzyme activity was visualized as clear zones due to hydrolysis of substrates. The strain with the highest activity was identified by 16S RNA gene sequencing. Plackett-Burman design was employed to find out the optimal medium constituents to enhance protease production. Five factors (glycerol, galactose, yeast extract, bacteriological peptone and NaCl) were evaluated in 12 experiments. Each factor was tested at two levels, high (+) and low (−). Minitab package version 15 was used. **Results:** A total of 47 strains were isolated, 10 were identified as protease producers and the strain with the highest activity was identified as Bacillus sp. based on 16S rRNA gene analysis. The diameters of zone of hydrolysis for this strain were 20, 18, 17 and 16 mm for gelatin, casein, skim milk and proteins from legumes, respectively. Plackett-Burman design revealed that galactose (12, 5 g / L) and yeast extract (9 g / L) significantly influenced the protease production ($p < 0,05$). The enhancement of protease from 139,52 U/mL to 337,49 U / mL was achieved with the optimization. **Conclusions:** *Bacillus sp*. isolated from Pilluana Salterns in San Martin, Peru produces an exoprotease that hydrolyze proteins from various sources. Plackett-Burman design allowed optimizing medium composition in order to increase protease production for the potential industrial applications.

Author Disclosure Block:

C. Flores-fernandez: None. **L. Alejandro Paredes:** None. **A. Zavaleta Pesantes:** None.

Poster Board Number:

FRIDAY-194

Publishing Title:

Metall (and Metalloid)-Binding of Different Metallophores from Bacteria and Fungi

Author Block:

M. Mehnert[1], G. Retamal M.[2], T. Heine[1], R. Schwabe[1], M. Schlömann[1], G. Levicán J.[2], D. Tischler[1]; [1]Technical Univ. Bergakademie, Freiberg, Germany, [2]Univ. de Santiago de Chile, Santiago, Chile

Abstract Body:

Background: Under iron-restricted conditions many bacteria and fungi produce metal chelating molecules called metallophores for scavenging metal ions from the environment. These compounds possess a high affinity and selectivity for ferric iron, but some of them are able to effectively bind other metals and metalloids as well. They find application in environmental and medical sector. In order to identify metallophores, which are able to bind strategically important metals we screened numerous bacteria and fungi for metallophore production and metal-affinity. **Methods:** A general and widely used method for metallophore detection is a colorimetric assay based on the utilization of chrome azurol S (CAS) [1]. We used a low phosphate minimal growth medium and a liquid CAS assay variant. The test has been optimized regarding to different metals and metalloids, ion concentrations and pH values in order have an applicative and useful screening method for different types of microorganisms. **Results:** We established the test for Ga, Al, As and V in microtiter plates and calibrate these CAS assay solutions as well as the available solutions for iron [2] and copper [3] with desferrioxamine B (DFOB) to calculate DFOB-siderophore-equivalents. Using these assay variants, we screened more than 35 stains of different bacteria and fungi for metallophores and their affinity to Fe, Al, V, As, Ga and Cu ions. **Conclusions:** The CAS assay with different metal solutions in microtiter plates was found to be a rapid and easy-to-perform method to test culture supernatants of different strains for metallophores and their affinity to different ions. The screening of various strains showed that the siderophores differ in their metal binding specificity. Indeed we determined, that the metallophore of A. niger bind Cupper and Vanadium much more effectively than other strains.

Author Disclosure Block:

M. Mehnert: None. **G. Retamal M.:** None. **T. Heine:** None. **R. Schwabe:** None. **M. Schlömann:** None. **G. Levicán J.:** None. **D. Tischler:** None.

Poster Board Number:

FRIDAY-195

Publishing Title:

Microorganisms for Industrial Antimicrobial Biosurfactant Production, a Review

Author Block:

D. SOLAIMAN, R. Ashby; ERRC, ARS, USDA, Wyndmoor, PA

Abstract Body:

There is a demand in the consumer product industry to address the emerging antibiotic resistance issue especially with regard to the cleaning products. Natural products such as glycolipid biosurfactants with antimicrobial activity synthesized by microorganisms could provide environmentally friendly bioproducts to fill this industrial demand. Accordingly, researchers had developed and applied screening methods such as colorimetric plate assay and nucleic-acid-based metagenomic screening to identify microorganisms capable of producing biosurfactants. Various antimicrobial assay methods were then applied to assay the antimicrobial activity of these biosurfactants. Fermentation system is constantly refined to lower the production costs of these biomaterials to improve their commercial viability. In this review presentation, emphasis will be placed on the antimicrobial sophorolipid and rhamnolipid biosurfactants with regard to their fermentative production and antimicrobial property.

Author Disclosure Block:

D. Solaiman: None. **R. Ashby:** None.

Poster Board Number:

FRIDAY-196

Publishing Title:

Resistance to Antibiotics and Lead of Bacteria Isolated from Sediments of St. Clair River

Author Block:

R. Gismondi, M. Fenner, D. Patel, S. M. Tiquia-Arashiro; Univ. of Michigan-Dearborn, Dearborn, MI

Abstract Body:

Bacteria are generally the first organisms affected by the increased presence of toxic compounds in the aquatic environments including antibiotics and heavy metals. The purpose of this study is to determine natural susceptibility levels of bacterial isolates to Pb and antibiotics. Two enrichment experiments (LB and R2A broths) containing 10 g of sediments and increasing concentrations of Pb(NO3)2 were carried out to isolate Pb-resistant strains. Cells that grew on enrichments containing the highest Pb concentrations (1500 mg/L for LB and 1250 mg/L for R2A) were isolated and characterized for their antibiotic resistance using eight different antibiotics. The results showed that the majority of the LB strains were resistant to chloramphenicol (80% of the isolates), erythromycin (71%), novobiocin (100%), penicillin G (100%) and tetracycline (73%). For the R2A strains, the majority were resistant to erythromycin (98% of isolates), neomycin (81%), novobiocin (100%), and penicillin G (100%). However, LB strains showed sensitivity to streptomycin (98% of isolates) while the R2A strains showed sensitivity to both streptomycin (75%) and chloramphenicol (72%). In general, Pb-resistant strains isolated from Saint Clair River sediments showed natural resistance to a variety of antibiotics. This result suggests that the combined expression of antibiotic resistance and metal tolerance is caused by selection resulting from the metals present in the environment. Bioremediation requires organisms that are tolerant to an array of toxins. As such, Pb-resistant strains isolated in this study could be successful candidates for bioremediation of Pb-contaminated sites in a more economical way in comparison to current chemical remediation methods.

Author Disclosure Block:

R. Gismondi: None. **M. Fenner:** None. **D. Patel:** None. **S.M. Tiquia-Arashiro:** None.

Poster Board Number:

FRIDAY-197

Publishing Title:

Optimized Production Conditions of Thermpohilic CMCase by a Novel "*Bacillus* TLW-3" Strain, Isolated from Local Oven Ash of Karachi

Author Block:

T. K. Awan, W. -. -. -. Asad, -, S. A. -. -. Rasool; Karachi Univ., Karachi, Pakistan

Abstract Body:

Cellulases have been reported as the third largest single industrial enzyme worldwide being employed in textile industries, paper recycling, detergent industry and food processing industries. A growing interest in the cellulases is obvious because of their utility in transportation i.e. fuel production, the economy driver of a country. For the production of extensively diversified cellulases, microorganisms (both fungi and bacteria) have been extensively exploited. Varieties of ecological niches were explored for the isolation of cellulolytic microorganisms. However, there have been scarce report on the hot oven ash cellulolytic bacterial flora. In this regard the newly isolated 37 *Bacillus* strains ("*Bacillus* TLW-1 to *Bacillus* TLW-37") from the local hot oven ash were screened for CMCase (carboxymethyl cellulase) production ability by plate screening method. Accordingly, 26 isolates could produce CMCase and, were subjected to submerged fermentation. Crude extract was obtained and assayed for CMCase titer by DNS (dinitrosalicylic acid) method. "*Bacillus* TLW-3" that shown the highest IU/ml/min was selected for further study. The enzyme production conditions and growth conditions were optimized for the highest CMCase yield. The "*Bacillus* TLW-3" showed the highest growth and enzyme production at 50ºC, pH 7 and after 72 hour of incubation at 150rpm. Moreever, the organisms showed higher enzymatic yield when grown on complex defined media as compared to minimal salt media. Studies on the different carbon and nitrogen sources effect on enzyme production revealed that CMC (1%), yeast extract (0.5%) and peptone (1%) significantly increased the CMCase yield compared to other tested sources. Tween-20, Tween-80 and EDTA acted as the inhibitors for the enzyme production. Furthermore, enzyme production regulation is not influenced by catabolite repression. The present study holds the conviction that the reported organism could directly be applied for the thermophilic cellulase production.

Author Disclosure Block:

T.K. Awan: None. **W.-.-.-. Asad:** None. **S.A.-.-. Rasool:** None.

Poster Board Number:

FRIDAY-198

Publishing Title:

Optimization of Pectinase Production from Indigenously Isolated Yeast Strain Using Response Surface Methodology

Author Block:

A. Ahmed; Karachi Univ., Karachi, Pakistan

Abstract Body:

Pectinases are hydrolases that degrade pectin molecules present in primary cell-wall and middle lamella of fruits and vegetables. In fruit-juice production, the presence of pectin may cause problem during the extraction, filtration and clarification by creating turbidity and viscosity. Pectinases have long been used to increase the juice yield and reduce the turbidity and viscosity. Though, several organisms are able to produce pectinases including plants, filamentous fungi, bacteria and some yeasts. Yeasts have a great potential to produce industrial enzymes and they offer an alternative source. Yeasts have many advantages over filamentous-fungi as they are unicellular, growth is simple, growth medium does not require an inducer and they usually don't produce undesirable pectinesterases. This study was conducted to isolate, screen and optimize indigenously isolated yeast strains for pectinase production. Different samples were used for isolation of yeast strain. Almost 30 strains were isolated. These isolated strains as well as some strains from departmental culture collection were qualitatively and quantitatively screened for pectinase production. The qualitative screening was carried out on mineral salt agar medium using ruthenium red. Our results showed that 48 strains out of 127 strains were pectinolytic. These 48 strains were quantitatively screened by performing enzyme assays based on determination of reducing sugar (galacturonic acid) using dinitrosalicylic acid. One strain, AA15, producing significant titer of pectinase was selected for further studies. Response surface methodology was employed for the optimization of fermentation conditions. Initial pH, inoculum size and substrate concentration were found to be the significant factors using Plackett-Burman design.The optimum fermentation conditions were determined by using Box-Behnken design in RSM. Inoculum size 3%, substrate concentration 0.75% and the initial pH 5.3 were determined as optimum conditions. The strain yielded 0.250 IU/ml pectinase activity under optimized conditions. It provides a merit to study this strain further as, yeasts generally do not produce the pectin methyl esterase which results in release of toxic alcohols so they have advantage over filamentous fungi therefore, it is worthwhile to explore the potential of this strain.

Author Disclosure Block:

A. Ahmed: None.

Poster Board Number:

FRIDAY-199

Publishing Title:

Potential Use of Dehydrogenase Activity as a Marker of Microbial Activity in Lake St. Clair and St. Clair River Sediments

Author Block:

R. Gismondi, M. Fenner, D. Patel, A. Oest, N. Bowman, D. Azzopardi, A. Alsaffar, S. M. Tiquia-Arashiro; Univ. of Michigan-Dearborn, Dearborn, MI

Abstract Body:

Dehydrogenase activity based on the reduction of 2,3,5-triphenyltetrazolium chloride (TTC) to red-colored formazan (TPF), was used in this study as a method to determine microbial activity from river and lake sediments. Three sites were selected from St Claire River (SC1, SC2, SC3) and three from Lake St. Clair (LC1, LC2, LC3). Bacterial and fungal counts and physico-chemical assays were carried out over three consecutive seasons to characterize the microbial and phyico-chemical properties of the sediments. Incubated sediments containing TTC (1 ml) and distilled water (2.5 ml) were extracted with methanol and the absorbance (485 nm) was used to determine the sediments' dehydrogenase activity. Sediments from sites LC2 and SC2 had little activity (0.0007-0.031 ug/TPFg) throughout spring, summer and fall seasons, which also corresponded to low bacterial count at these sites. Site LC1 had high activity only in spring (0.936 ug/TPF g). Sediments from LC3, SC1 and SC3 showed higher activities compared to LC1, LC2 and SC2 in summer, spring and fall. Dehydrogenase activity was correlated with fungal count, TOC and pH of the sediments. High dehydrogenase activity was reported for SC1 and SC3 sediments, which correlated with sediments having the highest overall bacterial and fungal counts. This trend was not consistent with sediments collected in Fall (SC3) and Spring (SC1), because dehydrogenase activity then correlated only with fungal counts, not with bacterial counts. The data indicate that dehydrogenase activity is at best a weak indicator of microbial activity in Lake St. Clair and the St. Clair River sediments.

Author Disclosure Block:

R. Gismondi: None. **M. Fenner:** None. **D. Patel:** None. **A. Oest:** None. **N. Bowman:** None. **D. Azzopardi:** None. **A. Alsaffar:** None. **S.M. Tiquia-Arashiro:** None.

Poster Board Number:

FRIDAY-200

Publishing Title:

Polyhydroxyalkanoates Production by Native Strains Isolated from Northeast Mexico

Author Block:

J. Villarreal-Chiu[1], O. López-Ayala[1], M. Salinas Santander[2], M. Garza-González[1], E. Blanco-Gámez[1]; [1]Univ. Autónoma de Nuevo León, San Nicolas de los Garza, Mexico, [2]Univ. Autónoma de Coahuila, Saltillo, Mexico

Abstract Body:

Background: Polyhydroxyalkanoates (PHA) are biopolymers synthesized by microorganisms as reserve materials for carbon and energy when cells are exposed to inorganic nutrient limitation coupled with an excess of carbon source. The composition and properties of PHA depend on the metabolic routes present in the microorganism as well as the monomers that are supplied to the cell.**Methods:** An initial bioinformatic study was carried out using a set of amino acidic templates representing the four distinct classes of PHA synthases to estimate their distribution in bacteria. 60 native bacteria isolated from northeast Mexico were tested under nutritional stress conditions using phosphorus and nitrogen-depleted minimal media enriched with acetate as carbon source.**Results:** The distribution of PHA synthase genes over the sequenced bacterial database contained on NCBI reached nearly 26% (868 de 3260). Analysis of the flanking regions of each homologue suggested that most of PHA synthases of class II (92%), which is related to the production of medium-chain length PHA, are controlled by the HTH-type transcriptional regulator *acrR*. Expression of *acrR* is increased under general stress conditions. 49 out of 60 native bacteria tested were confirmed as PHA producers when supplied with acetate. Four representatives bacteria were tested with 10 different nutrient-depleted agro-industrial residues supplied as carbon and energy source. Results indicated that all 4 native bacteria were capable of producing PHA at times ranging from 6 to 13 days with diverse monomer compositions, including hydroxybutyric and hydroxyvalerate acids.**Conclusions:** It is important to find new ways to decrease the cost of PHA production for industrial purposes. The use of agrodindustrial residues as carbon source successfully produced hydroxybutyric-hydroxyvalerate co-polymer.

Author Disclosure Block:

J. Villarreal-Chiu: None. **O. López-Ayala:** None. **M. Salinas Santander:** None. **M. Garza-González:** None. **E. Blanco-Gámez:** None.

Poster Board Number:

FRIDAY-201

Publishing Title:

Production of Bioactive Secondary Metabolites from Alkaliphilic Actinomycetes Against Various Multi Drug Resistant (MDR) Pathogens

Author Block:

S. Aslam, I. Sajid; Univ. of the Punjab, Lahore, Pakistan, Lahore, Pakistan

Abstract Body:

Background: The search for novel therapeutic agents for use in the pharmaceutical industry is driven by the need to combat the increase in the incidence of infection due to antibiotic resistant pathogens. Among the producers of commercially important metabolites, actinomycetes isolated from extreme environment are of prime importance as prolific producers of novel antibiotics having diverse biological activities. The present study was designed to screen the alkaliphilic actinomycetes for their antimicrobial potential against MDR bacterial pathogens.**Methods:** In the present study, forty different actinomycetes were isolated from the water and mud samples collected from a saline Kallar Kahar lake. The isolates exhibit significant tolerance to alkaline conditions and grow well at pH 9-11. The morphological, biochemical and genetic characterization of these strains was performed to identify them. The selected strains were cultivated on small scale, the resulting culture broth was freeze dried and the residue was extracted with ethyl acetate. In biological screening the activity of the extracts was determined against MRSA, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *K. pnuemoniae*, *E. coli*, *Enterobacter* spp., and *Acinetobacter baumanii*. In chemical screening the crude extracts were analyzed by TLC and HPLC-MS.**Results:** The current study revealed that majority of the actinomycete strains (90%) was belonging to the genera *Streptomyces*. Many lake actinomycetes showing remarkable antimicrobial activities against MDR bacterial pathogens. Among selected strains, $KL_1$4, $KL_2$1, $KL_3$2, $KL_3$7, $KL_4$1, $KL_6$3, $KL_7$2, $KL_7$3, $KL_8$1, $KL_9$3, $KL_9$6, $KL_9$8 and KL_{11}6 were found to be most biologically active as they exhibited promising activity against all the test strains of multi drug resistant pathogens. In thin layer chromatographic analysis of the crude extracts, appearance of various coloured bands indicating a unique pattern of different bioactive metabolites. HPLC-UV chromatograms of each crude extract of the isolates showed a number of different peaks at various retention times, which was also an indication of the presence of many valuable antimicrobial compounds.**Conclusions:** Alkaliphilic actinomycetes are an important resource of bioactive compounds, and can prove to be a goldmine of novel secondary metabolites in drug discovery.

Author Disclosure Block:

S. Aslam: None. **I. Sajid:** None.

Poster Board Number:

FRIDAY-203

Publishing Title:

Recovery & Characterization of Polyhydroxybutyrate Biopolymer Produced by *Bacillus cereus* Isolate P83

Author Block:

N. Elsayed, K. Aboshanab, M. Yassien, N. Hassouna; Faculty of pharmacy- Ain shams univerisity, cairo, Egypt

Abstract Body:

Background: Polyhydroxyalkanoates (PHA) biopolymers offer a valuable alternative to synthetic plastics in our daily life. Also, they are biocompatible and biodegradable giving rise to different applications in medical field. This study aimed at testing PHA production using *Bacillus cereus* isolate P83 in a laboratory 14L fermentor, studying different approaches of recovery and characterization of produced polymer. **Methods:** PHA production was tested using mineral salt medium containing 0.7% corn oil and 0.1 g/L ammonium chloride as carbon and nitrogen sources, respectively at 28°C, 200 rpm, 1 vvm and under uncontrolled pH. Various approaches of recovery of PHA were investigated using sodium hypochlorite, chloroform, dispersion of both of them and sonication. These approaches were evaluated by testing amount of PHA produced, molecular weight of polymer and its polydisperisty value. Statistical analysis was done using Graph Pad instat program. Characterization was done using transmission electron microscope, gel permeation chromatography, thin layer chromatography, IR and NMR spectroscopy. Appropriate universal heterologous primers were designed and used for PCR amplification followed by DNA sequencing of genes coded for PHA synthase enzyme and R subunit. **Results:** About 49% PHA per dry weight was produced after 24 h of incubation. Recovery using chloroform resulted in higher molecular weight of polymer ($2.15*10^4$ g/mole) and lowest polydisperisity (1.15). Characterization techniques revealed polymer identity which is Polyhydroxybutyrate (PHB). Melting point of the polymer was 180 °C. IR analysis confirmed the presence of key functional groups namely OH group at 3448 cm^{-1}, CH group at 2923 cm^{-1} and carbonyl at 1735 cm^{-1}. NMR analysis showed the resonance absorbance of CH_3 at 1.91ppm, CH at 4.52 ppm and CH_2 at 4.52 ppm. Analysis of PHA synthase (PhaC) and PhaR subunit PCR products indicated class IV PHA synthase. **Conclusion:** *Bacillus cereus* isolate P83 is a candidate for commercial production of PHB polymer due to cheap carbon source, high growth rate, high production level and comparable molecular weight of polymer with low polydispersity value. The low polydisperisty value extends use of PHB polymer in biomedicine.

Author Disclosure Block:

N. Elsayed: None. **K. Aboshanab:** None. **M. Yassien:** None. **N. Hassouna:** None.

Poster Board Number:

FRIDAY-204

Publishing Title:

Screening for Linear Plasmids in Actinomycetes Isolated From the Great Salt Plains of Oklahoma

Author Block:

C. Cornell, M. K. Fakhr; The Univ. of Tulsa, Tulsa, OK

Abstract Body:

One of the unique features of actinomycetes, especially the genus *Streptomyces*, other than the presence of a linear chromosome, is the linear extrachromosomal material seen in many of those microorganisms. These linear plasmids have a size range of 12 kb to upward of 600 kb, and are often termed as mega-plasmids. While many of the genes involved in secondary metabolite production reside in clusters on the chromosome, several studies have identified biosynthetic clusters on the large linear plasmids that produce important secondary metabolites including antibiotics. Due to the large size of the extrachromosomal DNA, most plasmid isolation kits and traditional gel electrophoresis do not allow separation of the plasmid(s) and chromosome. Pulse Field Gel Electrophoresis (PFGE) can be used to separate DNA fragments up to several mega-bases. There is a lack of large scale plasmid screening studies done using PFGE, therefore we developed a protocol by using several existing methods. PFGE was used to screen 176 actionmycete isolates collected from the Great Salt Plains of Oklahoma for the presence of plasmids. To determine the topology of the smaller plasmids present, the plasmids were treated with S1 nuclease to linearize any circular plasmids and ran against an untreated sample for comparison using PFGE. From the 176 strains screened, 78 showed the presence of plasmids, about 44% of the total sample number. Several of the strains contained more than one plasmid accounting for a total of 109 separate plasmids. Ten of the samples showed extrachromosomal DNA larger than 200 kb falling into the category of mega-plasmids. Based on the subset of samples treated with S1 nuclease to determine topology, all plasmids examined appeared to be linear. In conclusion, PFGE facilitated the detection of plasmids in different actinomycete strains found in the unique terrestrial environment of the Great Salt Plains of Oklahoma, with the majority of the plasmids being linear ranging from 54.7 kb up to 400 kb. Further studies are currently underway to molecularly characterize the large linear plasmids through next generation sequencing. DNA sequence will help in molecularly characterizing these large plasmids and in identifying the prospective encoded genes of secondary metabolites production and/or stress adaptation.

Author Disclosure Block:

C. Cornell: None. **M.K. Fakhr:** None.

Poster Board Number:

FRIDAY-205

Publishing Title:

Systematic Analyses of Transcriptome and Translatome Revealed the Regulation of Energy Conservation Metabolism in *Clostridium ljungdahlii*

Author Block:

J-N. Kim, M. M. Al-Bassam, J. K. Jiu, K. Zengler; Univ. of California San Diego, La Jolla, CA

Abstract Body:

The homoacetogen *Clostridium ljungdahlii* is an anaerobic bacterium that ferments sugars as well as other organic compounds. Additionally, the organism grows on carbon dioxide (CO_2) /hydrogen (H_2) and carbon monoxide (CO) (i.e. syngas). Syngas fermentation makes *C. ljungdahlii* an interesting microbial production platform for the production of commodity chemicals from syngas. In order to harness the full biosynthetic potential, it is important to understand the regulatory networks that orchestrate energy conservation in *C. ljungdahlii* and their response to autotrophic and heterotrophic conditions. We thus have integrated genome-scale measurements of the transcriptome and translatome at nucleotide resolution of *C. ljungdahlii*. ChIP-exo and transcription start site profiling techniques were used to determine RNA polymerase binding sites and elucidate transcription units and detailed promoter architectures. Integrating RNA-seq and ribosome profiling data revealed the translational efficiency of genes that are involved in energy conservation metabolism such as the Wood-Ljungdahl pathway under autotropic growth conditions. Overall, the multiomics approach provides insight into the regulation of energy metabolism of *C. ljungdahlii* between autotropic and heterotropic growth. These findings will enable the development of *C. ljungdahlii* as a chassis organism for commodity chemical production from cheap and renewable sources.

Author Disclosure Block:

J. Kim: None. **M.M. Al-Bassam:** None. **J.K. Jiu:** None. **K. Zengler:** None.

Poster Board Number:

FRIDAY-206

Publishing Title:

The Preservation Challenge Test Method Using Pool Inoculum Compatible with ISO11930

Author Block:

S. Hamada, H. Araki, H. Anan, N. Shigemune, M. Hasumi; Kao Corp., Tochigi, Japan

Abstract Body:

Background: Different preservation challenge test methods are commonly available in the cosmetic industry. As test inoculum, either pure or mixed cultures may be used. A challenge test method using pure culture is in the International Standard ISO11930. Pure culture challenge, although more time-consuming, will yield specific data on each microorganism employed. Mixed-culture challenge, on the other hand, can be used to obtain rapid pass-or-fail decisions on preservative adequacy and reduce the workload. However, antagonism among organisms may occur. In this study, we studied the antagonism among bacterial test strains to balance out the difference between pure and mixed cultures. With pool inoculum we constructed the bacterial challenge test method which is compatible with ISO11930 method.**Methods:**As test strains, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli* which are used in ISO 11930, and *Serratia marcescens*, *Burkholderia cepacia*, *Klebsiella pneumonia* which are known as contaminants of cosmetics, were used. The cell number in single and pooled inoculum was adjusted to 10^8 CFU/ml and viable bacteria were counted every few hours after preparation to confirm whether antagonism occurred or not. The challenge tests were conducted on 109 products including skin care, hair care and personal care products. The initial inoculum of approximately 10^8 CFU/ml was inoculated into 20g aliquots of each product formulation. At 7, 14 and 28 days post-inoculation, 0.1g aliquots were withdrawn from the inoculated samples to determine the recovery of viable test strains. Based on the results, the criterion of preservative efficacy compatible with ISO11930 was determined.**Results:**The viable cells in pooled cell suspension did not decrease within 24 hours after preparation. It suggested that pooled strains did not compete to kill each other. The log reduction observed in testing by our method tended to be more rapid than by ISO method, which might be due to antagonism. Based on the test results, we found that 4log reduction of pooled strains within 7day by our method met the preservative efficacy of the criterion B in ISO11930, 3log reduction of all 3 single strains within 14days.**Conclusions:**We investigated challenge test method using bacterial pool inoculum. With our method, the workload can be reduced and we can evaluate the product has preservative efficacy ISO11930 requires.

Author Disclosure Block:

S. Hamada: None. **H. Araki:** None. **H. Anan:** None. **N. Shigemune:** None. **M. Hasumi:** None.

Poster Board Number:

FRIDAY-207

Publishing Title:

Temperature Effects on Distributed Metabolisms of Partial Nitritation/Anammox (Pn/A) Reactor

Author Block:

A. S. Bhattacharjee[1], S. Wu[1], B. Dutilh[2], R. Goel[1]; [1]Univ. of Utah, Salt Lake City, UT, [2]Utrecht Univ., Utrecht, Netherlands

Abstract Body:

Background: Mitigation of Nitrogen (N) from wastewater is necessary because of the environmental impact of ammonia leading to eutrophication in the receiving bodies. The combination of partial nitritation & anammox (PN/A) process brought in an innovative biotechnological revolution in energy-efficient process with reduced greenhouse gas emissions for N removal from wastewater. PN process provides nitrite, which mainly acts as an electron acceptor for Anammox (AMX) process, wherein ammonia is oxidized directly to N_2 gas. Most PN/A reactors are maintained at mesophilic temperatures, despite the fact that AMX bacteria in natural ecosystems have been found to thrive below 10^{o}C, indicating the possibility of PN-AMX to be metabolically active even at low temperatures. In this study, we investigated the temperature effect by subjecting a pilot scale PN/A reactor to different gradients, as specific metabolic pathways underlying remains poorly understood. **Method:** To gain insight into the key interactions between PN-AMX, metagenome & metatranscriptomes were generated from biomass collected from a 9-L PN/A reactor, fed with reject water from anaerobically digested sludge. For investigating the temperature effect on PN/A metabolism, it was exposed to temperature gradients of 35, 21 & 13°C. Biomass was collected at each gradient post acclimatization period, wherein RNA & DNA were extracted to generate high throughput sequencing data. Briefly, DNA sequences were assembled into contigs using Omega & subsequently binned into genomes. RNA-Seq data were assembled into transcripts using trinity. **Results:** 11 near-complete genomes were recovered, 3 of which, *Planctomycete* sps., *Anaerolinea* sps. & *Ignavibacterium* sps. were estimated to be >90% complete based on single-copy gene analysis. Furthermore, from the transcriptomes generated we observed a significant down regulation of transcripts in case of low temperatures along with a 3 fold decrease in abundance of genes being expressed between gradients of 35 to 13°C. **Conclusions:** Low temperatures were found to drastically affect the distributed metabolism of the PN & AMX. Furthermore, metabolic pathways inferred from genome, & transcriptome annotations indicated that reactor organisms were metabolically versatile & exhibited novel opportunities for metabolic-cooperation**.**

Author Disclosure Block:

A.S. Bhattacharjee: None. **S. Wu:** None. **B. Dutilh:** None. **R. Goel:** None.

Poster Board Number:

FRIDAY-208

Publishing Title:

Using Microbial Populations in Concrete as Bio Indicators of Alkali-Silica Reaction

Author Block:

A. Treglia, J. Maresca; Univ. of Delaware, Newark, DE

Abstract Body:

Concrete is one of the world's most widely-used building materials and is the most used material in the world in terms of volume. In many areas, the alkali-silica reaction (ASR) damages concrete, reducing its lifespan. This reaction causes internal cracking in concrete that leads to deteriorating structural health and may result in structural failure. There are no current methods to detect ASR at an early stage before the reaction has occurred, but there are some options for mitigation, including limiting the alkali content of the concrete mix, and limiting or prohibiting the use of reactive aggregates. Here, we present a method for extraction of DNA directly from concrete, resulting in 9-900 ng DNA per g of concrete. Further, we use 16S amplicon sequencing to show that samples collected from inside concrete may have more than 2000 bacterial species, primarily Actinobacteria, and these species are not uniformly distributed inside the samples. We hypothesize that the changes in the chemical and physical properties of concrete over time will be reflected in the microbial populations, and use a three-year time series to test this hypothesis. To assess changes in microbial populations in ASR-affected concrete as damage progresses, we prepared concrete test cylinders using highly reactive materials provided by the Delaware Department of Transportation, and prepared parallel cylinders using the standard DelDOT ASR mitigation procedure, which replaces 50% of the cement powder with fly ash. These cylinders were placed outside to weather in the spring of 2013; since then, 1 cylinder from each series has been brought inside every 6 weeks for analysis of microbial population and assessment of ASR damage. This work demonstrates the feasibility of identifying microbes that can be used as bio-indicators for early diagnosis of structural problems in concrete.

Author Disclosure Block:

A. Treglia: None. **J. Maresca:** None.

Poster Board Number:

FRIDAY-209

Publishing Title:

Evolution of Bacteriophage T7 on Genetic Codes Expanded with Nonstandard 21st Amino Acids

Author Block:

M. J. Hammerling, D. E. Deatherage, **J. E. Barrick**; The Univ. of Texas at Austin, Austin, TX

Abstract Body:

The genetic code for translating DNA information in genomes into protein sequences has remained fixed for most of the history of life on Earth. Thus, while limited computational analysis of the organization of the code has been possible, it has been difficult to determine the extent to which the chemical alphabet consisting of the standard 20 amino acids is optimal for biochemistry and evolution versus an alternative set of amino acid side chains. We have begun to address this fundamental question by taking advantage of recently developed synthetic biology tools that enable the amber stop codon (TAG) to be efficiently recoded to incorporate a variety of nonstandard amino acids (nsAAs). We passaged 576 populations of a hypermutator T7 bacteriophage on nonevolving *Escherichia coli* hosts with the amber stop codon recoded to 3-iodotyrosine, 3-nitrotyrosine, 4-azidophenylalanine, 5-hydroxytryptophan, *o*-(2-nitrobenzyl)-tyrosine, or tyrosine (as a canonical control). After 1000 hours of continuous evolution as plaques in soft agar, we sequenced all of these populations to compare how their genomes and proteomes evolved with each different genetic code. In the resulting dataset of >100,000 amino acid substitutions and >1,000 amber substitutions, we are able to quantify the relative tolerance for neutral substitutions to each nsAA within the T7 proteome and also to find specific sites in proteins with statistical signatures that indicate that some nsAA substitutions improve phage fitness in unique ways. In particular, we observe sites in the T7 RNA polymerase and tail fiber proteins where substitutions of specific nsAAs occurred in parallel across many different populations. Our results illustrate how expanding the genetic code increases the evolvability of an organism, both by making new amino acid substitutions possible by converting a nonsense codon to a sense codon and by adding the potential for encoding proteins with a 21st amino acid that has a novel side chain.

Author Disclosure Block:

M.J. Hammerling: None. **D.E. Deatherage:** None. **J.E. Barrick:** None.

Poster Board Number:

FRIDAY-210

Publishing Title:

Methanogenic Paraffin Biodegradation Proceeds Via Alkane Addition To Fumarate By "*smithella*" Spp. Mediated By A Syntrophic Coupling With Hydrogenotrophic Methanogens

Author Block:

C. R. Marks[1], B. Wawrik[1], I. A. Davidova[1], M. J. McInerney[1], S. Pruitt[2], K. E. Duncan[1], J. M. Suflita[1], A. V. Callaghan[1]; [1]Univ. of Oklahoma, Norman, OK, [2]Oglethorpe Univ., Atlanta, GA

Abstract Body:

Background: Anaerobic biodegradation of recalcitrant and water-insoluble substrates, such as paraffins, poses unique metabolic challenges for microorganisms. We investigated the activation mechanism(s) and requisite organisms involved in the methanogenic mineralization of long chain *n*-paraffins.**Methods:**An anaerobic consortium that mineralizes long chain *n*-alkanes (C_{28}-C_{50}) under methanogenic conditions was enriched from San Diego Bay sediment. The phylogenetic composition and metabolic potential of the microbial community were interrogated via metagenomics and comparative analyses of binned draft *"Smithella"* genomes. Alkane activation by addition to fumarate was investigated via RT-PCR analysis of the catalytic subunit of alkylsuccinate synthase (*assA*).**Results:**Analysis of 16S rRNA genes indicated the dominance of Syntrophobacterales and Methanomicrobiales. Draft genomes were assembled of dominant community members belonging to the bacterial genus *Smithella* and the archaeal genera *Methanoculleus* and *Methanosaeta*. Five contigs encoding *assA* genotypes were detected in the metagenome, and mRNA transcripts for these genes, including a homolog binned within the *"Smithella"* sp. SDB genome scaffold, were detected via RT-PCR. Genomic analysis revealed a membrane-bound FeS oxidoreductase and putative formate dehydrogenases and hydrogenases within the "*Smithella*" sp. SDB scaffold.**Conclusions:**Metabolic reconstruction, transcript analyses, and comparison with genome scaffolds of uncultivated *n*-alkane degrading *"Smithella"* spp. suggest that syntrophically growing *"Smithella"* spp. activate *n*-paraffins by 'fumarate addition' and may achieve reverse electron transfer by coupling the reoxidation of ETF_{red} to a membrane-bound FeS oxidoreductase functioning as an ETF:menaquinone oxidoreductase. Subsequent electron transfer could proceed via a periplasmic formate dehydrogenase and/or hydrogenase, allowing energetic coupling to hydrogenotrophic methanogens such as *Methanoculleus*. These data provide fundamental insight into the energy conservation mechanisms that regulate interspecies interactions pertinent to methanogenic alkane mineralization.

Author Disclosure Block:

C.R. Marks: None. **B. Wawrik:** None. **I.A. Davidova:** None. **M.J. McInerney:** None. **S. Pruitt:** None. **K.E. Duncan:** None. **J.M. Suflita:** None. **A.V. Callaghan:** None.

Poster Board Number:

FRIDAY-211

Publishing Title:

A Long-chain Paraffin-degrading Consortium and the Potential for Steel Corrosion Under Defined Redox Conditions

Author Block:

I. A. Davidova, R. Liang, K. Duncan, B. Wawrik, J. Suflita; The Univ. of Oklahoma, Norman, OK

Abstract Body:

An archaeal and bacterial consortium was enriched for almost a decade in the absence of sulfate for its ability to mineralize large molecular weight paraffin hydrocarbons ($\geq C_{25}H_{52}$) to stoiciometrically expected amounts of methane. Molecular analysis of the consortium revealed predominant deltaproteobacterial families, including the *Syntrophaceae* that are incapable of reducing sulfate, as well as typical sulfate reducing taxa including, *Desulfobacteraceae, Desulfovibrionacea and Desulfobulbaceae.* Upon amendment, the consortium was able to reduce sulfate when $C_{28}H_{58}$ served as an electron donor. After three transfers, sulfate reduction far exceeded methanogenesis in either the absence or presence of the paraffin. The corrosivity of the consortium to carbon steel was evaluated by measurement of coupon weight loss, dissolved iron concentration and profilometry as a function of both electron donor (i.e. elemental iron or paraffin) and acceptor (sulfate) status. The mild steel corrosion rate increased from 1.2 ± 0.3 mpy (milli-inch per year) up to 9.6 ± 0.8 mpy when sulfate was available to the microorganisms. Other measures of corrosion were similarly increased in the presence of sulfate. When cultivated with iron as a potential electron donor, sequencing of 16S rRNA gene amplicons revealed that 69.9% of bacterial reads were affiliated with *Desulfobulbus* and 15.6% were classified as *Desulfovibrio.* Apparently, sulfate-reducing bacteria were capable of direct electron withdrawal from iron and to catalyze corrosion in the absence of any organic substrate. Efforts are underway to isolate the requisite organism(s).

Author Disclosure Block:

I.A. Davidova: None. **R. Liang:** None. **K. Duncan:** None. **B. Wawrik:** None. **J. Suflita:** None.

Poster Board Number:

FRIDAY-212

Publishing Title:

High Frequency of *Thermodesulfovibrio* spp. and *Anaerolineaceae* in Association with *Methanoculleus* spp. in a Long-Term Incubation of *n*-Alkanes-Degrading Methanogenic Enrichment Culture

Author Block:

B. Liang[1], B-Z. Mu[1], **J-D. Gu**[2]; [1]East China Univ. of Sci. and Technology, Shanghai, China, [2]The Univ. of Hong Kong, Hong Kong, China

Abstract Body:

Background: Alkanes are quantitatively the most significant components of petroleum hydrocarbons and they were considered hard-biodegradable in the absence of oxygen, nitrate or sulphate in petroleum reservoirs for a long time. Stable consotium obtained through enrichment culturing with long-term stability for methanogenic alkane degradation can eliminate the inactive members to larege extent so that the essential ones can be promoted. **Methods**: a methanogenic alkanes degrading consortium was established after enrichment culturing and long-term of incubation amended with mixture of n-alkanes as the sole sources of carbon and energy. The succession of microbial community and potential functional genes were analyzed using PCR and clone libraries coupling with quantitative PCR analysis in this study. Both degradation intermediates and product methane were also quantified. **Results**: A net increase of methane as the end product was detected in the headspace of the alkanes amendment enrichment cultures through hydrogenotrophic methanogenesis pathway; degradation intermediate metabolites including octadecanoate, hexadecanoate, isocaprylate, butyrate, isobutyrate, propionate, acetate and formate were measured in the liquid cultures. The composition of microbial community shifted through the enrichment transfer and a stable methanogenic alkanes-degrading microbial consortium was formed. **Conclusions**:Bacterial and archaeal 16S rRNA clone libraries and *mcrA* functional gene clone library analysis showed that bacteria of *Thermodesulfovibrio* (49.4%) and *Anaerolineaceae* (33.3%) syntrophically cooperated with the archaeal *Methanoculleus* (100%) as the dominant members performing the alkane degradation and methanogenesis. The presence of *assA* functional genes encoding the alkylsuccinate synthase α-subunit indicated that fumarate addition mechanism should be involved in the initial activation of alkanes.

Author Disclosure Block:

B. Liang: None. **B. Mu:** None. **J. Gu:** None.

Poster Board Number:

FRIDAY-213

Publishing Title:

Microbial Reduction of Co_2 and the Dominant Pathway of Methanogenesis in Production Water from High Temperature Oil Reservoirs Amended with C^{13} Labelled Bicarbonate

Author Block:

G-C. Yang[1], J-D. Gu[2], **B-Z. Mu**[1]; [1]East China Univ. of Sci. and Technology, Shanghai, China, [2]The Univ. of Hong Kong, Hong Kong, China

Abstract Body:

Background: The oil reservoirs are special ecosystem, which are natural anaerobic bioreactor with the potential ability to convert CO2 to methane with unique microorganisms. The objectives of this study were to assess the potential of bioconversion of CO2 to methane with indigenous microorganisms from oil reservoirs under microcosm conditions.**Methods:** The microcosm study was conducted with amendment of different concentrations of C13 labelled bicarbonate (0~90 mM) and ethanol to investigate microbial CO2 reduction to methane using the production water from high temperature oil reservoirs as inoculum.**Results:** Higher amounts of methane were detected in the treatments with higher bicarbonate addition, but little methane in the treatment without bicarbonate, indicating that bicarbonate was an important substrate for methanogenesis in the testing systems. The stable isotopic data showed that bicarbonate were converted to methane by methnogens through the CO2-reducing methanogenesis. The bicarbonate and ethanol addition impacted the microbial community differently. The dominant bacterial group in the initial inoculum and control treatment was Thermodesulfovibrio, but the majority in the treatments of bicarbonate additions was Synergistetes. The prevalent archaea also changed from Methanosarcinales in the inoculum to Methanobacteriales in all the amended treatments. **Conclusions:** The results showed that the dominant pathway for ethanol-driven CO_2 fixation in the microcosms was through the conversion of ethanol to acetate linked with syntrophic acetate oxidation and CO_2-reducing methanogenesis. These results suggest microbial CO_2 conversion and alternative biochemical pathways of methanogenesis in the presence of bicarbonate and ethanol in oil reservoirs.

Author Disclosure Block:

G. Yang: None. **J. Gu:** None. **B. Mu:** None.

Poster Board Number:

FRIDAY-214

Publishing Title:

Metagenomic Characterization of Soil Microbiota in Polluted Mangrove Swamp Using Bioinformatic Approach

Author Block:

C. C. NWANKWO, G. C. OKPOKWASILI, C. J. OGUGBUE; Univ. OF PORT HARCOURT, PORT HARCOURT, Nigeria

Abstract Body:

The quest for insights into soil microbial diversity using the metagenomics approach has emerged as a veritable tool for ecological study since majority of the microbes cannot be cultured in the laboratory using traditional techniques. Microbial population studies in the crude oil polluted Niger Delta ecosystem have relied mainly on traditional techniques rather than the metagenomics approach. In this study, a population-based understanding of the ecology of bioremediation was undertaken by investigating the diversity of microbial population and the patterns of their responses to hydrocarbon pollution and interactions in the mangrove swamp. Taxonomic study of three georeferenced crude oil contaminated swamps with known pollution history(Cawthornne Channel II, Bodo West and Bille) and three pristine sites with no pollution history were studied using Next Generation Sequencing technique to determine the nucleotide sequences of all microorganisms present in the swamp sample using the Genome sequencer 454 or Illumina, data sets generated were analyzed using bioinformatics. Results show that the polluted swamps had 23 bacterial classes whereas, 20 bacterial classes in the unpolluted swamp. In the polluted swamp samples, 6 classes had relative abundance greater than 2%; No-hits or potential novel bacteria (49.08%); Beta proteobacteria (30.78%); Gamma Proteobacteria (4.9 %); Uncultured bacteria (4.85%); Actinobacteria (3.29%) and Gamma proteobacteria (2.72%). On the other hand, 6 bacterial classes obtained in unpolluted swamps had a relative abundance greater than 2%: Bacilli (25.5%), No hits (24.3%), Beta proteobacteria (16.12%), Uncultured bacteria (12.83%), Actinobacteria (11.34%) and Alpha-proteobacteria (2.83%). Hierarchical clustering analysis based on overall patterns of variation showed distinct microbial communities in relation to geographical locations and this spatial species distribution was attributed to the monitored geochemical parameters of polluted swamps. The findings of this study have established that geochemical parameters influence the frequency, variability and microbial compositions in the different crude oil contaminated and pristine sites studied. Effective bioremediation strategies in the mangrove ecosystem will depend on the knowledge and manipulation of these geochemical parameters.

Author Disclosure Block:

C.C. Nwankwo: None. **G.C. Okpokwasili:** None. **C.J. Ogugbue:** None.

Poster Board Number:

FRIDAY-215

Publishing Title:

Population Dynamics And Community Composition Of Ammonia Oxidizers And Relationships With Nitrification Rates In Salt Marshes After The Macondo Oil Spill

Author Block:

A. E. Bernhard[1], R. Sheffer[1], J. Marton[2], B. Roberts[2], A. Giblin[3]; [1]Connecticut Coll., New London, CT, [2]Louisiana Universities Marine Consortium, Chauvin, LA, [3]The Ecosystems Ctr., Woods Hole, MA

Abstract Body:

The recent oil spill in the Gulf of Mexico had significant effects on microbial communities in the Gulf, but impacts on nitrifying communities in adjacent salt marshes has not been investigated. We studied persistent effects of oil on ammonia-oxidizing archaeal (AOA) and bacterial (AOB) communities and their relationship to nitrification rates and soil properties in Louisiana marshes impacted by the Deepwater Horizon oil spill. Sediments were collected at oiled and unoiled sites from Louisiana coastal marshes in July 2012, two years after the spill, and analyzed for community differences based on ammonia monooxygenase genes (*amo*A). TRFLP analysis of bacterial and archaeal *amo*A genes and DNA sequence analyses revealed significantly different AOA and AOB communities among the three regions, but few differences were found between oiled and unoiled sites. Community composition of nitrifiers was best explained by differences in soil moisture and nitrogen content. Despite the lack of significant oil effects on overall community composition, we identified differences in correlations of individual populations with potential nitrification rates between oiled and unoiled sites that help explain previously published correlation patterns. Our results suggest that exposure to oil, even two years post-spill, has led to subtle changes in population dynamics. How, or if, these changes may impact ecosystem function in the marshes, however, remains uncertain.

Author Disclosure Block:

A.E. Bernhard: None. **R. Sheffer:** None. **J. Marton:** None. **B. Roberts:** None. **A. Giblin:** None.

Poster Board Number:

FRIDAY-216

Publishing Title:

Dominance of the Genus, *Bacillus* in Soil Spiked with Polycyclic Aromatic Hydrocarbons

Author Block:

H. M. RAJI[1], J. B. AMEH[1], S. A. ADO[1], S. E. YAKUBU[1], G. WEBSTER[2], A. J. WEIGHTMAN[2]; [1]AHMADU BELLO Univ., ZARIA, ZARIA, Nigeria, [2]CARDIFF Univ., CARDIFF, United Kingdom

Abstract Body:

Soils contaminated with petroleum products are usually inhabited by bacteria and fungi adapted to the use of these hydrocarbons for their metabolic needs. Polycyclic aromatic hydrocarbons (PAHs) have the potential to be toxic and carcinogenic to living organisms, and they are persistent pollutants in soils, sediments and the aquatic environment. In this study, soil was obtained from a site close to a stream receiving petroleum refinery effluent, and spiked with PAHs. The possible effect of these hydrocarbons on the diversity of bacteria in the soil sampled was determined using phylogenetics and Shannon diversity index. Genomic DNA was extracted from soil at two depths from the site and the bacterial 16S rRNA gene analysed by PCR-DGGE, prominent bands were excised and sequenced. The Shannon diversity index was determined based on the presence and number of prominent bands on the DGGE profile. Similarity searches on the NCBI database revealed that all the 16S rRNA sequences from the study site were closely related to the genus, *Bacillus* (96%-100%). *Bacillus* species have been known to degrade many pollutants in the environment including PAHs, however there is no apparent reason for the selective dominance of this genus in the soil sampled. The soils spiked with PAHs had more prominent bands on DGGE gel than those samples not spiked with the PAHs, and they also produced better PCR amplicons. The soil sampled from a depth of up to 40cm had slightly more bacterial species than the soil sampled between 17 - 20 cm, with respect to the PAH treatments, the sample spiked with chrysene had significantly more bacterial species compared to the control and the soils spiked with either phenanthrene or benzo[a]pyrene.**Methods:**Extraction of genomic DNA, PCR-DGGE analysis of 16S rRNA gene, Determination of Shannon diversity index, Phylogenetic analysis of 16S rRNA sequences**Results:**The genus, *Bacillus* was the only phylum identified in the site based on similarity searches on the NCBI website. Majority of the 16S rRNA sequences identified were in the soil spiked with chrysene. The Shannon diversity index was found to be 2.4424**Conclusion:**The presence of pollutants such as PAHs in the soil could affect the diversity of bacterial species in such environments.

Author Disclosure Block:

H.M. Raji: None. **J.B. Ameh:** None. **S.A. Ado:** None. **S.E. Yakubu:** None. **G. Webster:** None. **A.J. Weightman:** None.

Poster Board Number:

FRIDAY-217

Publishing Title:

Microbial Response to Crude Oil Contamination of Freshwater Stream

Author Block:

J. A. SHENGE; Univ. of Ibadan, Ibadan, Nigeria

Abstract Body:

The impact of crude oil contamination emanating from oil exploration activities in Nigeria is devastating, affecting mostly aquatic lives, however, the effects on fish and other large aquatic forms are frequently studied, while the microbial population are less studied hence, underrepresented. We conducted microbial examination of freshwater samples in culture before and after contamination with crude oil and monitored for four weeks.Crude oil-free freshwater sample (temperature 26^{o} C; pH 7.0) was collected from upstream and downstream ends of Gbelede stream located at the University of Ibadan. Bacterial isolation was done using a Plate Count Agar and modified nutrient agar enriched with 1ml of crude oil to encourage the isolation of petroleum hydrocarbon degraders, at a dilution factor of 10^{-1} to 10^{-6} , total number of colonies were expressed as colony forming units per ml (Cfu/ml). Thereafter, stream was impacted with 500ml of Forcados crude oil obtained from Shell Petroleum Development Company, collection of contaminated sample was made daily at two hours interval, four times a day, for four weeks.Six bacterial species (*Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Enterococcus faecalis, Bacillus subtilis, and Streptococcus sp*) were isolated from uncontaminated freshwater samples (control). Four (66.6%); *Staphylococcus aureus, Escherichia coli*; *Enterococcus faecalis* and *Streptococcus sp,* were eliminated at 2hr (1.2m) after contamination, microbial population increased at 4hr, 6hr, and 8hrs at distances of 2.4m, 3.6m and 4.8m respectively. 2 (33.3%); *Pseudomonas aeruginosa and Bacillus subtilis* were proliferating suggesting that *P. aeruginosa and B. subtilis* are`petroleum hydrocarbon utilizers.Our results show that microbial species of a freshwater decreases with crude oil contamination, while oil utilizers among the species thrive under same condition. This study has pointed out the need for regular microbial examination and environmental impact assessment of freshwater, to understand microbial dynamics, preserve their population and prevent environmental degradation .This may help to identify hydrocarbon utilizers, their ability to degrade crude oil and exact microbes to introduce to utilize crude oil and clean up polluted freshwater environment.

Author Disclosure Block:

J.A. Shenge: None.

Poster Board Number:

FRIDAY-218

Publishing Title:

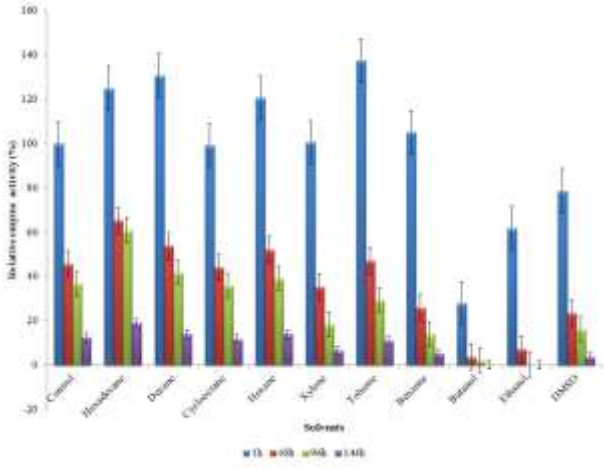
Purification and Characterization of a Solvent, Surfactant and Oxidizing Agent Tolerant Protease from *Bacillus cereus* Isolated from the Gulf of Khambhat, India

Author Block:

K. N. Shah[1], K. H. Mody[2]; [1]Gujarat Energy Res. and Management Inst., Gandhinagar, India, [2]Central Salt and Marine Chemicals Res. Inst., Bhavnagar, India

Abstract Body:

Twenty-eight organic solvent tolerant bacteria were isolated from crude oil contaminated samples, out of which, AK1871 isolate produced a solvent, detergent and oxidizing agent tolerant serine alkaline protease. Based on the morphological and biochemical characteristics, FAME analysis as well as 16S rRNA gene sequence, the isolate is identified as *Bacillus cereus*. A 58-fold purification of protease was achieved by a three-step purification procedure. This protease is active over a broad range of pH (6.0-9.0, optimum at 8.0); and temperature from 40 °C to 70 °C (optimum at 60 °C). Li^{+}, Ba^{2+}, K^{+}, Mg^{2+} and Mn^{2+} did not affect, while heavy metals like Cr^{3+}, Hg^{2+} and Cu^{2+} inactivated the enzyme. It is stable in the presence of non-ionic detergents (Triton X-100 and Tween 80), and oxidizing and bleaching agents (hydrogen peroxide). The protease exhibited noteworthy stability and activation in the presence of organic solvents with log *P* values equal to or more than 2.0. This protease could be used in detergent formulations, enzymatic peptide synthesis, biotransformation reactions and in the formulation of antifouling agent.Keywords: Organic solvent stable bacteria, *Bacillus cereus*, Organic solvent stable protease, Serine alkaline protease.



Author Disclosure Block:

K.N. Shah: None. **K.H. Mody:** None.

Poster Board Number:

FRIDAY-219

Publishing Title:

Microbial Community Structure in Mining and Drilling Impacted Appalachian Stream Sediments

Author Block:

L. J. Bird[1], T. Kondratyuk[2], K. H. Nealson[1], J. F. Stolz[2]; [1]Univ. of Southern California, Los Angeles, CA, [2]Duquesne Univ., Pittsburgh, PA

Abstract Body:

Drilling for natural gas has increased in the Appalachian region in recent years, particularly in West Virginia and Pennsylvania. Along with runoff from coal mining in the region, this boom in fossil fuel extraction activity has the ability to impact local waterways through small leaks and accidental spills of drilling fluids. We hypothesize that inputs from fossil fuel activity into nearby streams may impact the microbial community structure of freshwater sediments in the region. We used 16S rDNA sequencing to conduct microbial community structure analysis of 75 sites in West Virginia and Pennsylvania in the fossil fuel extraction zones. The sites are divided into un-impacted, impacted by mine drainage, impacted by drilling activity, or impacted by both mining and drilling. Sequencing results were combined with a variety of water quality parameters using correspondence analysis to determine the correlation between community differences among the collected sediments and water pH, ion concentrations, metal concentrations, and site history. We here report the correlations between community structure and diversity and aquatic chemistry parameters.

Author Disclosure Block:

L.J. Bird: None. **T. Kondratyuk:** None. **K.H. Nealson:** None. **J.F. Stolz:** None.

Poster Board Number:

FRIDAY-220

Publishing Title:

Culture Dependent Analysis of Bacterial Diversity Associated with Processed Salads

Author Block:

A. Bano; Univ. of the punjab, lahore, Pakistan

Abstract Body:

The main objective of the present study was to evaluate the biosafety of the raw-eaten fresh salad by exploring the general microbial diversity. For this purpose, more than hundred bacterial strains were isolated from the fresh salad of different localities in Lahore, Pakistan, by using variety of selective and enriched media. 16S rRNA gene sequencing showed the presence of bacterial strains belong to the genera of *Bacillus, Staphylococcus, Proteus, Macrococcus, Haemophilus, Escherichia, Citrobacter, Enterobacter, Salmonella, Shigella, Klebsiella* and *Acinetobacter.* Antibiotic susceptibility pattern against different antibiotics showed that majority of the strains were resistant against amoxicillin and ampicillin. Strains were also evaluated for biofilm formation, swarming motility, slime production and virulence inhibition. Maximum biofilm production in monocultures was observed by the strains *B. cereus* C35, *M. caseolyticus* C96, *S. saprophyticus* Ms99, *B. pumilis* C49, *K. pneumonia* Mc910. In the presence of carbon sources, *S. saprophyticus* Ms99 and *M. caseolyticus* C96 showed significant biofilm formation at 1 % and 3 % fructose, over control. Similarly, *H. influenza* C410 gave promising results at 3 % lactose. *P. mirabilis* L21 and *P. mirabilis* B33 were strongly positive for swarming motility; whereas *En. cloacae* E47, *B. cytotoxicus* B33, and *P. mirabilis* E11 were intermediate for slime production. In conclusion, fresh salads from different localities of Lahore were inhabited by different bacterial genera. Human potential pathogen like *E. coli* was isolated from salad which indicated fecal contamination. Some potential human pathogens such as *En. cloacae, K. pneumoniaea, B. cereus, S. dysentriae, H. influenza, S. saprophyticus* were also identified that make the biosafety of salad sources questionable. They can cause harmful health effects in their consumers.

Author Disclosure Block:

A. Bano: None.

Poster Board Number:

FRIDAY-222

Publishing Title:

Microbial Characterization of Pombe Samples Implicated in 75 Deaths in Mozambique, 2015

Author Block:

J. L. Brzezinski; United States Food and Drug Admin., Cincinnati, OH

Abstract Body:

In January, 2015, at least 75 people died and 177 were hospitalized following the consumption of a traditional beer called pombe in the village of Chitima in the Tete province of Mozambique. The symptoms included diarrhea, muscle aches, vertigo, nausea, dizziness, convulsions, aggression, agitation, and hallucinations. Deaths were preceded by coma and cardiovascular arrest. The cause of the illnesses and deaths were unknown. During the course of the investigation into this event, samples of the pombe and corn flour were obtained, and subjected to a battery of chemical and microbiological testing. The pombe and corn flour were enriched in trypticase soy broth and plated to a variety of selective and non-selective media, yielding a variety of bacteria and fungi, including *Bacillus, Pediococcus, Candida, Enterobacter* and *Rhizopus* species. After the identification of the bacterial toxin, bongkrekic acid, in the pombe and corn flour samples, targeted analysis revealed the presence of *Burkholderia gladioli*, the organism which produces this toxin, in the corn flour starting material. The symptoms of bongkrekic acid poisoning are consistent with those observed in the sickened patients. The identification of this organism was confirmed using 16S rDNA sequencing, and *in vitro* toxin production experiments determined that this strain is capable of producing bongkrekic acid. Taken together, these results suggest that the deaths in this case were caused by fatal bongkrekic acid intoxication, caused by contamination of the corn flour with *B. gladioli*. Foodborne bongkrekic acid poisoning had previously been reported in both China and Indonesia; this is the first reported incidence of bongkrekic acid poisoning in Africa.

Author Disclosure Block:

J.L. Brzezinski: None.

Poster Board Number:

FRIDAY-223

Publishing Title:

Analysis of Aflatoxin Content of Groundnut Cakes Sold in Ekiti State, Nigeria

Author Block:

R. I. Aboloma, Fajilade T.O and Oyebode J.A; The Federal Polytechnic, Ado-Ekiti, Nigeria

Abstract Body:

Ground nut cake(Kuli Kuli) is a fried product made from ground nut. It is a delicacy or snack which is consumed as an accompaniment to many foods. Ground nut has been implicated in mycotoxin contamination of food and feed stuff so it is thought that its use in production of kuli kuli could lead to contamination. Aflatoxin is a toxic metabolite produced by the mold *Aspergillus flavus* on the substrate which it grows. This toxin has been implicated in many disease conditions like hepatotoxicity, immune suppression, stunting in children etc. The present work was therefore undertaken to ascertain the safety or otherwise of Kuli kuli sold in the study area. Samples of Kuli Kuli were collected randomly from sellers in the 3 agricultural zones of the state. Samples were collected randomly from sellers in the 3 zones. They were then analysed for Aflatoxins B1, B2, G1, G2 using standard methods. The data obtained were subjected to statistical analysis of variance and means were separated using Duncan Multiple Range Test. The results showed that there was significant difference ($p<0.05$) in the values of Aflatoxin types in the kuli kuli samples. The levels of Aflatoxin B1 in all but one sample were higher than 20ppm which is the permissible level in food. There was no significant difference in Aflatoxin contents of Kuli Kuil from the 3 agricultural zones. It was concluded that Kuli Kuli produced and sold in Ekiti state is not fit for human and animal consumption. It was recommended that the food regulation agency(NAFDAC) should standardise production of Kuli kuli to ensure food safety.

Author Disclosure Block:

R.I. Aboloma: None.

Poster Board Number:

FRIDAY-224

Publishing Title:

Bacteriological Quality of Street Vended Foods in Ado-Ekiti, Ekiti State, Nigeria

Author Block:

R. I. Aboloma, T. O. Fajilade; The Federal Polytechnic, Ado-Ekiti, Nigeria

Abstract Body:

Unregulated food vending in public places has become a common practice in Ado-Ekiti with attendant increase in food borne diseases. This research was therefore undertaken to understand the role of food vendors in transmission of food borne bacterial pathogens. Samples of street vended foods were examined for presence of pathogens. These foods were randomly bought from fifty (50) food vendors as soon as they were prepared ready for sale in major public places like motor parks, markets and street corners. In the laboratory, the samples were stored in the refrigerator at 4°C until needed for analysis. Each of the food samples was cultured on Nutrient, Mackonkey and Deoxycholate Citrate agar, incubated at 37°C for 24 hours. The demographic data of the vendors were also obtained via questionnaires. The results obtained showed that many genera of bacteria contaminated the foods beyond acceptable levels. The most frequently occurring bacteria in order of occurrence were *Staphylococcus aureus*(90%),*Enterobacter aerogenes*(85%) *Bacillus cereus*(66%), *Escherichia coli*(54%), *Pseudomonas aeroginosa*(30%) *Salmonella* sp(18%), *Serratia marcescens* (10%). It was therefore concluded that with extra care most of these pathogens will be prevented from contaminating foods. Education of Food vendors on proper food handling methods was finally recommended.Keywords: Food, Bacteriological, Street-Vended

Author Disclosure Block:

R.I. Aboloma: None. **T.O. Fajilade:** None.

Poster Board Number:

FRIDAY-225

Publishing Title:

The First Detection of Florfenicol-Resistant Gene in Shiga-Like Toxin Producing *Escherichia coli* Isolated from Pork in Korea

Author Block:

J-S. Moon; Animal and Plant, Quarantine Agency, Anyang-si,Gyeonggi-do, Kosovo, Republic of

Abstract Body:

Background: Florfenicol is fluorinated derivative of chloramphenicol and represent highly protein inhibitors of bacterial protein biosynthesis. It is used in veterinary medicine or feed additives for pigs in Korea. The transfer of antibiotic resistance gene among the bacterial strains has become a problem worldwide, so drug resistant bacterial phenotypes should be identified. In this study, antimicrobial resistance and resistance gene were investigated for *Escherichia coli* (*E. coli*), especially shiga-like toxin producing *E. coli (*STEC) isolated from pork in Korea. **Methods:** We monitored 301 pork samples in slaughter houses and retail markets, and isolated 50 strains of *E. coli*. Among these isolates, six isolates resulted in STEC. Minimum inhibitory concentration (MIC) on six strains was performed for 14 antibiotics, ampicillin, amoxicillin/clavulanic Acid, cefoxitin, ceftiofur, cephalothin, florfenicol, ciprofloxacin, colistin, gentamicin, nalidixic acid, neomycin, streptomycin, tetracycline and trimethoprim/Sulphamethoxazole, and three strains showed high MIC to florfenicol and chloramphenicol (64 ug/mL). PCR was conducted to detect the florfenicol resistant gene (*floR*) and the chloramphenicol resistant gene (*cat*). **Results:** All of 3 strains contained the *floR*, while none of them had the *cat*. These PCR products were sequenced and aligned to obtain homology with other available genes in reference GenBank. A BLAST search showed that they contained sequences with homology to the *floR* gene of *E. coli* or *Salmonella enteric* serovar Heidelberg. **Conclusions:** This is the first report to detect *floR* gene in STEC isolated from slaughtered pigs in Korea. These results suggest that some STEC isolates in Korea carry florfenicol resistant gene and transfer this gene to other bacterial strains.

Author Disclosure Block:

J. Moon: B. Collaborator; Self; E. J. Heo, E. K. Ko, Y. J. Kim, H. J. Park, S. M. Oh.

Poster Board Number:

FRIDAY-226

Publishing Title:

Shiga Toxin (*stx*) Gene Analysis and Verotoxigenic Potentials of *Escherichia coli* Isolated from 'Bobozi'- Indigenous Nigerian Ready-To-Eat Fermented Cassava Chips

Author Block:

S. A. Enabulele[1], E. O. Daniel[1], R. O. Ohenhen[2]; [1]Benson Idahosa Univ., Benin City, Nigeria, [2]Ambrose Alli Univ., Ekpoma, Nigeria

Abstract Body:

Background: Many strains of *E. coli* have been identified as food borne pathogens inducing serious gastrointestinal diseases and even causing death in humans. Among these are those referred to as Shiga or Vero toxin producing *E. coli*. Bobozi is an Indigenous Nigerian ready to eat snack food made from cassava and is widely consumed in most parts of the country. The objective of this study was to Isolate and Identify *E. coli* from Bobozi, analyze their Shiga toxin (*stx*) gene profile and thereafter determine their verotoxin capabilities.**Methods:** A total of 248 samples of Bobozi were purchased from hawkers in the metropolis of Benin City, Edo state, Nigeria. Isolation and Identification was done using standard microbiological methods. Analysis for the presence of Shiga toxin (*stx1* and *stx2*) genes was done using PCR technique and for their verotoxigenic potentials using tissue culture assay on Vero cells.**Results:** Result of the investigation reveals that of the total 248 samples collected, 107(43.15%) had *E. coli* isolated from them. Total number of *E. coli* isolated from the 107 samples was 386. Shiga toxin gene analysis of the isolates reveal that 169(43.78%) had *stx1* gene alone, 112 (29.02%) had *stx2* gene alone, 3(0.78%) had both *stx 1* and *stx 2* genes while 102 (26.42%) had none of the two genes. Tissue culture assay on Vero cells indicate that there is a strong relationship between the presence of *stx* genes and their degree of cytotoxic effect on vero cells as over 80% isolates without the genes exhibited weak cytotoxic effect whereas over 80% of those with the genes exhibited more than strong cytotoxic effect.**Conclusions:** The findings from this study provide evidence of the presence of Shiga toxin producing *E. coli* in our food system and this can be of serious health challenge especially as Bobozi is a Ready to eat food that is consumed without further processing. It is recommended therefore that more measures be taken in monitoring the processing and sale of such food products to prevent potential food borne disease outbreak.

Author Disclosure Block:

S.A. Enabulele: None. **E.O. Daniel:** None. **R.O. Ohenhen:** None.

Poster Board Number:

FRIDAY-227

Publishing Title:

Characterization of Shiga Toxin-Producing *Escherichia coli* Isolates from Retail Meats in Korea

Author Block:

J. B. Lee, D. Han, H. T. Lee, J. W. Yoon; Coll. of Vet. Med. & Inst. of Vet. Sci., Kangwon Natl. Univ., Chuncheon, Korea, Republic of

Abstract Body:

Shiga toxin-producing *Escherichia coli* (STEC) is a zoonotic bacterial pathogen causing hemorrhagic colitis and a life-threatening hemolytic uremic syndrome. Consumption of raw or undercooked meat is the most common route of transmission of STEC. Recently, we isolated 7 STEC strains carrying one or both Shiga toxin genes (*stx1* or *stx2*) from 434 retail meat samples in Korea. Here, the seven STEC isolates were further characterized in terms of their serotypes, virulence potentials, and phylogenetic relatedness. We showed that all the STEC isolates belonged into the 4 different serotypes; O91:H14 (3 strains), O91:H21 (1), O121:H10 (2), and Ont:H20 (1). The two STEC O91:H14 isolates were non-motile although they had a functional copy of the *fliC* gene encoding the H antigen. The PCR analyses revealed that none of them carried the *eae*, *tir*, and *espB* genes, implying the absence of the LEE pathogenicity island encoding the Type 3 secretion system. Instead, the 2 STEC O91:H14 isolates had the *subAB* toxin genes with both *saa* and *iha* genes, previously known as the alternative adhesins for STEC. The STEC O91:H21 and Ont:H20 isolates possessed the *cdt-V* toxin gene encoding the cytolethal distending toxin. As expected, all the STEC isolates harbored plasmids of various sizes, but only 4 STEC isolates were PCR positive to the STEC O157:H7 pO157-encoded *ehxA*, *espP*, or *katP* genes required for full virulence of STEC. Notably, the L-glutamate-induced acid tolerance phenotypes of the STEC isolates were varied depending on the expression of GadA although no significant differences were observed in the RpoS-induced acid resistance. Both antibiogram and PFGE analyses demonstrated that all the STEC isolates were susceptible to the antimicrobials evaluated and one possible clonal set was identified between the two STEC O91:H14 isolates. Our results demonstrated the recent prevalence of STEC isolates among retail meats in Korea as well as their genetic and phenotypic properties. Further study might be needed to elucidate the *in vivo* virulence of the STEC isolates.

Author Disclosure Block:

J.B. Lee: None. **D. Han:** None. **H.T. Lee:** None. **J.W. Yoon:** None.

Poster Board Number:

FRIDAY-228

Publishing Title:

Prevalence of *Salmonella, Escherichia coli* O157 and Listeria monocytogenes Contamination of Chickens and Quails Eggs in Lagos State, Nigeria

Author Block:

O. O. Ishola, T. Olubade; Univ. of Ibadan, Ibadan, Nigeria

Abstract Body:

Background: The prevalence of Salmonella, Escherichia coli O157 and Listeria monocytogenes contamination in chickens and quail eggs from commercial farms and sold in markets in Lagos state, Nigeria was investigated. **Methods**: total of 510 eggs (240 chicken and 270 quail eggs) were randomly selected from 10 farms and 10 markets from the five Administrative Areas. Isolation of Salmonella, E. coli O157 and L. monocytogenes was done from enrichment samples using standard cultural and biochemical procedures. Antibiotics profiling, total aerobic counts and coliform counts were done using standard methods. **Results:**The chicken eggs had prevalence of 100% each for Salmonella, E. coli O157 and L. monocytogenes. The quail eggs sampled had prevalence of 100% each for Salmonella and E. coli O157 but with a prevalence of 61.8% for L. monocytogenes. Mean total aerobic count (Log CFU/ml) and mean total coliform count (Log CFU/ml) for the chicken eggs were 6.79 and 6.54, respectively. The mean total aerobic count for quail eggs from the farms was 6.67 while from the markets was 6.69. The mean coliform count (Log CFU/ml) from quail eggs from the farm was 6.30 while from the markets was 6.42. All the Salmonella and E. coli isolates were highly sensitive to Ofloxacin and Nalixic acid but resistant to Cotrimoxazole and Tetracycline. All the Listeria isolates were highly sensitive to Gentamycin and resistant to Cotrimoxazole. **Conclusions:** This study suggests that eggs from these farms and offered for sale in these markets were harbouring Salmonella, E. coli and Listeria. Poultry and personal hygiene should be improved to control these zoonotic pathogens of poultry and eggs.

Author Disclosure Block:

O.O. Ishola: None. **T. Olubade:** None.

Poster Board Number:

FRIDAY-229

Publishing Title:

Isolation and Characterization of Antimicrobial Resistant Non-Typhoidal *Salmonella enterica* Serovars from Imported Food Products

Author Block:

D. Bae, O. Kweon, A. A. Khan; U.S. FDA/Nat'l. Ctr. for Toxicol. Res., Jefferson, AR

Abstract Body:

Background: Unlike mild gastroenteritis by non-typhoidal *Salmonella* (NTS) infection, severe systemic infections including bacteremia or meningitis, can occur in immunocompromised persons. Fluoroquinolones and β-lactams are widely used to treat the life-threatening systemic infections. However, the emergence of multidrug resistant strains is a global health concern. The objective of this study was to determine antimicrobial resistance in 110 NTS isolates from food products imported into the US during the period from 2011 to 2013. **Methods:** One hundred ten NTS were isolated from a variety of imported food products (n = 3,840). The disk diffusion agar and the microdilution assays were performed for antimicrobial susceptibility testing using 10 antimicrobials. A combination of PCR, DNA sequencing, and plasmid analyses were performed to characterize antimicrobial resistance determinants. **Results:** Twenty-three out of 110 *S. enterica* isolates were resistant to various antimicrobial classes including β-lactam, aminoglycoside, phenicol, glycopeptide, sulfonamide, trimethoprim, and/or fluoroquinolone antimicrobials. Twelve of the isolates were multi-drug resistant strains. Antimicrobial resistance determinants, including *bla*$_{\text{TEM-1}}$, *bla*$_{\text{CTX-M-9}}$, *bla*$_{\text{OXA-1}}$, *tetA, tetB, tetD, dfrA1, dfrV, dhfrI, dhfrXII, drf17, aadA1, aadA2, aadA5, orfC, qnrS* and mutations of *gyrA* and *parC* were detected in one or more antimicrobial resistant NTS isolates. Plasmid profiles showed that 12 of the 23 antimicrobial resistant strains harbored plasmids having incompatibility groups IncFIB, IncHI1, IncI1, IncN, IncW, and IncX. **Conclusions:** Our study indicates that imported foods contaminated with multidrug resistant NTS may contribute to the spread of antimicrobial resistance genes and potentially compromise the therapeutic activity of antimicrobials. In addition, the data generated from the integrated research approaches reported in this study could be useful for investigations of foodborne illness outbreaks linked to domestic and international food facilities.

Author Disclosure Block:

D. Bae: None. **O. Kweon:** None. **A.A. Khan:** None.

Poster Board Number:

FRIDAY-230

Publishing Title:

***Campylobacter jejuni* Contamination of Raw Bovine Milk in Oyo State, Nigeria**

Author Block:

O. I. Olatoye, A. Ogunsemoyin; Univ. of Ibadan, Ibadan, Nigeria

Abstract Body:

Background: Dairy development and commercialization in Nigeria from local herds require microbial safety evaluation. This study determined the prevalence and antibiotic susceptibility of Campylobacter species isolated from milk of dairy herds in Oyo State. **Methods:** Bulk raw milk samples (n=300) were aseptically collected into Amies transport medium fromthree milk collection centres during June to September 2014. These were cultured for campylobacter and characterised using standard methods and further tested to commonly used antibiotics by breakpoint method.**Results:** Campylobacter species were isolated from 264 samples (88.0%) prevalence) comprising 349 strains of Campylobacter spp, out of which 70 (20.1%) were confirmed to be Campylobacter jejuni. The isolates were 100.0% resistance to nalidixic acid, gentamicin, erythromycin and 38.0% resistance to enrofloxacin.**Conclusions:** The high prevalence obtained couldresult from milking of mastitic cows, unhygienic milk handling andtransportation, while antibiotic resistance could be due tounregulated drug use in dairy herds. Hygienic milking, dairyproduction and processing education will ensure milk safety while Consumption of raw/improperly pasteurized milk should be discouraged.

Author Disclosure Block:

O.I. Olatoye: None. **A. Ogunsemoyin:** None.

Poster Board Number:

FRIDAY-231

Publishing Title:

Antimicrobial Resistance and Molecular Characterization of *Campylobacter coli* in Ceca and Carcass of Poultry in Lebanon

Author Block:

S. Fadlallah[1], R. Hajj[2], Z. Nasser[3], N. Ghosn[3], W. Ammar[3], **G. M. Matar**[1]; [1]American Univ. of Beirut, Beirut, Lebanon, [2]Lebanese Agriculture Res. Inst., Fanar, Lebanon, [3]Ministry of Hlth., Beirut, Lebanon

Abstract Body:

Background: *Campylobacter* is the primary bacterial cause of human intestinal infections worldwide; poultry products being the main source of contamination. This study assessed the prevalence of *Campylobacter* spp. in chicken carcass and ceca collected at two slaughterhouses in Lebanon, determining their antimicrobial resistance profiles and detecting genomic diversity within the species. **Methods:** Fifty one *Campylobacter* positive isolates (carcass: 13 and caeca: 38) were obtained from the Lebanese Agriculture Research Institute (LARI) through the Ministry of Health (MOH). *Campylobacter* isolates were identified biochemically according to the standard ISO 10272: 2006. Species identification was confirmed by the 16 S rRNA gene sequence analysis. Antimicrobial susceptibility testing (AST) was performed using a wide range of β lactam agents and tetracycline. PCR detection of the bla_{OXA-61} and *tet(0)* genes in resistant and susceptible isolates to ampicillin and tetracycline respectively was determined. Genomic diversity of the isolates was determined using Random Amplified Polymorphic (RAPD) and BIONUMERICS analysis. **Results:** Sequencing revealed that all tested isolates were *Campylobacter coli*. AST showed resistance in the isolates to cephalothin and aztreonam (100%), cefamandol and cefoxitin (98%), tetracycline (94%), ampicillin (49%), amoxicillin (46%), piperacillin (45%), carbenicillin (36%), ticarcillin (20%), ceftazdime (17%), cefotaxime (8%) and amoxdicillin-clavulanic acid (2%). All ampicillin-resistant isolates and 84% of the ampicillin-sensitive ones carried the bla_{OXA-61} gene. All tetracycline-resistant isolates were positive for the *tet* (O) gene with 96% encoded on plasmids. The tetracycline sensitive isolates were negative for the *tet* (O) gene. RAPD analysis revealed 9 distinct clusters with a minimum percentage of 43.5% genomic similarity. **Conclusion**: This study emphasizes the importance of surveillance in identifying and controling commonly circulating food borne pathogens and calls out for implementation of food safety measures.

Author Disclosure Block:

S. Fadlallah: None. **R. Hajj:** None. **Z. Nasser:** None. **N. Ghosn:** None. **W. Ammar:** None. **G.M. Matar:** None.

Poster Board Number:

FRIDAY-232

Publishing Title:

Prevalence and Antibiotics Resistance *Campylobacter jejuni* in Retail Chickens in Oyo State, Nigeria

Author Block:

A. Ogunsemoyin, O. Olatoye, J. Y. Adeseko; Univ. of Ibadan, Ibadan, Nigeria

Abstract Body:

Background: Increasing demand and production of animal protein in Nigeria require safety assessment of associated food borne pathogens for consumer protection. Unhygienic handling of chickens during processing is a common practice at retail markets and poultry slaughter slabs in Nigeria with possibility of foodborne pathogen contamination including Campylobacter. Published data on Campylobacter in poultry meat in Nigeria are scanty. **Methods:** In this study, Campylobacter jejuni were isolated from chicken samples obtained from retail markets in Ibadan to determine the prevalence and antibiotic susceptibility using Campylobacter standard culture technique. **Results:** Out of 252 chicken samples collected, Campylobacter jejuni was isolated in 242 (96.0% prevalence) samples. Susceptibility test indicated that the isolates were 100.0% resistance to nalidixic acid, gentamicin, and erythromycin and resistance of 38.0%, 46.0%, 50.0% and 58.0% to enrofloxacin, chloramphenicol, streptomycin and tetracycline respectively. **Conclusions:** Food safety risk could result from direct contamination and cross contamination of carcasses during evisceration and unhygienic rinsing of multiple carcasses from different farms. While the high resistance to commonly used antibiotics could be due to unregulated misuse of veterinary drugs commonly practiced in Nigerian poultry production. Prudence use of antibiotics in poultry, hygienic handling and thorough cooking of poultry products will ensure food safety.

Author Disclosure Block:

A. Ogunsemoyin: None. **O. Olatoye:** None. **J.Y. Adeseko:** None.

Poster Board Number:

FRIDAY-233

Publishing Title:

A Naturally Occurring MutY Mutation Promotes the Emergence of Spontaneous Oxidative Stress Resistant Mutants in *Campylobacter jejuni*

Author Block:

L. Dai, Q. Zhang; Iowa State Univ., Ames, IA

Abstract Body:

Campylobacter jejuni is a leading cause of foodborne illnesses worldwide. As a microaerophilic organism, *C. jejuni* has to defend against the oxidative stress encountered both in the host and in the environment. Recently we identified a *C. jejuni* mutator isolate CMT, with a naturally occurring mutation in MutY, promoting the emergence of antibiotic-resistant mutants. We also observed CMT conferred reduced susceptibility to oxidant treatment, but the underlying mechanisms remains unclear. This study was aimed to determine how CMT contributes to oxidative stress resistance in *C. jejuni*. *C. jejuni* isolates were assayed for oxidative stress susceptibility to a mixture of paraquat and H_2O_2. Spontaneous oxidative stress resistant (OX^R) mutants were subjected to qRT-PCR and sequencing for mutations in oxidative stress related genes. Site-specific reversion and EMSA were conducted to link the OX^R phenotype with *perR* mutations in *C. jejuni*. A promoter-reporter fusion P_{katA}-*cat* was utilized to determine spontaneous OX^R mutation frequencies of *C. jejuni* isolates. Results showed that CMT was more resistant to oxidant treatment than the wild-type *C. jejuni* isolate. Disk inhibition assay revealed the presence of spontaneous OX^R mutants in *C. jejuni* which are highly resistant to oxidant treatment. Notably, CMT demonstrated a spontaneous OX^R mutation frequency that was >100-fold higher than that of the wild-type strain. Transcription of *katA* and *ahpC* genes were significantly upregulated in these OX^R mutants, with over 500-fold increase for *katA* compared with that in the wild type. Sequencing results revealed that all *C. jejuni* OX^R mutants carried mutations in the transcriptional regulator gene *perR*. In the CMT background all *perR* mutations were G→T or C→A transversion, while in the wild-type background *perR* mutations were short (1~2 bp) deletions or insertions. Site-specific reversion and EMSA proved that the identified *perR* mutations were responsible for the OX^R phenotype. The mutator phenotype caused by a *mutY* mutation increases the loss-of-function mutation rate of *perR*, which results in the malfunction of PerR and the derepression of PerR-controlled oxidative stress defense genes, thereby promoting the emergence of OX^R mutants in *Campylobacter*.

Author Disclosure Block:

L. Dai: None. **Q. Zhang:** None.

Poster Board Number:

FRIDAY-234

Publishing Title:

Molecular Analysis and Toxigenic Potential of *Vibrio cholerae* Isolated from Hilsha Fish (*Tenualosa ilisha)*, Bangladesh

Author Block:

Z. Hossain[1], I. Farhana[1], S. Tulsiani[2], P. Jensen[2], A. Begum[1]; [1]Univ. of Dhaka, Bangladesh, Dhaka, Bangladesh, [2]Univ. of Copenhagen, Copenhagen, Denmark

Abstract Body:

Exposure to contaminated fish may upsurge the virulent strains of *Vibrio cholerae*, the deadly human pathogen in the households of rural and urban Bangladesh. Since *V. cholerae* spreading was reported from the Bay of Bengal, this study hypothesized that Hilsha (*Tenualosa ilisha)*, a marine and fresh water fish may serve as a transmission vehicle of potential emerging epidemic causing strains.We studied 9 toxigenic *V. cholerae* strains isolated from Hilsha fish including 6 *V. cholerae* O1 and 3 non O1/O139 serogroups for virulence associated genotype and their pathogenic potential on animal model and human cancer cell line. The study also analyzed clonality by genetic fingerprinting and *rpoB* gene sequencing.The *V. cholerae* O1 strains possessed diverse virulence genes but lacked some major toxin genes like *ctxA*, *tcp* etc. The non O1/O139 strains harbored genes for type III (T3SS) and type VI secretion systems (T6SS). Eight of the nine strains showed survivality up to 10% sodium chloride in broth culture which indicates their coastal origin. All nine isolates were able to accumulate fluid in rabbit ileal loops. Cell free culture supernatant of three O1 and two non O1 strains caused distinctive cell death in established HeLa cell line. Diverse polymorphic patterns were revealed in Random amplified polymorphic DNA (RAPD) fingerprinting except two non O1 isolates (I-49d and I-52a) showed considerable correlation in band patterns. Similar restriction fragment length polymorphism (RFLP) profile of *groEL*-I of *V. cholerae* chromosome 1 in all strains indicated homogeneity in species level. Hierarchical cluster analysis by β subunit of RNA polymerase gene (*rpoB*) sequencing showed that theses isolates did not cluster together and also distinct from clinical and environmental toxigenic strains.Our results indicate the indigenous *V. cholerae* strains associated with Hilsha fish possesses considerable virulence potentiality despite being quite diverse from current epidemic strains. In a cholera epidemic-prone region like Bangladesh, surveillance research on this fish species is vital to control the evolution of outbreak strains of *V. cholerae.*

Author Disclosure Block:

Z. Hossain: None. **I. Farhana:** None. **S. Tulsiani:** None. **P. Jensen:** None. **A. Begum:** None.

Poster Board Number:

FRIDAY-235

Publishing Title:

Detection and Molecular Characterization of *Vibrio parahaemolyticus* in the Clam *Phacoides pectinatus* and the Oyster *Crassostrea rhizophorae* from the Southwest Coast of Puerto Rico

Author Block:

K. E. Correa-Velez, H. M. Nieves-Rosado, M. Chaparro-Serrano, C. Rios-Velazquez; Univ. of Puerto Rico at Mayaguez, Mayaguez, PR

Abstract Body:

Bivalves such as clams and oysters are filtering mollusks that inhabit aquatic environments. These filter feeder organisms can accumulate bacterial species such as *Vibrio parahaemolyticus*. Recently, the Centers for Disease Control and Prevention estimated a higher incidence (7,880 cases/year) of *Vibrio* infections, where approximately 2,800 cases were associated with *V. parahaemolyticus*. The Food and Drug Administration (FDA) recognized this bacterium as leading cause of human gastroenteritis associated with seafood consumption. In Puerto Rico, there are no laws or regulatory agencies that assess bivalve quality for sale. Therefore, the Island does not have statistics on foodborne disease incidence caused by consumption of raw bivalves. This research sought the detection of *V. parahaemolyticus* in both raw bivalves consumed in PR, and established the potential of pathogenicity of the isolates. This study used standardized tests including molecular protocols established by the FDA in the Bacteriological Analytical Manual for the detection of *V. parahaemolyticus* strains in oyster and clam samples from the southwest coast. The enrichment on alkaline peptone water, the selection of *Vibrio* on Thiosulfate Citrate Bile Salts Sucrose and differentiation of species based on growth in different concentrations of NaCl were performed as standardized test. Finally, molecular analysis involved the use of Multiplex PCR to detect the presence of *V. parahaemolyticus* pathogenic associated genes. After 4 seasonal samplings, 58 strains of presumptive *V. parahaemolyticus* were isolated, where 36% were able to grow at 3%, 6% and 8% of NaCl. Also, 64% of the isolates grew in 10% of NaCl. Based on species specific marker amplicon, and the absence of pathogenic genes signal, 90% of the isolates in the study are *V. parahaemolyticus* and none of them are potentially pathogenic. This study has demonstrated the absence of pathogenicity markers in *V. parahaemolyticus* isolated from mollusk samples from the southwest coast of PR. Comparisons with isolates from patients with diarrhea associated to bivalve consumption will provide more information on potential foodborne disease associated to raw shellfish consumption from these waters.

Author Disclosure Block:

K.E. Correa-Velez: None. **H.M. Nieves-Rosado:** None. **M. Chaparro-Serrano:** None. **C. Rios-Velazquez:** None.

Poster Board Number:

FRIDAY-236

Publishing Title:

Evaluating the "Hot" Oyster: A Healthy Oyster Containing Elevated Levels of *Vibrio parahaemolyticus*

Author Block:

S. L. Klein, C. R. Lovell; Univ. of South Carolina, Columbia, SC

Abstract Body:

Outbreaks of the pandemic, emergent human pathogen *Vibrio parahaemolyticus* are increasing in size and frequency, and are occurring at higher latitudes. The United States Food and Drug Administration (FDA) estimates that there are approximately 4,500 cases of *V. parahaemolyticus* gastroenteritis per year in the United States; however, this could be a significant underestimate, as most cases are self-limiting and do not require hospitalization. Symptoms of *V. parahaemolyticus* gastroenteritis occur 24 to 72 h after ingestion of raw or undercooked seafood, usually oysters. Constant monitoring of *V. parahaemolyticus* densities within oysters is required due to recent *V. parahaemolyticus* outbreaks at locations usually considered to be too cold for *V. parahaemolyticus* (i.e.: Alaska, Chile, and the Baltic Sea). Relatively little is known regarding *V. parahaemolyticus* loads in individual oysters as monitoring protocols usually entail homogenizing several oysters together prior to analysis.A total of 110 oysters were harvested from two sampling sites, one a site of commercial oyster harvest (Whale Branch in Beaufort, SC) and the other a pristine comparison site (North Inlet in Georgetown, SC). The majority of oysters sampled (98%) contained levels of presumptive *V. parahaemolyticus* deemed safe for consumption by the FDA. However, two oysters from these sites contained presumptive *V. parahaemolyticus* densities that exceeded “safe levels” established by the FDA. After dissections, it was determined that the two “hot” oysters contained densities of presumptive *V. parahaemolyticus* within the gills that were approximately 100-fold higher than the average. Current FDA protocols for detection of *V. parahaemolyticus* require homogenizing a dozen oysters together, which might result in dilution of *V. parahaemolyticus* from the “hot” oysters to acceptable levels. *V. parahaemolyticus* gastroenteritis due to ingestion of the relatively rare “hot” oyster could explain why *V. parahaemolyticus* gastroenteritis is an illness that is sporadic and somewhat difficult to predict.

Author Disclosure Block:

S.L. Klein: None. **C.R. Lovell:** None.

Poster Board Number:

FRIDAY-237

Publishing Title:

Identification of Synergistic Antimicrobial Combinations to Control *Listeria monocytogenes* in an Acidified Broth System

Author Block:

S. M. Kozak, D. J. D'Amico; Univ. of Connecticut, Storrs, CT

Abstract Body:

Effective approaches using antimicrobials to reduce *Listeria monocytogenes* levels in ready-to-eat (RTE) foods and prevent pathogen growth have been identified. However, concerns with approved usage levels, cost, development of resistance, and negative sensorial changes can limit these applications. The identification of combinations of antimicrobials to produce additive or synergistic effects is desirable in an effort to limit usage levels without diminishing inhibitory or bactericidal effects. A broth micro-dilution assay was utilized to identify minimum inhibitory (MIC) and bactericidal concentrations (MBC) of antimicrobials when used alone or in binary combinations against an eight-strain cocktail of *L. monocytogenes* in growth media adjusted to pH 5.5. When applied singly, lauric arginate (LAE) and hydrogen peroxide (HP) were observed to have the lowest MICs at 10 and 50 ppm, respectively. Similarly, LAE, HP, and ε-polylysine (EPL) had the lowest MBCs at 20, 100, and 300 ppm, respectively. A total of six combinations resulted in reduced MICs while three combinations resulted in reduced MBCs. Fractional inhibitory and bactericidal concentration indices (FIC_I and FBC_I) from combination antimicrobial treatments were calculated to characterize these antimicrobial interactions. Of the four inhibitory combinations, the interaction of acidified calcium sulfate with lactic acid (ACSL)- octanoic acid (OA) was identified as synergistic ($FIC_I = 0.52$) while dihydroxybenzoic acid (DHBA)-LAE ($FIC_I = 0.67$) and ACSL-OA ($FIC_I = 0.62$) were moderately synergistic. The remaining interactions of ACSL-EPL, EPL-OA, and ACSL-HP were additive in the inhibition of *L. monocytogenes*. The bactericidal interactions of EPL- sodium octanoate (SO) ($FBC_I = 0.23$), ACSL-SO ($FBC_I = 0.19$), and HP-EPL ($FBC_I = 0.58$) were also identified as synergistic. In the present study the most effective inhibitory combination of ACSL-OA reduced the assay pH from 5.5 to 5.03 while a pH of 5.09 was noted for ACSL-SO. Reductions in pH resulting from antimicrobial combinations must be considered in future applications. Additional studies are necessary to identify the mechanisms of action for the combined treatments. The efficacy of antimicrobial combinations must also be determined when used as surface treatments and in the reformulation of RTE foods.

Author Disclosure Block:

S.M. Kozak: None. **D.J. D'Amico:** None.

Poster Board Number:

FRIDAY-238

Publishing Title:

***Staphylococcus aureus* Contamination in the Southern Brazilian Dairy Industry and Its Potential Health Risks**

Author Block:

K. K. Dittmann[1], L. T. Chaul[2], S. H. I. Lee[3], C. H. Corassin[3], C. A. F. de Oliveira[3], E. C. P. de Martinis[4], V. F. Alves[2], L. Gram[1], **V. Oxaran**[1]; [1]DTU - Technical Univ. of Denmark, Kgs. Lyngby, Denmark, [2]Federal Univ. of Goiás, Goiania, Brazil, [3]Univ. of São Paulo, Pirassununga, Brazil, [4]Univ. of São Paulo, Ribeirão Preto, Brazil

Abstract Body:

Staphylococcus aureus is one of the most common food-borne pathogenic bacteria and enterotoxigenic strains can cause food poisoning due to the production of heat-stable enterotoxins. *S. aureus* can contaminate several foods including dairy products. This is a challenge in the Brazilian dairy industry where soft cheese as Minas cheese, produced from either raw or pasteurized milk, is widely consumed and mainly produced in small dairy plants. The aim of this study was to evaluate the contamination rate of *S. aureus* in Minas cheese production plants and investigate if the contamination was caused by persistent clones or repeated introduction of new clones. Also we assessed the potential health risk of isolates. Five dairy plants were sampled during 8 months (421 samples in total). Using phenotypic tests and 16S rRNA gene sequencing, *S. aureus* (n=66) was found in 4 of 5 of the dairy plants. The contamination rate varied from 0% to 63.3% depending on the processing plant and the sampling date. Multi-Locus Sequence Typing was used to type and assess potential persisting Sequence Types (ST). Seven known ST (ST1, ST5, ST30, ST97, ST126, ST188 and ST398) were identified. Three new ST were identified and all belong to Clonal Complex (CC) 1 which was the dominant CC in the investigated dairy plants. However, there was no indication of reoccurring ST in the plants. The isolates were screened for resistance to 11 antibiotics recommended for control of *S. aureus* using the disk diffusion method. Only 16 isolates (24.2%) were resistant and only to 1 to 4 antibiotics. However, all of the isolates were methicillin sensitive *S. aureus* and presumptive non-vancomycin resistant. Despite a low level of antibiotic resistance of those isolates, the frequent isolation of CC1 strains, known to be an epidemic CC, indicates a potential risk for human health suggesting that surveillance of *S. aureus* should be improved.

Author Disclosure Block:

K.K. Dittmann: None. **L.T. Chaul:** None. **S.H.I. Lee:** None. **C.H. Corassin:** None. **C.A.F. de Oliveira:** None. **E.C.P. de Martinis:** None. **V.F. Alves:** None. **L. Gram:** None. **V. Oxaran:** None.

Poster Board Number:

FRIDAY-239

Publishing Title:

Vancomycin Non-Susceptibility and Co-Existing Virulence Determinants in *Enterococcus faecalis* Isolated from Traditionally Processed Fish Products of India

Author Block:

K. Biswas, S. R. Joshi; North Eastern Hill Univ., Shillong, India

Abstract Body:

Background: Fermented foods are frequently consumed in different parts of Northeast India. These are often reported to act as reservoir of pathogenic organisms that disseminate within the community complicating the treatment option. *Enterococcus faecalis* are often responsible for contamination of food products. The current study dealt with investigation of glycopeptide non susceptible enterococci found in traditionally processed foods and their co-existing virulence determinants.**Methods:** Susceptibility testing was performed for a total of 46 *E. faecalis* isolates obtained from fermented fish samples. Minimum inhibitory concentrations (MIC) of vancomycin was determined by the agar dilution method. PCR assays were performed targeting various vancomycin resistance genes and 6 virulence determinants (*efaAfs, cpd, esp, agg, cylA,* and *gelE*). Plasmids were isolated and purified from *vanA, vanC$_1$, esp* and *gelE* harbouring isolates. Transformation by heat shock method was carried out to assess plasmid transfer.**Results:** 82.6% (n=38) of *E. faecalis* isolates showed MIC of vancomycin at 124 µg/ml while 17.3% (n=8) of the isolates showed MIC of less than 4 µg/ml. Molecular characterization revealed presence of *vanA* (n=33), *vanB* (n=7), *vanC$_1$* (n=4) and *vanC$_{2/3}$* (n=2). Two of the isolates showed the presence of all the four resistance genes. *gelE, agg* and *esp* were present in 17, 13 and 5 isolates respectively. *cpd* and *efaAfs* was found in all the isolates whereas *cyl* could not be detected. *vanA, vanC$_1$, esp* and *gelE* were detected in plasmid DNA and these genes could be horizontally transferred from the host to *E. coli* DH5α. PCR analysis also yielded positive results for the presence of these genes on the plasmids of the transformants.**Conclusions:** The simultaneous occurrence of virulence determinants and vancomycin resistance genes in enterococci from the traditionally fermented fish products consumed in Northeast India pose a potential threat of transmission of these risk factors to humans through the food chain. This study, the first of its kind, highlights the importance of *E. faecalis* as a reservoir of resistance and virulence genes and their potential transfer to humans through consumption. Overall, these findings reopen the issue of food safety of enterococci prevalent in traditionally processed products.

Author Disclosure Block:

K. Biswas: None. **S.R. Joshi:** None.

Poster Board Number:

FRIDAY-240

Publishing Title:

Evaluating the Extraction Efficiency of Food Borne Pathogens on Automated Homogenizer Platforms

Author Block:

S. Garrett, J. Atwood, B. Easparro; Omni Intl., Kennesaw, GA

Abstract Body:

There is an ever increasing demand for food and beverage manufacturers to adhere to rigorous food safety regulations. As part of the global need for safe food, food and beverage manufacturers test their products for the presence of food borne pathogens. While safety regulations describe what pathogens should be controlled through testing there is no predefined method of sample preparation or detection. Typically, the first step in a food borne pathogen detection study is the disaggregation of the food product or the washing of the sample to release bacteria from the food surface using a Stomacher. While the later method is great for large masses of food stuffs, some researchers may require small amounts of product for analysis. Bead mill homogenizers, as well as rotor-stator homogenizers, can be used to aid in the sample preparation process. Processing can be completed in less than one minute while maintaining bacterial cell and analyte integrity. Depending on the pathogen of interest and the food matrix, the downstream results, including sensitivity can vary greatly based on the sample preparation method chosen. With this is mind, we analyzed two sample preparation techniques on commonly contaminated foods. Meat and spinach samples were inoculated with known levels of recombinant *Escherichia coli* expressing Green Fluorescent Protein. The inoculated samples were processed on a bead mill homogenizer and an automated rotor-stator homogenizer. The homogenates were grown on nutrient agar and the proportion of viable cell recovery was quantified. Limit of detections were determined by lysis of recovered homogenates, DNA purification and PCR amplification. It was determined that both homogenization techniques were able to process food samples for the extraction of live cells and plasmid DNA. The processing efficiency can deliver a 76% cell recovery with a PCR detection limit to $1x10^{-5}$ CFUs/ml. A linear relationship was demonstrated between the number of CFUs to intensity of DNA amplified via PCR. A detection limit of 10^{-5} CFUs is comparable to some food testing methods; however the vigorous processing of bead mill and rotor stator technology has the potential to eliminate the standard pre-enrichment step needed for the detection of food pathogens.

Author Disclosure Block:

S. Garrett: None. **J. Atwood:** None. **B. Easparro:** None.

Poster Board Number:

FRIDAY-241

Publishing Title:

Hyperspectral Images and Differences During Growth of *Salmonella enterica* Typhimurium Chemotypes

Author Block:

R. D. Riggs, K. Wade, I-H. Chen, J. M. Barbaree; Auburn Univ., Auburn, AL

Abstract Body:

BackgroundHyperspectral imaging (HSI) is currently being utilized in a variety of research and is being utilized for detection purposes. Eady and group used HSI to differentiate serotypes of *Salmonella* at various time points in order to characterize the surface chemistries of each of the serotypes.[1] Salmonella is an organism of particular interest in the food industry considering it is estimated to cause illness in 1 million people in the United States annually with symptoms of fever, severe cramping and diarrhea.**Methods**In this study we used the Cytoviva hyperspectral imaging system to elicit the individual hyperspectral profiles produced by *S. enterica* serotype Typhimurium lipopolysaccharide mutants at one hour post stationary phase, or 9 hours, and at 18 hours post inoculation. All organisms were cultured in Luria-Bertani (LB) broth, pelleted at 10,000x rpm and washed in 1x PBS buffer prior to imaging. 0.8 μL of each sample was transferred to a poly-l-lysine coated slide for immobilization and topped with a glass cover slip.Data is collected by the pushbroom approach during which an image is generated that contains both hyperspectral and spatial data by moving the sample pixel line by pixel line to generate distinct profiles for each specimen. The use of a patented enhanced darkfield condenser in conjunction with high resolution optical and hyperspectral imaging creates spectral profiles based upon the unique surface chemistries of each chemotype. The hyperspectral profiles of five cells were composed from each image through the ENVI software.**Results**All of the rough mutants were compared to the wild type and differences in the wavelength at which peak intensity of reflectance of the light were not seen at 1-hour post stationary phase as expected. However, differences in the overall hyperspectral curves produced by each were seen. Significance between chemotypes was noted at 18 hours when comparing one mutant to the smooth wild type through statistical analysis.**Conclusion**In conclusion, the Cytoviva HSI system was not capable of detecting differences in the lipopolysaccharide mutants of *S.* enterica Typhimurium at 9 hours; yet significance can be seen as early as the 18-hour time point.

Author Disclosure Block:

R.D. Riggs: None. **K. Wade:** None. **I. Chen:** None. **J.M. Barbaree:** None.

Poster Board Number:

FRIDAY-242

Publishing Title:

Microwave Cell Lysis and Dna Fragmentation of *Listeria monocytogenes*

Author Block:

T. Santaus, C. Geddes; Univ. of Maryland, Baltimore County, Baltimore, MD

Abstract Body:

The central hypothesis being addressed, is *can microwaves effectively and efficiently lyse cells of Listeria monocytogenes, and what is the effect on DNA fragmentation size (distribution) post heating.* Listeria monocytogenes is of particular interest because it is a deadly food borne bacterial pathogen with a mortality of 20-30% of those who get infected. People with a compromised immune system and pregnant women are more likely to suffer the effects of Listeriosis.[1] The Listeria bacterium can grow under extreme conditions such as low pH and high temperatures. Places that are most likely to transmit the bacterium are food processing plants. Once Listeria is contracted in the human body, it can cross in to the blood, through the blood brain barrier, and into the cerebral spinal fluid.[2] Most of the common DNA extraction and detection methods are slow, costly, and require multiple steps for cell lysis, DNA fragmentation and purification.The method used includes a standard Frigidaire 900 W microwave, a custom slide holder, and microscope slides equipped with an isolator for the sample and metallic microwave focusing geometries, optimized to condense microwaves into a small sample volume. A sample is heated at a specific power and time to lyse the cells and fragment the genomic DNA. Post lysis, a Varian Absorption Spectrometer and a Fluoromax 4P are used to analyze the annealing process of the DNA. Ethidium bromide and SYBR gold™ are the fluorescent molecules being used to study the changes in [*ss*DNA], as compared to [*ds*DNA] over a period of 3 hours. Both standard Gel electrophoresis and high resolution micro-gel techniques are used to determine the fragmentation patterns and distributions observed from microwave-based lysing.In this study we demonstrate that *Listeria monocytogenes* DNA can be efficiently and rapidly extracted and fragmented using metal microwave focusing lysing triangles and a conventional microwave. Post microwave lysis, solution DNA readily anneals forming *ds*DNA observed by an increase in ethidium bromide fluorescence. Our microwave based lysing approach[2] has the particular advantages in that it can fragment the Listeria genome to much smaller DNA fragments, which is ideal for rapid detection using the MAMEF (Microwave-Accelerated Metal-Enhanced Fluorescence) platform[3], as well as the potential for combining cell lysing and DNA fragmentation into one rapid and low cost step.

Author Disclosure Block:

T. Santaus: None. **C. Geddes:** None.

Poster Board Number:

FRIDAY-243

Publishing Title:

Pcr Time Real for Detection of *Salmonella* spp. and *Listeria monocytogenes* in Food of Markets of Guadalajara, México

Author Block:

Y. Lugo-Melchor[1], I. Higuera-Ciapara[1], M. Renteria-Ledezma[1], M. Novoa-Palazuelos[2]; [1]Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco, Guadalajara, Mexico, [2]Inst. Tecnológico y de Estudios Superiores de Occidente, Tlaquepaque, Mexico

Abstract Body:

Background: Foodborne diseases, caused by pathogenic microorganisms, are a major public health problem worldwide. In Mexico, the detection of food pathogens in routine laboratories is still mostly based upon traditional microbiological methods. Thus, the availability of reliable, rapid and accepted test systems to detect the presence or absence becomes increasingly important for the agricultural and food industry. Real-time PCR (RT-PCR) is currently used for the diagnosis of infectious agents, and there are reports of the application of RT-PCR for the direct detection of *Salmonella* spp. and *Listeria monocytogenes*. The aim of this study was to use RT-PCR for Detection of *Salmonella* spp. and *Listeria monocytogenes* in food of markets of Guadalajara, México.**Methods:** A total of seventy-four different samples of food from markets was evaluated for *Salmonella* and forty samples for *Listeria monocytogenes* using a commercial kit of RT-PCR. The results were confirmed by traditional methods based on the Mexican official standard.**Results:** Different types of samples as meat, sausage, vegetable and dairy products was analyzed. Only 1.35% (1/74) of the samples was positive for *Salmonella* spp. In case of *Listeria monocytogenes*, all samples showed absence of the pathogen.**Conclusions:** The findings of this study indicated the presence of several *Salmonella* spp. in food of the markets is a risk for health for consumers. The study showed that RT-PCR is a valuable alternative to determine Salmonella spp. in foods for their specifity and sensibility.

Author Disclosure Block:

Y. Lugo-Melchor: None. **I. Higuera-Ciapara:** None. **M. Renteria-Ledezma:** None. **M. Novoa-Palazuelos:** None.

Poster Board Number:

FRIDAY-244

Publishing Title:

Comparison of Immunomagnetic Separation Beads for Detection of Non-O157:H7 Shiga Toxin-Producing *Escherichia coli* in Complex Matrices

Author Block:

A. L. Kraft[1], W. L. Shelver[2], J. Sherwood[1], T. M. Bergholz[1]; [1]North Dakota State Univ., Fargo, ND, [2]USDA, Fargo, ND

Abstract Body:

An emerging concern in public health and food safety in the United States involves the need to accurately and efficiently detect six serotypes of Shiga toxin producing *Escherichia coli*, which include O26, O45, O103, O111, O121, and O145. Since these non-O157:H7 adulterants thrive in the GI tract of cattle, it is necessary to use reliable detection methods that will work effectively in various matrices such as cattle feces, ground beef and fresh produce, which can be exposed to these pathogens by fecal contamination. Immunomagnetic separation (IMS) used in conjunction with culture based methods has been a useful technique in the detection of pathogens. However, an assessment has not been performed on the extent that matrices or normal flora influence recovery of isolates of each serotype by IMS. Initially, samples of sterile phosphate buffered saline (PBS) were inoculated with a 1cfu/ml concentration mixture of isolates representing the six serogroups. After a 6h incubation at 37C, the samples were mixed with three different commercially available IMS beads and plated on eosin methylene blue agar with vancomycin, cefsulodin and cefixime (EMB-VCC). Three suspect *E. coli* colonies were selected from the EMB-VCC and multiplex PCR was used to determine the serotype. Next, IMS was used in samples of sterile feces in order to gauge the effects of a matrix. To evaluate the beads further by adding competing flora, IMS was performed with non-sterile fecal samples. Three replicates were performed for each bead type, STEC serogroup, and sample type. The most consistent results came from one IMS bead manufacturer with 100% correct identification for four serogroups in PBS and >88.9% correct identification in five serogroups in sterile feces. Conversely, rates of false positives rose for some serogroups in samples that had competing normal flora and a complex matrix such as for the O103 serogroup rising from 10%, 5.3%, and 10% in sterile feces to 21.7%, 40%, and 50%, respectively, in non-sterile feces for the three IMS bead manufacturers. These results suggest that matrix and IMS bead type markedly influence the ability to accurately recover these six serogroups. More research on specific sample preparation should be done to improve the efficacy of recovery of these serogroups with IMS from various matrices.

Author Disclosure Block:

A.L. Kraft: None. **W.L. Shelver:** None. **J. Sherwood:** None. **T.M. Bergholz:** None.

Poster Board Number:

FRIDAY-245

Publishing Title:

Using Multiplex-PCR to Detect Virulence Factor Genes from *vibrio Parahaemolyticus* Isolates from the Oyster *crassostrea Rhizophorae* from Puerto Rico

Author Block:

H. M. Nieves-Rosado, K. E. Correa-Velez, M. Chaparro, C. Rios-Velazquez; Univ. of Puerto Rico, Mayagüez, Puerto Rico

Abstract Body:

The detection of pathogens in food involves the use of diverse culture dependent and independent approaches such as selective-differential media, serological methods and molecular techniques. The Food and Drug Administration (FDA) has recommended the use of PCR, DNA hybridization and a modified version of PCR, known as Multiplex-PCR. This technique involves the use of more than one set of primers to amplify different DNA sequences simultaneously in one polymerase chain reaction. Recently, reports for the Center for Disease Control and Prevention indicate that the outbreaks of *V. parahaemolyticus* increases 52% compared with 2006-2008 incidences. In Puerto Rico, there are no regulatory agencies that control the handling and quality conditions for selling raw shellfish such as oysters. Detection of pathogenic strains of *V. parahaemolyticus* from environmental samples can allow us to potentially predict and compare outbreaks in PR with other countries and will develop awareness to local population about the health issues that can potentially be caused by eating raw seafood. Our goal is to determine the pathogenic levels of *V. parahaemolyticus* isolates from the oyster *Crassostrea rhizophorae* from the southwest coast of PR. DNA extractions were performed from presumptive *V. parahaemolyticus* strains previously isolated using TCBS media. The extracted DNA was processed following standardized multiplex-PCR protocol recommended by the FDA in the Bacteriological Analytical Manual. Multiplex PCR was performed for the amplification of *tdh* (270bp) and *trh* (500bp) genes associated with pathogenic activity; and *tlh* (450bp) a specie specific marker. *V. parahaemolyticus* O4:K12 strain 48057 (NR-21990) ATCC® was used as a positive control. From the analysis of a total of 9 isolates, all of them resulted positive to *V. parahaemolyticus* based on the presence of a *tlh* gene amplicon and none of them resulted to be pathogenic due to the absence of a *tdh* and *trh* amplicons by modified electrophoresis in a 3% agarose gel. While the isolates analyzed in this study lack pathogenic activity and do not represent a risk to population, future monitory is necessary using this molecular techniques to detect the pathogenic levels of any *V. parahaemolyticus* isolated from the coasts of PR.

Author Disclosure Block:

H.M. Nieves-Rosado: None. **K.E. Correa-Velez:** None. **M. Chaparro:** None. **C. Rios-Velazquez:** None.

Poster Board Number:

FRIDAY-246

Publishing Title:

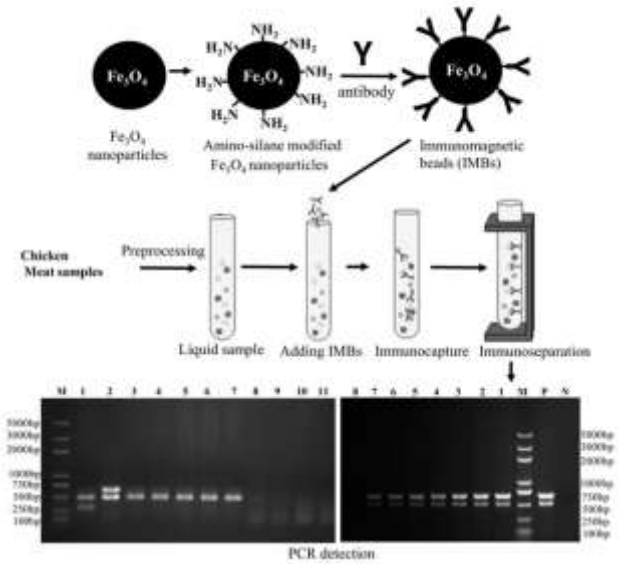
Immunomagnetic Separation Combined with Multiplex Polymerase Chain Reaction for Rapid Detection of *Salmonella* in Chicken Meat

Author Block:

B. Liu, C. Lei, A. Zhang, R. Xiang, H. Wang; Animal Disease Prevention and Food Safety Key Lab. of Sichuan Province, Coll. of Life Sci., Sichuan Univ., Chengdu, China

Abstract Body:

Background: Foodborne salmonellosis poses a threat to human health worldwide. The most common human nontyphoidal salmonellosis is caused by *S.* Typhimurium and *S.* Enteritidis, both of which are frequently found in contaminated meat. However many methods used to identify them are labor-intensive and time-consuming. Here we developed a method based on immunomagnetic separation combined with multiplex polymerase chain reaction (IMS-mPCR) for rapid detection of *Salmonella* and differentiation of *S.* Typhimurium and *S.* Enteritidis in chicken meat. **Methods:** The immunomagnetic beads (IMBs) were synthesized by immobilizing *Salmonella* polyclonal antibody onto the surface of the amino-silane modified Fe_3O_4 nanoparticles. Three pairs of primers targeting *S.* Typhimurium, *S.* Enteritidis and *Salmonella enterica* were designed for mPCR. The optimization of immunomagnetic separation system was performed. The sensitivity and specificity of the IMS-mPCR and the direct PCR were conducted. Both standard culture method and the IMS-mPCR procedure were used to detect *Salmonella* in 157 chicken meat samples. **Results:** When 150μL of IMBs (10 mg/ml) mixed with 1ml of samples to incubate for 30 min, the immunocapture efficiency was greater than 95%.The detection limit of IMS-mPCR was 10^1 CFU/g of *Salmonella* in chicken meat , which is significantly lower than that of the direct PCR (1×10^4CFU/g). Compared with standard culture method, the coincidence rates of the IMS-mPCR procedure for detecting *S.* Typhimurium, *S.* Enteritidis and *Salmonella enterica* were 99.4%, 98.1% and 98.1% respectively, but the latter only took less than 5 hours.Fig. 1. The flow chart for the detection of *Salmonella* by IMS-mPCR.



Author Disclosure Block:

B. Liu: None. **C. Lei:** None. **A. Zhang:** None. **R. Xiang:** None. **H. Wang:** None.

Poster Board Number:

FRIDAY-247

Publishing Title:

Evaluation of a Novel PCR Based System for the Detection of *Salmonella* spp. in a Variety of Food and Environmental Samples

Author Block:

H. P. Dwivedi, P. Chablain*, B. Nahlik, G. Devulder*, J. Mills, S. Bailey, R. Johnson; bioMerieux, Inc., Hazelwood, MO

Abstract Body:

Background: GENE-UP® is a novel PCR based molecular system for as early as same day detection of *Salmonella* in 25g and 375g samples using a very simplified workflow. The performance of GENE-UP molecular system for the detection of *Salmonella* was evaluated against USDA-FSIS reference methods in a variety of food and environmental matrices.**Methods:** The matrices including fresh raw ground beef (25g and 375g samples), fresh raw chicken breast, fresh raw fish, creamy peanut butter, vanilla ice cream, dry pet food (all 25g samples) and stainless steel (sponges) were separately inoculated using different *Salmonella enterica* strains as per AOAC guidelines and analyzed using GENE-UP method. The alternate method results were compared to the USDA-FSIS methods for *Salmonella* (MLG -4.08). The probability of detection (POD) analyses were conducted to compare the method performances.**Results:** All the samples were analyzed at three inoculation levels (un-inoculated, low, high). For samples with low contamination levels (n=20/ matrix), the difference in POD for confirmed alternate and reference methods (measured as $dPOD_C$ values) along with 95% confidence interval were -0.15 (-0.41,0.14) for 25g beef samples and -0.15 (-0.41,0.15) for 375g beef samples; 0.15 (-0.15, 0.41) for raw chicken breast; 0.05 (-0.24, 0.33) for raw fish; 0.15 (-0.13, 0.40) for creamy peanut butter; 0.10 (-0.19, 0.37) for vanilla ice cream; 0.15 (-0.15, 0.41) for dry pet food; and 0.05 (-0.23, 0.33) for stainless steel surface samples. As the confidence interval of all dPOD values contained zero, no statistically significant difference was observed between the alternate and reference methods. For all un-inoculated (n=5/ matrix) and high contamination level (n=5/ matrix) samples, $dPOD_{C\sim}$ values were 0, indicating no statistical difference between the candidate and reference methods.**Conclusion:** No statistical difference was found in the performance of GENE-UP *Salmonella* assay against the USDA reference method. The GENE-UP method offers a significant savings in terms of time and improved convenience when compared to the reference methods. It utilizes a simple one step enrichment, giving same day presumptive results for raw beef samples and next day results for processed food and environmental samples.

Author Disclosure Block:

H.P. Dwivedi: None.

Poster Board Number:

FRIDAY-248

Publishing Title:

Multi-Laboratory Validation of Abrin Detection Method in Foods

Author Block:

A. Minor, Z. Kuhl, B. Keavey; WVDA, Charleston, WV

Abstract Body:

Background: Abrin is an extremely potent bio-toxin produced from the seed of the tropical plant *Abrus precatorius*, commonly found in the southern U.S. and the Caribbean. Abrin toxin inhibits the synthesis of proteins in the cells of an exposed individual, causing severe illness and cell death. Due to the lethality and ease of accessibility, a screening method for the detection of abrin toxin in foods is necessary for biodefense purposes. This study's objective was to provide a multi-laboratory validated method, combining an optimized extraction and commercially available ELISA kit for the detection of abrin toxin in foods. **Methods:** Five participating Food Emergency Response Network / FSIS/ Cooperative Agreement Laboratories analyzed 3 sets, consisting of 18 x 5 g samples for abrin detection in hot dogs, liquid eggs, and liquid infant formula. Samples were homogenized with GBS and the toxins were extracted via centrifugation. Following extraction, the aqueous layers were analyzed in accordance with the ELISA manufacturer's instructions. The validation incorporated replicate sets in three fortification levels and analyzed in duplicate.**Results:**

	Specificity	Sensitivity (Low Fortification)	Sensitivity (High Fortification)	Test Efficiency
Hot Dogs	100% (48/48)	88% (42/48)	100% (48/48)	95.8% (138/144)
Liquid Eggs	100% (48/48)	58% (28/48)	96% (46/48)	84.7% (122/144)
Infant Formula	100% (48/48)	19% (9/48)	96% (46/48)	69.4% (100/144)

Conclusions: The sensitivity for the detection of abrin in all food matrices analyzed, at the high fortification levels, by all participating laboratories was 96% or greater. Low fortification levels were near the LOD for each food, and exhibited a much lower recovery rate. All foods analyzed demonstrated 0 false positives and a specificity of 100%. The data from this multi-laboratory study suggests that the optimized extraction procedure, combined with the ELISA detection assay, may offer a suitable method for the detection of abrin toxin ina variety of foods.

Author Disclosure Block:

A. Minor: None. **Z. Kuhl:** None. **B. Keavey:** None.

Poster Board Number:

FRIDAY-249

Publishing Title:

A Novel Nanotechnology-Based Antimicrobial Platform Using Engineered Water Nanostructures for Food Safety Applications

Author Block:

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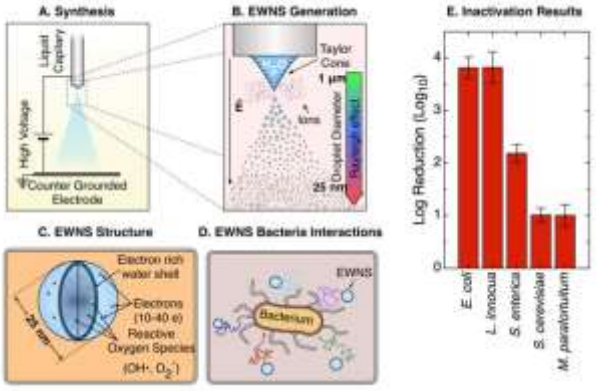
Abstract Body:

BACKGROUND: Microbial contamination is a leading cause of foodborne illnesses in the US each year[1] with the cost exceeding 15.6 billion USD.[2] There is a need for novel, antimicrobial interventions with the potential to be applied throughout the Farm-to-Fork chain for enhanced safety assurance. A chemical free, nanotechnology-based, antimicrobial platform using Engineered Water Nanostructures (EWNS), produced by electrospraying water, was recently developed. EWNS have high surface charge, are loaded with reactive oxygen species (ROS) and can interact with and inactivate an array of microorganisms of public health significance by causing damage to the cell envelope (Fig 1d). **METHODS:** EWNS were synthesized by concurrently electrospraying and ionizing highly purified water in a lab based EWNs generation system (Fig 1a, b). Characterization of EWNS properties (charge, size and ROS content) was performed using state-of-the art analytical methods[3] (Fig 1c). Microbial inactivation potential was evaluated using food related microorganisms (*E. coli, Salmonella enterica, Listeria innocua, Mycobacterium parafortuitum* and S*accharomyces cerevisiae),* spot inoculated on organic grape tomatoes. **RESULTS:** Microbial reductions were found to be microorganism dependent and ranged between 1.0 to 3.8 logs after only 45 mins of exposure to an EWNS aerosol dose of 40,000 #/cm^3. *E.coli* and *L. innocua* exhibited close to 4 log reductions, *S. enterica* a 2 log reduction while Mycobacterium and Saccharomyces showed a 1 log reduction (Fig 1e). **CONCLUSION:** This novel, low cost, antimicrobial platform is chemical-free, has low environmental impact, is easy to use and leaves no residues in the final food product.

Figure 1 – EWNs synthesis, Structure and Inactivation Results



Author Disclosure Block:

M. Eleftheriadou: None. **G. Pyrgiotakis:** None. **C. de Freitas Cirenza:** None. **P. Demokritou:** None.

Poster Board Number:

FRIDAY-250

Publishing Title:

Comparative Characterization of Botulinum Neurotoxin Subtypes F1 and F7 Featuring Differential Substrate Recognition and Cleavage Mechanisms

Author Block:

J. Guo[1], E. Chan[2], S. Chen[2]; [1]Hong Kong PolyU Shen Zhen Res. Inst., Shenzhen, China, [2]The Hong Kong Polytechnic Univ., Hong Kong, Hong Kong

Abstract Body:

Introduction: BoNT/F7, one of the seven subtypes of botulinum neurotoxin type F (F1 to F7)[1], is the second-most divergent subtype of this group. Despite sharing >60% identity with BoNT/F1 at both holotoxin and enzymatic domain levels, it requires an N-terminal extended peptide for efficient substrate cleavage[2], suggesting its unique substrate recognition mechanism but which remain to be elucidated. In order to probe the basis of discrepancy between the molecular mechanisms employed by BoNT/F1[3] and /F7 in recognizing substrate VAMP2, we dissected the substrate recognition mechanism utilized by BoNT/F7 in detail and compared it with that of BoNT/F1.**Method:** LC/F7 and VAMP2 were constructed and purified as previously reported[4]. LC/F7-VAMP2 complex structure was modeled and the possible interacting residues between LC/F7 and VAMP2 were predicted and further proved by performing linear velocity assays and enzyme kinetic analysis.**Results and Significance:** Our data revealed that VAMP2 (20-65) was likely a minimally effective substrate for LC/F7. LC/F7 recognized VAMP2 in a unique way, which differed significantly from that of LC/F1, although both share similar substrate binding and hydrolysis mode. LC/F7 utilizes distinct pockets for specific substrate binding and recognition in particular for the B1, B2 and S2 sites recognitions. Our findings provide insights into the distinct substrate recognition features of BoNT subtypes and useful information for therapy development for BoNT/F. This study also broadens our understanding of the general functional mechanisms of BoNTs and provides essential background knowledge for future development of antitoxins and novel approaches for detecting different subtypes of BoNTs.1.B. H. e. a. Raphael, *Appl Environ Microbiol* 2010, *76*. 4805-12, DOI: 10.1128/AEM.03109-09.2.S. R. Kalb, J. Baudys, C. Egan, T. J. Smith, L. A. Smith, J. L. Pirkle, J. R. Barr, *Appl Environ Microbiol* 2011, *77*. 1301-8, DOI: 10.1128/AEM.01662-10.3.S. Chen, H. Y. Wan, *Biochemical Journal* 2011, *433*. 277-284, DOI: 10.1042/bj20101310.4.J. Guo, S. Chen, *Protein expression and purification* 2015, *111*. 87-90, DOI: 10.1016/j.pep.2015.01.014.

Author Disclosure Block:

J. Guo: None. **E. Chan:** None. **S. Chen:** None.

Poster Board Number:

FRIDAY-251

Publishing Title:

Evaluation of 3m Molecular Detection Assay *Salmonella* and Ansr *salmonella* Methods for Rapid Detection of *Salmonella* from Egg Products

Author Block:

L. Hu[1], L. M. Ma[2], S. Zheng[3], H. Wang[1], E. W. Brown[1], T. S. Hammack[1], G. Zhang[1]; [1]U.S. Food & Drug Admin. (FDA), College Park, MD, [2]Oklahoma State Univ., Stillwater, OK, [3]Northeast Agricultural Univ., Harbin, China

Abstract Body:

Loop-mediated isothermal amplification (LAMP) is a novel simple detection technology that amplifies DNA with high speed, efficiency, and specificity under isothermal conditions. The objective of this study was to evaluate the effectiveness of 3M Molecular Detection Assay (MDA) *Salmonella* and ANSR *Salmonella* methods for detecting *Salmonella* from egg products in comparison with Food and Drug Administration (FDA) Bacteriological Analytical Manual (BAM) culture method and a modified culture method. Two *Salmonella* ser. Enteritidis (18579, PT4; CDC_2010K_1441, PT8), one *Salmonella* ser. Heidelberg (607310-1), and one *Salmonella* ser. Typhimurium (0723) isolates were used in this study. Seven wet egg products (e.g., liquid egg yolk, egg nog, etc.) and thirteen dry egg products (e.g., egg rolls cookies, macaroni & cheese, egg matzos, egg yolk powder, etc.) were inoculated with these strains individually at 1-5 CFU/25g. One set of sample processing followed FDA BAM procedures [with Lactose broth as preenrichment broth]. Another set of samples were processed using Buffered Peptone Water (BPW) as preenrichment broth as instructed by the two LAMP detection kits. The 3M and ANSR LAMP assays were 100% in agreement with their BPW based culture method results. The number of *Salmonella* positive test portions (80 tested), identified with the BAM, 3M, and ANSR methods were, 63, 61 and 59, respectively. In conclusion, both the 3M MDA and ANSR LAMP *Salmonella* methods were as effective as their BPW based culture method for the detection of *Salmonella* from egg products. These sensitive LAMP assays could be used as rapid detection tools for *Salmonella* in egg products when BPW was used as preenrichment broth.

Author Disclosure Block:

L. Hu: None. **L.M. Ma:** None. **S. Zheng:** None. **H. Wang:** None. **E.W. Brown:** None. **T.S. Hammack:** None. **G. Zhang:** None.

Poster Board Number:

FRIDAY-252

Publishing Title:

Development of a Novel Loop-mediated Isothermal Amplification (Lamp) Assay for the Detection of *Salmonella* ser. Enteritidis

Author Block:

L. Hu[1], L. M. Ma[2], S. Zheng[3], T. S. Hammack[1], E. W. Brown[1], G. Zhang[1]; [1]U.S. Food & Drug Admin. (FDA), College Park, MD, [2]Oklahoma State Univ., Stillwater, OK, [3]Northeast Agricultural Univ., Harbin, China

Abstract Body:

Loop-mediated isothermal amplification (LAMP) is a novel simple detection technology that amplifies DNA with high speed, efficiency, and specificity under isothermal conditions. The objective of this study was to develop a new LAMP assay for the detection of *Salmonella* ser. Enteritidis (SE) from foods. Prot6E gene encoding fimbrial biosynthesis protein was the target for detection. PrimerExplorer V4 software was used to design primers. Eight sets of primers were selected and evaluated for their effectiveness in detecting SE using isothermal master mix (OptiGene, UK), with SE isolates SE12 (Phage Type 14b), 18579 (PT4), and CDC_2010K_1441 (PT8). Ratio of outer and inner primers, amount of DNA template per reaction, reaction temperature and time were optimized using the 3 SE isolates described above. The following primers were determined to be effective in detecting SE: F3 5′-GTGAGGGCGAGGTTTGAAC-3′, B3 5′-GGTCATGGTCGGGTATTCGA-3′, FIP 5′-TCCTCCACTGACAGGATTCCCAAAGTGGAGCAGCTGAGCA-3′, BIP 5′-CAAACTGAACGTTTGGCTGCCTGCAGTGACTGGCATCAAGAA-3′. Inclusivity test using 24 SE isolates was 100% positive. Twenty four non-SE *Salmonella* isolates (24 serotypes) and 20 non-*Salmonella* isolates (10 species) were tested for exclusivity. They were 100% negative. More isolates will be tested. And effectiveness of these primers in detecting SE from egg products is being investigated. These preliminary data showed that the newly designed LAMP assay could be a rapid molecular tool for detecting SE.

Author Disclosure Block:

L. Hu: None. **L.M. Ma:** None. **S. Zheng:** None. **T.S. Hammack:** None. **E.W. Brown:** None. **G. Zhang:** None.

Poster Board Number:

FRIDAY-253

Publishing Title:

Charbohydrate-Active Enzymes from the Zygomycete *Lichtheimia ramosa* Strain h71D

Author Block:

M. Alvarez-Zúñiga[1], J. Ayala-Sumuano[2], C. Cano-Ramírez[1], M. Hidalgo-Lara[1]; [1]Centro de Investigación y Estudios Avanzados del Inst. Politécnico Natl., Distrito Federal, Mexico, [2]Univ. Natl. Autónoma de México, Querétaro, Mexico

Abstract Body:

Background: The phylum Zygomycota is a primitive and early diverging group of fungi that contains approximately 1% of the described species of true fungi (~900 described species). Zygomycetes surround us in our daily life, not only as agents of disease, but also as starters of fermentation in the preparation of food products, and as pioneer degraders in food spoilage. Since many members grow easily in axenic culture and show an impressive morphology, they have been subject of studies since the mid nineteenth century. However, Zygomycetes have been scarcely investigated both genetically and genomically. Fungi produce a variety of carbohydrate activity enzymes (CAZymes) for the degradation of plant polysaccharide materials to facilitate infection and/or gain nutrition. In our working group we are interested in the study of industrial enzymes. **Methodology:** The fungal strain H71D isolated from sugarcane bagasse was chosen because its ability to grow on a wide range of temperatures (heat-tolerant, with optimum growth temperature of 37°C) and present lignocellulosic activity. H71D was identified both morphologically and molecularly (ITS, and secondary structure of the ITS2) as *Lichtheimia ramosa*. The genome of *L. ramosa* was sequenced by Illumina HiSeq 2000 100bp pair-end. **Results:** DNA sequencing resulted in 57,199,250 raw reads. Filtered reads were assembled using SOAPdenovo2 resulting in a draft genome of a 15,915 scaffolds with a N50 size of 4,560, the GC content is 40% and 26,228 predicted protein-coding genes, which were analyzed with Cazymes Analysis Toolkit (CAT). CAZymes annotation resulted in 1160 glycoside transferases (GT), 613 glycoside hydrolases (GH), 322 carbohydrate esterases (CE), 198 carbohydrate-binding modules (CBM), and 146 auxiliary activities (AA). **Conclusion:** The *L. ramosa* genome encodes a collection of CAZymes different of that of other filamentous fungi like ascomycetes and basidiomycetes, *e.g.*, the *L. ramosa* genome encodes higher number of GT than GH compared to ascomycetes (*H. jecorina, C. globusum*, etc) and basidiomycetes (*U. maydis, P. chrysosporium*, etc), suggesting that zygomycetes present different strategies for polysaccharide degradation.

Author Disclosure Block:

M. Alvarez-Zúñiga: None. **J. Ayala-Sumuano:** None. **C. Cano-Ramírez:** None. **M. Hidalgo-Lara:** None.

Poster Board Number:

FRIDAY-254

Publishing Title:

Complete Genome Sequence of *Weissella jogaejeotgali* FOL01^{T}, Isolated from Traditional Korean Fermented Seafood

Author Block:

H-J. Ku, J-H. Lee; Inst. of Life Sci. and Resources, Kyung Hee Univ., Yongin-si, Korea, Republic of

Abstract Body:

Weissella is recognized as lactic acid bacteria and potential probiotics for promoting host health. Most recently, a novel species, *W. jogaejeotgali*, was reported. To understand genomic features of this new species, *W. jogaejeotgali* FOL01^{T} (= KCCM 43128^{T} = JCM 30580^{T}) was isolated from a jogaejeotgal, a traditional Korean fermented salted calm and its genome was completely sequenced and analyzed. The genome sequence of FOL01^{T} was determined using a SMRT® Technology PacBio RS II and analyzed using various bioinformatic programs including GeneMarkS, RAST, BLAST, InterProScan, and JSpecies. The genome consists of a circular chromosome consists of 2,114,163-bp with the GC content of 38.85% and a plasmid pFOL01 consists of 35,341-bp with the GC content of 39.14%. The chromosome has 2,016 open reading frame (ORF), 75 tRNA genes, 8 rRNA operons, and two additional 5S rRNA genes. Among the predicted ORFs, 1,583 ORFs were annotated to encode functional proteins (78.52%) and 433 ORFs were predicted to encode hypothetical proteins (21.48%). pFOL01 were predicted 51 ORFs, containing MobC and RepB genes for plasmid mobilization and replication. 16S rRNA sequence analysis and multilocus sequence typing (MLST) of FOL01^{T} showed that *W. thailandensis* is closely related, probably evolved from a common ancestor. Metabolic pathway analysis revealed that FOL01^{T} utilize heterofermentative sugar fermentation for energy production. In addition, osmotic stress response-related ORFs encoding proline/glycine betaine ABC transport were detected for adaptation in high-salt fermented foods. This is the first report for genome information of a newly identified *W. jogaejeotgali* providing its physiological and genomic properties for further understanding in its role in the fermented foods.

Author Disclosure Block:

H. Ku: None. **J. Lee:** None.

Poster Board Number:

FRIDAY-256

Publishing Title:

Identification of Background Flora in Ice Cream Associated with a Listeriosis Outbreak

Author Block:

C. J. GRIM, L. S. Burall, A. R. Datta; Food and Drug Admin., Laurel, MD

Abstract Body:

Recently, an outbreak of listeriosis was linked to ice cream products manufactured by a firm with plants in two different states. Enumeration of products from a single line linked to a cluster of illnesses in Kansas indicated that the *Listeria monocytogenes* (*Lm*) contamination had been present at a fairly consistent level for at least nine months. During enumeration work, direct plating of a portion of the samples on RAPID'*L.Mono*™ (RLM) agar, yielded a diverse range of colony phenotypes that did not match any of the typical *Listeria* phenotypes. The level of background flora observed varied and while it was not quantified, it generally appeared to exceed *Lm*. Subsequently, ice cream samples were plated on RLM and incubated at 5°C, room temperature and 37°C. Twenty colonies representing ten general colony phenotypes inconsistent with *Lm* were isolated for characterization via whole genome sequencing. The colonies were presumptively identified by comparing 16S RNA sequences to the NCBI and the Ribosomal Database Project (release 11) databases. The colonies were identified as matching 16S RNA sequences from *Bacillus paralicheniformis, Brevundimonas sp.*, two different *Microbacterium sp.*, isolates, *Microbacterium testaceum, Rhodococcus sp., Sanguibacter keddieii* and *Staphylococcus pasteuri*. We compared the draft genomes of the non-Lm organisms using tetramer frequency and found that, while multiple isolates matched to the same 16S RNA reference sequence, none appeared to have clonal relationships with each other or their reference strains' sequences. Evaluation of the non-*Lm* isolates demonstrated a broad diversity with Gram positive genera present from three different Orders, as well as a Gram negative isolate. Many of the species identified are typically associated with environmental sources, such as plants, soil, and water, as well as industrial settings, and are also known to grow in cold and various other stress conditions, overlapping some of *Lm* growth characteristics. Metagenomic sequencing of the microbiome of the ice cream samples confirmed our culture-based isolations, but revealed even greater diversity. This study helps to more clearly define the complicated nature of the background flora associated with a contaminated food production line that may affect the survival of the pathogen in the facility and the subsequent detection and enumeration of Lm in the samples.

Author Disclosure Block:

C.J. Grim: None. **L.S. Burall:** None. **A.R. Datta:** None.

Poster Board Number:

FRIDAY-257

Publishing Title:

Characterizing Methicillin Resistance in Methicillin Resistant *Staphylococcus aureus* of Swine

Author Block:

C. M. Logue, T. Cavender, D. W. Nielsen, N. Lima Barbieri; Iowa State Univ., Ames, IA

Abstract Body:

Background: Methicillin-associated resistance in *S. aureus* is encoded by the *mecA* gene, carried on the staphylococcal cassette chromosome *mec* (*SCCmec*) resulting in an altered penicillin binding protein (PBB) and reduced susceptibility to the β-lactam antibiotics including penicillin. Until now, detection of *mecA* or PBP was considered an indicator of methicillin resistance and confirmation of MRSA. Recently, new strains of MRSA resistant to methicillin but negative for *mecA* and PBP have been recognized. **Methods:** This study examined the prevalence of *mecA* and *mec* variants in a collection of MRSA and non-MRSA from swine and pork meat. Isolates examined were identified as methicillin resistant on media containing oxacillin. A total of 598 *S. aureus* and non-*S. aureus* strains from production swine at lairage and slaughter and 65 isolates recovered from retail pork were examined for *mec* genes including *mecA*, $mecA_{LGA251}$, *mecA1* and *mecC1* using PCR. Isolates were also subtyped for *SCCmec* using a multiplex PCR. **Results:** Overall most isolates identified as methicillin resistant were positive for the *mecA* gene (n = 521) however, a small collection (n=22) were identified as $mecA_{LGA251}$ positive, but they could not be confirmed as *mecC* using standard primers. Further typing using *mecA1* primers detected this variant in four isolates of both *S. aureus* and non-*aureus* species. Subtyping *mecA* positive isolates using the *SCCmec* typing scheme found the most common subtypes detected included II, IV and VI. A significant number could not be assigned to a subtype using the current scheme and will require further analysis. **Conclusions:** While the emerging *mecC* variant has not been detected in MRSA and non-MRSA of swine to date, our data suggests that there are other emerging novel variants of *mecA* and *mecA1* that would appear to be common. These novel variants may also be a source of, or recipient of resistance and could potentially pose a threat to animal or human health.

Author Disclosure Block:

C.M. Logue: None. **T. Cavender:** None. **D.W. Nielsen:** None. **N. Lima Barbieri:** None.

Poster Board Number:

FRIDAY-258

Publishing Title:

Food Plants are a More Efficient Reservoir Than Food Animals for Human Infections with *Salmonella* Newport

Author Block:

M. Yue, S. C. Rankin, D. M. Schifferli; Univ. of Pennsylvania Sch. of Vet. Med., Philadelphia, PA

Abstract Body:

Salmonella enterica remains the leading cause of bacterial foodborne diseases in the U.S. For the last 15 years, serovar Newport (*S.* Newport) has ranked among the three most frequent serovar for human infections. In a retrospective survey by the CDC, 14,811 non-human Newport isolates from 1968 to 2011, approximately half of the clinical isolates were of bovine origin, while nearly one third of the non-clinical strains were food plant and environmental isolates. These findings prompted an accentuated surveillance on bovines to better control the incidence of human infections with *S.* Newport. Surprisingly, our analysis of the 97 *S.* Newport outbreaks with confirmed sources of infection that occurred between 1998 and 2014 indicated that contaminated food plants (53%) were significantly more often the cause than products of land animal origins (32%). A minimum spanning tree based on multilocus sequence typing (MLST) separated 1,787 isolates in three clades that coincided with the origins of isolation (human, animal and environment). The constructed tree indicated that isolates of human origin were more directly related to environmental strains than to animal isolates ($P<0.01$). Moreover, a phylogenomic analysis of 1050 available *S.* Newport genomes clearly grouped over 90% of the land animal genomes together as a distinct cluster. In contrast, both the human and food plant isolates were randomly distributed among clades to the exclusion of the land animal clades, suggesting a greater role of plants than animals for *S.* Newport infections in humans. The environmental and aquatic isolates took an intermediary distribution by preferential associations with some specific clades. Finally, an extensive analysis of the antibiotic resistance profiles for 3,497 *S.* Newport isolates similarly separated human and plant strains from animal isolates of *S.* Newport. Taken together, our findings suggests that Newport joins an increasing number of food plant-associated *Salmonella* serovars that cause foodborne infections in humans. The relative trend in recent years of salmonellosis outbreaks due to food plants calls for improved surveillance methods.

Author Disclosure Block:

M. Yue: None. **S.C. Rankin:** None. **D.M. Schifferli:** None.

Poster Board Number:

FRIDAY-259

Publishing Title:

Novel Locus Mediating Metal Homeostasis in Listeria Monocytogenes

Author Block:

C. Parsons, S. Kathariou; North Carolina State Univ., Raleigh, NC

Abstract Body:

p *Listeria monocytogenes* is a ubiquitous facultative intracellular pathogen responsible for listeriosis, a severe foodborne illness in humans and other animals. One key element to *Listeria*'s survival in the environment is its ability to tolerate frequently high levels of heavy metals, especially cadmium and arsenic. Sequestration of essential metals or challenging with toxic metals are two mechanisms used by host organisms to combat infection. To better understand the functional role that genes associated with heavy metal homeostasis play in *Listeria*, a *mariner*-based mutant library was created for the cadmium and arsenic-resistant serotype 4b strain F8027 (originally isolated from celery) and screened for loss of heavy metal resistance. Four mutants with impaired tolerance to cadmium were isolated. Of those, two were found to have transposon insertions in a large chromosomal island harbored by F8027. For one of these mutants, E2G4, the transposon insertion localized to the intergenic space between two oppositely transcribed genes LMOSA_2450 (annotated as wall-anchored protein with the LPXTG motif) and LMOSA_2460-2470 (hypothetical proteins). While E2G4 was initially identified by its reduced tolerance to cadmium, this mutant also had reduced tolerance to zinc. Tolerance to arsenic and copper, as well as an array of antibiotics was not impacted. Genetic complementation of E2G4 with the LMOSA_2460-2470 cassette cloned into the integration vector pPL2 restored the cadmium resistance phenotype, while complementation with LMOSA_2450 restored the resistance to zinc. Consistent with the heavy metal resistance phenotype, this mutant was tested for growth on the surface of cantaloupe and biofilm formation, with no perceived inhibition. Virulence assessments were conducted in a *Galleria mellonella* model with no significant impact on virulence. Since LMOSA_ 2470 is co-transcribed with LMOSA_2460, they are in the process of being cloned individually to ascertain their specific contributions to the phenotype. Additionally, a deletion of LMOSA_2450 is also in progress to better understand what role this wall-associated protein may play in heavy metal tolerance and possibly virulence of *L. monocytogenes*. Quantitative real time PCR is also being pursued to assess potential induction of these genes in the presence of cadmium and zinc.

Author Disclosure Block:

C. Parsons: None. **S. Kathariou:** None.

Poster Board Number:

FRIDAY-260

Publishing Title:

Antimicrobiobial Resistance of Staphylococci Isolated from Bovine Mastitis in Argentina

Author Block:

M. E. Srednik[1], M. Archambault[2], L. Barnech[1], E. R. Gentilini[1]; [1]Univ. de Buenos Aires, Buenos Aires, Argentina, [2]Université de Montreal, St-Hyacinthe, QC, Canada

Abstract Body:

Background: Bovine mastitis causes important economic losses in the dairy industry. *Staphylococcus aureus* and coagulase-negative staphylococci (CNS) are commonly isolated from bovine mastitis. β-lactams and macrolides-lincosamides (ML) antibiotics are frequently used in intrammamary therapy. CNS can be considered reservoir of antimicrobial resistance genes. The genes *mec*A and *mec*C both confer resistance to all β-lactams antimicrobials. **Methods:** Eighty *S. aureus* and ninety CNS isolates from bovine mastitis milk samples from different farms in Argentina, were tested by disk diffusion test for susceptibility to penicillin, oxacillin, cefoxitin, erythromycin and clindamycin, and also were tested by PCR for the presence of resistance genes for β-lactams (*bla*Z, *mec*A and *mec*C genes) and ML (*erm*A, *erm*B, *erm*C, *mef*A, *msr*A, *mph*C and *lnu*A genes).**Results:** *S. aureus* resistance to β-lactams in Argentina is 23.1%. Among 80 *S. aureus* isolates, 32 (40%) were positive to *bla*Z gene and 15 (18.75%) to ML genes: *erm*B and *mef*A (4 isolates), *erm*B (2 isolates) *mef*A (2 isolates), *erm*B, *erm*C and *mef*A (1 isolate), *erm*A, *erm*B, *erm*C and *mef*A (1 isolate), *erm*B, *mef*A, *lnu*A and *msr*A (1 isolate), *erm*C, *erm*B *and lnu*A (1 isolate) and *erm*A (1 isolate). Among 90 CNS isolates, 12 (13.3%), 4 (4.4%), and 1 (1.1%) were positive for *bla*Z, *mec*A and *mec*C genes, respectively. Only in one isolate we found both, *bla*Z and *mec*A genes, and 6 (6.7%) were positive to ML genes: *erm*C (1 isolate), *erm*B and *erm*C (2 isolates), *erm*B, *erm*C and *mph*C (1 isolate), *mph*C (1 isolate), and *mph*C and *mrs*A (1 isolate). The recently described *mec*C gene has been detected by PCR in a few CNS of animal origin only around Europe. We describes for the first time a *mec*C positive isolate of CNS from bovine mastitis in Argentina. Identification of mastitis pathogens is important for selecting appropriate antimicrobial therapy. Reservoirs of antimicrobial resistance genes are potential threat to public health.**Conclusions:**The presence of a methicillin resistant (MR) CNS isolates in this study emphasize the importance of identification of CNS and its antibiotic susceptibility when an intramammary infection is present. Detection of MR should consider the inclusion of *mec*C gene because of the potential risk of lateral transfer of this gene between staphylococcal species.

Author Disclosure Block:

M.E. Srednik: None. **M. Archambault:** None. **L. Barnech:** None. **E.R. Gentilini:** None.

Poster Board Number:

FRIDAY-262

Publishing Title:

Multicenter Evaluation of Vancomycin MIC Results at 18 Hours for Staphylococci and Enterococci Using Microscan Dried Overnight Performance Evaluation Device Panel

Author Block:

P. C. Schreckenberger[1], J. Tjhio[1], M. P. Weinstein[2], C. R. Polage[3], R. M. Humphries[4], J. A. Hindler[4], M. Evans[5], **R. K. Brookman**[5], J. Y. Chau[5], D. Carpenter[5]; [1]Loyola Univ. Med. Ctr., Maywood, IL, [2]Rutgers Robert Wood Johnson Med. Sch., New Brunswick, NJ, [3]UC Davis Med. Ctr., Sacramento, CA, [4]UCLA David Geffen Sch. of Med., Los Angeles, CA, [5]Beckman Coulter Microbiol., West Sacramento, CA

Abstract Body:

Background: A multicenter study was performed to evaluate the accuracy of a revised formulation of vancomycin on a MicroScan Dried Gram Positive MIC (MSDGP) Panel when compared to CLSI broth microdilution reference panels.**Methods:** For efficacy, MSDCP panels were evaluated at four sites with 947 clinical isolates. For reproducibility, a subset of 11 organisms was tested on MSDGP panels at each site. MSDGP panels were inoculated using both turbidity and Prompt™* inoculation methods. Frozen reference panels, prepared according to ISO/CLSI methodology, were inoculated using the turbidity inoculation method. All panels were incubated at 35 ±2ºC and visually read. Frozen reference panels were read and reported at 24 hours for all organisms. Read times for the MSDGP panels were 18 hours for all species. FDA/CLSI breakpoints (µg/ml) were used for interpretation of MIC results.**Results:** When compared to frozen reference panel results, essential and categorical agreements for isolates tested in Efficacy are as follows:

Read Method	**Essential Agreement %**		**Categorical Agreement %**		**Very Major Errors %**		**Major Errors %**		**Minor Errors %**	
	T	**P**	**T**	**P**	**T**	**P**	**T**	**P**	**T**	**P**
Visual	99.4 (941/947)	96.2 (911/947)	99.5 (942/947)	99.2 (939/947)	0.0 (0/62)	0.0 (0/62)	0.0 (0/882)	0.1 (1/882)	0.5 (5/947)	0.7 (7/947)
WalkAway	99.5 (942/947)	95.8 (907/947)	99.6 (943/947)	99.2 (939/947)	0.0 (0/62)	0.0 (0/62)	0.0 (0/882)	0.0 (0/882)	0.4 (4/947)	0.8 (8/947)

autoSCAN-4	99.0 (938/947)	96.2 (911/947)	99.4 (941/947)	99.3 (940/947)	0.0 (0/62)	0.0 (0/62)	0.1 (1/882)	0.0 (0/882)	0.5 (5/947)	0.7 (7/947)
T = Turbidity inoculation method, P = Prompt™* inoculation method										

Reproducibility among the four sites were greater than 95% for all read methods for both the turbidity and Prompt™* inoculation methods.**Conclusion:** This multicenter study showed that vancomycin MIC results read at 18 hours for *Enterococcus* and *Staphylococcus* species obtained with the MSDGP panel with a revised vancomycin formulation correlate well with MICs obtained using frozen reference panels read at 24 hours.* PROMPT is a registered trademark of 3M.Beckman Coulter, the stylized logo and the Beckman Coulter product and service names mentioned herein are trademarks or registered trademarks of Beckman Coulter, Inc. in the United States and other countries.

Author Disclosure Block:

P.C. Schreckenberger: None. **J. Tjhio:** None. **M.P. Weinstein:** None. **C.R. Polage:** None. **R.M. Humphries:** None. **J.A. Hindler:** None. **M. Evans:** None. **R.K. Brookman:** None. **J.Y. Chau:** None. **D. Carpenter:** None.

Poster Board Number:

FRIDAY-263

Publishing Title:

Multicenter Evaluation of Daptomycin Mic Results for *Staphylococci* Using Microscan Dried Gram Positive Mic Panels

Author Block:

P. C. Schreckenberger[1], J. Tjhio[1], R. Humphries[2], J. A. Hindler[2], C. R. Polage[3], M. P. Weinstein[4], M. Evans[5], R. K. Brookman[5], J. Y. Chau[5], **D. Carpenter**[5]; [1]Loyola Univ. Med. Ctr., Maywood, IL, [2]UCLA David Geffen Sch. of Med., Los Angeles, CA, [3]UC Davis Med. Ctr., Sacramento, CA, [4]Rutgers Robert Wood Johnson Med. Sch., New Brunswick, NJ, [5]Beckman Coulter Microbiol., West Sacramento, CA

Abstract Body:

Background: A multicenter study was performed to evaluate the accuracy of a revised formulation of daptomycin on a MicroScan Dried Gram Positive MIC (MSDGP) Panel when compared to frozen CLSI broth microdilution reference panels.**Methods:** For efficacy, MSDGP panels were evaluated at four sites with a total of 784 *Staphylococcus* spp. and 169 *Enterococcus* spp. clinical isolates using the turbidity and Prompt™* methods of inoculation. For reproducibility, a subset of 12 organisms was tested on MSDGP panels at each site. MSDGP panels were incubated at 35 ±2°C and read on the WalkAway System, the autoSCAN-4 instrument, and read visually. Read times for the MSDGP panels were at 16-20 hours. Frozen reference panels, prepared according to ISO/CLSI methodology; were inoculated using the turbidity inoculation method, incubated at 35 ±2°C, and read visually. Frozen reference panels were read and reported at 18 hours for all organisms. CLSI breakpoints (µg/ml) used for interpretation of MIC results were: *Staphylococcus* spp. ≤ 1 S; and *Enterococcus* spp. ≤ 4 S.**Results:** When compared to frozen reference panel results, essential and categorical agreements for isolates tested in Efficacy are as follows:

Read Method	**Essential Agreement %**		**Categorical Agreement %**		**Categorical Errors %**	
	T	**P**	**T**	**P**	**T**	**P**
Visual	99.3 (946/953)	93.6 (892/953)	99.7 (950/953)	99.4 (947/953)	0.3 (3/953)	0.6 (6/953)
WalkAway	98.6 (940/953)	94.2 (898/953)	99.5 (948/953)	99.0 (943/953)	0.5 (5/953)	1.0 (10/953)
autoSCAN-4	97.0 (924/953)	93.4 (890/953)	99.5 (948/953)	99.2 (945/953)	0.5 (5/953)	0.8 (8/953)

T = Turbidity inoculation method, P = Prompt™* inoculation method

Reproducibility among the four sites were greater than 95% for all read methods for both the turbidity and Prompt™* inoculation methods.**Conclusion:** This multicenter study showed that daptomycin MIC results for *Enterococcus* and *Staphylococcus* spp. obtained with the MSDGP panel with a revised daptomycin formulation correlate well with MICs obtained using frozen reference panels.For Export Only. Not for sale in the US* PROMPT™ is a registered trademark of 3M.

Author Disclosure Block:

P.C. Schreckenberger: None. **J. Tjhio:** None. **R. Humphries:** None. **J.A. Hindler:** None. **C.R. Polage:** None. **M.P. Weinstein:** None. **M. Evans:** None. **R.K. Brookman:** None. **J.Y. Chau:** None. **D. Carpenter:** None.

Poster Board Number:

FRIDAY-265

Publishing Title:

Molecular Characterization Of Methicillin-Susceptible *staphylococcus Aureus* Blood Isolates With High Vancomycin Mics

Author Block:

Y. R. Kang[1], D. R. Chung[1], S. H. Kim[1], Y. E. Ha[2], C-I. Kang[2], K. R. Peck[2], J-H. Song[1]; [1]Asia Pacific Fndn. for Infectious Diseases, Seoul, Korea, Republic of, [2]Samsung Med. Ctr., Sungkyunkwan Univ. Sch. of Med., Seoul, Korea, Republic of

Abstract Body:

Background: High MIC of vancomycin in methicillin-resistant *Staphylococcus aureus* (MRSA) has been considered as one of the causes of vancomycin treatment failure. Among methicillin-susceptible *S. aureus* (MSSA), however, strains with high vancomycin MICs are often found. We characterized 71 MSSA blood isolates with high vancomycin MICs. **Methods:** Vancomycin susceptible MSSA with vancomycin MIC ≥ 1 mg/L was selected among the *S. aureus* collection from the previous nationwide surveillance study on bacteremia in Korea during the periods 2006-2007 and 2012-2013. MSSA isolates with vancomycin MIC < 1 mg/L were randomly selected in the ratio of 1:1 as the control. Isolates were characterized by *spa* typing, multilocus sequence typing (MLST), *agr* group and the distribution of 7 staphylococcal enterotoxin (SE) genes. The presence of hVISA was screened by Etest and growth on brain heart infusion agar with 4 mg/L vancomycin (BHIV4). hVISA was confirmed by using a modified PAP-AUC method.**Results:** Among 242 MSSA isolates, a total of 71 isolates (29.3%) with vancomycin MIC ≥ 1 mg/L were identified. A total of 25 *spa* types and 10 STs were identified. The most predominant clonal type was ST6-t207 (14.1%). In control group, 31 *spa* types and 21 different STs were identified with distribution of ST188-t189 (14.1%). All *agr* types were represented in the isolates of MSSA with high vancomycin MIC : 63.4% were *agr*-group I, 5.6% *agr*-group II, 26.8% *agr*-group III and 4.2% were non-typable (NT). The control strains were grouped as *agr*-group I (52.1%), *agr*-group II (15.5%), *agr* group III (29.6%), *agr* group IV (1.4%) and 1.4% were NT. SEI was the predominant enterotoxins in both MSSA with high vancomycin MIC (45.1%) and MSSA with low vancomycin MIC (55%). Six isolates were positive by hVISA screening, however none of those were not hVISA by confirmation test.**Conclusions:** We investigated the molecular characteristics of MSSA blood isolates with high vancomycin MICs. ST6-t207 was frequently found in MSSA with high vancomycin MICs compared to MSSA with low vancomycin MICs, of which ST188-t189 was predominant. Multiple combinations of enterotoxin genes were seen in both groups. No hVISA was detected among tested isolates.

Author Disclosure Block:

Y.R. Kang: None. **D.R. Chung:** None. **S.H. Kim:** None. **Y.E. Ha:** None. **C. Kang:** None. **K.R. Peck:** None. **J. Song:** None.

Poster Board Number:

FRIDAY-266

Publishing Title:

Genetic Alteration Responsible for Reduced Vancomycin Susceptibility in *Staphylococcus aureus* of Sequence Type 72

Author Block:

J. Y. Baek[1], D. R. Chung[2], S. H. Kim[1], S-J. Yang[3], C-I. Kang[2], K. S. Ko[4], K. R. Peck[2], J. H. Song[1]; [1]Asia Pacific Fndn. for Infectious Diseases, Seoul, Korea, Republic of, [2]Samsung Med. Ctr., Sungkyunkwan Univ. Sch. of Med., Seoul, Korea, Republic of, [3]Sch. of Bioresources and BioSci., Chung-Ang Univ., Anseong, Korea, Republic of, [4]Samsung BioMed. Res. Inst., Sungkyunkwan Univ. Sch. of Med., Suwon, Korea, Republic of

Abstract Body:

Background: Previously, we reported the first case of vancomycin treatment failure due to development of vancomycin intermediate resistance in a patient with community genotype ST72-methicillin-resistant *Staphylococcus aureus* (MRSA)- SCC*mec* IV infection. We obtained two isogenic MRSA isolates from the bacteremia patient who experienced treatment failure with vancomycin and rifampin. We investigated the genetic alteration that confers to vancomycin resistance by tracking the mutations in these strains using whole genome sequencing (WGS). **Methods:** To identify the genetic changes that confer vancomycin intermediate resistant *S. aureus* (VISA) phenotype, WGS of vancomycin-susceptible and intermediate resistant *S. aureus* isolates (VSSA303 and VISA072, respectively) was completed. The mutations between two isolates were introduced into VSSA303 to test the effect of mutation on VISA phenotype. The strains were tested for antimicrobial susceptibilities, population analysis under the curve (PAP/AUC) and transmission electron microscopy (EM). **Results:** Five non-synonymous mutations were identified by genome sequence data, which included *agrC* (K389stop), *dprA* (G196C), *rpoB* (H481Y), *vraR* (E127K), and a gene encoding a UDP-N-acetylmuramoylpentapeptide-glycine glycyltransferase (F92C). Introduction of the *vraR* (E127K) mutation was accompanied by increase of vancomycin MIC, and acquisition of hetero-VISA phenotype confirmed by PAP/AUC, which are partially responsible for vancomycin resistance. EM of strains with mutated *vraR* showed increased cell wall thickness. **Conclusion:** We identified 5 alleles that were potentially associated with VISA phenotype developed in clinical MRSA strain of the ST72 background. The presence of a unique mutation in the *vraR* gene (E127K) may contribute to vancomycin resistance, which is supported by cell wall thickness and PAP analysis. Further studies are needed to elucidate the molecular effects of these genetic changes which confer or partially contribute to resistance.

Author Disclosure Block:

J.Y. Baek: None. **D.R. Chung:** None. **S.H. Kim:** None. **S. Yang:** None. **C. Kang:** None. **K.S. Ko:** None. **K.R. Peck:** None. **J.H. Song:** None.

Poster Board Number:

FRIDAY-267

Publishing Title:

Trends in Antimicrobial Resistance of *Streptococcus pyogenes* and Its Association with *emm* Types

Author Block:

T. ABRAHAM, S. Sistla; Jawaharlal Inst. of Postgraduate Med. Ed. and Res., Puducherry, India

Abstract Body:

Background: In Penicillin allergic patients, macrolides and tetracyclines are the most common antibiotics employed for the treatment of streptococcal infections. A decline in the resistance rate of these antibiotics has been observed in different parts of the world. The present study was undertaken to evaluate the antibiotic resistance pattern, molecular basis of resistance and its association with *emm* types among *Streptococcus pyogenes* isolates in Pondicherry, India**Methods:**One hundred and seventy three *Streptococcus pyogenes* isolates were characterized by antimicrobial susceptibility, resistance genotype, macrolide resistance phenotype (using double- disc test) and *emm* genotyping (M typing)**Results:**All the isolates were susceptible to penicillin and vancomycin. Erythromycin resistance was found in 51.4%, with MIC_{50} &MIC_{90} of ≥256 μg ml^{-1}. Inducible phenotype was common (iMLS, 67.4%, 60 strains) followed by the M phenotype (32.5%, 29 strains). Among these isolates, 65.1% harboured *erm*B and 32.5% *mef*A as sole macrolide resistance gene, whereas presence of *erm*B plus *mef*A (2.2%) was also observed. Of the erythromycin resistant strains, 42.6% were invasive. The prevalent *emm* types among resistant strains were *emm* 63 (11.2%), *emm* 44 (6.7%), *emm* 42 (5.6%), and *emm* 75.3, *emm* 82, *emm* 85, *emm* 92, *emm* 111.1(4.4% each). Statistically significant association was observed between *emm* 63, *emm* 44 and erythromycin resistance (P-value < 0.05). Tetracycline resistance was found in 59.5%, with MIC_{50} &MIC_{90} of 24 μg ml^{-1} and 32 μg ml^{-1} respectively. Among these isolates, 96.1% were positive for *tet*M, 2.9% were positive for *tet*O and one strain positive for both *tet*M and *tet*O. Of these tetracycline resistant strains, 23.4% were invasive. . The prevalent *emm* types among resistant strains were *emm* 63 (9.7%), *emm* 44 (5.8%), *emm* 92 (4.8%), and *emm* 75.3, *emm* 82.1, *emm* 85, *emm* 106 (3.8% each). Statistically significant association was not observed between emm types and tetracycline resistance.**Conclusion:**Erythromycin and tetracycline resistance continues to exist at high levels in Pondicherry, may be attributed to the over prescription and use of these antibiotics. This study suggests that the variation in antibiotic resistance among *Streptococcus pyogenes* is, with a few exceptions independent of *emm* type distribution

Author Disclosure Block:

T. Abraham: None. **S. Sistla:** None.

Poster Board Number:

FRIDAY-268

Publishing Title:

Intestinal Colonization of VanA Genotype Vancomycin-Resistant E*nterococcus faecium* in Western Nepal

Author Block:

S. Hosuru Subramanya[1], N. Kishor Sharan[1], B. Prasad Baral[1], N. Nayak[1], I. Bairy[2], S. Gokhale[1]; [1]Manipal Coll. of Med. Sci., Pokhara, Nepal, [2]Melaka Manipal Med. Coll., Manipal, India

Abstract Body:

Background: The gastrointestinal colonization by multi drug resistant *Enterococcus* has been increasing worldwide. The present study was dedicated to determine the rate of fecal carriage of multi drug resistant *Enterococcus* spp. in hospitalized patients and in individuals living in the community and to determine the predominant vancomycin resistance genotype in western Nepal.**Materials and Methods:** A cross sectional study was conducted. Stool specimens were collected from 141 community subjects and 129 hospitalized patients. Isolates were identified by conventional methods and Vitek based identification parameters. Antibiotic susceptibility testing was performed using Kirby-Bauer disk diffusion method. The Minimum inhibitory concentration [MIC] for vancomycin was determined by both E-test strips and micro broth dilution method. Genotypic characterization of VRE was performed by multiplex PCR assays targeting VanA, VanB and VanC genes.**Results:** Out of a total 270 study subjects, 142 (52.59%) yielded *Enterococci* spp. amongst these, 99 (69.71%) were from community subjects and 43(30.28%) were from hospitalized patients. Of these 142; 58 (40.84%) were *E faecalis,* 46 (32.39%) *E faecium* and 38 (26.76) were other *Enterococcus* species. The ampicillin resistance that was as high as 80.98% was quite alarming. A total of 47 (33.1%) out of 142 *Enterococci* were MDR. 31 (72.1%) of 43 hospitalizes individuals were colonised with MDR *Enteroccocci*, as compared to only 16 (16.2%) of 99 community individuals ($\chi^2 = 42.35$; $p < 0.001$).The overall prevalence rate of fecal VRE colonization was 6.33% (9/142). Whereas, 18.6% (8/43) of the hospitalised patients were colonised with VRE, only 1 (1.01%) out of 99 community subjects was colonised with VRE ($\chi^2=15.63$; $p< 0.001$). Quantification of VRE from 9 of the colonized patients showed that the colony counts ranged between 10^2 to 10^5 CFU/g of stool. All 9 VRE isolates were found to be *E faecium* and positive for *vanA* genotype, no *vanB or vanC* genotypes were found.**Conclusion:** High rate of colonization by MDR *Enterococcus* and VRE is alarming. Further studies are warranted to elucidate the origin and the epidemiology of vancomycin resistance in this geographical area.

Author Disclosure Block:

S. Hosuru Subramanya: None. **N. Kishor Sharan:** None. **B. Prasad Baral:** None. **N. Nayak:** None. **I. Bairy:** None. **S. Gokhale:** None.

Poster Board Number:

FRIDAY-269

Publishing Title:

Activity of Ceftaroline Tested Against Different Clonotypes of Methicillin-Resistant *Staphylococcus aureus* Collected from Two Nationwide Studies in Spain

Author Block:

E. Cercenado[1], A. Vindel[2], O. Cuevas[1], M. Marín[1], F. Román[2], E. Bouza[1], Staphylococcus Study Group; [1]Hosp. Gen. Univ.rio Gregorio Marañón, Madrid, Spain, [2]Inst. de Salud Carlos III, Majadahonda, Spain

Abstract Body:

Background: Ceftaroline (CPT) is a new cephalosporin active against methicillin-resistant *Staphylococcus aureus* (MRSA). Previous studies have demonstrated variable activity of CPT against different MRSA clonotypes and geographical differences regarding CPT MICs due to high prevalence of clonotypes ST228, ST239, and ST247. We evaluate the in vitro activity of CPT tested against MRSA isolates from different clonotypes collected in Spain. **Methods:** A total of 311 MRSA isolates collected in two nationwide prevalence studies performed in Spain in 2010 (152 isolates) and in 2014 (159 isolates) were studied. The presence of the *mecA* gene was determined by PCR. CPT susceptibility testing was performed by the gradient diffusion (GD) method (epsilon test, Liofilchem, Italy). CLSI and EUCAST breakpoints were applied for CPT (susceptible ≤1 mg/L). *S. aureus* ATCC 29213 and *E. faecalis* ATCC 29212 were used as control strains. Molecular characterization was achieved by SCC*mec* typing, *spa* typing, BURP, and MLST.**Results:** Among the 311 MRSA isolates, 212 corresponded to clonal complex (CC) 5 (ST5, ST125, ST225, ST146, and ST228), 38 to CC8 (ST8), 31 to CC22 (ST22), 9 to CC30 (ST36, ST30), 8 to CC398 (ST398), 5 to CC1 (ST1), 4 to ST72, 2 to ST273, 1 to ST146, and 1 to ST45. A total of 297 isolates (95.5%) carried SCC*mec* type IV (with its variants), 7 SCC*mec*II, 6 SCC*mec*V, and 1 SCC*mec* I. CPT inhibited 98% of isolates at an MIC of ≤1 mg/L, with MIC50/90 values of 0.38/1 mg/L. Only one isolate belonged to clonotype ST228. Comparative CPT activity (MIC, mg/L) against the different clones is shown in the table.**Conclusions:** This study shows potent in vitro activity of CPT against all MRSA clonotypes isolated in Spain with no differences in activity against isolates belonging to different CC. The very low frequency or absence of ST228, ST239, and ST247 in our country could determine a higher activity of CPT against MRSA.

Clonal complex (No. isolates)	**CPT MIC50**	**CPT MIC90**	**Range**
All CC (n=311)	0.38	1	0.064-2
CC5 (n=212)	0.38	1	0.064-2

CC8 (n=38)	0.25	1	0.19-1
CC22 (n=31)	0.5	1	0.12-1.5
CC30 (n=9)	0.75	0.75	0.38-1
CC398 (n=8)	0.38	0.75	0.19-0.75
CC1 (n=5)	-	-	0.25-1
ST72 (n=4)	-	-	0.25-0,5
ST273 (n=2)	-	-	0.75-1
ST146 (n=1)	-	-	0.25
ST45 (n=1)	-	-	0.38

Author Disclosure Block:

E. Cercenado: None. **A. Vindel:** None. **O. Cuevas:** None. **M. Marín:** None. **F. Román:** None. **E. Bouza:** None.

Poster Board Number:

FRIDAY-270

Publishing Title:

Antimicrobial Susceptibility Testing of Outbreak Associated *Listeria monocytogenes*, 1983-2015

Author Block:

C. Smith[1], J. Chen[1], Z. Kucerova[2], K. Jackson[2], K. Roache[3], C. Scheel[2]; [1]IHRC, Inc., Atlanta, GA, [2]Ctr.s for Disease Control & Prevention, Atlanta, GA, [3]ORISE, Oak Ridge, TN

Abstract Body:

Listeria monocytogenes is a bacterial foodborne pathogen causing an estimated 1600 cases of invasive listeriosis infections annually in the United States. Exposure to *L. monocytogenes* typically occurs through consumption of contaminated foods. Among foodborne illnesses, listeriosis has the highest mortality rate of around 20%. Treatment for invasive listeriosis consists of antibiotic therapy, usually an aminopenicillin and gentamicin or trimethoprim-sulfamethoxazole for patients with a β-lactam allergy. Antibiotic resistance is a growing concern with many enteric pathogens; however, little resistance has been shown in *L. monocytogenes*. In this study, 152 *L. monocytogenes* isolates representing over 90 different outbreaks from 1983-2015, were selected for antimicrobial susceptibility testing. Broth microdilution using the Thermo Scientific Sensititre™ Gram positive susceptibility panel comprised of 17 antimicrobials was performed and then interpreted using Clinical Laboratory Standards Institute (CLSI) M45 or European Committee of Antimicrobial Susceptibility Testing (EUCAST) criteria. No resistance to ampicillin, erythromycin or trimethoprim-sulfamethoxazole was detected, and gentamicin MICs were all ≤4 μg/mL. Seven isolates (5%) showed tetracycline MICs >16 μg/mL; the other 145 showed tetracycline MICs ≤4 μg/mL. Whole genome sequencing confirmed that all isolates with high tetracycline MICs possessed the *tetM* gene. Antimicrobial susceptibility should continue to be monitored in *L. monocytogenes* to identify changing trends in antimicrobial susceptibility.

Author Disclosure Block:

C. Smith: None. **J. Chen:** None. **Z. Kucerova:** None. **K. Jackson:** None. **K. Roache:** None. **C. Scheel:** None.

Poster Board Number:

FRIDAY-271

Publishing Title:

Colonization with Ca-Mrsa St5-Scc*mec*iv and St30-Scc*mec*iv Clones in Healthy Horses from Argentina

Author Block:

P. Gagetti[1], G. Giacoboni[2], C. Lopez[3], M. Kienast[2], M. Maldonado[4], D. Faccone[1], **A. Corso**[1]; [1]Antimicrobial Div. Natl. Reference Lab. INEI-ANLIS "Dr. Malbran", Buenos Aires, Argentina, [2]Faculty of Vet. Sci, UNLP, Buenos Aires, Argentina, [3]Faculty of Vet. Sci, UBA, Buenos Aires, Argentina, [4]Antimicrobial Div. Natl. Reference Lab. INEI-ANLIS, Buenos Aires, Argentina

Abstract Body:

The emergence of MRSA in horses, pet animals and productive livestock has risen worldwide. Colonized horses may transmit MRSA to other horses and people. We evaluated the MRSA nasal colonization in horses from Argentina, characterized the resistance profile and the clonal relation between isolates**Methods:** During May, September and October of 2015, 96 healthy thoroughbreds horses belonging to the La Plata Race track were screened for MRSA. Swabs were taken from nasal vestibulum and plated on chromogenic agar (CHROMagar™ MRSA). Nine suspicious strains were confirmed as *S. aureus* by conventional tests. Antibiotic susceptibility were done by disk diffusion test (CLSI M100S25). D-test was performed to evaluate inducible or constitutive MLSb-phenotype. Detection of macrolide resistance genes *ermA* and *msrA*, *mecA* and PVL were performed by PCR, SCC*mec* type was determined by multiplex PCR, and molecular typing by *Sma*I PFGE and MLST**Results:** Nine strains were resistant to cefoxitin and *mecA* positive. The prevalence of MRSA was 9.4% (9/96). All were susceptible to ceftaroline, gentamicin, ciprofloxacin, tetracycline, chloramphenicol, trimethoprim-sulfamethoxazol, rifampin, teicoplanin, linezolid and nitrofurantoin, but seven (78%) were resistant to erythromycin and clindamycin. All MRSA isolates carried the SCC*mec*IV. Two clonal types were observed by PFGE. Most of them (7/9) belonged to clonal type A the already described ST5-SCC*mec*IV responsible of the emergence of community associated MRSA in human infections in our country between 2000-08. All MRSA ST5-SCC*mec*IV were PVL negative, showed inducible MLSb phenotype, carried the *erm*A gene and were negative to *msrA* gene. Isolates from clone B (2/9) were PVL positive and belonged to ST30-SCC*mec*IV (South West Pacific clone), responsible for the half of the CA-MRSA infections in humans in our country nowadays**Conclusions:** This is the first study of prevalence of nasal MRSA colonization in healthy horses from Argentina. All the strains belonged to ST5-SCC*mec*IV or ST30-SCC*mec*IV, the two dominant community-associated clones in Argentina

Author Disclosure Block:

P. Gagetti: None. **G. Giacoboni:** None. **C. Lopez:** None. **M. Kienast:** None. **M. Maldonado:** None. **D. Faccone:** None. **A. Corso:** None.

Poster Board Number:

FRIDAY-272

Publishing Title:

In Vitro* Activity of Tigecycline and Minocycline Against Vanm-type Vancomycin-resistant *Enterococcus faecium

Author Block:

C. Chen, Y. Guo, F. Hu, X. Xu; Huashan Hosp., Fudan Univ., Shanghai, China

Abstract Body:

Background: Vancomycin-resistant enterococci (VRE) have become one of the most important nosocomial pathogens worldwide due to limited adequate treatment options and increasing occurrence of high-level resistance to most antimicrobial drugs. Tigecycline is one of the last-line treatment options left to combat multidrug-resistant bacteria including VRE. In previous study, we found that VanM is the most prevalent genotype in vancomycin-resistant *Enterococcus faecium* (VREm) in Shanghai. Most VanM-type VREm isolates were susceptible to doxycycline, but their susceptibilities to two other tetracycline antibiotics minomycline and tigecycline remain unknown. In this study, we investigated the in vitro activity of minocycline and tigecycline against VanM-type VREm and to provide guidance for clinical treatment on VRE infections. **Methods:** Forty five VREm clinical strains were obtained from hospitals in Shanghai between 2006 and 2014. Species and vancomycin resistance genotype were identified by PCR and sequencing. Minimal inhibitory concentrations (MICs) of ten antimicrobial agents (vancomycin, teicoplanin, tigecycline minocyline, linezolid, ampicillin, erythromycin, levofloxacin, gentamicin, and rifampicin) were determined by agar dilution or broth microdilution.**Results:** All the 45 isolates were VanM-type vancomycin-resistant *Enterococcus faecium,* and all of them were resistant to vancomycin with high MICs of 128 to >256 mg/L, 71.1% of the strains were resistant to teicoplanin. No isolates were resistant to tigecycline. The resistance rates to minocycline, gentamycin and rifampicin were 15.6%, 64.4% and 82.2%, respectively. Almost all isolates showed resistance to levofloxacin (100%) and ampicillin (97.8%).**Conclusions:** Though minocycline resistant VREm strains emerged, tigecycline exhibit excellent *in vitro* activity against these VanM-type VREm isolates. Tigecycline can be a promising treatment option for VRE infections.**Table 1. MICs of 10 antimicrobial agents against VanM-type VREm isolates (mg/L)**

Antimicrobial agents	**VanM-type VREfm(n=45)**			
	MIC range	**MIC_{50}**	**MIC_{90}**	**Resistance rate/%**
Vancomycin	128~>256	>256	>256	100
Teicoplanin	0.5~>256	64	128	71.1

Linezolid	1~2	1	2	0
Tigecycline	0.032~0.125	0.064	0.094	0
Minocycline	≤0.06~32	0.125	16	15.6
Ampicillin	0.5~>256	>256	>256	97.8
Levofloxacin	32~>256	64	128	100
Erythromycin	0.125~>256	>256	>256	91.1
Rifampicin	≤0.06~16	8	16	82.2
Gentamicin	2~>256	>256	>256	64.4

Author Disclosure Block:

C. Chen: None. **Y. Guo:** None. **F. Hu:** None. **X. Xu:** None.

Poster Board Number:

FRIDAY-273

Publishing Title:

Ceftaroline Activity Against *S. aureus* Clinical Isolates from Argentina: Association with Different Mrsa Clonal Types

Author Block:

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Abstract Body:

MRSA is an increasing problem worldwide, both in hospitals (healthcare-associated-HA) and in the community (community-associated-CA). In a previous study, we demonstrated that higher rates of MRSA were mainly associated with the spread of two CA-MRSA clones ST5-IV (32%) and ST30-IV (32%) and one HA-associated ST5-I Cordobes/Chilean (19%) (*Egea et al.* Int J Med Microbiol 2014; 304:1086-99). The aim of the present study was to evaluate in the same collection. ceftaroline (CPT) susceptibility and its association with the different MRSA clonal types **Methods**: 591 consecutive *S. aureus* clinical isolates were collected in 66 hospitals from 20 provinces and BA City in Argentina, 322 were MRSA (54.5%). Susceptibility to CPT was performed by agar dilution and interpreted according to CLSI M100-S25. The 322 MRSA isolates (231 CA and 91 HA) were previously studied by SCC*mec*, spa-typing, PVL, PFGE and MLST by standard procedures **Results:** The prevalence of intermediate (I) resistance to CPT (MIC 2mg/L) was 6.4% (38/591) for *S. aureus*, $MIC_{50/90}$ 0.25/1 mg/L, range 0.06-2 mg/L. All MSSA isolates were susceptible to CPT with $MIC_{50/90}$ 0.25 mg/L, range 0.06-0.25 mg/L. Among MRSA, 11.8% (38/322) were CPT-I. CPT resistant isolates were not detected ($MIC \geq 4$ mg/L). The susceptibility profiles of CA and HA-MRSA clones are shown in Table. **Conclusions:** CA-MRSA isolates were CPT susceptible independently of the clone involved. In contrast, in HA-MRSA isolates, CPT susceptibility was strongly associated to the clonal type, being ST5-I

Cordobes/Chilean the main clone associated with CPT-I isolates

Table. Susceptibility profiles to ceftaroline (CPT) of MRSA clones.

MRSA	Clones	N°	n (%) CPT Susceptible MIC ≤ 1 mg/L	n (%) CPT Intermediate MIC 2 mg/L	$CIM_{50/90}$ (mg/L)	Range (mg/L)
CA-MRSA	ST5-IV	103	103 (100)	0	0.5 / 0.5	0.25 - 1
	ST30-IV (South West Pacific)	103	103 (100)	0	0.5 / 0.5	0.25 - 1
	ST72-IV (USA 700)	10	10 (100)	0	0.5 / 0.5	0.25 - 0.5
	ST8-IV (USA 300)	5	5(100)	0	0.5 / 0.5	0,5
	Other	10	10 (100)	0	0.5 / 0.5	0.25 - 0.5
	All CA-MRSA	**231**	**231 (100)**	**0**	**0.5 / 0.5**	**0.25 - 1**
HA-MRSA	ST5-I (Cordobés/Chilean)	63	27 (42.9)	36 (57.1)	2 / 2	1 - 2
	ST239-IIIA (Brazilian)	7	5 (71,4)	2 (28,6)	1 / 2	1 - 2
	ST100-IVnv (Pediatric)	21	21 (100)	0	0.5 / 0.5	0.25 - 0.5
	All HA-MRSA	**91**	**53 (58.2)**	**38 (41.8)**	**1 / 2**	**0.25 - 2**
Total MRSA		**322**	**284 (88.2)**	**38 (11.8)**	**0.5 / 2**	**0.25 - 2**

Author Disclosure Block:

A. Corso: None. **P. Gagetti:** None. **P. Ceriana:** None. **C. Lucero:** None. **A. Egea:** None. **D. Barcudi:** None. **J. Bocco:** None. **C. Sola:** None.

Poster Board Number:

FRIDAY-274

Publishing Title:

Isolation, Identification and Differentiation of *Staphylococcal* Isolates from the Patients of Wound Infections

Author Block:

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Abstract Body:

Background: *Staphylococcus species* are the notorious pathogens causing serious infections. They also have acquired resistance against a wide range of the antibiotics. Methicillin Resistant *Staph. aureus* (MRSA)and Methicillin Resistant *Staph, epidermidis* (MRSE) are becoming more prevalent in current scenario and thus difficult to treat. **Objective:** The present study was designed to investigate the prevalence of Staphylococcus among the infected wounds, burns and the surgical sites and further, to evaluate the frequency of the Staphylococcus spp on the basis of Methicillin resistance. Antimicrobial assay was also performed against other available antibiotics of different groups including, Penicillins, Cephalosporins, Quinolones, Aminoglycocides, beta-lactamase inhibitors, Tetracyclines, Carbapenems, Macrolides, lincosamides and others antistaphylococcal drugs. **Methods:** A total of 200 pus and burn wound samples were taken and were processed in the Microbiology laboratory at Mayo hospital Lahore, Pakistan. Isolation, identification and differentiation of the *Staphylococcus* species were performed according to the standard protocol. (Monica Cheesebrough, 2006).**Results:** From a total of 200 pus samples, *Staphylococcus spp* were observed to be 43.1% among the positive cultures. Staphylococci were isolated from 49 (62.8%) of the pus samples and 29 (37.2%) from the burn wound swabs. MRSA was found to be dominating among the staphylococcal isolates, with the overall rate of 53.8%. This increased percentage of Methicillin Resistance was also observed among the cases of Staph. epidermidis. Risk factors associated with such increased rate of antibiotic resistance among the infected individuals were the irrational use of the antibiotics and the inadequacies in the alertness among the infected patients. Antibiogram assay showed variable resistance pattern, however, Carbapenems, Aminoglycosides, Linezolids and Vancocins were found superiors as compared to others. ($P<0.05$) **Conclusion:** *Staph. aureus* was found to be prevalent 66.6% among the other staphylococcal isolates, however, *Staph. epidermidis* MRSE established more resistance i.e. 57.6% as compared to MRSA that was 53.8% along with other antibiotics as well.

Author Disclosure Block:

T. Ijaz: None. **M. Afzal:** None. **N. Ijaz:** None.

Poster Board Number:

FRIDAY-275

Publishing Title:

Phenotypic and Genetic Diversity of Nasopharyngeal *Streptococcus pneumoniae* Isolates from Children at the Age of Six Weeks and Nine Months from Selected Health Centres of Addis Ababa, Ethiopia

Author Block:

W. T. Sime; Armauer Hansen Res. Inst., Addis Ababa, Ethiopia

Abstract Body:

Background: *Streptococcus pneumoniae* (Pneumococcus) among the most important human pathogens, with high morbidity and mortality rates. Nasopharyngeal colonisation is the necessary first step in the pathogenesis of associated invasive pnuemococcal diseases. Ethiopia, introduced the ten-valent pneumococal conjugate vaccine (PCV110) since October, 2011 without adequate baseline information on epidemiological factors for subsequent impact assessment. The aim of this study was to determine phenotypic and genotypic diversity nasopharyngeal isolates of *Streptococcus pneumoniae*.**Method**: A total of 789 newborn babies were enrolled at the age of six weeks when they came for the first PCV10 vaccine, and 206 were re-sampled at the age of nine months and 201 at two years after final dose of PCV10. Nasopharyngeal swabs were taken for bacteriological analysis before vaccination at the age of six weeks, and after completion at the age of nine months and two years. Isolates were tested for 13 commonly used antibiotics by disc diffusion method and those isolates that showed resistance for penicillin and erythromycin the minimum inhibition concentration were determined by E-test A total of 325 pneumococcal isolates were serotyped and characterized by Pulsed Field Gel Electrophoresis and 12 isolates were analyzed by multilocus sequence typing.**Results**: The carriage rate of *Streptococcus pneumoniae* at the age of six weeks, nine months and two years was 26.6%, 56.8% and 47.6% respectively. A total of 59 serotypes of *S. pneumoniae* were identified from 325 isolates, and 6A, 11A, 15B, 23F,15A and 19F dominated in decreasing order. The proportion of serotypes covered by PCV10 vaccine among the isolates at 6 weeks and 9 months were 20.2% and 11.1% respectively. Molecular typing further showed a presence of high genetic diversity.The antibiotic test indicated that resistance rates were ranging from 4.3% for chloramphenicol to 27.7% for Trimethoprim/sulfamethoxazole.**Conclusion**: This study highlights the presence of very diverse serotypes in the country, and PFGE and MLST result indicates case of a possible capsular switching event. This work were conducted in Addis Ababa only, it is necessary to scale up the investigation to other part of the country and include clinical cases to have a complete picture.

Author Disclosure Block:

W.T. Sime: None.

Poster Board Number:

FRIDAY-276

Publishing Title:

Utilization of Sensititre(C) to Evaluate Antibiotic Susceptibilities of *Aerococcus* Species

Author Block:

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Abstract Body:

Aerococcus species are being identified with increasing frequency in urine cultures, but it is unclear if this is due to improved identification technologies or to a better recognition of the bacterium as a urinary tract pathogen. From October to December 2015, we observed that ~1% of positive urine cultures included *Aerococcus urinae* and in 55% of cases it was the only organism present. Recently, CLSI has approved antimicrobial susceptibility testing guidelines and interpretive breakpoints for *Aerococcus* species. In the present study, we compared standard broth microdilution MIC determinations to MICs identified using commercially available Sensititre© MIC Susceptibility plates recommended for *Streptococcus pneumoniae*. Sensititre© plates were selected for comparison to manual broth microdilution plates due to the excellent overlap of drugs between the antibiotics on the plate and those with CLSI interpretive breakpoints for *Aerococcus* species; Ciprofloxacin is the only drug with CLSI interpretive breakpoints not included in Sensititre© plates. To date, 22 clinical isolates have been tested by manual broth microdilution and Sensititre© with few significant discrepancies. Major discrepancies were described as changes in interpretation between the methods from sensitive to resistant or vice versa and are as follows: Penicillin - 4.5%, Cextriaxone - 5%, and Levofloxacin - 10.5%. When utilizing Sensititre© alone, all isolates were susceptible to Meropenem, Linezolid, and Vancomycin and most were susceptible to Tetracycline (93%), Cextriaxone (93%), Cefotaxime (96%), and Penicillin (96%); for Trimethoprim/Sulfamethoxazole 75% were susceptible and 25% were intermediate. The only appreciable resistance was to Levofloxacin (25% resistant). In conclusion, susceptibility testing of *Aerococcus* species using commercially available Sensititre© microdilution plates may enhance ease of testing, increase clinical treatment options, and assist in efficiently gaining insight into the susceptibility patterns of these strains in the clinical laboratory.

Author Disclosure Block:

K.L.J. Smith: None. **S.R. Petry:** None. **D.J. Hardy:** None.

Poster Board Number:

FRIDAY-277

Publishing Title:

Kinetic of Eosinophil Count and Correlation with Effective Antibiotic Treatment

Author Block:

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Abstract Body:

Background: Eosinophil count has been in the center of debates for decades as a criterion of SIRS (Systemic Inflammatory Response Syndrome). Furthermore, some authors claimed that a cut-off value<40 to 50/mm3 could help physician in the diagnosis of sepsis.Our present work evaluates the kinetic of eosinophil count according to antibiotic treatment and its correlation with patient's response. **Methods:** We gathered prospectively all data of patients hospitalized with sepsis + eosinopenia (<100/mm^3) on day (D) 0 confirmed by microbiology (i.e. blood culture, urinalysis etc.)Patients were divided in 2 groups:- Number 1: effective antibiotic treatment since day 0 (D0)- Number 2: delayed effective antibiotic treatment after D1We compared temperature, leukocyte count and C - reactive protein (CRP) during the first 5 days of hospitalization.**Results:** Mean age and comorbidities were not significantly different between both groups.We noted 17 urinary tract infections, 11 bloodstream infections, 8 pneumonias, 5 skin and soft tissue infections and 4 digestive tract infections.

	Day	Group number 1 (n=30)	Group number 2 (n=15)	P-value (alpha=5%)
Mean temperature (°C)	0	38.8±1	38.5±1	Non-significant (NS)
	1	38.7±1	38.2±1	NS
	3	37.8±1	37.7±1	NS
	5	37.5±1	37.2±1	NS
Mean value of polymorphonuclear neutrophils (PMN) count (/mm^3)	0	10475±7000	7440±3800	NS
	1	9900±5000	7550±4100	NS
	3	6450±3580	3210±1580	<0.0001
	5	6680±3150	4000±2230	NS
Mean value of eosinophil count (/mm^3)	0	9±8	7±12	NS
	1	44±21	9±15	<0.0001

	3	110±61	140±94	NS
	5	166±100	130±78	NS
Mean value of CRP (mg/L)	0	220±110	105±70	0.0006
	1	245±120	145±120	NS
	3	120±60	79±80	NS
	5	70±50	44±34	NS

Table 1: data characteristics of studied patientsStatistical analysis showed a significantly higher eosinophil count at D1 ($p<0.0001$) in the group Number 1, meanwhile CRP and PMN were comparable. (Table 1).However, CRP was significantly lower at D0 in group Number 2, which may explain why physician in charge delayed the antibiotic therapy. PMN count was significantly lower at D3 in group Number 2, which may also be consecutive to a delayed antibiotic treatment.Clinical outcome was favourable in all cases after an effective antibiotic regimen.**Conclusions:** Kinetic of eosinophil count appears to normalize faster than CRP and PMN if patient is under the appropriate antibiotic therapy. Resolution of eosinopenia seems to predict a quick recovery.

Author Disclosure Block:

S. Makhloufi: None. **A. Dinh:** None. **R. Calin:** None. **O. Senard:** None. **J. Salomon:** None. **B. Davido:** None.

Poster Board Number:

FRIDAY-278

Publishing Title:

Plasma Neutrophil Gelatinase-Associated Lipocalin (NGAL) as a Predictor of Acute Kidney Injury in Patients Treated with Colistin

Author Block:

J. LEE, S. PARK, J. Eom, J- . Park, Y-S. Ju; Kangdong Scred Heart Hosp., Hallym Med. Coll., seoul, Korea, Republic of

Abstract Body:

Background: We hypothesized that plasma neutrophil gelatinase-associated lipocalin (NGAL) would be an early biomarker for acute kidney injury (AKI) in patients treated with colistin. **Methods:** We performed a prospective cohort study in patients receiving colistin. All 109 patients aged ≥20 years who received intravenous colistin from March 1, 2014 to November 30, 2015 were eligible for the cohort and 23 patients were enrolled. The primary outcome was AKI which was defined according to a Kidney Disease: Improving Global Outcomes (KDIGO) criterion. We measured the serum creatinine at baseline and daily after initiation of colistin up to day 7. We collected blood samples at baseline and 8 hours after the beginning of colistin therapy, every 8 hour for the third day, and then every 12 hour for 4 days for measuring NGAL.**Results:** We studied 23 patients with a mean age was 61.3 ± 16.1 years, and 65.2% were males. At the baseline, plasma NGAL level was 233.0 ± 135.4 ng/mL. Overall, 13/23 (56.5%) patients fulfilled an AKI definition. The mean duration of colistin therapy before the onset of AKI was 78.15 ± 30.49 hours. Based on the primary outcome, we classified patients into those with and without AKI. There was no statistically significant difference (*P*=0.218) in baseline NGAL levels between patients with AKI (264.0 ± 167.3 ng/Ml) and patients without AKI (192.7 ± 65.3 ng/mL). The amount of NGAL in plasma at 64 hours after colistin treatment was the most powerful predictor of AKI. For concentration in plasma of NGAL at 64 hour, the AUC curve was 0.840 (95% [CI], 0.672 to 1.000; *P*=0.006), sensitivity was 76.9%, and specificity was 80.0% for a cutoff value of 288 ng/mL. **Conclusions:** In the current study, plasma NGAL is a powerful immediate early biomarker for AKI, preceding any increase in serum creatinine by 12-28 hours. Plasma NGAL represents sensitive and specific predictive early biomarkers for AKI in patient treated colistin.

Author Disclosure Block:

J. Lee: None. **S. Park:** None. **J. Eom:** None. **J. Park:** None. **Y. Ju:** None.

Poster Board Number:

FRIDAY-279

Publishing Title:

Hyperferritinemia As A Marker For Severe Fever With Thrombocytopenia Syndrome

Author Block:

U. KIM, **B-s. Kim**, S. Kim, S. Kang, H-C. Jang, K-H. Park, S. Jung; Chonnam Natl. Univ. of Med. Sch., Gwangju, Korea, Republic of

Abstract Body:

Background: Eversince the first identification in China in 2009, severe fever with thrombocytopenia syndrome(SFTS) is an emerging infectious disease in many rural areas of Asia. Its increasing prevalence and overall mortality as high as 46% puts emphasis on early diagnosis of SFTS to prevent both transmission and mortality of the disease. Our aim was to determine biomarker that can help early diagnosis of SFTS in emergency setting before the confirmed diagnosis with PCR sequencing.**Methods:** Patients aged 18 years or older with serologically confirmed SFTS or microbiologically confirmed community-onset bacteremia with thrombocytopenia (blood platelet count $\leq 130{,}000/mm^2$) at presentation between June 2013 and August 2015 were included from two tertiary university hospitals in Republic of Korea. Data on age, gender, residence, and medical history, clinical manifestations on admission and laboratory test results were collected by a retrospective review of electric medical records.**Results:** From June 2013 to August 2015, data of 9 SFTS and 62 bacteremia with thrombocytopenia were collected. In laboratory findings, white blood cell count and absolute neutrophil count were significantly lower in SFTS group. SFTS group had significantly higher serum level of aspartate aminotransferase (AST), lactate dehydrogenase (LDH), creatine kinase (CK), ferritin. Inflammatory markers such as C-reactive protein (CRP), procalcitonin and fibrinogen were lower in SFTS group. Calculated AUC of AST, LDH, CK, ferritin were above 0.5, indicating high discriminative power of the biomarkers. Among these markers, serum ferritin had an AUC of 1.0. In addition, the optimal cut off value of ferritin was 3835.9 ng/ml with a sensitivity of 100% and specificity of 100%.**Conclusion:** This study highlights the importance of serum level of ferritin as a diagnostic tool in differentiating SFTS from bacteremia.Table 1. Comparison of basal characteristics and laboratory findings in patients with SFTS and bacteremia with thrombocytopenia.

	STFS (N=9)	Bacteremia (N=62)	P-value
Demographic Data			
Age	74 (65-79)	68 (59-75)	0.254
Male	2 (22.2)	25 (40.3)	0.467
Rural area resident	7 (77.8)	26 (41.9)	0.072

Comorbidities	5 (55.6)	59(95.2)	0.004
Clinical manifestation			
Fever on admission (>38℃)	9 (100)	37 (59.7)	0.022
Neurologic symptoms	4 (44.4)	10 (16.1)	0.068
Mortality	2 (22.2)	7 (29.0)	1.000
Laboratory results			
White blood cell ,/mm^3	1600 (950-2200)	11850 (7625-19375)	<0.001
Platelet, $x10^3/mm^3$	43.0 (34.5, 61.5)	69.5 (46,7, 97.8)	0.158
Fibrinogen, mg/dL	193 (183-223)	464 (350-687)	<0.001
AST, U/L	149.0 (67.0, 620.5)	46.5 (23.0, 84.5)	0.002
CRP, mg/dL	1.0(0.0-1.5)	19.0 (13.75-26.25)	<0.001
LDH, U/L	1071.0 (627.0, 2574.0)	593.0 (419.3, 872.8)	0.011
CK, IU/L	796.0 (261, 2219)	117.5(45.0,308.0) (60/62)	0.002
Ferritin, ng/mL	11539(4674-35123) (n=8)	596.7 (302.9, 1169.4)	<0.001
Procalcitonin, ng/mL	0.50 (0-1.0) (n=6)	22(4.5-77)(n=37)	<0.001

Author Disclosure Block:

U. Kim: None. **B. Kim:** None. **S. Kim:** None. **S. Kang:** None. **H. Jang:** None. **K. Park:** None. **S. Jung:** None.

Poster Board Number:

FRIDAY-280

Publishing Title:

Utility of Procalcitonin in Oncology Patients with Bacteremia

Author Block:

D. Y. C. Yii, **W. H. L. LEE**, Y. Liew, S. S. L. Tang, A. L. Kwa; SINGAPORE GENERAL Hosp., SINGAPORE, Singapore

Abstract Body:

Background: Diagnosis of bacterial infections in oncology patients can be challenging as symptoms are often non-specific. Procalcitonin (PCT) may be a useful biomarker in such cases. We aim to determine the diagnostic value of PCT in early identification of oncology patients with bloodstream infections (BSI).**Methods:** This is a retrospective study of all adult oncology patients with ≥1 PCT level done during their hospital admission between January 2013 and December 2014. BSI was defined as any clinically significant bacterial growth in blood cultures taken at symptom presentation or start of antibiotics. We evaluated the median of biomarkers measured at this point, using cutoffs of PCT 0.5mcg/L and CRP 50mg/L as research supports increased likelihood of bacterial infection above these values. Patients were placed in the control group if the physician deemed sepsis unlikely based on clinical and microbiological data.**Results:** A total of 258 patients were included - 107 with BSI and 151 in the control group. Baseline PCT levels were significantly higher in those with BSI (2.0mcg/L vs 0.12mcg/L, $p<0.001$). Of note, 80 (75%) patients with BSI but only 8 (5%) controls had PCT≥0.5mcg/L (, $p<0.001$). CRP levels were also more likely to be elevated in patients with BSI - 64 (75%) had CRP>50mg/L vs 52 (50%) in patients with no infection ($p<0.001$); median CRP in BSI group was 125mg/L (IQR 50-194) vs. 49mg/L (IQR 17-85) in the controls ($p<0.001$). There was no statistically significant difference in PCT levels by type of causative pathogen. In distinguishing BSI from no infection, the area under ROC curve was 0.92 (95% CI 0.88-0.96, $p<0.001$) for PCT and 0.72 (95% CI 0.64-0.79, $p<0.001$) for CRP. Optimal cutoffs were PCT 0.28mcg/L, with 87% sensitivity and 81% specificity; and CRP 76mg/L which had 65% sensitivity and 71% specificity. In comparison, values of PCT 0.5mcg/L had 75% sensitivity and 95% specificity, and CRP 50mg/L showed 75% sensitivity and 51% specificity.**Conclusions:** These findings support the use of PCT over CRP for detection of BSI in oncology patients, given its superior specificity.

Author Disclosure Block:

D.Y.C. Yii: None. **W.H.L. Lee:** None. **Y. Liew:** None. **S.S.L. Tang:** None. **A.L. Kwa:** None.

Poster Board Number:

FRIDAY-281

Publishing Title:

Clinical Significance of Low $Cd4^+/Cd8^+$ Ratio in Patients with Immune Thrombocytopenic Purpura: A Single Centre Study

Author Block:

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Abstract Body:

Background: Immune thrombocytopenic purpura (ITP) is an autoimmune clinical syndrome in which decreased number of circulating platelets leads to epistaxis, bruises and bleeding. The T cells play an important role in the pathogenesis of ITP. CD4 co-receptor assists the T cell receptor in communicating with an antigen presenting B-cells resulting in development of clones of plasma cells secreting antibodies against the platelets glycoprotein as antigens. Decrease in the helper and cytotoxic T cells ratio is generally observed in immune conditions like ITP.**Methods:** A total of 50 ITP patients and 30 healthy volunteers were included in this study as control. Informed consent was taken from all the patients. About 21 were male while 29 were females. Complete blood count was performed on automated hemo-analyzer XN-1000 and morphological examination of blood was done in duplicate by two different microscopists. The $CD4^+/CD8^+$ ratio was determined by BD Tri-testCD4 FITC/ CD8 PE/ CD3 PerCP (Cat Number: 340298) antibody cocktail using BD FACS Calibur flow-cytometer. The data was acquired and analyzed by using Multiset version 3.0. Mean platelet count and $CD4^+/CD8^+$ were calculated using SPSS version 17. **Results:** Out of total 50 patients, 21(42%) were males and 29 (58%) were females. The mean age of the patients was 35 years (range : 3-80 years).Platelet anisocytosis and decreased platelet count was observed. The mean platelet count was 28.2x103/µl and 328x103/ µl for ITP patients and controls respectively. The mean CD4/CD8 ratio was 0.8 in patients while it was $\geq$1.09 in controls. It was observed that patients with low CD4/CD8 ratio ($\leq$ 0.3) exhibited poor treatment response. Prolonged thrombocytopenia was observed in these patients. **Conclusions:** In ITP,T-cell plays an important role in pathogenesis. In the present study decreased platelet count and low CD4/CD8 ratio were observed. This decreased ratio can be use as a marker in evaluating the immune-suppressive drug's efficacy/ response in ITP patients. More studies are needed to confirm significant involvement of T-cell in pathogenesis of ITP.

Author Disclosure Block:

A. Arshad: None. **S.N. Mukry:** None. **M. Saud:** None. **I. shamim:** None. **T.S. Shamsi:** None.

Poster Board Number:

FRIDAY-282

Publishing Title:

A Comparison of Two Assays for Quantification of Hepatitis B Surface Antigen in Patients with Chronic Hepatitis B

Author Block:

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Abstract Body:

Background: Serum hepatitis B surface antigen (HBsAg) levels correlate with hepatitis B virus intrahepatic covalently closed circular (ccc) DNA and may be useful to predict treatment response. Currently there are two commercial platforms available for HBsAg quantification in clinical practice: the Roche HBsAg QT and Architect HBsAg Quant. This study aimed to compare the results of both assays. **Methods:** HBsAg levels were measured in 50 serum samples from chronic hepatitis B patients and subsequently analysed for HBsAg levels using both assays.**Results:** Correlation between Roche and Architect results was high ($r=0.989$, $p<0.05$). By Bland Altman analysis, agreement between two assays was close (mean difference between Roche and Architect 0.001 log IU/mL, limit of agreement -0.20 log IU/mL (95% CI: -0.25 - -0.15) to 0.21 log IU/mL (95% CI: 0.15-0.26). There were 2 (4%) samples with far agreement (difference 0.42 and 0.30 log IU/mL). This discrepancy is hypothesized to be caused by HBsAg mutants.**Conclusions:** There is a high correlation and close agreement between quantitative HBsAg measurement by Roche and Architect. Clinical prediction rules derived from data from one platform can be applied on another. Both assays can be used interchangeably in clinical practice.

Author Disclosure Block:

T. Adilistya: None. **N. Indrasari:** None.

Poster Board Number:

FRIDAY-283

Publishing Title:

Identification of Novel Diagnostic Antigens for Human Granulocytic Anaplasmosis

Author Block:

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Abstract Body:

Human granulocytic anaplasmosis (HGA), an emerging tick-borne infectious disease is caused by a gram-negative obligatory intracellular bacterium, *Anaplasma phagocytophilum*. HGA is accompanied by fever, chills, headache, myalgia, malaise, leucopenia, thrombocytopenia, and elevation of hepatic aminotransferase activity. Because the clinical manifestation is not specific, the diagnosis of HGA is highly depends on the laboratory tests. *A. phagocytophilum*-infected cell-based indirect immunofluorescence assay (IFA) is the standard method to serologically assist diagnosis of HGA, but the interpretation of result relies on the well-trained clinical laboratory personnel. The enzyme-linked immunosorbent assay (ELISA) is an alternative serological test for detection of *A. phagocytophilum* infection by providing convenience and accuracy, but the peptide antigen derived from *A. phagocytophilum* outer membrane protein P44 offers lower sensitivity of detection in current ELISA kit compared to IFA. In this study, we attempted to identify new protein antigens aiming to increase the sensitivity of ELISA for detection of *A. phagocytophilum* infection. Genes encoding 24 *Anaplasma*-specific proteins were amplified by PCR, cloned into prokaryotic expression vector pET28a(+) and expressed in *E. coli*. Recombinant proteins were purified with Nickel affinity chromatography, and subjected to western blot analysis using human serum against *A. phagocytophilum* as primary antibody. Comparable to P44, 6 proteins were found to strongly react with the positive serum. Further study showed 2 of them reacted with sera which are positive for *A. phagocytophilum*, but negative for P44 in western blot analysis. Here these two proteins are named as *A. phagocytophilum* antigen 1 (Apa-1) and 2 (Apa-2), respectively. ELISA with peptides derived from Apa-1 and Apa-2 was found to have high sensitivity of detection. Furthermore, besides reacting with sera, which contain anti-P44 antibody, the peptides also reacted with sera, which contain anti-*A. phagocytophilum* antibody, but no detectable anti-P44 antibody. Taken together, we identified novel *A. phagocytophilum* antigens, which may increase the sensitivity of ELISA in assisting diagnosis of HGA.

Author Disclosure Block:

M. He: None. **W. Xu:** None. **L. Zhang:** None. **Y. Li:** None. **S. Wu:** None. **R. Huang:** None. **H. Niu:** None.

Poster Board Number:

FRIDAY-284

Publishing Title:

Detection of Antibodies to *Borrelia Garinii* and *Borrelia Afzelii* by Immunoblot

Author Block:

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Abstract Body:

Lyme disease (LD) occurs following infection with a *Borrelia burgdorferi* (*Bb*) sensu lato complex member. Certain complex members, including *B. afzelii* (*Ba*) and *B. garinii* (*Bg*), are geographically confined in Europe, while *Bb* is endemic in both Europe and the United States (US). LD diagnostic assays in the US target antibodies specific to the *Bb* and may not detect antibodies to *Ba* or *Bg*, possibly leading to missed diagnoses among travelers who acquired LD abroad. Currently no US reference laboratory offers testing specific to *Ba* or *Bg*. We evaluated the *Borrelia* ViraStrip IgM and IgG (Viramed Biotech AG, Planegg, Germany) immunoblots for detection of antibodies to *Ba* and *Bg* from patients with culture confirmed infection in comparison to the US two-tiered algorithm (TTA) for LD.Thirty serum samples from patients with culture confirmed *Ba* (n=23) or *Bg* (n=7) infections were tested by the C6 ELISA (Immunetics Inc., MA, USA) and the US *Borrelia* B31 and European *Borrelia* IgM/IgG ViraStrip immunoblots (Viramed Biotech AG, Planegg, Germany). European blot specificity was evaluated using 20 C6 ELISA negative samples, 50 donor samples from a US LD endemic region, 49 samples seropositive for *Bb*, and 61 samples positive for antibodies to other infectious agents.The C6 ELISA was positive in 93% (28/30) of the *Ba* and *Bg* culture confirmed serum samples. The B31 IgM/IgG blots were positive in 47% (14/30) of cases compared to 87% (26/30) by the European IgM/IgG blots. Of the 20 C6-negative samples and 50 donor samples, the European IgM/IgG blots had a specificity of 95% and 90%, respectively. The European IgM and IgG blots were positive in 63% (12/19) and 97% (29/30) of samples previously seropositive for IgM and IgG by the B31 assays, respectively. Finally, the European IgM and IgG blots were positive in 2/10 CMV IgM and 1/5 *Anaplasma* positive (C6 negative) specimens, respectively.The current assays used for the LD TTA in the US are specific to *Bb* and this study confirms that these assays are not sensitive enough to detect antibodies to the European species associated with LD, including *Ba* and *Bg*. As international travel increases, the need has arisen for US reference laboratories to offer serologic assays specific to these LD agents to ensure that LD diagnoses are not missed.

Author Disclosure Block:

D. Granger: None. **F. Strle:** None. **E.S. Theel:** None.

Poster Board Number:

FRIDAY-285

Publishing Title:

A Comparative Analysis of Indoleamine 2,3-Dioxygenase Activity in Plasma of Cystic Fibrosis Patients with and without Exacerbation

Author Block:

M. Betancourt, **E. Manavathu**, C. Forseen, J. Vazquez; Med. Coll. of Georgia/Augusta Univ., Augusta, GA

Abstract Body:

Background: Indoleamine 2,3-dioxygenase (IDO) catalyzes the conversion of tryptophan (T) to kynurenine (K). Elevated levels of IDO have been detected in association with respiratory diseases, including cystic fibrosis (CF). The increased levels of IDO modulates the immune response against microbial pathogens in chronic infections, including respiratory diseases such as CF. Since active infection in CF patients is associated with exacerbation (EXBN) we hypothesized that elevated levels of IDO would be detected in the plasma of CF patients during an EXBN. We therefore examined the level of IDO activity by determining the relative amounts of K and T in plasma of CF patients with and without EXBN. **Methods:** Heparinized blood was collected from control (CONT) and EXBN CF patients. Plasma was collected, treated with trichloroacetic acid, centrifuged and the clear supernatant was used to determine K and T levels. K and T were separated on a C18 RP column (4.5 mm x 150 mm) using a Shimadzu HPLC system (mobile phase: 15 mM acetic acid-sodium acetate (pH 4.0) with 2.7% acetonitrile, 1.0 ml/min). HPLC grade K, T and 3-nitro-L-tyrosine were used as external and internal standards. K (360 nm) and T (278 nm) were detected using a UV detector. The K and T levels were determined from standard curves. The K/T ratio which represents the relative activity of IDO was calculated from the K and T levels for each patient. **Results:** Detectable levels of K and T were observed in 14 patients each in the CONT and EXBN groups. The mean K levels in the CONT and the EXBN patients were 1.29±0.17 µM (range 1.01-1.70 µM) and 1.47±0.55 µM (range 1.02-3.08 µM), respectively. Similarly, the mean T levels in the CONT and the EXBN patients were 21.38±5.72 µM (range 12.60-36.34 µM) and 17.38±6.72 µM (range 6.55-30.03 µM), respectively. The mean K/T ratio for the CONT and the EXBN patients were 0.0359±0.0115 (range 0.0213-0.0585) and 0.0556±0.0341 (range 0.0221-0.1346), respectively. A comparison of the K/T ratio for the CONT and the EXBN patients showed that it is significantly higher (p=0.0504) in the EXBN patients. **Conclusions:** The K/T ratio which represents the relative activity of IDO was significantly higher in CF patients with EXBN compared to non-EXBN patients. The plasma level IDO activity has the potential to be used as a biomarker for the onset of EXBN in CF patients.

Author Disclosure Block:

M. Betancourt: None. **E. Manavathu:** None. **C. Forseen:** None. **J. Vazquez:** None.

Poster Board Number:

FRIDAY-286

Publishing Title:

Evaluation Of Ehrlichiosis In Northeastern Oklahoma Canines

Author Block:

J. M. Corley, D. E. Kirk, S. A. Ruskoski; Northeastern Oklahoma State Univ., Broken Arrow, OK

Abstract Body:

Background: Certain *Ehrlichia* species can be found in ticks which can cause world-wide ehrlichiosis in humans and other mammals. The lone star tick is the primary host for the bacteria. *Ehrlichia* spp. reproduces in mammalian leukocytes. In ticks, organisms remain dormant in the salivary glands until transferred to secondary hosts, such as canines, during feeding. The purpose of this study was to evaluate exposure to *Ehrlichia* spp. in Northeast Oklahoma canines currently exhibiting tick-borne disease symptoms, such as lethargy, anorexia, fever, and depression.**Materials:** Local veterinary hospitals supplied 51 blood samples from canines exhibiting tick-borne disease symptoms between September 2014 and September 2015. Sera were tested for antibodies to *Ehrlichia* spp. using an indirect immunofluorescent antibody assay (IFA). DNA was extracted from EDTA-treated whole blood from positive IFA samples and end-point polymerase chain reaction (PCR) was employed to confirm the presence of the organism.**Results:** Antibody testing revealed that 33 (65%) canines had positive titers to *Ehrlichia* spp. From the positive IFA samples, 17 (52%) tested positive for the *Ehrlichia* spp. DNA while 16 (48%) were negative.**Conclusion:** These data support the conclusion that while symptomatic canines may test positive for antibodies to *Ehrlichia* spp., it does not necessarily indicate a current infection with the organism.

Author Disclosure Block:

J.M. Corley: None. **D.E. Kirk:** None. **S.A. Ruskoski:** None.

Poster Board Number:

FRIDAY-287

Publishing Title:

Development of Elisa Kit for Serological Diagnostics of the Ruminants' Bluetongue

Author Block:

S. Nychyk, M. Sytiuk, V. Spyrydonov, G. Kovalenko; Inst. of Vet. Med. of the NAAS, Kyiv, Ukraine

Abstract Body:

Bluetongue virus (BTV) belongs to genus *Orbivirus* family *Reoviridae* consisting of 24 serotypes (BTV 1-24). The virus has small (diameter - 90 nm) icosahedral capsid containing segmented genome with 10 segments of double stranded RNA. Each segment encodes a different viral protein. The internal capsid layer consists of 5 structural proteins: 3 minor (VP1, VP4, VP6) and 2 major (VP3, VP7). The external capsid layer includes 2 major proteins (VP2, VP5). VP2 antigen determines serotype diversity of BTV, and VP7 antigen is responsible for serotype specificity. Therefore, VP7 is an appropriate protein for bluetongue diagnostics in ruminants. In our previous work, we produced transgenic tobacco plants expressing functional recombinant virus-specific capsid antigen VP7 that we used as a part of the developed ELISA kit for BTV antibody detection in serum samples.As of today, no bluetongue cases have been registered in Ukraine, but it should be taken into account that several outbreaks of this disease were observed a year ago in Europe, namely in France, Germany and Poland. BTV serotypes 6 and 8 were detected in Europe. That was the reason that we had performed validation of a developed ELISA kit with the use of reference samples and panels from animals experimentally infected with BTV serotypes 6 and 8 (Friedrich Loeffler Institute).For the validation of ELISA specificity and reproducibility, 19 positive and 89 negative sera were analyzed in triplicate. The average optical density (OD_{450}) of the positive samples was 1.150±0.488 and for the negative ones - 0.04±0.01.As a result of the experiments, it has been shown that the developed ELISA is characterized by high sensitivity, specificity and reproducibility. The developed ELISA kit has been recommended for use by the State Veterinary and Phytosanitary Service of Ukraine to monitor the spread of ruminants' bluetongue throughout Ukraine.

Author Disclosure Block:

S. Nychyk: None. **M. Sytiuk:** None. **V. Spyrydonov:** None. **G. Kovalenko:** None.

Poster Board Number:

FRIDAY-288

Publishing Title:

Use of Recombinant *Salmonella* Flagellar Hook Protein (Flgk) for Detection of Anti-*Salmonella* Antibodies in Chickens by Automated Capillary Immunoassay

Author Block:

H-Y. Yeh[1], A. Acosta[2], K. Serrano[2], R. J. Buhr[1]; [1]USDA/ARS/PMSPRU, Athens, GA, [2]Univ. of Puerto Rico, Mayaguez, PR

Abstract Body:

Background: Conventional immunoblot assays are a very useful tool for specific protein identification, but are tedious, labor-intensive and time-consuming. An automated capillary electrophoresis-based immunoblot assay called "Simple Western" has recently been developed that enables protein separation, blotting and detection in an automatic manner. This technology has been used by pharmaceutical industries to identify and characterize biophamarceutical products. However, this technology has not been used in clinical diagnosis. The flagellar hook protein **(**FlgK) is required for flagellar filament formation, plays a role in intestinal inflammation, and is the most immune-reactive flagellar protein. Therefore, it is a potential target for host immune response. In this communication, we evaluated whether the Simple Western system could be used to detect *Salmonella* FlgK antibodies from chicken sera. **Methods**: The recombinant *Salmonella* FlgK (rFlgK) protein was cloned, expressed and purified using commercial kits. Chickens were immunized with rFlgK emulsified in Freund's incomplete adjuvant at the ages of one and three weeks. Appropriate controls were included. Immunoblot analyses were performed in two ways: (1) conventional Western blot analysis, and (2) Simple Western immunoblot analysis according to the manufacturer's instructions. Chicken sera were collected from field. **Results**: The rFlgK protein reacted strongly to sera from the immunized chickens by both conventional Western blot and Simple Western analysis. Sera from un-immunized chickens and commercial specific pathogen free chickens did not react rFlgK. Further, we tested 66 individual chicken sera collected from a single flock raised similar to commercial conditions, and observed 63 out of 66 sera reacted at various degrees to the rFlgK protein by both methods. **Conclusion**: The *Salmonella* FlgK protein was expressed in and purified from the *E. coli* expression system. This protein induced strong immune response in chickens. The Simple Western immunoassay was successfully used for detection of antibodies against *Salmonella* rFlgK from chicken sera. These results provide a rational for further evaluation of the Simple Western immunoassay as a tool using *Salmonella* rFlgK in *Salmonella* outbreak investigation.

Author Disclosure Block:

H. Yeh: None. **A. Acosta:** None. **K. Serrano:** None. **R.J. Buhr:** None.

Poster Board Number:

FRIDAY-289

Publishing Title:

Recovery of Four Healthcare Associated Organisms from Hospital Surfaces Using Sponge Sticks

Author Block:

H. Houston, L. J. Rose, R. West-Deadwyler, J. A. Noble-Wang; CDC, Atlanta, GA

Abstract Body:

As healthcare-associated infections (HAI) become an increasing concern, we need a better understanding of the environmental reservoirs and the role of fomites in transmission. Collecting surface samples during HAI investigations or evaluation of cleaning and disinfection procedures is common, but no standard sampling protocol exists and little is known about the recovery efficiency of the sampling tools. We investigated the efficiency of the Sponge Stick (3M™), in recovering *Acinetobacter baumannii* (AB), *Clostridium difficile* (CD), carbapenem-resistant *Klebsiella pneumoniae* (KPC), and vancomycin-resistant *Enterococcus faecalis* (VRE) from three healthcare surface types (steel, laminate, and acrylic). Surfaces (50 in^2) were inoculated with each organism suspended in phosphate buffer (BB) or Artificial Test Soil (ATS) ($n \geq 6$ for each variable) to simulate body fluids, and allowed to dry before being sampled with a Sponge Stick. The organisms were eluted from the Sponge Stick, cultured and percent recovery (%R) was calculated. We compared: 1) %R of organisms in BB spread evenly across the surfaces to those suspended in ATS; 2) %R of inocula uniformly spread to a 4 cm^2 "hot spot" on the surface; and 3) %R of 10^4 and 10^6 CFU evenly spread and as a "hot spot." When spread evenly, %R was significantly higher if cells were suspended in ATS matrix ($p<0.005$). The highest %R for each organism (in ATS) was: CD from acrylic (58.9, SD 12.7), VRE "hot spot" on steel (62.8, SD 9.7), KPC spread on steel (8.9, SD 3.9) and AB spread on acrylic (21.5, SD 9.9). The lowest %R for each (all spread evenly in BB) was: CD on steel (26.3, SD 9.6), VRE on acrylic (3.2, SD 8.8), KPC on acrylic (0.3, SD 0.3), and AB on acrylic (6.9, SD 2.2).The %R was generally higher for VRE and AB as "hot spots" as compared to the spread inocula on surfaces ($p<0.0005$) except AB on acrylic ($p>0.05$). When comparing inoculum levels, %R was higher for 10^4 from steel ($p<0.05$), but no difference was seen from acrylic. The %R varied with organism, surface, matrix and deposition method. Additional studies are needed to investigate the role of cell and surface characteristics in attachment, sampling and recovery. The low %R for some organisms suggests current sampling and culture methods may not serve as a true indicator of bioburden and therefore underestimate the potential for transmission involving surfaces.

Author Disclosure Block:

H. Houston: None. **L.J. Rose:** None. **R. West-Deadwyler:** None. **J.A. Noble-Wang:** None.

Poster Board Number:

FRIDAY-290

Publishing Title:

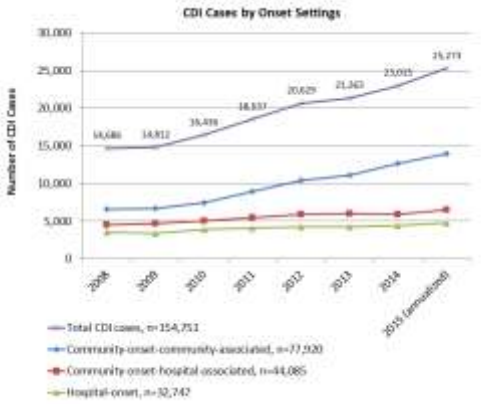
Trend of *Clostridium difficile* Infections by Onset Settings: A Multicenter Study

Author Block:

Y. P. Tabak[1], **C. A. DeRyke**[2], V. Gupta[1], X. Sun[1], R. S. Johannes[1], S. W. Marcella[2]; [1]Becton, Dickinson & Company, Franklin Lakes, NJ, [2]Merck & Co. Inc., Rahway, NJ

Abstract Body:

Background: A historically hospital-onset infection, C. *difficile* infection (CDI) may be increasingly community-onset. We sought to examine the trend of CDI by onset settings. **Methods:** We analyzed automated clinical data in 154 USA hospitals from 1/1/2008 to 9/30/2015. A CDI case was defined as a positive result of C. *difficile* toxin or molecular assay of a stool specimen obtained from a patient without a positive assay in the previous 8 weeks. Cases were classified based on the positive specimen collection time and setting: **a) Community-onset-community-associated**: in an outpatient setting or ≤3 calendar days after hospital admission AND no overnight hospital stay in the prior 12 weeks; **b) Community-onset-hospital-associated**: in an outpatient setting or ≤3 calendar days after hospital admission AND with overnight hospital stay in the prior 12 weeks; **c) Hospital-onset**: >3 calendar days after hospital admission.**Results:** From 2008 to 2015 (2015 cases annualized), the total number of CDI cases increased from 14,686 to 25,273 (72% increase, $p<0.01$). Setting specific rates were: community-onset-community-associated, from 6,586 to 13,975 (112%); community-onset-hospital-associated, from 4,545 to 6,524 (44%); hospital-onset, from 3,555 to 4,775 (34%) (all $p<0.01$). The community-onset-community-associated cases accounted for half of overall cases and proportionately increased from 45% in 2008 to 55% in 2015 ($p<0.01$). The combined community onset cases with or without prior hospital stay accounted for 81% of all cases in 2015. **Conclusions:** The overall CDI cases increased significantly over time for all three settings. The community-onset cases accounted for vast majority of all CDI cases and increased in proportion over time.



Author Disclosure Block:

Y.P. Tabak: D. Employee; Self; BD. **C.A. DeRyke:** D. Employee; Self; Merck & Co. Inc. **V. Gupta:** D. Employee; Self; BD. **X. Sun:** D. Employee; Self; BD. **R.S. Johannes:** D. Employee; Self; BD. **S.W. Marcella:** D. Employee; Self; Merck & Co. Inc..

Poster Board Number:

FRIDAY-291

Publishing Title:

Molecular Epidemiological Analysis of *Clostridium difficile* Pcr Ribotype 018 Isolates from a University Hospital Over 5 Years

Author Block:

M-R. Seo, J. Kim, J. Kang, **H. Pai**; Med Coll Hanyang Univ., Seoul, Korea, Republic of

Abstract Body:

Background: *Clostridium difficile* PCR ribotype 018 (RT018) is the most prevalent strain in Korea, Japan and Italy. RT018 was known to be highly resistant to antibiotics. Objective of this study was to analyze a change of clonality and minimum inhibitory concentration (MIC) of antibiotics among annual isolates of RT018 from 2009 to 2013 in a tertiary hospital.**Methods:** A total of 207 *C. difficile* RT018 isolates were obtained from patients at the Hanyang Univeristy Hospital from January 2009 to December 2013. Multilocus variable-number tandem repeat analysis (MLVA) using six selected *C. difficle* repeat (CDR) loci was performed. The susceptibility of these isolates to clindamycin (CLI), moxifloxacin (MOX), metronidazole (MET), vancomycin (VAN), rifaximin (RFX) and piperacillin/tazobactam (PTZ) was determined.**Results:** Minimum-spanning-tree analysis of the MLVA data from 207 RT018 strains identified 78 MLVA types: 21 in 2009, 27 in 2010, 22 in 2011, 23 in 2012 and 10 types in 2013. The 78 MLVA types of RT018 isoates were categorized into 6 clonal complexes containg 2 or more isolates. The largest cluster, CC-I was composed of 51 MLVA types from 148 isolates (71.5%), and distributed in: 83.8% (31/37) in 2009, 93.3% (56/60) in 2010, 42.2% (19/45) in 2011, 59.6% (31/52) in 2012 and 84.6% (11/13) in 2013. CC-II composed of 10 MLVA types from 36 isolates distributed mostly in 2011: 0 in 2009, 3.3% (2/60) in 2010, 51.1% (23/45) in 2011, , 21.2% (11/52) in 2012 and 0 in 2013. Among 207 RT018 isolates, resistance rates to CLI and MOX were 97.6% and 98.6% respectively, while 1.4% presented VAN resistance. All strains were susceptible to MET or PTZ, with MIC_{90} values of 0.094 and 16ug/ml. Resistance rate to RFX was 8.2% ($MIC_{50,}$ 0.0038 and MIC_{90}, 0.5ug/ml). Comparing the MIC value of each antibiotic among annual isolates of RT018, MICs of CLI, MOX, PTZ, RFX and MET increased during 5 years (*P* for trend: <0.0001, <0.0001, <0.0001, <0.0001, and 0.006).**Conclusions:** MLVA types of RT018 changed over the years in a hospital, and several antibiotics' MICs of annual isolates increased.

Author Disclosure Block:

M. Seo: None. **J. Kim:** None. **J. Kang:** None. **H. Pai:** None.

Poster Board Number:

FRIDAY-292

Publishing Title:

Clinical and Bacterial Characterization of Nosocomial *Clostridium difficile* Infection in Pediatric Ward in Japan

Author Block:

Y. Nukui[1], T. Chino[1], R. Saito[2], C. Tani[2], G. Igawa[2], Y. Kamiichi[2], A. Kasai[2], S. Ogihara[3], E. Sawabe[1], S. Tohda[1], R. Koike[1]; [1]Med. Hosp. of Tokyo Med. and Dental Univ., Tokyo, Japan, [2]Tokyo Med. and Dental Univ., Tokyo, Japan, [3]Yamanashi Univ. Hosp., Yamanashi, Japan

Abstract Body:

Background: *Clostridium difficile* is an important cause of antibiotic-associated diarrhea and one of the most common healthcare-associated infections among children. This study investigated the molecular epidemiology and antimicrobial susceptibility of *C. difficile* infecting children.**Methods:** From January 2012 to October 2015, we analyzed 25 non-repetitive *C. difficile* strains isolated from children under eighteen years old who suffered diarrhea in the medical hospital of Tokyo Medical and Dental University. Multilocus sequence typing for molecular epidemiology and multiplex PCR for detecting the toxin genes were performed. The minimum inhibitory concentrations of antibiotics were determined by the agar dilution method.**Results:** The incidence of *C. difficile* infection in children <18 years was 1.58 cases per 1,000 in-patients. The most common risk factors were immunosuppression (96.0%), use of antibiotics (92.0%), use of proton pump inhibitors and/or histamine-2 receptor antagonists (40.0%). The clinical course was mild in most cases; however, 60% of the cases experienced recurrence despite metronidazole treatment. Nineteen toxigenic strains (76.0%) (including eleven *tcdA*-positive, *tcdB*-positive, and *cdtA/cdtB*-negative strains ($A^+B^+CDT^-$) and eight $A^+B^-CDT^-$ strains) and six (24.0%) non-toxigenic strains were classified into eleven and four sequence types, respectively. Of these, sequence type (ST) 109 (20.0%) was the most predominant. Antibiotic susceptibility rates to clindamycin, vancomycin, and metronidazole were 8, 92, and 100%, respectively.**Conclusions:** The role of *C. difficile* infection in immunocompromised children, including those with malignancies, hematopoietic stem cell transplants, and other humoral and cellular immunodeficiencies, has been increasingly recognized. In addition, the recurrence rates among these patients were higher than expected. Continuous surveillance for antibiotic susceptibility of *C. difficile* is very important for the administration of the appropriate antimicrobial therapy.

Author Disclosure Block:

Y. Nukui: None. **T. Chino:** None. **R. Saito:** None. **C. Tani:** None. **G. Igawa:** None. **Y. Kamiichi:** None. **A. Kasai:** None. **S. Ogihara:** None. **E. Sawabe:** None. **S. Tohda:** None. **R. Koike:** None.

Poster Board Number:

FRIDAY-293

Publishing Title:

Impact of Presence of Malignancy and Exposure to Chemotherapy on Clinical and Microbiological Characteristics of *Clostridium difficile* Infection

Author Block:

M. Chung, J. Kim, **J. Kang**, H. Pai; Med Coll Hanyang Univ., Seoul, Korea, Republic of

Abstract Body:

Background: Purpose of the study was to investigate the impact of malignancy and chemotherapy on clinical and microbiological characteristics of *Clostridium difficile* infection (CDI).**Methods:** We performed a retrospective study with the patients of CDI from February 2009 through January 2013. CDI patient with history of malignancy within 5 years was defined as cancer group. Among the cancer group, patient who received chemotherapy within 2 months was regarded as chemotherapy group. Characteristics of patients were compared between the groups according to presence of malignancy and exposure to chemotherapy.**Results:** Of 580 patients with CDI, 159 cases (27.4%) belonged to cancer group and 421 (72.6%) to non-cancer group, and 69 of 159 cases were in subgroup of chemotherapy. More patients in cancer group were hospitalized within prior 2 months (P<0.001). Leukocytosis was more common in non-cancer group (P = 0.034), and infection by PCR ribotype 017 strains occurred more frequently in cancer group with marginal significance (P =0.07). Recurrence rate was higher in cancer group than non-cancer group (20.4% vs. 9.5%, P =0.005), and the presence of malignancy was consistently a significant risk factor for recurrence of CDI in multivariate logistic regression (OR=2.665, 95% CI 1.343-5.289, P =0.005). Age significantly contributed to recurrence of CDI as well (OR=1.032, 95% CI 1.004-1.060, P =0.026). However, chemotherapy did not effect on clinical course of CDI significantly.**Conclusions:** Malignancy and old age were independent risk factors for recurrence of CDI. Cancer patients require careful observation for recurrence after treatment of CDI.

Author Disclosure Block:

M. Chung: None. **J. Kim:** None. **J. Kang:** None. **H. Pai:** None.

Poster Board Number:

FRIDAY-294

Publishing Title:

Risk Factors for *Clostridium difficile* Infection in Patients with HIV

Author Block:

H. Imlay, D. R. Kaul, K. Rao; Univ. of Michigan, Ann Arbor, MI

Abstract Body:

Background: *Clostridium difficile* infection (CDI) is a healthcare-associated infection resulting in significant morbidity. Immunosuppression is a significant risk factor for both acquisition of CDI and adverse outcomes. However, the epidemiology of CDI in HIV-infected patients has been little studied in the era of antiretroviral therapy (ART). This study's goal was to identify the risk factors, including HIV-specific ones, for acquisition of CDI in HIV-infected patients.
Methods: A retrospective, propensity score-matched case/control study design was employed, with patients selected from our institution's outpatient HIV clinic. CDI cases were defined as having positive stool testing for either the toxin(s) A/B or the *tcdB* gene plus an appropriate clinical presentation. The propensity score was generated via multiple logistic regression from year of HIV diagnosis, age at first contact, duration of follow up, gender, and initial CD4 count. Chart review extracted variables.**Results:** The 46 cases included were matched up to 1:4 by propensity scores to a total 180 controls. The final model is presented in Table 1. Adjusting for covariates, including age, prior antibiotic treatment was a significant predictor of CDI (odds ratio [OR] 13, 95% confidence interval [CI] 3.49-48.8, *P* <.001) as was number of hospital admissions in the preceding year (OR 4.02, CI 1.81-8.94, *P* <.001). Neither PPI use nor low CD4 count (<200 cells/µL) were independent predictors of CDI, but if both were present an interaction existed that conferred a significantly increased risk of CDI in the multivariable model (OR 15.17, CI 1.31-175.9, *P* = .021).**Conclusions:** As in the general population, frequent hospitalizations and exposure to antimicrobials are independent predictors of CDI acquisition in patients with HIV. Intriguingly, the HIV-specific risk factor of a low CD4 count combined with PPI use significantly increased the risk of CDI and, as potentially modifiable characteristics, these present targets for preventative interventions in future studies.

Table 1. Multivariable model for acquisition of *Clostridium difficile* infection with HIV

Variable	**β coefficient ± standard error**	***P***
Exposure to antimicrobials	2.57 ± 0.67	<.001
Number of hospitalizations in the preceeding year	1.39 ± 0.41	.001
Exposure to proton pump inhibitors (PPIs)	−0.74 ± 0.88	.4
CD4 count <200 cells/µL	−0.87 ± 0.97	.369
CD4 <200 cells/µL + PPI exposure	3.46 ± 1.67	.039

Author Disclosure Block:

H. Imlay: None. **D.R. Kaul:** None. **K. Rao:** None.

Poster Board Number:

FRIDAY-295

Publishing Title:

Multi-laboratory Evaluation of Storage Conditions and Stability of Purified Spore Suspensions of *Clostridium difficile*

Author Block:

J. A. HASAN, K. M. Japal, R. M. Pines; US Environmental Protection Agency, FT. MEADE, MD

Abstract Body:

The US Environmental Protection Agency (EPA) regulates antimicrobial pesticides including products with claims for use against spores of *Clostridium difficile* on hard non-porous surfaces. ASTM Standard Test Method (E2839-11) is recommended for production of *C. difficile* spores for use in efficacy testing. The method does not currently specify storage conditions for spore suspensions. Six laboratories participated in a study to evaluate two storage conditions over a twelve week period; spore suspensions stored at -80°C, and, spores dried on test carriers and stored under desiccation at room temperature (RT). The log densities (LD) of the spore suspensions on control carriers and the log reduction (LR) values of the spores when exposed to laboratory grade sodium hypochlorite (NaOCl) at 5,000 ppm, 2,500 ppm and 1,500 ppm available chlorine were evaluated every two weeks over a twelve week period. The OECD Quantitative Method (ASTM E2197-11) was used to measure the LD and LR values for control and treated carriers, respectively. For four of the six labs, the LD values of control carriers inoculated with spores and stored in a desiccator at RT dropped below the required minimum of six logs of spores per carrier necessary to evaluate the LR values for the NaOCl treatments. The LD values of control carriers inoculated with spores stored at -80°C demonstrated stable counts across all labs over the 12 week period ($p = 0.797$). Spores stored at -80°C and exposed to the NaOCl solutions exhibited low variability for the 5,000 ppm and 1,500 ppm treatments, but higher variability for the 2500 ppm treatment over the study period. The mean LR values for all three treatments are statistically significantly different ($p \leq 0.010$) indicating the responsiveness of the OECD method at each point in time. The trends of the mean LR were not statistically significantly different across treatments ($p \geq 0.577$), suggesting the suitability and stability of the spores when stored at -80°C. EPA will utilize the data in considering whether to modify the guidance used for developing efficacy data for antimicrobial products against *C. difficile.*

Author Disclosure Block:

J.A. Hasan: None. **K.M. Japal:** None. **R.M. Pines:** None.

Poster Board Number:

FRIDAY-296

Publishing Title:

Effect of Testing Methods with Real-Time Clinical Surveillance Tools in Patients with *Clostridium difficile* Infections

Author Block:

H. Burgess, **M. Cooper**, R. Burks, D. Smith, A. Nelson, S. Fraker, J. Kramer; HCA Corporate, Nashville, TN

Abstract Body:

Background: Despite effective antibiotic therapy, *Clostridium difficile* infections (CDIs) are associated with a high rate of mortality. Moreover, patients who survive the disease can experience high morbidity and be left with debilitating effects. The utilization of polymerase chain reaction and nucleic acid amplification tests (PCR/NAAT) for diagnostic testing has been suggested as an opportunity for antimicrobial stewardship programs to improve antimicrobial use and clinical outcomes in patients with CDIs. Additionally, real-time clinical surveillance tools (RTCSTs) can be utilized to alert pharmacists to opportunities for intervention. The aim of this study was to determine the impact of PCR/NAAT and RTCST compared to conventional CDI testing and RTCST on time to appropriate antimicrobial therapy. **Methods:** This retrospective study looked at patients treated at one of 83 hospitals from November 1, 2014 through June 30, 2015 who had RTCST alerts for a positive *C. difficile* test who had not been started on therapy prior to the result being reported as positive. The primary outcome was frequency of appropriate antimicrobial therapy administration within 4 hours of RTCST alert activation. 2-sample t-tests, Chi-square tests, and Mann-Whitney-Wilcoxon tests were used for statistical analysis of the data. **Results:** 1748 patients were included in the analysis. For the primary outcome, 41.9% of patients in the PCR/NAAT group received an appropriate medication within 4 hours of RTCST activation, compared with 27.4% in the conventional group ($p<0.001$). In those patients who received appropriate therapy within 4 hours, time from collection to results was significantly shorter in the PCR/NAAT group when compared to the conventional group, resulting in faster time to therapy (6.6 hours vs. 16.1 hours, respectively [$p<0.001$]). When looking at the group as a whole and comparing PCR/NAAT and conventional testing, no differences were found in length of stay or mortality. **Conclusions:** RDT testing shows benefit over conventional diagnostic testing in patients with CDIs. In this study, it led to faster diagnostic results which, in turn, resulted in earlier administration of appropriate antimicrobial therapy.

Author Disclosure Block:

H. Burgess: None. **M. Cooper:** None. **R. Burks:** None. **D. Smith:** None. **A. Nelson:** None. **S. Fraker:** None. **J. Kramer:** None.

Poster Board Number:

FRIDAY-297

Publishing Title:

In Vitro Activities Of Fidaxomicin, Op-1118, And Surotomycin Against Ribotype 027 (Nap1/Bl) *clostridium Difficile* Isolated From Canadian Patents: Can-Diff 2013-2015

Author Block:

J. Karlowsky[1], H. Adam[1], Z. Narrandes[2], G. Golding[3], K. Nichol[1], T. Kosowan[3], M. Baxter[2], N. Laing[2], D. Hoban[1], G. G. ZHANEL[2], CARA; [1]Diagnostic Services Manitoba, Winnipeg, MB, Canada, [2]Univ. of Manitoba, Winnipeg, MB, Canada, [3]Natl. Microbiol. Lab., Winnipeg, MB, Canada

Abstract Body:

Background: Clinical microbiology laboratories do not routinely culture toxin-positive (TP) stool specimens for *C. difficile* (CD), or perform antimicrobial susceptibility testing (AST) or ribotyping on TP-CD isolates. Therefore, periodic assessments are informative. The current study determined genotypes and the in vitro activities of 6 routinely tested anti-anaerobic agents as well as the oral narrow-spectrum macrocyclic antimicrobial, fidaxomicin, its active metabolite OP-1118, and surotomycin, an oral cyclic lipopeptide against TP-CD isolates collected in Canada from 2013 to 2015.**Methods:** To date, 983 isolates of CD have been cultured from TP stool specimens using CDMN agar. Each isolate's identity was confirmed by accepted laboratory methods. AST was performed using the agar dilution method (CLSI, M11-A9, 2015). The presence of *tcdA* (toxin A), *tcdB* (toxin B), *tcdC* (negative regulator of toxin production) and *cdtB* (binary toxin) were determined by PCR methods. Genotyping was performed by an accepted PCR ribotyping method.**Results:** >99.9% of CD were susceptible to metronidazole, amoxicillin-clavulanate, and tigecycline; isolate susceptibilities to clindamycin (4.8%), ceftriaxone (11.9%), and moxifloxacin (66.7%) were lower. MIC ranges/MIC_{90}s (µg/mL) were ≤0.015-1/0.5, 0.25-32/16, 0.12-8/2, and ≤0.25-4/2, respectively, for fidaxomicin, OP-1118, surotomycin, and vancomycin. 027 (25.7% of isolates), 014 (8.5%), 020 (5.9%), and 106 (5.3%) were the most common ribotypes identified. Ribotype 027 accounted for 15.3, 27.9, and 34.7% of isolates from western Canada (BC, Alberta, Manitoba), Ontario, and eastern Canada (Quebec, Nova Scotia), respectively, as well as 18.1, 22.9, and 40.2% of isolates from patients aged ≤64, 65-79, and ≥80 years. Isolates of ribotype 027 had higher MIC_{90}s for moxifloxacin and vancomycin (>32 and 2 µg/ml), respectively, than other ribotypes (2-16 and 1 µg/ml).**Conclusion:** Fidaxomicin, OP-1118, and surotomycin demonstrated potent in vitro activity against all genotypes of TP-CD including ribotype 027 (NAP1/BI).

Author Disclosure Block:

J. Karlowsky: None. **H. Adam:** None. **Z. Narrandes:** None. **G. Golding:** None. **K. Nichol:** None. **T. Kosowan:** None. **M. Baxter:** None. **N. Laing:** None. **D. Hoban:** I. Research

Relationship; Self; Abbott, Astellas, Cubist, Galderma, Merck, Pharmascience, Sunovion, The Medicines Co, Tetraphase, Zoetis. **G.G. Zhanel:** I. Research Relationship; Self; Abbott, Astellas, Cubist, Galderma, Merck, Pharmascience, Sunovion, The Medicines Co, Tetraphase, Zoetis.

Poster Board Number:

FRIDAY-298

Publishing Title:

***In Vitro* Activity of Vancomycin (VA), Metronidazole (ME), Clindamycin (CL), Moxifloxacin (MX), Fidaxomicin (FX), and Rifaximin (RX) Against *Clostridium difficile* (CD) Isolates Recovered from Patients Enrolled in a Global Clinical Trial of Actoxumab and Bezlotoxumab**

Author Block:

D. M. Citron[1], E. J. C. Goldstein[1], S. Sambol[2], M. H. Wilcox[3], M. Dorr[4]; [1]R.M. Alden Res. Lab., Culver City, CA, [2]VA Med. Ctr., Hines, IL, [3]Leeds Inst. of BioMed. and Clinical Sci., Leeds, United Kingdom, [4]Merck & Co., Kenilworth, NJ

Abstract Body:

Background: MODIFY I and II were global clinical trials of actoxumab and bezlotoxumab (+ standard of care) for prevention of recurrent CD infection, conducted over 3 years (2012-15) at 382 sites in 30 countries in N. and S. America, Europe, Asia, and including Israel and S. Africa. **Methods**: CD positive samples came from 297 (93.6 %) of 317 study sites that sent specimens and yielded 1776 unique Visit 1 isolates. Susceptibility testing was performed with 6 antimicrobial agents using an agar dilution method. Isolates were also shipped to two laboratories for REA and ribotyping (RT). **Results**: 42.8% of isolates came from US, while Spain had 7.8%, Canada 7.5%, Poland and Israel each 4.1%, Japan 3.7%, Czech Republic 3.6%, and Italy 3.1%. The remaining countries each had &lt3% of the total. Decreased susceptibility to VA (MIC 4-8 µg/ml) was seen in 144/1776 isolates (8.1%), predominantly from USA and Israel. The most common RTs were 027 and 137, corresponding to REA groups BI and BN, respectively. Resistance to MX (MIC &gt2µg/ml) was also present in 88.2% of these (127/144), isolates. Decreased susceptibility to ME (MIC 4-8 µg/ml) was found in 46/1776 strains (2.6%), which also showed resistance to MX (95.7%) and were recovered largely from sites in Chile, Poland, and USA, mostly RTs 027 and 001 (REA groups BI and J, respectively). RX resistance (MIC &gt32µg/ml) was seen in 12.9% of isolates overall; over half of these (116/229) were RT 027, mostly from the US, and Poland and almost half the isolates (26/53) from Italy were RX-resistant with RTs 356 and 018 (REA group W) predominating. Two strains with FI MIC &gt8 µg/ml were found, one each from US and France. **Conclusions:** Worldwide resistance of CD is increasing. RT 027 strains are multi-resistant, including decreased susceptibility to VA (22.8%) and ME (6.2%), and resistant to MX (89.1%) and RX (35.8%); other highly resistant RTs include 137 (Israel) and 001 (Europe and S. America). 99.9% of the isolates were susceptible to FI at &lt2 µg/ml. New therapies to improve outcomes are needed for this increasingly problematic pathogen.

Author Disclosure Block:

D.M. Citron: None. **E.J.C. Goldstein:** H. Research Contractor; Self; Merck& Co., Actelion, Cutis Pharma. J. Scientific Advisor (Review Panel or Advisory Committee); Self; Summit Pharmaceuticals. L. Speaker's Bureau; Self; erck & Co.. **S. Sambol:** None. **M.H. Wilcox:** None. **M. Dorr:** D. Employee; Self; Merck % Co.

Poster Board Number:

FRIDAY-299

Publishing Title:

Genotypes and Antifungal Susceptibility of *Cryptococcus neoformans* Species Complex Causing Invasive Diseases in China: Predominance of *C. neoformans* var. *Grubii* Sequence Type 5, and Emergence of Isolates Non-Susceptible to Fluc

Author Block:

M. Xiao[1], X. Fan[1], S. Chen[2], F. Kong[2], H. Wang[1], Y-C. Xu[1]; [1]Peking Union Med. Coll. Hosp., Beijing, China, [2]Westmead Hosp., Westmead, Australia

Abstract Body:

Background: There are limited data on cryptococcosis in China. Here we investigated the nationwide genetic structure and antifungal susceptibility of the *Cryptococcus neoformans* species complex over a five-year period.**Methods:** A total of 312 *C. neoformans* species complex isolates from patients with cryptococcosis collected from 10 hospitals from August 2009 to July 2014 in China were studied. The isolates were identified by sequencing of the rDNA internal transcribed spacer (ITS) region, and multilocus sequence typing was used for genotyping of the species complex. The ability of two MALDI-TOF MS systems to identify *C. neoformans* complex isolates were also evaluated. *In vitro* susceptibility to six antifungal drugs was determined by the Sensititre YeastOne method.**Results:** Of 312 isolates, 211 (67.6%) were isolated from cerebrospinal fluid (CSF), followed by blood cultures (n = 74, 23.7%). *C. neoformans* was the most common species (n = 305, 97.8%), of which *C. neoformans* var. *grubii* sequence type (ST) 5 were predominant (n = 272, 89.2%), followed by ST31 (n = 31, 6.1%), while other *C. neoformans* var. *grubii* STs (n = 12 in all, 3.9%) and *C. neoformans* var. *neoformans* (n = 2, 0.7%) were rare. Molecular serotyping found all *C. neoformans* var. *grubii* were serotype A, and the two *C. neoformans* var. *neoformans* isolates were serotype AD heterozygote. *C. gattii* were also less common (n = 7, 2.2%). Two MALDI-TOF MS showed good capacity in identifying *C. neoformans* var. *grubii* and var. *neoformans* isolates (>99%) but less satisfying in identifying *C. gattii* (Bruker Biotyper 71.4%, Vitek MS, 0%). Amongst *C. neoformans* var. *grubii* isolates, the proportion of fluconazole non-wild-type isolates were significantly higher in the fourth year (23.9%) comparing to previous three years (0-2.1%), including five fluconazole highly non-susceptible isolates (minimum inhibitory concentrations ≥ 32 mg/L) that all belonged to *C. neoformans* ST5.**Conclusions:** In summary, *C. neoformans* var. *grubii* ST5 was the most predominant in China. The emergence of fluconazole highly non-susceptible isolates and their geographic difference warrant continuous surveillance.

Author Disclosure Block:

M. Xiao: None. **X. Fan:** None. **S. Chen:** None. **F. Kong:** None. **H. Wang:** None. **Y. Xu:** None.

Poster Board Number:

FRIDAY-300

Publishing Title:

Evaluation of *In Vitro* Amphotericin B and Triazole Activity Against *Coccidioides* species from United States Institutions

Author Block:

G. Thompson, III[1], B. M. Barker[2], N. P. Wiederhold[3]; [1]UC-Davis, Sacramento, CA, [2]Tgen, Flagstaff, AZ, [3]UTHSCSA, San Antonio, TX

Abstract Body:

Background: Large scale testing of *Coccidioides* isolates to define population susceptibility has not previously been performed, nor has the frequency of clinical isolates with elevated amphotericin B (AMB) or triazole MICs been evaluated. **Methods:** *Coccidioides* isolates ($n \geq 380$) submitted to the UT Health Science Center Fungus Testing Laboratory were queried for AMB, fluconazole (FLC), itraconazole (ITC), posaconazole (PSC), and voriconazole (VRC) MICs. Isolates from across the United States were included in this assessment. Antifungal susceptibility testing was performed by broth macrodilution according to the CLSI M38-A2 standard. The MIC range, MIC50, MIC90, and geometric mean (GM) MIC values were determined, and differences in GM MIC values were assessed for significance by ANOVA with Tukey's post-test for multiple comparisons.**Results:** Of the antifungals evaluated, VRC had the most potent *in vitro* activity. The VRC GM MIC (0.106 μg/ml) was significantly lower than that observed for AMB (0.243 μg/ml), FLC (7.586 μg/ml), and ITC (0.244 μg/ml) ($p < 0.0001$ for all comparisons). The PSC GM MIC (0.140 μg/ml) was similar to that of VRC, and was significantly lower than that of AMB, FLC, and ITC ($p < 0.0001$). Interestingly, there was a wide range of potency for each antifungal with several isolates having elevated MIC values for FLC (MIC $\geq$ 16 μg/ml; 37.2% of isolates), ITC ($\geq$ 2 μg/ml; 1.0%), PSC ($\geq$ 1 μg/ml; 1.1%), and VRC ($\geq$ 2 μg/ml; 1.2%), AMB ($\geq$ 2 μg/ml; 2.8%).**Conclusions:** Mould active triazoles exhibited low MICs for the majority of isolates tested with MICs likely to confer clinical resistance uncommon. However elevated fluconazole MICs were commonplace. Further work focusing on patient outcomes and the molecular mechanisms responsible for elevated triazole MICs should be performed as resistance and clinical failure remain uncharacterized in coccidioidomycosis.

Author Disclosure Block:

G. Thompson: None. **B.M. Barker:** None. **N.P. Wiederhold:** None.

Poster Board Number:

FRIDAY-301

Publishing Title:

Development of a Risk Score for Fluconazole Failure among Patients with Invasive Candidiasis

Author Block:

L. Ostrosky-Zeichner[1], **R. Harrington**[2], N. Azie[2], H. Yang[3], N. Li[3], J. Zhao[3], V. Koo[3], E. Wu[3]; [1]UTHlth., Houston, TX, [2]Astellas, Northbrook, IL, [3]Analysis Group, Boston, MA

Abstract Body:

Background: Resistance to fluconazole (FLU) has become a growing concern. We developed a prediction model to identify patients with invasive candidiasis (IC) at high risk of failing FLU. **Methods:** Adult patients with IC (defined by positive microbiology blood culture) who received FLU during hospitalization were selected from Cerner Health Facts database (2005-2013). FLU failure was defined as switching from or adding other antifungal to FLU, a positive culture for *Candida* ≥ 10 days after FLU initiation, or death during hospitalization. Patients were randomly split into modeling and validation samples (2:1 ratio). A least absolute shrinkage and selection operator regression was used to select predictors for estimating individual risk of FLU failure. The list of potential predictors evaluated included patient demographics, *Candida* species, initiation of FLU before positive culture and after admission, co-morbidities, procedures, and treatments during the period from 6-month before admission to initiation of FLU. The performance of the selected prediction model was evaluated using the validation sample.**Results:** A total of 987 patients were identified, including 488 with FLU failure and 499 without failure. The final prediction model for FLU failure was composed of 9 risk factors: days to start FLU from admission, *C. glabrata* infection, *C. krusei* infection, hematological malignancies, venous thromboembolism (VTE), enteral nutrition, use of non-operative intubation or irrigation, use of mechanical ventilation, and use of other antifungals. All factors, except VTE, were associated with a higher risk of FLU failure. The c-statistic of the model was 0.65. Model performance using a predicted risk score threshold ranging from 0.3 to 0.7 to define FLU failure was summarized in Table 1.**Conclusion:** The prediction model could identify patients with a high risk of FLU failure, illustrating the potential value and feasibility of personalizing IC treatment.**Table 1.** Model performance

Threshold	**Sensitivity**	**Specificity**	**Positive Predictive Value**	**Negative Predictive Value**
0.3	0.97	0.17	0.53	0.85
0.4	0.78	0.42	0.57	0.66
0.5	0.56	0.68	0.63	0.61

0.6	0.33	0.84	0.66	0.56
0.7	0.15	0.95	0.74	0.53

Author Disclosure Block:

L. Ostrosky-Zeichner: C. Consultant; Self; Astellas, Merck, Scynxis, Cidara. E. Grant Investigator; Self; Merck, Astellas, Pfizer, Scynexis, T2 Biosystems, Meiji, Immunetics. L. Speaker's Bureau; Self; Merck, Pfizer. **R. Harrington:** D. Employee; Self; Astellas Medical Affairs, Americas. **N. Azie:** D. Employee; Self; Astellas Medical Affairs, Americas. **H. Yang:** D. Employee; Self; Analysis Group. **N. Li:** D. Employee; Self; Analysis Group. **J. Zhao:** D. Employee; Self; Analysis Group. **V. Koo:** D. Employee; Self; Analysis Group. **E. Wu:** D. Employee; Self; Analysis Group.

Poster Board Number:

FRIDAY-302

Publishing Title:

Echinocandin (Ec) Agent-Specific Spontaneous and *fks* Mutation Rates in *Candida glabrata* (Cg)

Author Block:

R. K. Shields, M. H. Nguyen, E. G. Press, C. J. Clancy; Univ. of Pittsburgh, Pittsburgh, PA

Abstract Body:

Background: EC resistance in Cg is mediated by point mutations in *FKS1* and *FKS2* hot spots (HS). Our objective was to measure spontaneous (SMR) and *FKS* mutation rates among Cg exposed to anidulafungin (ANF), caspofungin (CSP), and micafungin (MCF). **Methods:** 20 *FKS* wild-type (WT) Cg with varying EC MICs from pts w/ or w/o prior EC exposure were selected. SMR was calculated as ratio of Cg on SDA plates w/ or w/o ECs at 3x MIC. Minimum fungicidal (MFC) and mutation prevention concentrations (MPC) were the lowest EC concentrations inhibiting ≥99% and 100% of Cg, respectively. MICs and *FKS* genotypes were determined for colonies growing at ≥MFC for each EC. **Results:** Median ANF, CSP, and MCF MICs were 0.06 (range: 0.03 - 0.12), 0.12 (0.03 - 1), and 0.03 (0.015 - 0.03), respectively. Corresponding median MFC/MPCs were 0.25/0.5, 1/2, and 0.06/0.06, respectively. CSP MFCs and MPCs were higher than other ECs (P<0.0001 for each). Median Cg SMRs in ascending order were 3.1E-9 (0 to 1.1E-7), 3.5E-7 (2.7E-8 to 3.9E-6), and 1.0E-5 (5.3E-7 to 3.5E-3) for MCF, ANF, and CSP, respectively (P<0.0001). SMR did not vary by CSP MIC or prior EC exposure. 159, 74, and 14 strains growing at ≥MFC of CSP, ANF, and MCF, respectively, were collected; 20% had no change in MIC compared to parent strains. Among the remaining 80%, CSP, ANF, and MCF MICs were increased by ≥2-fold in 89%, 60%, and 44%, respectively. All strains selected on MCF-agar had increased MICs to all agents; however, strains selected on ANF or CSF-agar demonstrated agent-specific MIC increases (P<0.004). 33% of MFC mutants (41% of mutants w/ ≥2-fold MIC increase) had *FKS* mutations. *FKS* mutations were detected among 30%, 30%, and 86% of strains recovered on CSP, ANF, and MCF-containing agar, respectively (P<0.001). 78%, 16%, and 6% of *FKS* mutations were in FKS2-HS1, FKS2-HS2, and FKS1-HS1, respectively. Mutations at S629 (FKS1) or S663 (FKS2) were more common among strains exposed to ANF or MCF (26%) compared to CSP (4%; P=0.006). 3% of MFC mutant strains had ≥4-fold MICs to all agents, but no *FKS* mutations. **Conclusions:** MFC and MPC are highest for CSP. Across agents, SMR and *FKS* mutations are inversely related. In rank order, SMR are CSP>ANF>MCF; however, the percentage of mutant strains harboring *FKS* HS mutations was MCF>ANF>CSP. *FKS* mutations also differ by EC. A small, meaningful % of strains demonstrate increased MICs to all ECs, but do not harbor *FKS* HS mutations.

Author Disclosure Block:

R.K. Shields: I. Research Relationship; Self; Astellas, Merck. **M.H. Nguyen:** I. Research Relationship; Self; Astellas, Merck, T2 Biosystems. **E.G. Press:** None. **C.J. Clancy:** I. Research Relationship; Self; Astellas, Merck, Pfizer, T2 Biosystems.

Poster Board Number:

FRIDAY-303

Publishing Title:

In Vitro Antifungal Activity of Echinocandins Against 198 *Candida* spp Strains

Author Block:

N. Kiraz; Istanbul Univ. Cerrahpasa Faculty of Med., Istanbul, Turkey

Abstract Body:

Background: The echinocandins anidulafungin (ANF), caspofungin (CSF), micafungin (MCF) represent a new antifungal group with potent activity against *Candida* species. A commercially prepared dried colorimetric microdilution panel (Sensititre YeastOne, TREK Diagnostic Systems, Cleveland, OH, USA) is able to determine the susceptibility of echinocandins and demonstrated excellent corelation with the reference method M27-A3 from the Clinical Laboratory Standards Institute (CLSI). Resistance rates were determined by recently revised CLSI antifungal breakpoints. The aim of this study is to evaluate the *in vitro* activity of ANF, CSF and MCF against clinically significant *Candida* strains by YeastOne colorimetric microdilution panel.**Methods:** Isolates were checked of purity and were identified by classical morphological tests including germtube formation in human serum at 37°C for 3 h, blastoconidia, pseudohyphae, truehyphae and chlamydoconidia formation on corn meal agar-Tween 80, integrating with the results of chromogenic agar (CHROMagar, HiMedia, India) and of API 20C AUX kit (Bio Mérieux, France). Identification kit procedure and colorimetric microdilution tests were performed according to the manufacturer's instructions. Quality control was ensured by testing CLSI-recommended strains *C. parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258.**Results:** Among 198 Candida isolates, 112 were C. albicans, 11 were C. glabrata, 45 were C. parapsilosis, 14 were C. tropicalis, seven C. krusei, three of each C. kefyr and C. lusitaniae, two C. guilliermondii, and one C. pelluculosa. Regarding to the MIC values obtained, resistance to ANF was observed in one C. albicans, two C. tropicalis, five C. parapsilosis strains; resistance to CSF was observed in one C. albicans and one C. parapsilosis strains; and resistance to MCF was observed in two C. albicans, one C. tropicalis and fiveC. Parapsilosis strains. There remaining strains were in vitro susceptible to all echinocandins tested.**Conclusions:** Based on newly established breakpoints for species-specific interpretive criteria, the results of this study affirmed that echinocandins exhibit excellent activity against the *Candida* secies most frequently involved in human infections.

Author Disclosure Block:

N. Kiraz: None.

Poster Board Number:

FRIDAY-304

Publishing Title:

World-Wide Epidemiology of FKS Mutations in Candida: A Systematic Review of the Literature

Author Block:

M. Al Obaidi[1], D. Thye[2], L. Ostrosky-Zeichner[1]; [1]UTHlth., Houston, TX, [2]Cidara, San Diego, CA

Abstract Body:

Background: Echinocandins are the agents of choice for treatment of candidemia. Echinocandin failures and FKS mutations are being reported more frequently. We describe the world-wide epidemiology of FKS mutations.**Methods:** We performed a systematic review of the literature from 2006-2015 using Pubmed. We included publications in English with the following keywords: FKS candida, FKS1, and FKS2, excluding duplicate isolates by screening for any publication with overlapping sources, including the SENTRY database. Data at the individual strain level were collated and descriptive statistics were performed.**Results:** We found 54 publications that met criteria reporting 535 *Candida* isolates with FKS mutations. The number of reported isolates fluctuated throughout the years with a peak in 2014. *C. glabrata* dominated the number of reported FKS mutations with 326 (60.9%) isolates (68% FKS2 and 32% FKS1), followed by *C. albicans* with 71 (13.27%), *C. krusei* 14 (2.6%), *C. tropicalis* 12 (2.2%), *C. kefyr* 5(0.9%), *C. lusitaniae* 4(0.7%), *C. parapsilosis* 4(0.7%), *C. orthopsilosis* 3(0.56%), and *C. dubliniensis* 2 (0.37%). All non-glabrata isolates had only FKS1 mutations. North America accounted for the highest proportion of the reported isolates with a total of 252 (47%), with Europe reporting 111 (20.7%) isolates. Only 20/535 (3.7%) isolates harbored a heterozygous mutation. 282/535 (52.7%) of the reported mutations were within the classic hotspot regions (96.8% HS1 and 3.19% HS2). Amino Acid substitutions analysis showed S663 was the commonest with 137/535 (33.7% of *C. glabrata* isolates). S629P was the second most frequent substitution seen in 35 *C. glabrata* isolates and 1 *C. albicans*. **Conclusions:** Our study is limited by publication and search strategy bias; nevertheless, our systematic review confirmed an increasing number of isolates with FKS mutations being reported in the literature since 2006. Most mutations are being reported in North America and Europe with *C. glabrata* and *C. albicans* accounting for the majority of strains. FKS2 mutations are seen exclusively in *C. glabrata*. Most mutations are homozygous and the high frequency of certain amino acid substitutions may indicate clonal/regional spread. Continued surveillance of these epidemiological trends is warranted as echinocandin use extends.

Author Disclosure Block:

M. Al Obaidi: None. **D. Thye:** D. Employee; Self; Cidara. **L. Ostrosky-Zeichner:** C. Consultant; Self; Astellas, Merck, Scynexis, Cidara. E. Grant Investigator; Self; Merck, Astellas, Pfizer, Scynexis, T2 Biosystems, Meiji, Immunetics. L. Speaker's Bureau; Self; Merck, Pfizer, T2 Biosystems.

Poster Board Number:

FRIDAY-305

Publishing Title:

Incidence, Clinical Characteristics And Sensitivity Profile Of Candidemia In A Teaching Hospital Located In The Southeast Of Brazil

Author Block:

H. M. S. Canela[1], M. E. S. Ferreira[1], B. Cardoso[1], R. Martinez[2], L. H. Vitali[2]; [1]Sch. of Pharmaceutical Sci. of Ribeirão Preto, Ribeirão Preto, Brazil, [2]Clinical Hosp. of Ribeirão Preto Med. Sch., Ribeirão Preto, Brazil

Abstract Body:

Background: *Candida* spp. are the seventh leading cause of nosocomial bloodstream infections in Brazil and are related to high mortality rates. Therefore, the aim of this work was to characterize 79 bloodstream isolates of *C. albicans*, *C. glabrata*, *C. orthopsilosis*, *C. parapsilosis* and *C. tropicalis* from patients of a Brazilian hospital. **Methods:** The Minimal Inhibitory Concentration (MIC) of amphotericin B, fluconazole and voriconazole was determined by broth microdilution CLSI guidelines. The clinical characteristics were analyzed using patients files.**Results:** *C. albicans* was the predominant species (44%), followed by *C. glabrata* (19%), *C. tropicalis* (19%), *C. parapsilosis* (14%) and *C. orthopsilosis* (4%). Amphotericin B mean MIC was 0.85 (±0.31). Since CLSI has no breakpoints for this drug, the results were not classified in resistant or susceptible. All *C. glabrata* isolates were susceptible dose-dependent to fluconazole. One *C. albicans* isolate was susceptible dose-dependent to voriconazole. One *C. albicans* isolate showed high MIC values against fluconazole (512 μg/mL) and voriconazole (128 μg/mL). The incidence was 1.52 per 1,000 admissions and the mortality rate was 52%. *C. glabrata* (73%) showed the highest mortality rate, followed by *C. tropicalis* (53%), *C. albicans* (49%), *C. parapsilosis* (36%) and *C. orthopsilosis* (33%). The majority of the patients was male (57%) and the mean age was 42 (±27), however, the mean age of *C. orthopsilosis* patients was significantly lower (0.58±0.32). Patients were hospitalized on medical units (42%), Intensive Care Units (ICU) (34%) and surgery units (24%). Though, all *C. orthopsilosis* patients belonged to ICU and most of *C. glabrata* (47%) and *C. parapsilosis* (45%) were hospitalized in surgery units. The main risk factor was antibiotic therapy (86%), followed by urinary catheter (68%), central venous access (60%), surgeries (54%), parenteral nutrition (42%) and neutropenia (14%). Solid cancer was the main underlying disease (28%), followed by diabetes (23%), gastrointestinal diseases (20%) and liver diseases (15%).**Conclusions:** These results are useful to bring a better comprehension of candidemia in the studied hospital.

Author Disclosure Block:

H.M.S. Canela: None. **M.E.S. Ferreira:** None. **B. Cardoso:** None. **R. Martinez:** None. **L.H. Vitali:** None.

Poster Board Number:

FRIDAY-306

Publishing Title:

Prevalence,Clinical Relevance, and Azole Resistance of Cryptic *Aspergillus* Section *Fumigati* Isolates: A 2000-2013 Survey in a Montreal Hospital

Author Block:

M. Parent-Michaud[1], L. de Repentigny[1], S. F. Dufresne[2]; [1]Univ. of Montreal, Montreal, QC, Canada, [2]Maisonneuve-Rosemont Hosp., Montreal, QC, Canada

Abstract Body:

Cryptic species of *Aspergillus* section *Fumigati* are an emerging cause of invasive aspergillosis, and are of growing concern because many isolates display intrinsic *in vitro* resistance to triazole antifungals, associated with treatment failure. However, the prevalence of these cryptic species can vary widely across institutions, due to varying methods in detection/identification, different patient populations, and differing strategies in antifungal prophylaxis. We therefore conducted a retrospective survey of 1183 stored clinical isolates of *Aspergillus fumigatus*, collected from 2000 to 2013 at Maisonneuve-Rosemont Hospital. The isolates were cultured on Sabouraud-dextrose agar, and Czapek-Dox agar, to ascertain their macroscopic and microscopic morphology, and thermotolerance was determined at 49^{o}C. Twenty-one atypical isolates (delayed sporulation and/or no growth at 49^{o}C) were further identified by sequencing of the β-tubulin gene, yielding *A. fumigatus* (N=9; 9 patients), *Aspergillus turcosus* (N=4; 4 Pts.), *Neosartorya pseudofischeri* (N=7; 1 Pt.), and *Neosartorya hiratsukae* (N=1; 1 Pt.). Antifungal susceptibility to itraconazole, voriconazole and posaconazole was determined using the CLSI M38-A2 broth microdilution procedure. *Aspergillus* isolates were considered resistant if they showed an MIC above the epidemiological cutoff values (ECVs) for *A. fumigatus*: >1 µg/ml for itraconazole and voriconazole, and >0.25 µg/ml for posaconazole. All seven *N. pseudofischeri* isolates were resistant to voriconazole (MIC=4 µg/ml), while the two *A. turcosus* isolates also showed reduced susceptibility (MIC=2 µg/ml). *Cyp51A* gene sequencing of these isolates is underway. Review of medical records revealed that all four *A. turcosus* isolates were associated with airway colonization, that *N. hiratsukae* caused chronic cavitary aspergillosis, and that *N. pseudofischeri* was the cause of disseminated pulmonary, nasal sinus and cerebral aspergillosis in an allogeneic hematopoietic stem cell transplant recipient. These findings confirm the overall low prevalence (1%) of cryptic species of *Aspergillus* section *Fumigati* among clinical isolates, but also emphasize the need for active surveillance of these emerging resistant species.

Author Disclosure Block:

M. Parent-Michaud: None. **L. de Repentigny:** None. **S.F. Dufresne:** None.

Poster Board Number:

FRIDAY-307

Publishing Title:

A Multi-Site Evaluation of Isavuconazole on the Sensititre YeastOne Test Plate with the Frozen Reference CLSI M27-A3 and ISO 16256 Micro Broth Dilution Methods for Antifungal Susceptibility Testing A Multi-Site Evaluation of Isavuconazole on the Sensititre YeastOne Test Plate with the Frozen Reference CLSI M27 A3 and ISO 16256 Micro Broth Dilution Methods for Antifungal Susceptibility Testing

Author Block:

N. M. Holliday[1], C. C. Knapp[1], S. B. Killian[1], D. N. Zollos[1], E. Scopes[2], M. A. Ghannoum[3], R. N. Jones[4], J. M. Streit[4], M. Castanheira[4], R. R. Dietrich[4], S. A. Messer[4], K. Lagrou[5], S. Patteet[5]; [1]Thermo Fisher Scientific, Oakwood Village, OH, [2]Thermo Fisher Scientific, Basingstoke, United Kingdom, [3]Case Western Reserve Univ., Cleveland, OH, [4]JMI Lab., North Liberty, IA, [5]UZ Leuven, Leuven, Belgium

Abstract Body:

Background: Isavuconazole (Astellas Pharma US, Inc., Northbrook, IL) is a triazole antifungal that is currently approved for use in the treatment of invasive mucormycosis and aspergillosis. A multi-site evaluation was undertaken to determine the performance of the Thermo Scientific™ Sensititre® YeastOne® susceptibility system (Thermo Fisher Scientific, Cleveland, OH) with Isavuconazole (ISA) compared to the CLSI M27 and ISO16256 (2012)(E) Broth Microdilution Methods (BMD).**Materials and Methods:** 400 clinical (100 per site) and 100 challenge strains along with 10 reproducibility isolates of *Candida* spp. were tested at 4 sites comparing the performance of ISA on the Sensititre YeastOne plate with CLSI and ISO BMD. The range tested for ISA was 0.004-8ug/ml. CLSI quality control (QC) organisms were tested daily and were within the CLSI expected QC ranges. **Results:** Clinical isolate comparisons of the Sensititre YeastOne plate to the CLSI and ISO BMD resulted in 99% and 92% essential agreement (EA) +/- 2 $\log_2$ dilutions. Challenge isolates resulted in 99% and 89% EA +/- 2 $\log_2$ dilutions. YeastOne modal reproducibility MICs +/- 2 fold dilutions between sites for ISA resulted in 98.6% agreement at 24 hours. **Conclusions:** This evaluation indicates that the Sensititre YeastOne plate with ISA is equivalent to the CLSI and ISO BMD and is a potential method for susceptibility testing of ISA.

Author Disclosure Block:

N.M. Holliday: None. **C.C. Knapp:** None. **S.B. Killian:** None. **D.N. Zollos:** None. **E. Scopes:** None. **M.A. Ghannoum:** None. **R.N. Jones:** None. **J.M. Streit:** None. **M. Castanheira:** None. **R.R. Dietrich:** None. **S.A. Messer:** None. **K. Lagrou:** None. **S. Patteet:** None.

Poster Board Number:

FRIDAY-308

Publishing Title:

Evaluation of the Sensititre Yo-9 Plate for Antifungal Drug Susceptibility Testing of Molds

Author Block:

R. C. WALCHAK, A. Abbenyi, A. A. Milone, N. L. Wengenack; Mayo Clinic Rochester, Rochester, MN

Abstract Body:

Background: The performance of the commercially available Sensititre YeastOne YO-9 plate (ThermoFisher/Trek Diagnostics) was compared with the broth dilution reference method (CLSI M38-A) performed at a reference laboratory in order to evaluate the potential utility of the YO-9 plate for mold antifungal susceptibility testing.**Methods:** Well-characterized clinical isolates of molds including *Aspergillus* spp. and *Fusarium* spp. were tested in parallel using both the reference method and the YO-9 plate against 9 antifungal drugs including several azoles, amphotericin B, and three echinocandins. The MIC or MEC for each drug were determined by following the guidelines in the CLSI M38-A document.**Results:** The agreement between the reference laboratory and the YO-9 plate was 98.9% using an essential agreement acceptance range of +/- 2 doubling dilutions. There was a single discordant fluconazole result for a *Fusarium* sp. isolate.**Conclusions:** The Sensititre YeastOne plate compared favorably with the reference method in terms of essential agreement for each drug tested across a variety of mold genera and species. Using the Sensititre YeastOne plates also provides a simplified workflow compared with the reference method for implementation into a clinical laboratory.

Author Disclosure Block:

R.C. Walchak: None. **A. Abbenyi:** None. **A.A. Milone:** None. **N.L. Wengenack:** None.

Poster Board Number:

FRIDAY-309

Publishing Title:

Comparison of Copan THIOL Broth to F.A.B and THIO W/O Indicator Broths for the Enrichment of Aerobic, Anaerobic Bacteria and Fungi

Author Block:

R. Paroni, L. Conter, S. Castriciano; Copan Italia, Brescia, Italy

Abstract Body:

Background: Different enriched broths, currently available on the market, are used for the cultivation of facultative and obligatory anaerobic microorganisms present in clinical specimens. Copan developed the THIOL, an enrichment broth that contains Vitamin K and Hemin as growth supplement for many fastidious anaerobic bacteria. THIOL, (T1) a ready to use LBM device, contains 4 ml broth in a PET tube with a cap with a plastic sealant to maintain anaerobic conditions during incubation. The objective of this study was to compare the Copan THIOL broth to LABM F.A.B (T2) and THIO W/O indicator (T3) broths for the enrichment of anaerobes and aerobes and fungi. **Methods:** In this comparison study 6 anaerobic ATCC strains (*B. Fragilis*, *B. Vulgatus, P. ana*, *P. mel*, *C. perfrigens*, and *C.difficile*) and 9 aerobic including 2 fungi, (*E.faecalis* 12022, *P.auroginosa* 27853 and 27853, *Salmonella* 14028, *Shigella sonnei* 9290, *C.albicans* 10211 and 24433, S.*aureus* 6538, 29213 and 25923, *E.coli* 25922) were used to inoculate the T1, T2 and T3 for enrichment. A 0.5McFarland dilution, made with fresh culture of each strain, was used to prepared countable dilutions of 25-250ufc /100 ul for the anaerobes and $10^{4,}$ 10^{5} dilutions for the aerobes and used for testing. Ten tubes were inoculated with 400ul of each strain; one for T0, 2 for T24, and T48h, vortexed and incubated at 35°C. Aliquots of 100ul from T0 tubes was plated on Blood agar plates and incubated at 35°C for 24/48H. Same procedure was followed for 24 and 48h incubations. Culture and turbidity results were recoded.**Results:** The T0 results for the 3 broths were compared toT24 and T48 incubations. The anaerobic strains growth was present only at T0,in T2 and T3 broths and no growth or turbidity after 24 and 48h, while for the T1 the growth and turbidity incremented from countable to TNTC. In the aerobic strains only a few colonies were present at T0 for both dilutions used for all 3 media and an exponential growth of TNTC and turbidity was present at both 24 and 48h.**Conclusions:** The results demonstrated that the Copan THIOL supports both anaerobic and aerobic bacteria growth confirmed by the turbidity compared to the no growth with the F.A.B. and THIO W/O indicator broths. Copan THIOL can be used for anaerobic and aerobic bacteria and fungi growth with manual and WASP™ and WASPLab™ automation broth inoculation and culture streaking.

Author Disclosure Block:

R. Paroni: None. **L. Conter:** None. **S. Castriciano:** None.

Poster Board Number:

FRIDAY-310

Publishing Title:

A System-Wide Initiative to Increase Blood Culture Volumes Correlates with Increased Positivity Rates

Author Block:

R. Khare, S. Juretschko; Northwell Hlth.Lab., Lake Success, NY

Abstract Body:

Background: Collection of adequate blood volume is highly associated with an increased yield of pathogens from blood cultures. Blood culture volumes from >260,000 bottles from 9 hospital sites were analyzed before and after implementation of a large-scale improvement initiative. **Methods:** Blood culture volumes and culture results were evaluated from aerobic bottles for a period of 12 months using the Becton Dickinson automatic blood volume monitoring system (BVMS), software version 6.60. **Results:** From January to June 2015, the average blood culture collection volume per bottle was 3.13 ml with an overall positivity rate of 3.7%. A system-wide initiative to increase blood culture collection volumes was implemented in July 2015. After a period of 6 months, 9 out of 9 hospitals achieved statistically significant increases in collection volume (range: 14-94%). The average volume increased to 4.36 ml, and the average positivity rate increased to 4.4%. In the post-implementation phase the rates of detection for potential contaminants and Gram negative bacilli increased, while detection rates of *S. aureus* and *Enterococcus* sp. remained constant. **Conclusions:** All hospital sites in this study showed small but significant increases in blood culture fill volumes. This correlated with an overall increase in blood culture positivity rate, although an increase in number of contaminants also occurred. Further education, monitoring and feedback needs to be performed to correctly collect an adequate volume of blood.

Author Disclosure Block:

R. Khare: None. **S. Juretschko:** None.

Poster Board Number:

FRIDAY-311

Publishing Title:

Evaluation Of The BD MAX™ Staphsr Assay For Detecting MSRA And MSSA in ESwab™ Collected Wound Samples

Author Block:

A. Gostnell, **S. Silbert**, C. Kubasek, R. Widen; Tampa Gen. Hosp., Tampa, FL

Abstract Body:

The BD MAX™ StaphSR assay performed on the BD MAX™ System is a FDA-cleared molecular test for detection of *S. aureus* (SA) DNA and MRSA DNA from nasal swab specimens collected from patients at risk of nasal colonization. This PCR based test can provide results in approximately 2.5 hours. The objective of this study was to evaluate the BD MAX StaphSR assay for the detection of SA and MRSA from ESwab (Copan Diagnostics Inc., USA) collected wound samples, and to compare the results to culture, our standard of care procedure. **Methods:** A total of 250 wound samples collected by ESwab were included in this study. Samples were submitted for traditional wound culture and after processed, residual ESwab samples were tested by PCR on the BD MAX System. Briefly, an aliquot of 200µL of the residual ESwab sample was transferred to the BD MAX Sample Buffer Tube. Extraction and multiplex PCR were performed on the BD MAX System, using the BD MAX StaphSR assay. Discrepant result samples were retested by an in-house *mecA*/*femA* real-time PCR assay and by the Xpert® MRSA/SA SSTI assay on the GeneXpert® System (Cepheid, Sunnyvale, CA). In addition, an enrichment culture was processed by inoculating 100µL of the remaining ESwab sample into TSB with 6.5% NaCl (BD Diagnostics). After incubation at 37°C for 24h, TSB was plated on CHROMagar *S. aureus* and CHROMagar MRSA II (BD Diagnostics) for another 24h at 35-37°C. Results from traditional and enrichment culture were combined and compared to PCR results. **Results:** Out of 250 samples tested, 194 were negative and 54 were positive for MRSA, and 159 were negative and 83 were positive for *S. aureus* by both PCR and culture methods. Nine samples had discrepant results between culture and PCR. Out of them, 5 were false positive for SA (Ct values ≥ 38) and 1 was false negative for MRSA by the BD MAX StaphSR PCR. **Conclusion:** The BD MAX StaphSR assay and culture displayed an excellent overall agreement for the detection of MRSA (99.2%) and SA (96.8%) from ESwab collected wound samples. Moreover, the BD MAX StaphSR assay has the ability to yield faster results than culture and can potentially facilitate earlier appropriate treatment for wound infections.

Author Disclosure Block:

A. Gostnell: None. **S. Silbert:** None. **C. Kubasek:** None. **R. Widen:** None.

Poster Board Number:

FRIDAY-312

Publishing Title:

Prevalence and Impact of Blood Volume on the Recovery of True Pathogens and Contaminants from Pediatric Blood Cultures

Author Block:

J. Dien Bard, J. Mestas, J. Lee, P. Pannaraj; Children's Hosp. Los Angeles, Los Angeles, CA

Abstract Body:

Background: Blood volume remains the most critical variable that directly influences blood culture (BC) yield. Insufficient volume is an ongoing issue that is particularly prevalent in the pediatric setting. There are currently limited data on the optimal collection volume in children as most studies are devoted to the adult population. We sought to assess the prevalence and impact of adequate blood volume on blood culture positivity in a large pediatric hospital.**Methods:** A total of 4073 BacT/Alert Pediatric FAN BC bottles collected from pediatric patients were analyzed. Bottles were initially weighed in the microbiology lab prior to distribution and reweighed upon arrival back in the lab. The final BC weight was determined by subtracting the initial from final bottle weight plus 0.16 to account for bottle volume depletion. Blood volume > 1mL and < 5mL were defined as “adequate” whereas volume outside the range were considered “inadequate.” Blood volumes were compared between positive and negative culture results.**Results:** 406 (10%) blood cultures were positive; 313 (77%) Gram-positive, 73 (18%) Gram-negative, and 20 (4.9%) yeast. Probable contaminant organisms were recovered from 52% (215/406) of all positives. The mean blood volume was 2.2 mL and ranged from 0.2 to 12.0 mL. In total, 83.3% of BC were adequate in blood volume and there were no significant difference between adequate blood volume and BC positivity (83.5% neg vs 81.3% pos , p=0.261). Overall, positive BC had a lower mean blood volume compared to negative BC (2.02 mL vs 2.24 mL, p=0.005) and the proportion of positive BC decreased with each mL of blood obtained. Further removal of contaminants from the analysis resulted in no significant difference (2.3 mL vs 2.2 mL, p=0.465). BC growing true pathogens had a mean blood volume of 3.4 mL compared to contaminants at 2.2 mL (p=0.0008).**Conclusions:** In this study, the yield of positive BC in pediatric BC bottles inversely correlated with blood volume obtained. Although the mean difference was < 1mL, the findings were statistically significant. Removal of contaminants from the analysis resulted in no significant correlation between positive BC and blood volume. Low blood volume directly increases recovery rate of BC contaminants.

Author Disclosure Block:

J. Dien Bard: None. **J. Mestas:** None. **J. Lee:** None. **P. Pannaraj:** None.

Poster Board Number:

FRIDAY-313

Publishing Title:

Current Pattern of Antibiotic Resistance in Clinical Isolates of *Acinetobacter baumannii* from Intensive Care Units of Tertiary Care Hospital

Author Block:

I. Bashir[1], I. Khan[2]; [1]Univ. of Karachi, Karachi, Pakistan, [2]SUNY Albany, Albany, NY

Abstract Body:

Purpose: To determine the pattern of antibiotic resistance in clinical isolates of *Acinetobacter baumannii* from ICU's of tertiary care hospital in Karachi.**Study Design:** A case control study.**Methods:** Three hundred and fifteen clinical isolates of *Acinetobacter baumannii* collected from different ICUs were evaluated during 1 year period. The isolates were identified by morphology, growth and biochemical characteristics, susceptibility to a panel of antimicrobial agents in disc diffusion assay and molecular characterization by PCR using *gltA* and *gyrB genes*.**Results:** 94% of *Acinetobacter* spp were detected as multi drug resistant (MDR) and sensitive to Polymixin B only. About 6% *Acinetobacter* spp were also MDR but sensitive to Polymixin B, Meropenem and Salbactum + Cefoperazone. All tested isolates exhibited differing resistance representation, as establish by medium incorporation-replica method, against different tested antibiotics, as follows: Amoxicillin-Calvulanic acid, Tazobactam, Ceftriaxone, Ceftazidime, Meropenem, Imipenem, Gentamicin, Amikacin, Chloramphenicol, Co-trimoxazole, Tobramycin, Salbactam, Cefoperazone, Gentamicin and Amikacin. All the isolates of *Acinetobacter baumannii* were PCR positive for *glt* A and gyrB.**Conclusions:** *Acinetobacter baumannii* is the most frequently isolated and alarming pathogen in the health care system specifically for the patients in intensive care units (ICUs). Its survival in hospital environment is because of tolerance the antibiotics and antiseptic pressures. Multi drug resistance made this pathogen the lethal pathogen of this century to infect debilitated patients. There is a strict need to monitor the surveillance of global clones at institutional and or intra-institutional level for accurate treatment, precise prevention and batter control.**Keywords:** Multidrug resistance (MDR), housekeeping genes

Author Disclosure Block:

I. Bashir: None. **I. Khan:** None.

Poster Board Number:

FRIDAY-314

Publishing Title:

A New GN Broth for Selective Enrichment of Gram Negative Bacteria Especially *Salmonella* and *Shigella*

Author Block:

L. Conter, R. Botrugno, B. Massetti, S. Castriciano; Copan Italia, Brescia, Italy

Abstract Body:

Background: Selective enrichment broths are essential to enhance the isolation of some Gram Negative bacilli (G-) like *Salmonella* and *Shigella* (S&S), isolated from stool specimens, highly contaminated with enteric flora (EF) limiting the growth of other coliform G-. Copan is producing the GN broth (Hajna's formula) with an increased amount of mannitol to accelerate the growth of *S&S* and contains sodium citrate and deoxycholate to inhibit gram positive growth in the first 8h. GN broth, a Copan LBM device, is available in 12x80mm plastic tubes with 4ml broth, and can be used for manual and WASP™ automation broth inoculation and agar plating. The objective of this study was to validate the performance of the GN broth for the selective enrichment of SA and SH using clinical isolated strains **Methods:** In this validation study were used 171 clinical isolated strains of G- and G+ bacteria, *Salmonella* (29) and *Shigella (*23) strains were used. GN selectivity at 6-8h was tested with 11 *Proteus,* 7 *Bacteroides,* 16 *Enterobacter and* 19 *E.coli.* G+ strains were used for selective and inhibitory action of GN broth until 24h like 12 *S. aureus, 6 Bifidobacterium, 12 Lactobacillus, 19 E.Faecium and* 17 *E.faecalis.*From a fresh culture for each strain, a 0.5McFarland inoculum was prepared and diluted to a countable dilution of 25-250ufc/100ul. Aliquots of 100ul of each strain were inoculated in GN broth. All tubes were vortexed and 100ul were plated in appropriate agar plates. All tubes were incubated at 35°C and after 6-8 h the broths were checked for turbidity and 100ul were manually plated on appropriate agar plates and WASP™ automation plated with a 10ul loop. All GN broth were re-incubated at 35°C and at 24h incubation were plated manually and on the WASP™ as above and plates incubated at 35°C for 24h.**Results:** In the results obtained using the 52 isolates S&S after both 6-8h and 24h incubation turbidity was present in all GN broth tubes. The selectivity of the GN broth was confirmed by the inhibition of the 53 isolates of enteric coliform flora after 6-8h and 66 G+ bacteria after 24h incubation.**Conclusions:** The results obtained demonstrated that Copan GN broth enriches and supports the growth of *Salmonella* and *Shigella*; inhibits the growth up to 6-8h of EF and inhibits G+ growth up to 24h. GN broth can be used for the selective enrichment of stool specimens for the detection of S&S with manual and WASP™ automation.

Author Disclosure Block:

L. Conter: None. **R. Botrugno:** None. **B. Massetti:** None. **S. Castriciano:** None.

Poster Board Number:

FRIDAY-315

Publishing Title:

A New Amies Medium +20% Glycerol Device for the Collection and Long Term Frozen Storage of Clinical Specimens for Bacteria Culture

Author Block:

R. Botrugno, L. Conter, B. Massetti, R. Paroni, S. Castriciano; Copan Italia, Brescia, Italy

Abstract Body:

Background: Research studies to monitor bacteria changes during infection due to vaccination and antibiotics require to freeze original specimens to culture bacteria at a later date. After requests from microbiologists, Copan developed a new LBM device, the Amies Liquid Medium+20%glycerol (ALMG), a tube with 1.0 ml medium and a regular FLOQSwab™, for the collection and frozen storage of clinical specimens for bacteria culture. The objective of this study was to validate the performance of the new Amies Liquid Medium+20%glycerol, for the isolation of bacteria after long term frozen storage at -20°C and -80°C using a panel of ATCC bacteria strains. **Method:** In this study these ATCC bacteria strains were used: *P.aeruginosa* (BAA-427) *S.pyogenes* (19615), *S. pneumoniae* (6305), *H. influenzae* (10211), *B. fragilis* (25285), *P. anaerobius* (27337), *P. acnes* (6919), *N. gonorrhoeae* (43069) MRSA (43300), *E.coli* (25922), *S.aureus* (6538). Starting from a 0.5McF dilution of fresh cultures for each strain, two dilutions were prepared, one with countable CFUs (200-500/100ul) and another with uncountable CFUs. Tubes of ALMG were inoculated with 100ul of each dilution per each strains and the swab was broken inside the tube. Two sets of 12 tubes for each bacteria and dilutions were prepared, one tube was used at zero time as baseline, and the other sets were stored at -80°C and -20°C; stability was tested monthly up to 1 year. At each storage time, 10ul of each tube inoculated with different bacteria was manually plated using a micropipette and spreaders and WASP automated plated using a 10ul loop on the appropriate agar plates. All plates were incubated for 24/48h at 35^0C at the appropriate conditions. CFUs were counted for each plate and results recorded for all culture plates. The results were expressed as percentage of recovery. **Results:** No loss of bacteria viability was detected with the new ALMG devices inoculated with all the bacteria strains when comparing the zero time CFUs counts to the CFUs counts obtained after one to six and nine months storage at -80°C and -20°C. **Conclusions:** The data obtained suggests that the new Copan ALMG device, supports the viability of a wide range of aerobic, anaerobic and fastidious bacteria and can be used for the collection, transport and long term frozen storage of specimens for clinical and research studies.

Author Disclosure Block:

R. Botrugno: None. **L. Conter:** None. **B. Massetti:** None. **R. Paroni:** None. **S. Castriciano:** None.

Poster Board Number:

FRIDAY-316

Publishing Title:

Validation of COPAN LIM Broth for the Detection of *Streptococcus* Type B

Author Block:

S. Castriciano, L. Conter, R. Paroni; Copan Italia, Brescia, Italy

Abstract Body:

Background: The CDC guidelines for screening for type B streptococcal colonization recommend the collection of vaginal/rectal swab in pregnant women at 35 to 37 weeks of gestation. After collection specimens are inoculated in an enrichment broth. Copan is producing the LIM broth, another LBM device, a tube with 2 ml of LIM broth for the enrichment of group B Streptococcus (GBS).The objectives of this study were to: 1) Compare the performance of the Copan LIM Broth to the BD for pre-enrichment of GBS. 2) Validate the LIM broth ability to support *S.agalactiae* growth during shelf life.**Methods:** In this validation the ATCC 12386 *of S.agalactiae* was used to compare the performance of the Copan LIM (Todd Hewitt+ CNA) broth to the BD Todd Hewitt (BDTH) broth. 3 lots of LIM at 6 and 1 month prior and 1 after expiry date were also tested for growth of SA. Starting from a fresh culture of *SA a* 0.5McF dilution (1.5 x 10^8 UFC/ml) was prepared in PBS. Serial dilutions were prepared up to a concentration of 10^2 UFC/ml. Five sets tubes of LIM and BDTH and the 3 different LIM lots of broths were inoculated with each SA dilution using a 1:10 inoculum ratio in order to obtain a final concentration of 10^1 UFC/ml for 0, 2, 4, 6, 8, 10, 12, 14, 16, 18 and 24 hours incubations at 35^0C. At zero time 100 ul of the T0 set were inoculated in triplicate on blood agar plates (BioMèrieux) as a baseline and repeated after each incubation time only 10 ul were inoculated as above. All plates were incubated at 35^0C for 24 hours, colonies were counted and recorded on an excel file.**Results:** The LIM broth had a log more growth of *S.agalactiae* than the BD Todd-Hewitt broth starting from 2 hours incubation time. No *S.agalactiae* difference was noted in the LIM at 6 and 1 month prior and 1 after expiry date. The LIM broth tubes are compatible with WASP™ automation and are safe to handle compared to the large glass tubes of the BD Todd-Hewitt broth , that are not suitable for automation and prone to bread if accidental dropped.**Conclusions:** Better *S.agalactiae* growth was detected with the Copan LIM broth compared to the BD Todd-Hewitt broth. LIM support S.*agalactiae* growth even after one month expiry date. The Copan LIM broth is suitable for the collection of clinical specimens for type B streptococcal colonization screening and compatible with both manual and WASP™ automation inoculation methods and is safe to handle.

Author Disclosure Block:

S. Castriciano: None. **L. Conter:** None. **R. Paroni:** None.

Poster Board Number:

FRIDAY-317

Publishing Title:

Comparison of Recovery Rates of *Clostridium difficile* from Stool Using Chromogenic Agar versus a Classic Culture Method

Author Block:

S. Shannon, D. Gustafson, R. Segner, A. Ali, B. Schieffer, K. Boelman, J. Mandrekar, R. Patel; Mayo Clinic, Rochester, MN

Abstract Body:

Introduction: The goal of this study was to compare performance characteristics of a conventional medium, taurocholate, cycloserine, cefoxitin, fructose agar (TCCFA) with CHROMagar *C.difficile* (ChromAGAR Paris , France) for recovery of *C. difficile* from stool. **Methods:** 50 stool specimens in Cary Blair that were known to be *C. difficile* PCR positive were subcultured in parallel to CHROMagar and TCCFA agars. Specimens were plated directly to both selective agars without processing prior to inoculation, and incubated in anaerobe jars with anaerobiosis attained using an AnaeroPack anaerobic gas generating system (Mitsubishi Gas Chemical America, Inc. New York, NY). The CHROMagar medium was examined at 24 hours in accordance with the manufacturer's recommendation, and tested within 6 hours of examination. Identification was performed by MALDI-TOF mass spectrometry directly from the CHROMagar plate. TCCFA medium was examined at 48 (±6) hours, in accordance with standard protocols. Identification was performed by MALDI-TOF mass spectrometry directly from selective plates, except in circumstances where subculture was required for isolation of colonies resembling *C. difficile*. Sensitivities of the two tests were compared using McNemar's test and detection duration was compared using a log rank test.**Results:** CHROMagar media provided a 94% recovery rate of *C. difficile* on the first day of examination. Two specimens were positive on the first day, but were subcultured and identified on the second day. Three specimens did not yield *C. difficile* on CHROMagar media; *C. difficile* was recovered from one of the three specimens on TCCFA. Organisms other than *C. difficile* were not recovered on CHROMagar. TCCFA agar provided a 68% recovery rate of *C. difficile*, requiring up to 4 days of incubation and occasional subculture to yield final results. Non-*C. difficile* bacteria were isolated on TCCFA.**Conclusion:** For culture-based screening of stool for *C. difficile*, the CHROMagar medium was more sensitive than the TCCFA medium ($p=0.0008$), with faster detection ($p<0.0001$).

Author Disclosure Block:

S. Shannon: None. **D. Gustafson:** None. **R. Segner:** None. **A. Ali:** None. **B. Schieffer:** None. **K. Boelman:** None. **J. Mandrekar:** None. **R. Patel:** E. Grant Investigator; Self; nanoMR, BioFire, Check-Points, Curetis, 3M, Merck, Actavis, Hutchison Biofilm Medical Solutions,

Accelerate Diagnostics, Allergan, The Medicines Company. N. Other; Self; Dr. Patel has a patent Bordetella pertussis/parapertussis PCR with royalties paid to TIB, a patent Device/method for sonication with royalties paid to Samsung, and a patent anti-biofilm substance issu, non-financial support from bioMérieux, Bruker, Abbott, Nanosphere, Siemens, BD, other from Curetis.

Poster Board Number:

FRIDAY-318

Publishing Title:

***Propionibacterium acnes* Detection Rates are Lower from Swab Specimens and Higher with Longer Incubation Duration**

Author Block:

A. O. Miller, J. DiPietro, J. P. Kleimeyer, O. A. Shonuga, M. W. Henry, F. Zodda, M. Klein, R. F. Warren, B. D. Brause; Hosp. for Special Surgery, New York, NY

Abstract Body:

Background: *Propionibacterium acnes* is increasingly recognized as a troublesome pathogen, and a common cause of indolent shoulder and spine hardware infections - as well as a problem in neurosurgical and cardiac surgical infections. Because the microbe typically requires at least 5 days to grow, and grows best in enriched anaerobic media, *P. acnes* infections are likely underdiagnosed. Proper collection methods and incubation times are not clear. We sought to identify optimal practices to detect *P. acnes.* **Methods:** Over a single two-month period, 1,039 consecutive anaerobic thioglycollate broth cultures from orthopedic surgery patients were observed for an extended duration (39 cultures for 28 days, and 1000 for 42 d). Samples were anonymized so that cultures positive after 10 d were not linkable to patients. Data on anatomic site and specimen type (SP Brand CultureSwab swab, solid tissue, or synovial fluid) were collected. Positive cultures were identified by turbidity, Gram stain, and biochemical identification.**Results:** In total, 34/1039 cultures grew *P. acnes*; 8/34 (23%) took more than 10 days to grow. Only 3 CultureSwab specimens yielded positive cultures, and these specimens grew after 15-21 days. All positive non-swab cultures (solid tissue and fluid cultures) grew in 14 days or less (n=31, range 3-14 days). No cultures (0/1039) grew *P. acnes* after 21 days. When multiple positive broth cultures with identical date, site, and type were grouped together and analyzed as if from a single patient, 6/18 (33%) of such patients had *P. acnes* isolates that took over 10 days to grow.**Conclusions:** Swab specimens lead to slower growth of *P. acnes* than direct tissue and fluid samples. Swab specimens are also probably less sensitive for *P. acnes* in a clinically significant manner, regardless of incubation time. A significant percentage of *P. acnes* infections may be missed when isolates are incubated for less than 10 d. In summary, swab cultures should be avoided, and anaerobic broth cultures should be incubated for 14 d, in cases where *P. acnes* is a pathogen of interest, such as surgical hardware infections.

Author Disclosure Block:

A.O. Miller: None. **J. DiPietro:** None. **J.P. Kleimeyer:** None. **O.A. Shonuga:** None. **M.W. Henry:** None. **F. Zodda:** None. **M. Klein:** None. **R.F. Warren:** None. **B.D. Brause:** None.

Poster Board Number:

FRIDAY-319

Publishing Title:

The Importance of Gram Stained Smears in the Diagnosis of *Nocardia* Infections

Author Block:

S. Olmez[1], O. Tuncer[1], B. Altun[1], B. Sancak[1], N. Gursoy[2], B. Otlu[3], **B. Sener**[1]; [1]Hacettepe Univ., Ankara, Turkey, [2]Inonu Univ., Malatya, Turkey, [3]Malatya Univ., Malatya, Turkey

Abstract Body:

Background: Nocardiosis can be a cause of disease in patients with underlying risk factors such as immunosuppression, chronic lung disease and malignancies. The overall correlation between Gram staining of the clinical specimens and subsequent organism identified is usually fair to moderate. This study was aimed to emphasize the utility of the microscopic examination of Gram stained smears for the diagnosis of *Nocardia* infections.**Methods:** The study included cases whose specimens yielded isolation of *Nocardia* spp. between November 2014-December 2015 in a university hospital in Turkey. Following the examination of Gram stained smears, the incubation period of the cultures which yielded morphology consistent with *Nocardia* spp., were prolonged. The relevant colonies that grew in ≥72 h of incubation, were identified by standard microbiological methods. Species level identification of the *Nocardia* isolates was done by 16S rRNA sequence analysis and antimicrobial susceptibility testing by gradient strip method.**Results:** A total of 19 *Nocardia* spp. strains were isolated from 8 patients. Three cases exhibited repeated growth of *Nocardia* spp. up to a period of nine months. All of the *Nocardia* positive specimens revealed growth following prolonged incubation. The most frequently isolated species was *N.cyriacigeorgica,* identified in three cases. The other species identified were *N.asteroides, N.transvalensis/wallace, N.farcinicia, N.asciatica/arthritidis.* All of the isolates were found to be susceptible to amikacin, ceftriaxone, imipenem, linezolid and trimetoprim-sulphametoxazole. All *N.cyriacigeorgica* were resistant to ciprofloxacin and except one also to clarithromycin.**Conclusion:** Proactive suspicion of nocardiosis based on Gram stain results may aid to isolation of *Nocardia* spp. from routine cultures. Thus longer incubation with a preceeding Gram stained smear should be encouraged in clinical practice for the early diagnosis of nocardiosis. *N.cyriacigeorgica* is the most common and most antibiotic resistant species in the study population. Increasing information about the *Nocardia* species specific antimicrobial susceptibility profiles is necessary for the establishment of successful empirical treatment protocols.

Author Disclosure Block:

S. Olmez: None. **O. Tuncer:** None. **B. Altun:** None. **B. Sancak:** None. **N. Gursoy:** None. **B. Otlu:** None. **B. Sener:** None.

Poster Board Number:

FRIDAY-320

Publishing Title:

Optoelectronic Detection of Bacterial Growth in Different Media Types

Author Block:

U. Aurbach[1], E. Michael[2], S. Lim[2], P. Rhodes[2], **H. Wisplinghoff**[3]; [1]Wisplinghoff Lab., Cologne, Germany, [2]Specific Technologies, Mountain View, CA, [3]Inst. for Med. Microbiol., Univ. of Cologne, Cologne, Germany

Abstract Body:

Background: Optoelectronic detection and identification of microorganisms based on an inexpensive, printed, disposable colorimetric sensor array (CSA) responsive to the volatiles emitted into the headspace of culture bottles during growth has been shown to have the potential to shorten time to result in blood cultures. Similarly, using sensors placed in the lids of blood agar plates, it has been possible to identify presence and species during growth on blood agar plates.**Methods:** This study was conducted to evaluate the reportedly faster time to detection exhibited by this paradigm to improve efficiency in routine clinical microbiology. A total of 20 clinically relevant bacteria including E. coli and other Enterobacteriaceae, P. aeruginosa and other non-fermenting gram-negatives, Enterococcus spp., Staphylococcus spp., and Streptococcus spp. were incubated at concentrations ranging from 10^2 to 10^6 on different selective and non-selective solid media. All samples were run in triplicate.**Results:** All bacteria could be identified on all media (if the bacterium was able to grow on the respective medium). Overall, time to positivity ranged from 4.2 to 11 hours, varying between different bacteria/media combinations. Sensitivity of species identification (monomicrobial cultures) at 1, 2 and 3 hours after initial detection of growth was as high as 99.4%. Accuracy varied between different bacteria/media combinations and could be improved by adaption of algorithms to the respective media.**Conclusions:** In our study, CSA-based optoelectronic detection could be adopted to be used largely independent of different types of solid media and seems to be a promising addition to the routine clinical microbiology laboratory with the potential to significantly shorten the time to positivity, time to negativity, and / or species identification.

Author Disclosure Block:

U. Aurbach: None. **E. Michael:** D. Employee; Self; Specific Technologies. **S. Lim:** D. Employee; Self; Specific Technologies. **P. Rhodes:** D. Employee; Self; Specific Technologies. **H. Wisplinghoff:** I. Research Relationship; Self; Beckman Coulter, BioMerieux, Bruker Daltonics, Cepheid, Hologic, iSense, r-biopharm, Siemens, Specific Technologies. L. Speaker's Bureau; Self; Beckman Coulter, Bruker Daltonics.

Poster Board Number:

FRIDAY-321

Publishing Title:

Evaluation Of Oxyrase(R) Agar Media for Growth and Selection of Anaerobic Isolates

Author Block:

L. M. Koeth[1], J. DiFranco-Fisher[1], A. Windau[2]; [1]Lab. Specialists, Inc., Westlake, OH, [2]Microtech Lab., Inc., Westlake, OH

Abstract Body:

Background: Oxyrase® (Oxyrase, Inc., Mansfield, OH) is an enzyme system that removes dissolved oxygen from liquid, gas or semisolid products. Oxyrase® is included in agar media that are typically used to grow and select for anaerobic organisms. Oxyrase allows the plate to remain reduced on the bench up to 2 hours. This study was performed to evaluate the growth of anaerobic bacteria on Oxyrase Brucella and Oxyrase Schaedler agar plates compared to conventional media. Oxyrase selective media was also evaluated for growth of specific anaerobic organisms from mixed isolate cultures. **Methods:** The study isolates were stock strains from clinical sources that included various Gram-positive and Gram-negative aerobes and anaerobes. Within the anaerobe chamber, inocula were prepared and plated onto control media (Remel brucella agar w/5% sheep blood, hemin, vitamin K and Becton Dickinson tryptic soy agar with 5% sheep blood). In addition, inocula were plated onto Oxyrase media (Schaedler OxyPlate, Brucella OxyPRAS, BBE, KVL, AnaSelect and PEA) outside the anaerobe chamber. All plates were incubated under anaerobic conditions for 48 hours. Phase I consisted of single isolate cultures plated onto control media. Phase II consisted of 3 separate mixed isolate cultures (anaerobic and aerobic) plated onto various selective media. **Results:** As shown in the table, bacteria concentration was similar on all tested media. The colony sizes were also comparable. In Phase II, the selective media also showed good growth and selection/inhibition of specified organisms according to manufacturer specifications.

		CFU/mL Range		
Organism	n	Remel Brucella	Brucella OxyPRAS	Schaedler OxyPlate
Bacteroides spp.	4	2 - 5.4 x 10^7	8 x 10^7 - 2.6 x 10^8	1.8 x 10^7- 1.5 x 10^8
Clostridium spp.	3	1 x 10^6 - 9.9 x 10^7	7 x 10^6 - 7.6 x 10^7	1 x 10^6 - 1.5 x 10^7
Eubacterium lentum	1	3.8 x 10^8	4.4 x 10^8	2.1 x 10^8
Fusobacterium nucleatum	1	6.8 x 10^7	4.4 x 10^7	4.8 x 10^7
Peptostreptococcus spp.	2	4 x 10^6- 5 x 10^7	2.6 - 3.6 x 10^7	6 - 8.0 x 10^6
Prevotella spp.	2	1.6 x 10^8 - 3.1 x 10^8	1.4 - 2.1 x 10^8	1.9 x 10^{8*}

Propionibacterium acnes	1	2 x 10^6	1.2 x 10^7	1.4 x 10^7
*One isolate (*Prevotella bivia*) had no colonies on Schaedler OxyPlate after 48 hours incubation				

Conclusions: Count and size of colonies on the Schaedler OxyPlate and the Brucella OxyPRAS plate (inoculated aerobically and incubated anaerobically) were similar to control plates (inoculated and incubated anaerobically). The Oxyrase selective media (BBE, KVL, AnaSelect and PEA) allowed for growth of specified organisms.

Author Disclosure Block:

L.M. Koeth: None. **J. DiFranco-Fisher:** None. **A. Windau:** None.

Poster Board Number:

FRIDAY-322

Publishing Title:

Impact of Urine Gram Stain Order Availability on Utilization and Test Performance

Author Block:

A. BRYAN, N. G. Hoffman, B. T. Cookson; Univ. of Washington, Seattle, WA

Abstract Body:

The utility of urine Gram stain in addition to urine culture is unclear given rapid urinalysis, transport time, and empiric therapy. The laboratory can alter ordering practices by altering availability in computer-provider-order-entry (CPOE). To reduce over-utilization, urine Gram stains were eliminated from the urine culture panel and offered as a separate test in our inpatient and emergency department CPOE. In the year after the order changes, the portion of urine isolates with an associated Gram stain decreased from 50 to 2% for ED patients and inpatients and 38 to 30% ($p < 0.0001$) for outpatients. Isolates were considered more likely clinically significant if antimicrobial susceptibility testing (AST) was performed per protocol; possible urosepsis isolates were designated as those with the same organism isolated from the blood within 7 days. These sub-groups revealed decreases in the fraction of isolates accompanied by Gram stains: 40 to 16% for AST isolates and 65 to 14% for possible urosepsis. Separately ordered Gram stains accounted for a small fraction of the performed stains. Interestingly, Gram stain sensitivity for orders associated with a urine culture panel differed from independent orders. Isolate Gram results were counted as concordant if a urine stain from the same day appropriately identified the phenotype (GNRs, GPCs, GPRs, or yeast); allowing for multiple phenotypes to be observed by stain. Culture results that reported extensively mixed or Gram positive flora were excluded unless a specific isolate was reported. The sensitivity of Gram stains ordered with a culture panel was 51.4% (95% CI: 50.6 - 52.1%), while the sensitivity of separately ordered stains was 63.4% (95% CI: 57.3 - 69.2%) ($p < 0.0001$). When limiting separately ordered Gram stains to isolates where AST was performed, the sensitivity was 68% versus 35% for non-AST isolates ($p < 0.0001$). These results demonstrate the dramatic impact uncoupling urine culture and Gram stain orders panels can have to reduce over-utilization, as well as the greater sensitivity of Gram stains for organisms most likely to be of clinical significance. To prevent under-utilization and improve test performance, future work is needed to establish ordering practices that facilitate Gram stain ordering in populations where it is diagnostically useful and to elucidate pre-analytic factors that impact test performance.

Author Disclosure Block:

A. Bryan: None. **N.G. Hoffman:** None. **B.T. Cookson:** None.

Poster Board Number:

FRIDAY-323

Publishing Title:

Detection of *Clostridium difficile* Toxin in Patient Samples by Cell Culture Cytotoxicity Neutralization Assay (CCNA) Using Isolated Colonies of *C. difficile* versus Stool Samples

Author Block:

P. P. Patel, L. Edwards; Covance Central Lab Services, Indianapolis, IN

Abstract Body:

Background: Clostridium difficile toxin is a common cause of antibiotic associated diarrhea (AAD) and pseudomembranous colitis (PMC). The toxigenic culture method in which organisms are cultured and the stool is tested by the Cell Culture Cytotoxicity Neutralization assay (CCNA) remains the diagnostic "gold standard" for detection of toxigenic C difficile. The CCNA consists of two reactions - detection of toxin and toxin neutralization by the C.difficile antitoxin. The method is extremely sensitive and can detect toxin levels as low as 1 picogram. This testing should be performed using stool specimen filtrate however our evaluation shows that when a pure isolate of C.difficile is used as an alternative specimen, the sensitivity of the test is higher than the raw stool specimen.**Method:** Stool samples tested - preserved samples in Cary-Blair transport medium (stored ambient) and unpreserved samples (stored @ -70°C). Preserved stool from Cary-Blair medium was cultured anaerobically for growth of C.difficile. 27 stool cultures that yielded C. difficile were tested. Isolated colonies for these 27 samples along with C. difficile strains ATCC9689 (toxin producing) and ATCC700577 (non-toxin producing) were tested. 2 to 3 colonies of C. difficile isolate were inoculated into cooked meat broth and thioglycolate broth. CCNA using the Diagnostic Hybrids Cytotoxicity Assay was performed on both broth types after 48 hours of incubation (anaerobically). For the preserved samples that were positive by culture, the corresponding frozen stool sample was also tested for CCNA.**Results:** 9 out of the 27 unpreserved stool samples were cytotoxin-positive and 18 were negative on direct testing. Of the 27 C.difficile isolates; 22 confirmed cytotoxin production with both broths; 1 tested positive in the thioglycolate broth but was negative in cooked meat broth and frozen stool sample; remaining 4 were cytotoxin-negative. The ATCC strains performed as expected.**Conclusions:** A delay in transport of unpreserved stool samples can lead to degradation of toxin in samples leading to a false negative result. CCNA testing of C.difficile isolates from preserved stool samples (enriched in cooked meat broth or thioglycolate broth) provides better sensitivity than that obtained with direct testing of unpreserved stool samples.

Author Disclosure Block:

P.P. Patel: None. **L. Edwards:** None.

Poster Board Number:

FRIDAY-324

Publishing Title:

Is There a Relationship between Bacteriology Report Time in the Morning and Length of Stay in Hospital after the Report?

Author Block:

K. H. RAND, M.D., S. G. Beal, G. Lipori; Univ. of Florida, GAINESVILLE, FL

Abstract Body:

Background: Physicians are under continuing pressure to discharge patients early to lower length of stay (LOS). Since decisions are often made on AM rounds, logically the sooner microbiology reports are available the sooner discharge decisions can be made.**Methods:**We studied the last bacteriology culture report times (BCRT) before discharge from 10/1/2014–9/30/2015 that were within 168 h (1 week) of a patient's actual time of discharge, assuming that after 1 week the BCRT would be unlikely to influence discharge plans. University HealthSystem Consortium (UHC) risk-adjusted expected LOS on admission was also included https://www.uhc.edu/26295. We calculated the mean LOS following the BCRT for each ½ h time period between 6 AM and 1PM (N=14), representing 94% of all results, and tested the relationship to LOS after BCRT for significance by correlation and t-test. http://graphpad.com/quickcalcs/ttest2/**Results:** As shown in Figure, for patients whose total LOS was less than 168 h (N=6,895), there was a highly significant positive linear relationship between the BCRT and LOS following the last report (r=0.8686, p = 0.000056): the later the report the longer the LOS after BCRT. For patients with total hospital LOS > 168 h (N=2,434), the LOS after BCRT was actually longer between 6 – 8 AM than between 8-10 AM; the 6–8 AM patients also had a higher UHC risk-adjusted expected LOS on admission than those reported later when divided into ½ h time cohorts (r= -.6494, p=0.01196, 2 tailed).**Conclusions:** We conclude that the relationship between BCRT and use of this information by physicians in discharging patients is complex, but for certain subgroups there is a strong relationship between an earlier report and earlier patient discharge.

Author Disclosure Block:

K.H. Rand: None. **S.G. Beal:** None. **G. Lipori:** None.

Poster Board Number:

FRIDAY-325

Publishing Title:

Routine Tissue Culture vs. the Use of Blood Culture Bottles for Suspected Cases of PJI

Author Block:

E. J. Davis, S. E. Sharp; Kaiser Permanente - Northwest, Portland, OR

Abstract Body:

Background: The culturing of multiple tissue specimens is becoming the standard of care for patients suspected of having prosthetic joint infections (PJI). These specimens consisting of both aerobic and anaerobic cultures significantly increase the workload for the microbiology laboratory. We investigated the incubation of these tissue specimens in blood culture bottles in order to compare the results to those of routine culture.**Methods:** 37 patients with a total of 198 specimens (4-7 per patient) were routinely cultured and were also added to a set of blood culture bottles. The specimens consisted of tissues from 16 hips, 13 knees, 6 shoulders, 1 ankle and 1 elbow. All specimens were incubated aerobically and anaerobically for 14 days unless they were positive for growth before that time.**Results:** A total of 113 tissues (57%) and 11 patients (30%) had cultures that were negative for microbial growth after 14 days of incubation for all culture media. 10 patients (27%) showed growth only of a contaminating organism ($\leq$ 1 positive specimen). 16 patients (43%) showed potentially significant culture results ($\geq$ 2 positive specimens) in either the routine culture (94%) and/or the blood culture bottles (88%); 1 of these patients had a 2nd significant organism in routine cuture only. These 17 organisms consisted of: S.aureus (5), P.acnes (4), S.epidermidis (3), S.mitis (2), and 1 each of B.fragilis, S.lugdunensis, and F.magna. Of the 87 specimens from these 16 patients, the recovery of significant organisms per patient in blood culture bottles compared to culture was 75% and 63%, respectively. 11 of these 16 patients had the same organism cultured from both the routine media and the blood culture bottles. The remaining 5 patients had varying discordant results as shown in the table.

	Routine Culture	Blood Culture Bottles
1	5/5 NG	2/5 P.acnes
2	2/5 P.acnes	1/5 P.acnes; 1/5 Micrococcus
3	3/6 F.magna; 2/6 S.epidermidis	4/6 S.epidermidis
4	2/6 P.acnes; 1/6 CoNS	1/6 CoNS
5	1/6 S.aureus	5/6 S.aureus

Conclusions: This study shows that utilizing blood culture bottles in place of routine cultures is an acceptable method for culturing tissue specimens submitted from patients with suspected PJI.

This method would also save technologist time on the bench as approximately half of cultures remain negative and would not require manipulation of media.

Author Disclosure Block:

E.J. Davis: None. **S.E. Sharp:** None.

Poster Board Number:

FRIDAY-326

Publishing Title:

Chromogenic Media is Selective and Differential but Not Specific for the Detection of Carbapenem-Resistant *Enterobacteriaceae*

Author Block:

C. Young, W. LeBar, M. Bachman, D. W. Newton; Univ. of Michigan Hlth. Syst., Ann Arbor, MI

Abstract Body:

Background: Infection preventionists request surveillance cultures in clinical care units when there is concern of carbapenemase-resistant *Enterobacteriacae* (CRE) colonization in their patient cohort. The CDC broth method is time consuming requiring 4 days while chromogenic media is now available. We evaluated the use of HardyCHROM CRE (H-CRE), MALDI identification and detection of resistance determinants using Verigene BC-GN directly from H-CRE.**Method:** Previously characterized *Enterobacteriaceae* were grown on MacConkey (Mac) and H-CRE, incubated in ambient air at 35C for 24 hours and evaluated for growth and color on H-CRE. In addition, perirectal swabs were obtained from ICU patients and inoculated on H-CRE. Organisms growing on H-CRE were identified by MALDI (Bruker) followed by direct isolate testing on Verigene BC-GN for resistance determinants (KPC, NDM, OXA, IMP, VIM, CTXM). MALDI scores from isolates recovered from Mac were compared to those obtained from isolated recovered from H-CRE.**Results:** Fifty well-characterized *Enterobacteriaceae* from clinical specimens were evaluated for growth on H-CRE. Of 32 confirmed CRE, 28 exhibited the appropriate color change on H-CRE (25 KPC, 2 NDM and 1 OXA) and 17 non-CRE were also chromogenic on H-CRE (16 *Enterobacter sp.* and 1 *Klebsiella sp*). In addition, there were 4 CRE which did not grow on H-CRE (3 KPC and 1 NDM). Results from 139 perirectal swabs demonstrated no growth (86), haze or pinpoint (33), cream (13) and chromogenic (7) colonies. The 7 chromogenic colonies were tested on Verigene which identified 3 *Acinetobacter sp.* and 1 each K. *pneumoniae, K. oxytoca, Escherichia coli* and *Citrobacter sp*. Of these, one KPC was detected in *Citrobacter sp.* Eighteen of twenty three isolates from Mac and 23/23 isolates from H-CRE had MALDI scores >2.0.**Conclusion:** We show that Hardy-CRE is a selective and differential media that can be used for surveillance cultures; however, it does appear to lack specificity. Direct identification of chromogenic colonies on Hardy CRE can be performed on Bruker MALDI followed by detection of resistance determinants with Verigene BC-GN. When the chromogenic colony is a genus/species not on Verigene, another method must be used to determine whether the isolate is CRE.

Author Disclosure Block:

C. Young: None. **W. LeBar:** None. **M. Bachman:** None. **D.W. Newton:** None.

Poster Board Number:

FRIDAY-327

Publishing Title:

A Multicenter Assessment of Errors in Culture Reports

Author Block:

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Abstract Body:

Background: Review of bacteriology culture work up is a standard daily practice in some clinical microbiology laboratories; however, there is a paucity of information regarding laboratory practices of review and error rates. Futhermore, because the practices are not standardized between laboratories a benchmark for error rates has never been established. The goal of this study was to describe and compare the culture review practices and error rates for four large laboratories affliliated with tertiary care academic medical centers. **Methods:** Aerobic bacteriology culture reports from sterile sites including joint fluids, tissues, cerebrospinal fluids (CSF), body fluids and aspirates were evaluated based on the current review practices and standard operating procedures specified by each individual laboratory. Laboratories that did not currently practice a process of secondary review implemented it for the purposes of this study. **Results:** A total of 2304 laboratory reports were reviewed, with an overall positivity rate of 19% for all sites. Two-hundred and sixteen (9%) errors were found, with 149 (65%) being characterized as clerical errors and 67 (35%) being characterized as technical errors. Overall, technical errors were more likely to have a clinical impact on patient care. **Conclusions:** Establishing a benchmark error rate for culture review is a challenging process due to variability in laboratory practices. A process of secondary culture review is a resource intensive but useful tool for uncovering systematic laboratory error and for routine quality control review of daily processes.

Author Disclosure Block:

A. Harrington: None. **L. Samuel:** None. **J. Balada-Llasat:** None. **R. Cavagnolo:** None.

Poster Board Number:

FRIDAY-328

Publishing Title:

Application of Eubacterial Molecular Detection to Clinical Routine

Author Block:

A. E. Budding[1], M. Hoogewerf[1], C. M. J. E. Vandenbroucke-Grauls[1], P. H. M. Savelkoul[2]; [1]VU Univ. Med. center, Amsterdam, Netherlands, [2]Maastricht Univ. Med. center, Maastricht, Netherlands

Abstract Body:

Background: The past decade has seen an increasing application of molecular techniques -most notably qPCR- for the detection of bacterial pathogens in clinical samples. Advantages of qPCR over culture are its speed and the ability to detect species when culture cannot. However, qPCR is typically directed at specific species. Thus, anything that is not explicitly searched for will be missed. Universal PCR followed by sequencing is an alternative. However, this significantly increases cost, complexity and turnaround time. Here we investigated the application of a rapid universal bacterial molecular assay, IS-pro, to clinical practice in 220 patient samples.**Methods:** IS-pro is a fully automated and internally controlled eubacterial assay based on the detection and categorization of 16S-23S Interspace regions, that are specific for each microbial species. IS-pro was performed on 220 clinical samples from a range of bodily locations that are normally sterile (pleural cavity, joints, liver abscesses etc). Results were compared to culture and clinical records.**Results:** Of the 220 samples that were analysed, 30% of samples that were negative by culture were positive by IS-pro. Species found included many common pathogens, such as *Staphylococcus aureus*, *Streptococcus pyogenes* and *Escherichia coli*, but also many species less established as pathogens, such as *Faecalibacterium prausnitzii* and *Ailstipes putredinis*. The finding of cultivable species with IS-pro while culture was negative could often -but not always- be explained by administration of antibiotics prior to sampling. The species that were less well established as pathogens were often species that were refractory to standard culture techniques. These species commonly derived from endogenous microbiota.**Conclusions:** Here we demonstrate that the application of a broad range molecular detection technique, IS-pro, can be of great benefit to clinical diagnostics. We conclude that 1. IS-pro can very often detect pathogens when culture cannot (30% of culture negative samples). 2. Not only uncultivable, but also common pathogens that are missed by culture are detected by IS-pro. 3. Additionally detected species are highly clinically relevant: IS-pro results often guided clinical decisions.

Author Disclosure Block:

A.E. Budding: A. Board Member; Self; IS-Diagnostics Ltd.. K. Shareholder (excluding diversitied mutual funds); Self; IS-Diagnostics Ltd.. **M. Hoogewerf:** None. **C.M.J.E.**

Vandenbroucke-Grauls: None. **P.H.M. Savelkoul:** A. Board Member; Self; IS-Diagnostics. K. Shareholder (excluding diversitied mutual funds); Self; IS-Diagnostics.

Poster Board Number:

FRIDAY-329

Publishing Title:

Detection Of Carbapenemase-Producing Organisms in Rectal Swabs: Comparison Of The Check-Direct Cpe Assay with the CDC Broth Enrichment Method

Author Block:

I. Martin, B. Opene, T. Tekle, J-A. Miller, K. Carroll, A. Milstone, P. Simner; The Johns Hopkins Med. Inst.s, Baltimore, MD

Abstract Body:

Background: This study evaluated the performance of the Check-Direct CPE assay (CA)—a multiplex PCR assay for detection of carbapenemase genes (CG) (*bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{OXA-48-like}$, *bla*$_{VIM}$)—directly from rectal swabs in comparison to the CDC broth enrichment method (CDCM) in detecting carbapenemase-producing organisms (CPO).**Methods:** 213 remnant VRE surveillance rectal ESwabs from 191 inpatients and 10 characterized isolates containing CG were included in this study. The CA was performed using 25 µl of ESwab broth on the BD MAX instrument. The CDCM was performed using a 10µg ertapenem disk for selection. ID and AST of isolates were performed with the BD Phoenix and MALDI-TOF MS. CG production was confirmed by the CarbaNP and Check-MDR CT103 XL assays.**Results:**

Specimen Type:	**Remnant VRE surveillance rectal ESwabs (N=213)**		**Previously Characterized Isolates (N=10)**
Method:	**CDCM**	**CA**	**CDCM and CA**
Number of swabs (%)			
Turbid Broths	168 (78.9)	NA	10 (100)
GNB Recovery	83 (39.0)		10 (100)
CRO Recovery	13 (6.1) 6 *S. maltophilia* 4 *E. cloacae* *2 P. aeruginosa* *1 K. pneumoniae*		10 (100)
CPO Recovery	2 (0.9) 1 KPC-producing *E. cloacae* 1 KPC *K. pneumoniae*		10 (100)
CG positive direct from ESwab broth	NA	10 (4.7) 7 *bla*$_{KPC}$ 2 *bla*$_{VIM}$	3 *bla*$_{KPC}$ 3 *bla*$_{OXA-48-like}$

		1 *bla*$_{\text{OXA-48-like}}$	2 *bla*$_{\text{VIM}}$ 2 *bla*$_{\text{NDM}}$
Other Considerations			
Hands-on-time	30 - 45 mins	5 mins	
TAT	24 hrs - 5 days	3 hrs	
Costs	$1 - 15	~$50	
NA: not applicable; GNB: Gram-negative bacilli; CRO: carbapenem resistant organism			

Of the 10 CG detected by CA, only 2 (20%) were recovered by CDCM. Discrepant analysis recovered 4 additional CPOs (data not shown). Failure to recover these CPOs by CDCM was due to the overgrowth of meropenem susceptible *P. aeruginosa*. No growth was observed by the CDCM method or by discrepant analysis for 2 *bla*$_{\text{VIM}}$ (Ct 39.7 & 40.8), 1 *bla*$_{\text{KPC}}$ (Ct 40.5) & 1 *bla*$_{\text{OXA-48-like}}$ (Ct 40.6).**Conclusions:** The CA is more sensitive for detection of CPO than the CDCM. However, the significance of results with Ct values > 35 requires further evaluation. The advantage of greater sensitivity and more rapid TAT of the CA is mired by its high cost. The CDCM yielded many turbid broths which were negative for CRO. However, CDCM was able to detect many non-carbapenemase-producing CRO.

Author Disclosure Block:

I. Martin: None. **B. Opene:** None. **T. Tekle:** None. **J. Miller:** None. **K. Carroll:** None. **A. Milstone:** None. **P. Simner:** None.

Poster Board Number:

FRIDAY-330

Publishing Title:

Comparison of chromid CPS Elite Media to Conventional Culture Media for Urine Specimens

Author Block:

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Abstract Body:

Objective: Urine specimens are among the most common sample submitted to microbiology laboratories. The objective of our study was to compare chromID CPS Elite (bioMérieux), a chromogenic medium, to conventional primary culture medium for urine specimens.**Methods:** Remnant urine specimens (n=200) submitted to the Barnes Jewish Hospital Microbiology Laboratory (St. Louis, MO) were evaluated. Using a 1 uL calibrated loop, urine was inoculated to blood and MacConkey agars (Hardy) (conventional media) and to the chromID CPS Elite agar (chromID). Cultures were evaluated after 22-24 hours of incubation. The chromID medium was evaluated per the package insert. Organism identification was obtained with VITEK MS v2.0. Time to identification and consumables used were documented for both methods. For accuracy analysis, the conventional media was used as the gold standard for comparison.**Results:** Of the 200 cultures, 61 were no growth, 67 demonstrated clinically insignificant growth, 16 were contaminated, and from 56, clinically significant pathogen(s) were recovered. Of the 56 clinically significant cultures, chromID classified 46 (82.1%) as significant. Overall, the rate of uropathogen agreement between conventional and chromogenic media was 76.8%. In discrepant specimens, organisms that were not recovered on chromID included Group B *Streptococcus*, *Lactobacillus*, and coagulase negative *Staphylococcus.* The time interval between plating and final organism identification of *E. coli* was statistically significant between conventional and chromID ($p<0.0001$). Notably, the number of consumables used was significantly higher with conventional media versus chromID ($p=0.0013$) (Table 1).**Conclusions:** The use of chromID CPS Elite for urine cultures may reduce consumable use. chromID may expedite the identification of *E. coli*, the most commonly recovered uropathogen in clinical specimens.**Table 1. Comparison of Conventional Culture Media to chromID CPS Elite**

	Conventional Medium Mean (SD)	**chromID CPS Elite** Mean (SD)	*p*
Number of consumables used	2.23 (3.694)	1.58 (3.460)	0.0013
Time from plating to final organism identification (h), All positive cultures (n=44)	26.56 (1.283)	25.44 (1.686)	<0.0001

Time from plating to final organism identification (h), *E. coli* (n=18)	27.10 (0.822)	24.37 (1.229)	<0.0001

Author Disclosure Block:

M. Wallace: None. **M. Yarbrough:** None. **C. Marshall:** None. **E. Mathias:** None. **C. Burnham:** None.

Poster Board Number:

FRIDAY-331

Publishing Title:

Ultraviolet Germicidal Irradiation of Influenza-contaminated N95 Filtering Facepiece Respirators

Author Block:

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Abstract Body:

An influenza pandemic could lead to a shortage of Filtering Facepiece Respirators (FFRs), leaving health care workers unprotected. Past research on FFR decontamination and reuse (FFR-DR) strategies indicate ultraviolet germicidal irradiation (UVGI) is effective at killing influenza on FFRs. This study expands this research by 1) optimizing UVGI dose for influenza kill in the presence of soiling agents, 2) optimizing the dose to reduce exposure time, and 3) evaluating 15 FFR models to understand universal application of the technology.For UVGI dose optimization, triplicate 3M 1870 coupons were contaminated with influenza and either sebum or mucin. Contaminated coupons were exposed to 254-nm UV-C dose ranging from $1 \times 10^3 – 2 \times 10^6$ $\mu J/cm^2$. Influenza virus was extracted and quantified using a median tissue culture infectious dose ($TCID_{50}$) assay. A UVGI device was built for applying the optimized UV dose to an intact FFR in ~1 min. Triplicate samples of 15 FFR models were contaminated as described above on three facemask locations and one strap location per FFR. After UV treatment, coupons were cut from the FFRs, and remaining viable virus extracted and quantified.The optimized dose was determined to be $\sim 1 \times 10^6$ $\mu J/cm^2$. Whole FFR exposure demonstrated high variability in UVGI decontamination between FFR models and surface type, with log reductions ranging from 0.00 – 4.85 $\log_{10}$ $TCID_{50}$. The mean log reduction on the facemask portion of all 15 FFR models was 3.42 ± 1.08 $\log_{10}$ $TCID_{50}$ and 2.48 ± 1.29 $\log_{10}$ $TCID_{50}$ for straps. Only two of the FFR models demonstrated $\geq$ 3-mean log reduction for all contaminated surfaces. FFRs with hydrophilic surfaces correlated with the lowest log reduction values, and vice versa for hydrophobic surfaces.These data suggest that FFR-DR using UVGI is possible, but not universal. Some FFRs are not compatible, presumably due to material incompatibilities. These data are critically important for regulators and hospitals to understand in the event FFR-DR technologies are deployed during a pandemic. Studies are currently underway to evaluate FFR durability and performance following multiple UVGI cycles and to evaluate logistical parameters for implementing UVGI-based FFR-DR technology into health care settings.

Author Disclosure Block:

D. Mills: None. **C. Lawrence:** None. **M. Sandoval:** None. **D.A. Harnish:** None. **B.K. Heimbuch:** None.

Poster Board Number:

FRIDAY-332

Publishing Title:

Stability Of Hydrogen Peroxide Antimicrobial Activity In Silica Hydrogels: A Pilot Study

Author Block:

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Abstract Body:

Hydrogen peroxide is an effective, environmentally friendly disinfectant. However, it decays rapidly into water and oxygen, limiting its practical applications. Sol-gel chemistry can be used to microencapsulate H_2O_2 in silica hydrogels, in which the H_2O_2 binds strongly with the silica gel surface. Changing the pH and sodium concentration during hydrogel generation allows fine control of release and decomposition rates through variations in the surface area, pore volume, and pore size. This process will allow H_2O_2 hydrogels to be developed for specific applications through optimization of room temperature stability and decomposition rates. The resultant hydrogels have potential usefulness in wound treatment, hand sanitization, and other disinfection applications.In this study, freshly prepared and 4 month old gels at 0, 10, and 20 wt% were compared for activity against *S. aureus* and *E. coli* using a standard agar well diffusion method. Experimental conditions included: duplicate plates, 0.5 MacFarland density for bacteria (~1.5 x 10^8 CFU/mL), agar depth of 15 mL, 6 mm wells, and 0.04 ±0.01 g of gel. Each gel was tested in 2 separate runs. Average zones of inhibition (ZOI) are shown in the Table.Table. Average zones of inhibition (ZOI) for freshly prepared and 4 month old H_2O_2 hydrogels.

	Hydrogel Weight%	***S. aureus* ZOI (mm) (SD)**		***E. coli* ZOI (mm) (SD)**	
Freshly Prepared Hydrogel	0	6	0.0	6	0.0
	10	51	1.7	44	1.3
	20	54	0.5	45	0.5
4 Month Old Hydrogel	0	6	0.0	6	0.5
	10	34	1.0	28	0.8
	20	39	0.8	34	1.7

Zone sizes are consistently larger for *S. aureus* than *E. coli*, suggesting greater activity, however this must be further tested. The average difference in activity between 10 and 20 wt% is 4% for fresh gel and 16% for 4 month old gel. This suggests a non-linear relationship between gel wt% and activity, however the difference is greater for the older gel, implying that a higher

concentration might be more effective for long-term storage. Antimicrobial activity diminished by an average of 30% over 4 months, but significant activity remained. H_2O_2 can be stabilized in silica hydrogels for at least a 4-month period. Further investigations into ideal concentrations, stability, release rates, and antimicrobial activity are needed.

Author Disclosure Block:

H. Mohamed: None. **N. Orbey:** None. **N. Goodyear:** None.

Poster Board Number:

FRIDAY-333

Publishing Title:

One to One Counseling is Highly Effective in Increasing Influenza Vaccination among Doctors

Author Block:

Y. Jung, J. Song, M-H. Kwon; Konyang Univ. Hosp., Daejeon, Korea, Republic of

Abstract Body:

Introduction: The influenza vaccination rate among healthcare workers (HCW) remains suboptimal. We tried to increase the vaccine uptake in the 2015-2016 season by non-mandatory measures including one to one counseling. **Methods:** Since 2004, our hospital (a university affiliated, 830-bed acute care center) have given free influenza vaccinations to HCW for three days at a designated location, and, after that period in the clinics. This year (2015-2016), to increase uptake, we used a stepwise approach including text messaging, extending the vaccination period, education and, personal counseling for unvaccinated HCW.**Results:** The number of potential HCW for influenza vaccination was 1454. First, we reminded the unvaccinated HCW of the vaccination by text messaging on the last day of vaccination. By the end of the last day, the uptake rate was 82.2% (1196/1454), lower than in the previous year (85%), so we decided to extend vaccination by three days. One month after starting the vaccination, 89% (1296/1454) of the HCW were vaccinated, but only 78.1% (207/275) of the doctors, the lowest rate among the subgroups. Next, we provided lectures to the doctors three times over two weeks but no one had been vaccinated by one week later. Lastly, we counselled all but one of the unvaccinated doctors (67/68) by telephone or personally, and recommended the influenza vaccine and informed them of the relevant location and time, or made an appointment for vaccination. After that intervention, 63.2% (43/68) of the unvaccinated doctors were vaccinated, thus achieving 91.3% (251/275) compliance, higher than the 58.4% (157/259) in the previous year.**Conclusions:** Influenza vaccination rate is lower among doctors than among other HCW subgroups. One to one counseling is highly effective in increasing influenza vaccination among doctors.

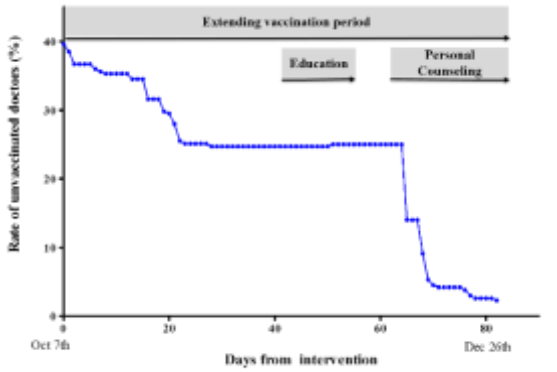

Figure. Changes in unvaccinated doctors with stepwise interventions in 2015-2016

Author Disclosure Block:

Y. Jung: None. **J. Song:** None. **M. Kwon:** None.

Poster Board Number:

FRIDAY-334

Publishing Title:

Towards a Novel Method for Detecting Microbial Biofilms on Indwelling Intravascular Catheters in Real Time

Author Block:

R. Donlan, M. A. Mazher, H. Moura, j. barr; CDC, Atlanta, GA

Abstract Body:

Background: Intravascular catheters (IC) colonized with bacterial biofilms are a major source of healthcare-associated infections. Bacteria produce extracellular proteins (EP) during growth and metabolism, and EP profiles for different organisms are unique. Nanoflow liquid chromatography-electrospray ionization-tandem mass spectrometry (nLC-ESI-MS) can detect and identify unique EP from microorganisms. We hypothesized that EP could be developed into biomarkers to detect and identify biofilm microorganisms in IC in real time. **Methods:** Biofilms of *Escherichia coli* were formed on human serum-pretreated silicone coupons in 12-well plates in RPMI 1640 medium at 37^{o}C. After 24 h, coupons were harvested and biofilms were recovered. Biofilms of *Pseudomonas aeruginosa* were developed using the same medium and growth conditions as for *E. coli*. Glass wool was used instead of silicone coupons to increase EP yield. Samples from the spent medium and from the recovered biofilm suspension for both organisms were centrifuged, and the proteins in the supernatants (biofilm EP) were trypsin digested. The resulting peptides were fractionated, ionized, and analyzed using nLC-ESI-MS. Proteins were identified using the MASCOT search engine and validated using Scaffold. **Results:** Flagellin (GI:120312) was the most abundant protein detected from biofilms of 4 different *E. coli* strains, and was selected as a putative biomarker for this organism. *E. coli* biofilm viable counts on silicone surfaces were approximately 10^5 - 10^7CFU/coupon for all strains, demonstrating the ability of this analytical platform to detect biofilm EP in the presence of more highly abundant proteins (e.g. serum albumin), suggesting a wide dynamic range for this method. The most abundant EP in 4 different strains of *P. aeruginosa* was Cpn60 groEL (GI:15599581), demonstrating that *P. aeruginosa* and *E. coli* had unique EP that could be developed into specific biomarkers. **Conclusions:** These results demonstrate the ability of this analytical approach to detect and differentiate biofilm organisms based on their specific protein signatures, and provide preliminary data for the development of a method to detect biomarkers for biofilms on intravascular catheters.

Author Disclosure Block:

R. Donlan: None. **M.A. Mazher:** None. **H. Moura:** None. **J. barr:** None.

Poster Board Number:

FRIDAY-335

Publishing Title:

Hepatitis C Continuum of Care in France: A 20-Year Cohort Study

Author Block:

C. Hermetet[1], F. Dubois[2], C. Gaudy-Graffin[2], Y. Bacq[2], B. Royer[3], C. Gaoborit[2], L. D'Alteroche[2], J. Dessenclos[4], P. Roingeard[2], **L. Grammatico-Guillon**[5]; [1]Service of Publ. Hlth., Univ. Hosp. of Tours, TOURS, France, [2]Univ. Hosp. of Tours, TOURS, France, [3]IRSA, Lariche, France, [4]Agence de Santé Publique, France, St maurcie, France, [5]Univ. Hosp. of Tours, TOURS, France

Abstract Body:

Background: Hepatitis C virus (HCV) requires a specific continuum of care (CoC) from screening to treatment. However, many patients do not move through the CoC. We assessed the CoC of HCV patients in France in a longitudinal study.**Methods:** A cohort study of subjects undergoing a preventive consultation in one French regional medical centre from 1993 to 2013 was built, providing 12,993 HCV screenings. The HCV CoC was described as followed: Stage 1- HCV screening; Stage 2- HCV RNA infection confirmation; Stage 3- continuing care; Stage 4- antiviral treatment. HCV CoC was first described and Cox multivariate analysis was performed to identify factors favoring CoC, calculating Hazard Ratios (HR and 95%CI).**Results:** In total, 12,993 HCV tests were performed and 478 outpatients were found to be HCV-seropositive. We included 405 seropositive patients, after excluding false positives and patients lost to follow-up. The baseline characteristics were: sex ratio (M/F) 1.4; mean age 38.5 years; intravenous drug use (IDU) in 55%; 28% of precarious situations. Antiviral treatment was initiated for 179 (42.9%) of the 379 (90.9%) patients attending specialist consultations. CoC was associated with screening after 1997 (HR 2.0, 95%CI 1.4-2.9), age >45 years (HR 1.5, 95%CI 1.02-2.3), patient acceptance of care (HR 9.3, 95%CI 5.4-16.10), specialist motivation for treatment (HR 10.9, 95%CI 7.4-16.0), absence of cancer (HR 6.7, 95%CI 1.6- 27.9). Other comorbid conditions, such as depression and IVDU, were not associated with CoC.**Conclusions:** This 20-year overview of CoC of HCV infection in France showed that a substantial proportion of patients have access to antiviral treatment. However, this proportion should be increased, as new direct-acting antiviral drugs are highly effective and well-tolerated.

Author Disclosure Block:

C. Hermetet: None. **F. Dubois:** None. **C. Gaudy-Graffin:** None. **Y. Bacq:** C. Consultant; Self; abbvie. **B. Royer:** None. **C. Gaoborit:** None. **L. D'Alteroche:** None. **J. Dessenclos:** None. **P. Roingeard:** None. **L. Grammatico-Guillon:** C. Consultant; Self; speaker at a meeting for Abbvie.

Poster Board Number:

FRIDAY-336

Publishing Title:

***Helicobacter pylori* Prevalence in Dental Plaques of Diabetic and Gastritis Patients, as a Possible Source of Re-Infection**

Author Block:

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Abstract Body:

Background: *Helicobacter pylori* infection is considered as one of the most prevalent infectious diseases throughout the world. Oral infection with *H. pylori* is usually associated with *H. pylori* infection of the stomach therefore it has been speculated that oral bacteria are responsible for stomach re-infection. The objective of this study is to elucidate the prevalence of *H pylori* infection in dental plaques of type 2 diabetic subjects with and without gastritis. **Methodology:** Supragingival and subgingival samples were collected from 70 patients with chronic periodonitis, 15 of whom were also suffering from gastritis , 26 from diabetic, 9 from both gastritis and diabetic, and 2 from ischemic heart disease, diabetic and gastritis. The samples were analyzed by PCR using two Random sequence nt 4835-5041 primer. DNA extraction was done using the Gentra PureGene DNA isolation Kit **Results:** *H. pylori* was detected in 40% of the patients. The prevalence of *Helicobacter pylori* in dental plaques was 60% in gastritis patients and 31% in patients with diabetes only. Patients with gastritis and diabetes had 56% PCR positivity in gastritis patients with ischemic heart diseases and 100% with diabetes, compared to 24% among the control patients (healthy with no gastritis, diabetes or heart diseases). Interaction of age and infection was not significant but the prevalence of *H. pylori* in dental plaque of both diabetic and gastritis male patients were higher than among female patients. **Conclusion:** The prevalence of *H. pylori* in the dental plaque of gastritis patients was high and the alterations of glucose metabolism in diabetes seems to promote the *Helicobacter pylori* colonization in the dental plaques of the diabetic patients. The oral cavity may be a reservoir for *H pylori* infection, it is therefore suggested that professional plaque removal and oral hygiene procedure be performed, along with the antibiotic treatment of *H. pylori*.

Author Disclosure Block:

J.A.A. Qasem: None. **A.A. Qasem:** None. **J. Thomas:** None.

Poster Board Number:

FRIDAY-337

Publishing Title:

Validation of Quality Indicators to Measure the Appropriateness of Antibiotic Use in a University Hospital

Author Block:

L. Boix Palop[1], C. Nicolas[1], M. Xercavins[2], C. Badia[1], P. Arcenillas[1], L. Gómez[1], E. Calbo[1]; [1]Hosp. Univ.ri Mútua de Terrassa, Terrassa, Spain, [2]Microbiol., Catlab, Terrassa, Spain

Abstract Body:

Background: An important requirement for an effective antibiotic stewardship program is the ability to measure appropriateness of antibiotic prescription (AP). To succesfully design effective and targeted interventions to improve antibiotic prescribing, it is first necessary to better understand the factors that influence appropriate prescribing. The aim of this study was to evaluate the quality of AP in a university hospital using a set of quality indicators (QI) previously described by Van den Bosch et al. **Methods:** A cross-sectional study was conducted (June-November-2015). All adult inpatients receiving systemic antibiotic therapy (SAT) were included in a monthly point-prevalence survey (PPS). Patients on SAT were identified through the Pharmacy Department Database. Patients from ICU, Psychiatry, Paediatrics and Emergency Department were not included. QIs previously described were adapted (Table 1). Three trained Infectious Disease Consultants collected QI checking data from medical records. The 3 evaluators simultaneously collected 10% of the sample in order to evaluate the interobserver concordance. **Results:** 505 patients were on SAT during the study period. 142 were excluded (130 discharge before evaluation or suspension of SAT, 12 antifungal or prophylactic SAT). 363 patients were finally analyzed. An average of 84.2 patients was included per PPS. The results are shown in Table 1. Kappa index for interobserver concordance was 0.67. **Conclusions:** Evaluation of QIs designed to measure the appropriateness of SAT has identified several areas for improvement including culturing, switching to oral therapy, stopping unnecessary SAT and directing therapy to culture results.Table 1

Quality indicators	Numerator description	Denominator description	Results
Empirical systemic antibiotic therapy (EAT) prescribed according to the local guideline	Number of patients (NP) with EAT according to the local guideline	NP with EAT and who had a clinical syndrome described in the local guideline	71.7%
Before starting systemic antibiotic (SAT) therapy 2 sets	NP with 2 blood cultures taken before starting SAT	NP who started with SAT	38%

of blood cultures should be taken			
When starting SAT, specimens for culture from suspected sites of infection should be taken	NP with SAT and in whom cultures from suspected sites of infection were taken	NP with SAT and who had a cultivable site of infection	75.5%
EAT should be changed to pathogen-directed therapy if culture results become available	NP with changing to pathogen-directed therapy done	NP with EAT whose culture became positive with clinical significance	73.5%
Dose adapted to renal function	NP with a compromised renal function with a dosing regimen adjusted to renal function (defined as eGFR <50 mL/min/1.73 m2)	NP with SAT and a compromised renal function	79.7%
SAT should be switched from intravenous to oral antibiotic therapy within 48-72 h on the basis of the clinical condition and when oral treatment is adequate	NP with intravenous antibiotics for 48-72 h, with changing to oral antibiotic therapy on the basis of clinical condition	NP with intravenous antibiotics for 48-72 h, in whom changing to oral antibiotic therapy was indicated	51.6%
No discontinuation of EAT after 5d of treatment without clinical and/or microbiological evidence of infection	NP with EAT ≥ 5d and without clinical and/or microbiological evidence of infection	NP with EAT ≥ 5d	23.2%
Therapeutic drug monitoring	NP with at least 1 serum drug level measurement	NP who received aminoglycosides for >3 d and/or vancomycin for >5 d	33.3%

Author Disclosure Block:

L. Boix Palop: None. **C. Nicolas:** None. **M. Xercavins:** None. **C. Badia:** None. **P. Arcenillas:** None. **L. Gómez:** None. **E. Calbo:** None.

Poster Board Number:

FRIDAY-338

Publishing Title:

Development of a Bacterial Consortium of *Lysinibacillus sphaericus* Strains as Biocontrol Agents Against *Aedes aegypti* Larvae

Author Block:

J. Silva, J. Dussán; Univ. de Los Andes, Bogota, Colombia

Abstract Body:

Background: One of the actual problems in public health is the increasing transmission of diseases via mosquito vectors in tropical regions. In this study, we focused on the mosquito *Aedes aegypti* (**Ae**). Biocontrol is a promising solution because it consists of using natural antagonists against target organisms. The bacterium *Lysinibacillus sphaericus* (**Ls**) has been reported to cause mortality in mosquito larvae. Three **Ls** strains were used on this project: the WHO strain 2362 and OT4b.25 and III(3)7 isolated in Colombia, a country with recent reports of dengue, Chykungunya and Zika virus. Previous studies proposed **Ls** as a candidate to control *Culex* and *Anopheles* populations but not for *Aedes*.The objective of this study was to produce a bacterial consortium efficient to control Ae populations in vitro. All bacterial consortia evaluated contained the strain 2362 since it has good toxicity by itself and because it is a reference strain worldwide. **Methods:** Bacterial strains of Ls used during this project were OT4b.25, III(3)7 and 2362. On the other hand, the WHO strain Ae Rockefeller was tested.All larvicidal bioassays were tested according to the parameters stated by the WHO and using four different initial inocula concentrations per assay in order to calculate the LC50 as an efficiency estimator. Bacterial consortia were tested as follows: 2362 + III(3)7, 2362 + OT4b.25, and III(3)7 + OT4b.25 + 2362. We determined statistical differences among lethality counts for the inocula concentrations evaluated on each bioassay with a Kruskal-Wallis test. **Results:** All bioassays showed larval mortality and results were validated by the effectiveness of the positive and negative controls. We found that the consortium of Ls strains 2362 + III(3)7 displayed the most efficient LC50 (8.67e8*) when compared to bioassays: 2362 + OT4b.25 (7.82e9*), and III(3)7 + OT4b.25 + 2362 (9.32e10*) and individual 2362 (1,11e9*) and III(3)7 (1,04e9*). Note: * = means bacterial inoculum units cfu/mL. **Conclusions:** We found that the bacterial consortium of **Ls** strains 2362 + III(3)7 is a good candidate consortium for biocontrol of **Ae** populations *in vitro*. Future *in situ* studies are required to verify the liability of this consortium under both environmental conditions and **Ae** populations.

Author Disclosure Block:

J. Silva: None. **J. Dussán:** None.

Poster Board Number:

FRIDAY-339

Publishing Title:

F1-V Antigen Lead to Elevated Immune Response Against *Yersinia pestis* Together with Chlolera Toxin B Sub-Unit on Mice

Author Block:

J. JEON, O-K. PARK, S-Y. CHOI, G-E. RHIE, J-y. YU; Korea NIH, Osong, Korea, Republic of

Abstract Body:

Yersinia pestis is known as causative agent of plague which is a highly fatal infectious disease to human and certain animals. Despite of the seriousness of infection with *Y. pestis*, an effective plague vaccine is still not available. In this study, we investigated the effect of Cholera toxin B (CTB) subunit on immune response against *Y. pestis* F1-V antigen which has known to potent vaccine candidate for plague and on protection against lethal respiratory challenge with *Y. pestis* strain KIM5. BALB/c mice were immunized with His6-tagged purified F1, V, F1-V, V-F1, and F1+V proteins together with alum or cholera toxin B sub-unit. Immunization with F1-V together with CTB induced elevated immune responses to F1 or V compared to F1, V, F1-V, V-F1, and F1+V together with alum-immunized group. Furthermore, F1-V plus CTB-immunized mice showed a higher survival rate against lethal challenge with *Y. pestis* than F1, V, F1-V, V-F1, and F1+V together with alum-immunized mice. Taken together, these results suggest that CTB could serve as an effective candidate adjuvant for plague vaccination.

Author Disclosure Block:

J. Jeon: None. **O. Park:** None. **S. Choi:** None. **G. Rhie:** None. **J. Yu:** None.

Poster Board Number:

FRIDAY-340

Publishing Title:

Wipe Cleaning Method for Restoring Antibacterial Activities of Soiled Surfaces of an Antibacterial Stainless Steel

Author Block:

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Abstract Body:

Background: Introducing antibacterial metals into frequently hand-touched places, such as handrails and doorknobs, has been considered to reduce risks of outbreaks of hospital acquired infections. Antibacterial activities of antibacterial metals will be deteriorated by surface soiling. The object of this study is to study practical wipe cleaning method for restoring antibacterial activities of antibacterial stainless steel surfaces, which were deteriorated by soiling. **Methods:** The sample metal used in this study was an antibacterial stainless steel containing about 3.8 mass% copper. Bovine serum albumin (BSA) and triolein were employed as model protein and oily contaminants, respectively. The contaminants were spread over a test surface of a coupon specimen. The specimen was then subjected to wipe cleaning using a crockmeter. Detergents used for the wipe cleaning were sodium hypochlorite aqueous solutions (NaOCl aq.) and ethyl alcohol aqueous solutions (EtOH). In some cases, surfactant (sodium dodecyl sulfate(SDS)) was added to these detergents. Antibacterial tests of wiped test surfaces were carried out in accordance with ISO 22196, using *Escherichia coli* (NBRC 3972) and *Staphylococcus aureus subsp. aureus* (NBRC 12732). **Results:** NaOCl aq. was effective against the BSA surface contamination, but less effective against the triolein surface contamination. EtOH was, contrary to NaOCl aq, effective against the triolein surface contamination but less effective against the BSA surface contamination. **Conclusion:** The practical wipe cleaning method effective against both protein and oily surface contamination was as follows: firstly wipe the soiled surfaces with wipes loaded with NaOCl aq. containing surfactant and then wipe the surfaces again with wipes loaded with distilled water to remove the surfactant remaining on the surfaces.

Chemical compositions of samples [mass%]

Sample	C	Si	Mn	Cr	Ni	N	Cu
Cu-SS	0.011	0.52	1.78	18.14	9.37	0.011	3.78
type304	0.04	0.05	0.91	18.08	8.09	-	-

Author Disclosure Block:

H. Kawakami: None. **K. Hirayama:** None. **Y. Sato:** None. **Y. Kikuchi:** None.

Poster Board Number:

FRIDAY-341

Publishing Title:

Multi-Laboratory Evaluation of the Oecd Quantitative Method for Assessing the Effectiveness of Antimicrobial Products on Hard Surfaces

Author Block:

S. F. Tomasino, R. M. Pines; US EPA, Ft. Meade, MD

Abstract Body:

The US EPA is working with the Organization of Economic Co-operation and Development (OECD) on the development of a standard method for assessing the efficacy of liquid antimicrobial products on hard non-porous surfaces. The method under consideration is based on the OECD Guidance Document on Quantitative Methods for Evaluating the Activity of Microbicides. In the method, 1 cm diameter stainless steel disks (carriers) are inoculated with 10 µL of a suspension of the test microbe and a three-part soil load. After drying, each carrier is placed in a vial and the inoculum is exposed to 50 µL of the antimicrobial agent. Control carriers receive 50 µL of phosphate buffered saline. Following a 5 min contact time, 10 mL of neutralizer is added to the vial, contents vortexed, serially diluted, and viable bacteria are enumerated using filtration and direct plating. The differences between the mean log density (LD) for control and treated carriers are used to calculate a mean log reduction (LR) value. Multi-laboratory studies were conducted on *Staphylococcus aureus, Pseudomonas aeruginosa* and *Mycobacterium terrae* to assess method performance within (repeatability) and between (reproducibility) labs. Sodium hypochlorite solutions and several antimicrobial liquids with a range of efficacy were tested in a blinded and replicated fashion. Repeatability (S_r) and reproducibility (S_R) standard deviations were calculated for the mean control LD and mean treated LR values. Across microbes, the control LDs exhibited S_r from 0.16 to 0.26 and S_R from 0.24 to 0.35. For *S. aureus* tests, the S_r for mean LRs ranged from 0.12 to 0.54 while the S_R ranged from 0.23 to 0.85. For tests involving *P. aeruginosa*, higher S_r (0.19 to 0.84) and S_R (0.28 to 1.2) for mean LRs were observed compared to *S. aureus*. The mean LRs for tests involving *M. terrae* exhibited similar ranges of S_r (0.25 to 0.49) and S_R (0.27 to 1.2) across the treatments. The S_r and S_R for treatments resulting in low LRs (0 to 1.5) or high LRs (4.5 to 6) were smaller with elevated variability occurring with moderately effective treatments. The studies provided the data necessary to further explore the use of the method for regulatory purposes.

Author Disclosure Block:

S.F. Tomasino: None. **R.M. Pines:** None.

Poster Board Number:

FRIDAY-342

Publishing Title:

The Role of Sodium Percarbonate as an Antimicrobial Ingredient of Laundry Detergents

Author Block:

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Abstract Body:

Background: In order to save energy there is a trend towards lower washing temperatures. Especially in countries where it is not common to use chlorine bleach, hygiene problems might be expected due to the lack of temperature as one major antimicrobial impact factor. In general, the decrease in temperature can be compensated for by other washing parameters, such as the use of activated oxygen bleach (AOB), which can be found in solid all-purpose detergents. Although AOB is well known as a biocidal active, it remains unclear to what extent it contributes to the antimicrobial efficacy of the washing process, since other factors such as detergency effects or the mechanical action of the washing machine do also lead to a reduction of microorganisms on the textile. **Methods:** To better understand the impact of the different parameters, we systematically investigated the antimicrobial efficacy of AOB in presence or absence of detergents with regards to time and temperature using quantitative suspension testing according to DIN EN 1276 and DIN EN 1650. **Results:** It turned out that AOB was able to increase the reduction of the chosen test organisms (*Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Trichophyton mentagrophytes* and *Candida albicans*) in a time and temperature dependent manner, although the effect strongly depended on the observed organism. While the inactivation of the bacterial strains and *Trichophyton* could be increased by up to three logarithmic steps when using AOB even at temperatures below 40°C, an additional temperature effect was needed for *Candida*. Together with the finding that the addition of detergents did not considerably increase the reduction of microorganisms in suspension, it must be assumed that the removal of bacterial and fungal cells from the textile (driven by either mechanical action or detergency effects) plays an important role in the antimicrobial effect of laundering.**Conclusions:** The results of our study help to understand the role of chemistry (such as AOB and detergents), time, temperature and mechanics for the inactivation of microorganisms in the laundry process and show the necessity for comprehensive investigations when assessing the antimicrobial efficacy of laundering.

Author Disclosure Block:

B. Brands: None. **S. Bloomfield:** None. **D.P. Bockmühl:** None.

Poster Board Number:

FRIDAY-343

Publishing Title:

Infectious Diseases (Id) Telemedicine: Characteristics of the First 6 Months of an Electronic Id Consultation Service at a Tertiary-care VA Medical Center

Author Block:

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Abstract Body:

Background: Electronic medical record systems and other digital technologies have made it possible to deliver quality healthcare in novel ways. Limited data is available regarding provision of ID expertise by these methods. In 2013, we added a physician-led, chart-based, rapid-turnaround, ID electronic consultation (E-consult) service to our pre-existing standard ID inpatient and outpatient consult services. We hypothesized that this service might be useful for particular types of infectious conditions, thus expanding capability in the standard consult arena. **Methods:** A retrospective analysis of patients undergoing ID E-consult for the first six months of our program was conducted. Patient characteristics, medical conditions, location of requesting healthcare providers, reasons for consultation, times to consult completion, and efficacy of consultation (as measured by the need for a further unexpected ID visit, either electronic or physical, within 30 days) were tabulated and expressed as a percentage of the whole, to construct a descriptive profile of this initial cohort of patients. **Results:** 105 ID E-consults were performed. The average age of the subjects was 63; 79% were Caucasian and 95% were male. 48% of consults came from clinics or nursing homes that were physically remote from the main hospital and our ID practice. The most common distinct reason for ID E-consult was management of urinary tract infection (UTI) (20%), followed by interpretation of culture data (14%), vaccine-related questions (13%), management of methicillin-resistant *Staphylococcus aureus* (MRSA) infection (10%) or *Clostridium difficile* infection (9%), concern for syphilis (5%), and travel medicine questions (4%). Average time to consult completion was 1.8 days, and only 6% of subjects required unexpected re-consultation or a physical visit within 30 days. **Conclusion:** We found electronic provision of rapid infectious diseases consultation services to be very efficacious, especially for particular ID syndromes. Such E-consults can successfully be deployed to expand the accessibility of quality ID expertise to patients and primary care providers. These findings could positively impact provision of ID care in busy, resource-poor, or physically remote healthcare settings.

Author Disclosure Block:

M.J. Skalweit: None. **U. Stiefel:** None.

Poster Board Number:

FRIDAY-344

Publishing Title:

Assessment Of Half-Mask Elastomeric Respirator And Powered Air-Purifying Respirator Reprocessing For An Influenza Pandemic

Author Block:

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Abstract Body:

Many health care agencies are considering the use of reusable respiratory protection devices (RPDs) to mitigate an RPD shortage due to an influenza pandemic. US regulators are also considering stockpiling reusable RPDs for a pandemic event, but limited data exists on cleaning and disinfection of these devices. This study focuses on 1) determining if standard protocols provided by the Occupational Safety and Health Administration (OSHA) and manufacturers are capable of disinfecting influenza-contaminated reusable RPDs and 2) assess the durability and performance of these devices after being treated up to 150 times using inert aerosol filtration testing and manikin-based face piece fit procedures. Five half-mask elastomeric respirator (HMER) and three powered air-purifying respirator (PAPR) models were contaminated with influenza and artificial skin oil (sebum) on five unique surfaces. Triplicate RPDs per model were cleaned, or cleaned and decontaminated using standard protocols. Presence of viable influenza was determined via swab sampling and a median tissue culture infectious dose ($TCID_{50}$) assay with Madin-Darby Canine Kidney cells. Filtration efficiency testing and manikin-based fit assessments were performed on HMERs and PAPRs after 75 and 150 cleaning and decontamination cycles. No detectable influenza was found on all models of HMERs and PAPRs regardless of inoculation location or treatment. The mean log reduction was ~5-log $TCID_{50}$ for the HMER/PAPR bodies and on the PAPR hoods, but was lower on some straps (~3-log) due to lower extraction efficiencies. Treated HMERs and PAPRs showed no significant degradation in filtration efficiency or fit performance. These data provide the first evidence that HMERs and PAPRs contaminated with influenza (and sebum) are capable of being disinfected using OSHA or manufacturer-defined cleaning protocols. Cleaning alone was shown to be sufficient for removing/killing influenza. Fit and filtration performance test data indicate the devices would be acceptable for use after 150 cleaning and disinfection treatments. These combined data should provide confidence to hospitals that HMERs and PAPRs can be effectively reprocessed. Time and logistics required for RPD reprocessing may be significant; future work will focus on evaluating automated methods.

Author Disclosure Block:

C. Lawrence: None. **D. Mills:** None. **M. Sandoval:** None. **D. Harnish:** None. **B. Heimbuch:** None.

Poster Board Number:

FRIDAY-345

Publishing Title:

Carrying a Buddy to the Gym: Molecular Epidemiological Characterization of *Staphylococcus aureus* in Multiple Gym Facility Types

Author Block:

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Abstract Body:

Staphylococcus aureus is a commonly found bacterium of the nose and throat of healthy individuals and can lead to infections and death. Methicillin-resistant *Staphylococcus aureus* (MRSA) invasive infections cause 11,000 yearly deaths with approximately 1 in 3 people asymptomatically colonized with *S. aureus* and ~2% colonized with MRSA. Initially hospital patients were the main risk group for MRSA carriage and infection; though places of close contact such as gym facilities have emerged as risk factors. We investigated environmental contamination of gym facilities with *S. aureus* in order to determine molecular types and antibiotic susceptibility profiles of isolates, which may have the ability to be transmitted to facility patrons. Environmental swabs (n=270) were obtained from several gym facilities across Northeast Ohio including cross-fit type (n=4), traditional iron gyms (n=5), community centers (n=4), and hospital-associated facilities (n=3). Samples were taken from 18 different surfaces per facility and processed within 24 hours using typical bacteriological methods. Five colonies per positive sample were then subjected to antibiotic susceptibility testing and molecular characterization (multilocus sequence, PVL and *mec*A PCR, and *spa* typing). The overall prevalence of *S. aureus* on environmental surfaces in gym facilities was 37.4% (101/270). The most commonly colonized surfaces were the weight ball (8.9%) as well as the treadmill handle (7.9%) and weight plates (7.9%). Interestingly, the bathroom levers and handles were the least colonized in both males and female facilities (1-3%). Of note, traditional iron gyms (44%) and community gyms (39%) had the highest colonization prevalence among sampled surfaces with hospital associated (33%) and crossfit (30%) the least colonized. All samples were resistant to penicillin and several were multidrug resistant, with preliminary results indicating an intermediate vancomycin resistant *S. aureus* isolate present. Additional characterization is ongoing. Our pilot study indicates that a diverse number of surfaces are contaminated by *S. aureus* and that additional studies are needed to better characterize the metagenomic structure of both the surfaces and the patrons that come to these differing facility types.

Author Disclosure Block:

M.R. Dalman: None. **H. Naimi:** None. **S. Bhatta:** None. **D. Thapaliya:** None. **T.C. Smith:** None.

Poster Board Number:

FRIDAY-346

Publishing Title:

Pneumococcal Multiple Serotype Carriage Following Vaccination with the 13-Valent Pneumococcal Conjugate Vaccine

Author Block:

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Abstract Body:

Background: *Streptococcus pneumoniae* is a Gram-positive pathogen that frequently colonizes the human nasopharynx, particularly children up to six years old. There are over 90 different capsular types (or serotypes) that are recognized by the immune system. Colonization with multiple serotypes, or co-colonization, is frequent and an important driving force for competition and evolution of the species, either by competition for nutrients or by promoting gene exchange between strains. The study of co-colonization is also important to understand how the pneumococcal population is changing with vaccination. In this study we aimed to determine the impact of the 13-valent pneumococcal conjugate vaccine (PCV13) on multiple serotype carriage.**Methods:** Nasopharyngeal samples from fully vaccinated pneumococcal carriers (4 doses of PCV13, n=141, aged 18-72 months) or from non-vaccinated pneumococcal carriers (0 doses of any PCV, n=140, same age group) were analyzed. Co-colonization was evaluated by DNA hybridization with a molecular serotyping microarray that detects all known serotypes.**Results:** Vaccinated children presented a lower prevalence of co-colonization than the non-vaccinated group (20.6% vs 29.3%, p=0.097), and a significantly lower proportion of PCV13 serotypes (6.4% vs 38.5%, p=0.0001). PCV13 serotypes found among vaccinated children were frequently detected as a minor serotype in co-colonization with a more abundant non-vaccine serotype. Also, vaccinated children were colonized by a significantly higher proportion of commensal non-pneumococcal *Streptococcus spp*. (58.2% vs 42.8%, p=0.012). In vaccinated children there were significantly less non-vaccine type (NVT) co-colonization events than expected based on the distribution of these serotypes in non-vaccinated children.**Conclusions:** The results suggest that vaccinated children have lower pneumococcal co-colonization prevalence due to higher competitive abilities of non-vaccine serotypes expanding after PCV13 use. This might represent an additional benefit of PCV13, as decreased co-colonization rates translate into decreased opportunities for horizontal gene transfer and might have implications for the evolution and virulence of pneumococci.

Author Disclosure Block:

C. Valente: None. **J. Hinds:** None. **K.A. Gould:** None. **H. de Lencastre:** None. **R. Sá-Leão:** E. Grant Investigator; Self; Pfizer. L. Speaker's Bureau; Self; Pfizer.

Poster Board Number:

FRIDAY-347

Publishing Title:

Optimization of Cluster Analysis Based on Antimicrobial Resistance Profile of Methicillin-resistant *Staphylococcus aureus* (Mrsa) Isolates

Author Block:

H. Mikamo, H. Tani, N. Nishiyama, Y. Koizumi, Y. Yamagishi; Aichi Med. Univ., Aichi, Japan

Abstract Body:

Background: Pulsed-field gel electrophoresis (PFGE) is the gold standard used for the epidemiological analyses of methicillin-resistant *Staphylococcus aureus* (MRSA), but this method has many disadvantages including procedural complexity, the long period of 5 days or more required to obtain results, and the need for special equipment. New techniques such as multi-locus sequence typing, phage open reading frame typing, and repetitive sequence-based PCR are difficult to perform in routine laboratories. Antimicrobial susceptibility testing is routinely performed in hospital laboratories and produces a wide range of results, there have been limited reports of the use of drug susceptibility testing for epidemiological analysis.**Method:** We examined 402 MRSA strains isolated from clinical specimens in our hospital between 2010 and 2011 to evaluate the similarity between cluster analysis of drug susceptibility tests and PFGE.**Results:** The 402 strains tested were classified into 27 PFGE patterns (151 subtypes of patterns). Cluster analyses of drug susceptibility tests with the cut-off distance yielding a similar classification capability showed favorable results - when the MIC method was used, and MIC values were used directly in the method, the level of agreement with PFGE was 74.2% when 15 drugs were tested. the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) method was effective when the cut-off distance was 16. Using the SIR method in which susceptibility (S), intermediate (I), and resistance (R) were coded as 0, 2, and 3, respectively, according to the CLSI criteria, the level of agreement with PFGE was 75.9% when the number of drugs tested was 17, the method used for clustering was the UPGMA, and the cut-off distance was 3.6. In addition, to assess the reproducibility of the results, 10 strains were randomly sampled from the overall test and subjected to cluster analysis. This was repeated 100 times under the same conditions. The results indicated good reproducibility of the results, with the level of agreement with PFGE showing a mean of 82.0%, standard deviation of 12.1%, and mode of 90.0% for the MIC method and a mean of 80.0%, standard deviation of 13.4%, and mode of 90.0% for the SIR method.**Conclusions:** Cluster analysis for drug susceptibility tests would be useful for the epidemiological analysis of MRSA.

Author Disclosure Block:

H. Mikamo: None. **H. Tani:** None. **N. Nishiyama:** None. **Y. Koizumi:** None. **Y. Yamagishi:** None.

Poster Board Number:

FRIDAY-348

Publishing Title:

Microbiological Characteristics of Group B *Streptococcal* Isolates (GBS) from Enhanced Surveillance of Invasive Disease in Young Infants, UK and Ireland, 2014-2015

Author Block:

D. Patel[1], T. Lamagni[1], P. T. Heath[2], C. O'Sullivan[2], V. J. Chalker[1], G. Kapatai[1], A. Reynolds[3], R. Cunney[4], L. Doherty[5], A. Efstratiou[1]; [1]Publ. Hlth.England, London, United Kingdom, [2]St George's, Univ. of London, London, United Kingdom, [3]Hlth.Protection Scotland, Glasgow, United Kingdom, [4]Hlth.Protection Surveillance Ctr., Dublin, Ireland, [5]Publ. Hlth.Agency, Belfast, United Kingdom

Abstract Body:

Infant GBS disease surveillance is essential to assess the impact of current prevention guidelines and guide vaccine development and implementation programmes. To update our estimates of incidence, risk factors, outcome and microbiology, enhanced infant GBS disease surveillance was established across the UK and Ireland during 2014/15.Cases diagnosed from 1st April 2014 to 30th April 2015 were identified through paediatric and microbiology surveillance networks and defined as infants aged <90 days from whom GBS was isolated from a normally sterile site. Isolates were characterised by serotyping, multilocus sequence typing (MLST) and antimicrobial susceptibility testing.GBS were characterised from 399 of 817 confirmed cases, 208 were early onset cases (age <7 days; EOD) (52%) and 191 late onset (age 7-89 days; LOD) (48%). Serotypes Ia, Ib, II, III and V (n=386) comprised 94% of isolates. Serotype III (n=246) and Ia (n=69) were the most prevalent serotypes. The majority of serotype II (94%) and V isolates (78%) were from infants with EOD. Isolates from EOD cases (n=186) were assigned to 29 MLST sequence types (ST) (index of discrimination (D)=0.81) and from LOD (n=160) to 21 STs (D=0.60). ST17 was the most prevalent (n=173, 42%), associated with 67% of EOD and 58% of LOD meningitis cases. Serotype II and V displayed the highest resistance to clindamycin (cli) (33% and 41%) and erythromycin (50% and 63% respectively). Overall 16% of EOD and 20% of LOD isolates were cli resistant but resistance among serotype V isolates from EOD was 26% and 83% in LOD.Differences in serotype, MLST and antimicrobial susceptibilities between EOD and LOD isolates were identified. ST17, serotype Ia and III were common amongst invasive GBS infections indicating protection with a pentavalent vaccine containing these serotypes may potentially provide protection against up to 94% of cases. This surveillance data will contribute towards future clinical guidelines for GBS prevention and to vaccine implementation.

Author Disclosure Block:

D. Patel: None. **T. Lamagni:** None. **P.T. Heath:** None. **C. O'Sullivan:** None. **V.J. Chalker:** None. **G. Kapatai:** None. **A. Reynolds:** None. **R. Cunney:** None. **L. Doherty:** None. **A. Efstratiou:** None.

Poster Board Number:

FRIDAY-349

Publishing Title:

Evidence For Horizontal Transmission Of *streptococcus Mutans* Among Children And Their Family Members By Rep-Pcr

Author Block:

S. S. Momeni, J. Whiddon, S. A. Moser, N. K. Childers; Univ. of Alabama at Birmingham, Birmingham, AL

Abstract Body:

Streptococcus mutans is frequently associated with dental caries. Epidemiological studies of *S. mutans* typically focus on vertical or horizontal transmission, with mother-to-child transmission most commonly considered the primary route of transmission. However, studies evaluating both routes of transmission are limited. In this study, horizontal and vertical transmission of *S. mutans* isolates among index children (IC) and with their household family members (HH) were evaluated by repetitive extragenic palindromic PCR (rep-PCR). *S. mutans* isolates from 119 African American IC (N=7,494) and 414 of their HH (N=5,651) were evaluated by DiversiLab rep-PCR. For each rep-PCR genotype, IC were evaluated as having either no match with any HH or matching with one or more HH. IC with no match were classified as horizontal while IC sharing a genotype with one or more HH were classified as vertical transmission. The mean number of genotypes per IC was 2.69 (range 1-9). Twenty-nine IC (24%) shared no genotype with any HH while 33 IC (28%) shared all genotypes within the household. There were 86 IC (72%) that had at least one genotype not shared with any HH. Since IC can have multiple genotypes, analysis was done for individual genotypes. This resulted in 157 cases among 21 genotypes where the IC did not share the GT with any HH. For these cases, 53 had only 1 isolates (transient), 57 had 2-9 isolates (borderline), and 47 had >10 isolates (established). There were 158 cases among 21 genotypes where an IC and one or more HH shared the genotype. Of these, 87 cases (55%) had genotypes shared with more than one HH. IC most commonly shared a genotype with mother (54%), siblings (46%), and/or cousins (23%). While this study is consistent with mothers as a primary route of transmission for *S. mutans*, it also presents compelling evidence for horizontal transmission among children. This is the largest know study of extra-familial horizontal cases evaluating both horizontal and vertical routes of transmission in the same study. The data is more robust as it considers transient and established genotypes as well as evaluates multiple HH. These findings indicate the importance of considering horizontal, as well as vertical, acquisition of *S. mutans* in prevention strategies for dental caries.

Author Disclosure Block:

S.S. Momeni: None. **J. Whiddon:** None. **S.A. Moser:** None. **N.K. Childers:** None.

Poster Board Number:

FRIDAY-350

Publishing Title:

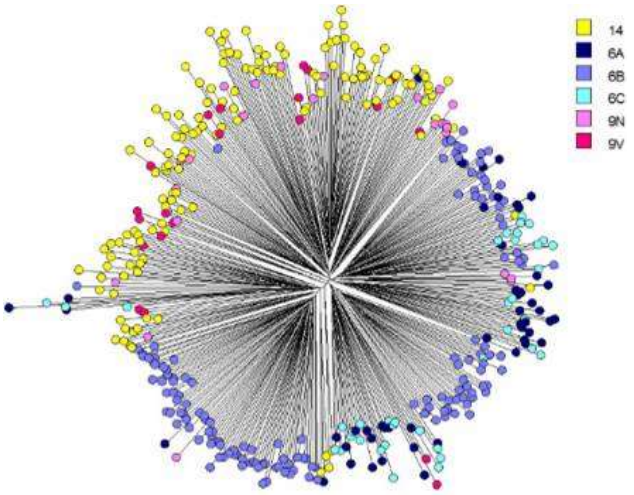
Maldi-Tof Ms for Capsular Typing of *Streptococcus pneumoniae* Isolates

Author Block:

T. C. A. Pinto, N. S. Costa, L. F. S. Castro, R. L. Ribeiro, J. M. Peralta, L. M. Teixeira; UFRJ, Rio de Janeiro, Brazil

Abstract Body:

Streptococcus pneumoniae can be classified in more than 90 capsular types, as determined by Quellung reaction or PCR. Such methods, however, can be expensive, laborious or unable to accurately discriminate among certain serotypes; thus, determination of capsular types, which can predict some virulence and resistance traits, is mainly restricted to research laboratories, being rarely performed in the clinical setting. In the present study, MALDI-TOF MS was evaluated as an alternative tool to characterize 421 pneumococcal isolates belonging to types 6A, 6B, 6C, 9N, 9V or 14. Capsular types were determined by Quellung reaction and/or multiplex PCR. For MALDI-TOF MS analysis, each isolate was submitted to an in-house extraction protocol using formic acid and acetonitrile before applying to the target plate and covering with CHCA matrix. Measurements were performed with a Microflex LT mass spectrometer using the default parameters and generating spectra in the range of 2,000-20,000 *m/z*. Spectra were analyzed with BioNumerics software v7.5. Number of detected peaks (or biomarkers) varied from 22 (in serotype 6A isolates) to 38 (in serotype 6C isolates). Isolates were distributed mainly according to the type in a Neighbor Joining tree and capsular types were discriminated by the presence/absence of 5 selected biomarkers (Graphic 1). The results suggest that MALDI-TOF MS is a promising alternative for typing pneumococcal strains, highlighting its usefulness for rapid and cost-effectiveness routine application in clinical laboratories.



Biomarker*/ serotype	4,213	6,269	6,642	6,671	8,395
6A	Absent	Absent	Present	Absent	Present
6B	Absent	Absent	Present	Absent	Absent
6C	Absent	Absent	Absent	Absent	Present
14	Absent	Absent	Absent	Present	Present
9V	Absent	Present	Absent	Present	Present
9N	Present	Absent	Present	Absent	Absent

*Position in the spectra (m/z) using a tolerance of ± 0,002

Author Disclosure Block:

T.C.A. Pinto: None. **N.S. Costa:** None. **L.F.S. Castro:** None. **R.L. Ribeiro:** None. **J.M. Peralta:** None. **L.M. Teixeira:** None.

Poster Board Number:

FRIDAY-351

Publishing Title:

***Streptococcus pneumoniae* of Serogroups 6 and 19 Circulating in Brazil Over a Period of 24 Years**

Author Block:

L. Oliveira[1], L. Castro[1], A. Souza[1], S. Chochua[2], P. Hawkins[2], L. McGee[2], T. Pinto[1], L. Teixeira[1]; [1]Univ.e Federal do Rio de Janeiro, Rio de Janeiro, Brazil, [2]CDC, Atlanta, GA

Abstract Body:

Streptococcus pneumoniae (*Sp*) is a leading human pathogen that can be characterized into more than 90 capsular types, based on the composition of its polysaccharide capsule. *Sp* strains belonging to certain serotypes, including those of serogroups 6 and 19, can be highlighted by their high prevalence and association with antimicrobial resistance. In this study, 488 *Sp* isolates of serogroups 6 and 19 recovered from patients and healthy carriers in Brazil over a period of 24 years, were subjected to serotyping, multilocus sequencing typing and antimicrobial susceptibility testing to penicillin. Serotype 6B was the predominant serotype (163 strains; 33.4%), followed by serotypes 19F (146 strains; 30.5%, 0.6% being serotype 19Fvar), 19A (61 strains; 12.5%), 6A (58 strains; 11.9%) and 6C (56 strains; 11.5%). Serotypes 6A and 19F were uniformly distributed over the period of time investigated, while serotype 6B has been decreasing and serotypes 6C and 19A have been increasing. Occurrence of penicillin non-susceptible pneumococci (PNSP; MICs of 0.12-4 µg/ml), was higher among serogroup 6 (especially serotype 6B) isolates (81 out of 278 isolates; 29%), and serogroup 19 (46 out of 210 strains; 22%). The highest penicillin MICs were observed for serotype 19A isolates. A total of 45 and 13 different STs were, respectively, detected among serogroup 6 (Simpson's Index of Diversity, SID of 0.952) and serogroup 19 (SID of 0.818) isolates. ST748 was the most common among serogroup 6 isolates, being associated with PNSP and circulating in our country since 1991. ST733 was the most common among serotype 19A isolates, associated with penicillin susceptibility and has been circulating in our setting since 2008. Portugal^{19F}-ST177 was most frequent among serotype 19F isolates, being detected since 1997. Other international clones, including Greece^{6B}-22, Spain^{6B}-2 and Taiwan^{19F}-14, were also identified. Our results demonstrate the genetic diversity among serogroups 6 and 19 circulating in Brazil over a large period of time, revealing the presence of both regional and international clones, some of them highly associated with penicillin non-susceptibility, reinforcing the need for constant surveillance of such serogroups.

Author Disclosure Block:

L. Oliveira: None. **L. Castro:** None. **A. Souza:** None. **S. Chochua:** None. **P. Hawkins:** None. **L. McGee:** None. **T. Pinto:** None. **L. Teixeira:** None.

Poster Board Number:

FRIDAY-352

Publishing Title:

Distribution of *psp*a and Pilus Type I Genes Among *Streptococcus pneumoniae* Strains Isolated Over a Period of 22 Years in Brazil

Author Block:

A. Souza, N. Costa, S. Pina, T. Pinto, J. Peralta, L. Teixeira; Univ.e Federal do Rio de Janeiro, Rio de Janeiro, Brazil

Abstract Body:

Streptococcus pneumoniae (*Sp*) is a leading cause of vaccine-preventable infections. The polysaccharide capsule is the major virulence factor and the basis for currently licensed vaccines. In Brazil, the 10-valent pneumococcal conjugate vaccine (PCV10) has been available since 2010. In addition, surface proteins such as PspA and pilus type 1 (PI-1) have more recently been proposed as serotype-independent vaccine targets, but data on their prevalence and diversity are still lacking in many areas. In this study, the presence of genes coding for PspA and PI-1 was evaluated among 404 *Sp* strains belonging to 24 serotypes and recovered from different clinical specimens between 1988 and 2012, in Brazil. All isolates had *psp*A genes; 36% and 63% of the strains had, respectively, *psp*A genes of families 1 (mostly clade 1) and 2 (mostly clade 3). Family 3 *psp*A genes (clade 6) were detected in 1% of the strains, all of them being non-typeable, penicillin susceptible pneumococci (PSP) and recovered from asymptomatic nasopharyngeal colonization (NPC). *psp*A genes of family 2 were the most common among isolates belonging to both PCV10 serotypes (65%) and non-PCV10 serotypes (61.3%), as well as they were the most prevalent among isolates from both invasive pneumococcal disease (IPD; 65.5%) and NPC (60.7%). *psp*A genes of family 2 were also highly prevalent among penicillin non-susceptible pneumococci (PNSP; 80%), while family 1 genes were more common among PSP strains (50.5%). A total of 44% of the strains also had PI-1 genes, which were commonly found among PNSP strains (72.5%), isolates belonging to PCV10 serotypes (53.2%), and strains recovered from IPD (70%); but were more rarely detected among PSP (28%) and isolates belonging to non-PCV10 serotypes (6.3%). PI-1 genes were also more common among isolates presenting *psp*A genes of family 2 (92%) when compared to family 1 (8%), while no isolate of family 3 had PI-1 genes. Occurrence of strains harboring PI-1 genes has been increasing along the years, while prevalence of isolates presenting *psp*A genes of family 1 has been decreasing. Our results shows that *psp*A genes of family 2 and PI-1 genes are highly prevalent and have been increasingly detected among *Sp* strains isolated in Brazil since the late 80's, being especially associated with PCV10 serotypes and PNPS.

Author Disclosure Block:

A. Souza: None. **N. Costa:** None. **S. Pina:** None. **T. Pinto:** None. **J. Peralta:** None. **L. Teixeira:** None.

Poster Board Number:

FRIDAY-353

Publishing Title:

Emergence of *Streptococcus pneumoniae* Serotype 6c After Pcv10 Implementation on Brazil

Author Block:

J. Caierão[1], **G. Rosa da Cunha**[1], M. P. Mott[1], P. A. d'Azevedo[1], L. McGee[2], C. A. G. Dias[1];
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Abstract Body:

Background: Childhood immunization against *Streptococcus pneumoniae* decreased the incidence of pneumococcal diseases around the world; despite of reports, in different regions, of emergence of non-vaccine serotypes, such as 6C. We aimed to analyze frequency of occurrence and molecular epidemiology of serotype 6C before and after PCV10 vaccination in Brazil.**Methods:** *S. pneumoniae* were recovered from invasive and non-invasive sites of patients attended in Porto Alegre, South Brazil during 2007-2015. Identification was performed as described elsewhere (SPELLERBERG & BRANDT, 2011). Isolates were serotyped using multiplex PCR (Dias et al, 2007) and/or Quellung reaction. MLST was performed for molecular analysis (Enright & Spratt,1998).**Results:** From 2007 to 2015, 586 *S. pneumoniae* were serotyped, 202 before and 394 after vaccination; 582 out of 586 had defined site on medical records: 465 pneumococci were recovered from invasive sites, mainly blood, and 117 from other sites. Among the 572 isolates recovered from patients that we had information considering age, 52 were 5 years old or younger, whereas 156 had 65 years or more. Of note, 247 isolates belonged to PCV10 serotypes. Serogroup 6 represented 8.8% (52/586) of all serotypes: 6B (20/586 - 3.4%), 6A (17/586 - 2.9%), and 6C (15/586 - 2.5%). All 6C isolates were recovered after PCV10 implementation and 9 out of 15 came from invasive sites. One isolate of serotype 6C (6.6%) was recovered from vaccine-target population and 40% were related to elderly. MLST results demonstrated a considerable genetic diversity, with six different ST (3390, 2185, 172, 6373, 7288, 9007) and four other representing a new combination of alleles. ST 3930 was the most common ST representing 33.3% of all, being associated with other serotype 6C pneumococci from other region of Brazil before vaccination.**Conclusions:** Serotype 6C has not suffered cross protection of vaccination targeting serotype 6B. Although with a predominant ST, dissemination of serotype 6C does not seem to have a clonal profile, which could suggest distinct genetic origins. Such emergence may be a result of selective pressure exerted by PCV10, but other causes should be considered.

Author Disclosure Block:

J. Caierão: None. **G. Rosa da Cunha:** None. **M.P. Mott:** None. **P.A. d'Azevedo:** None. **L. McGee:** None. **C.A.G. Dias:** None.

Poster Board Number:

FRIDAY-354

Publishing Title:

Comparison of Snp-based and Cgmlst-based Typing of *Listeria monocytogenes* Isolates from Seeliger's Historical "Special Listeria Culture Collection"

Author Block:

P. Hayden[1], **C. J. GRIM**[2], A. Pietzka[1], M. Blaschitz[3], A. Indra[3], C. W. Sensen[4], F. Allerberger[1], T. Rattei[5], W. Ruppitsch[1]; [1]Austrian Agency for Hlth.and Food Safety, Vienna, Austria, [2]Food and Drug Admin., Laurel, MD, [3]Austrian Agency for Hlth.and Food Saftey, Vienna, Austria, [4]Graz Univ. of Technology, Graz, Austria, [5]Univ. of Vienna, Vienna, Austria

Abstract Body:

Background: Whole genome sequencing has emerged as the ultimate tool for comparison of bacterial isolates for surveillance and outbreak investigation. In this study we sequenced 12 serotype 4b isolates of Seeliger's historical 'Special Listeria Culture Collection' (SLCC) and compared typing results obtained by mapping of read data against a reference genome followed by single nucleotide polymorphism (SNP) analysis to a de novo assembly of read data followed by a gene-by-gene comparison of a defined core genome (cgMLST).**Material/methods:** Genomic DNA was isolated from SLCC isolates grown overnight and Nextera XT libraries were sequenced on a MiSeq (Illumina). Reads were processed, de-novo assembled with Velvet v.1.1.08 and cgMLST allelic schemes were extracted using SeqSphere+ v2. For SNP based analysis, the CFSAN SNP pipeline was used to produce a SNP matrix by mapping unprocessed reads on the reference genome of strain J1-220 (GenBank CP006047.2). Phylogeny was calculated using RAxML v8.2.2. Visualization was performed on itol.embl.de.**Results:** The Velvet assemblies had a median N50 value of 229,641 bp. Both assemblies and read mappings returned an average coverage of 92x. The SNP-analysis for 12 serotype 4b SLCC isolates resulted in a total of 3,307 single nucleotide variant positions distributed over the reference genome. In the core genome MLST, 205 out of 1,701 genes had more than one sequence type. All isolates could be differentiated by SNP and cgMLST analysis,**Conclusions:** Comparison of 12 serotype 4b isolates by NGS using a standardized cgMLST based typing scheme for Listeria monocytogenes with a SNP analysis pipeline reveals no major differences of these two methods. Thus, although SNP based typing provides higher resolution than cgMLST and might be useful in certain situations, the defined cgMLST scheme has a similar discriminatory power, allows the global exchange of data, and is easier to standardize than mapping followed by SNP calling.

Author Disclosure Block:

P. Hayden: None. **C.J. Grim:** None. **A. Pietzka:** None. **M. Blaschitz:** None. **A. Indra:** None. **C.W. Sensen:** None. **F. Allerberger:** None. **T. Rattei:** None. **W. Ruppitsch:** None.

Poster Board Number:

FRIDAY-355

Publishing Title:

Molecular Comparison of Vancomycin-Resistant *Enterococcus faecium* Bloodstream and Stool Isolates from a Hematology Unit in Salt Lake City, Utah

Author Block:

M. A. GAZDIK, Z. Burr, J. Coombs, B. Lopansri, C. Ford; LDS Hosp., Salt Lake City, UT

Abstract Body:

Background: *Enterococci*, most commonly *E. faecium* and *E. faecalis*, are one of the most frequently isolated organisms in hospital-acquired infections in the US. Patients on hematology/oncology and stem cell transplant units are at high risk of gastrointestinal tract colonization by vancomycin-resistant *E. faecium* (VREfm), a risk factor for development of VREfm bloodstream infection (BSI). The purpose of our study was to identify features of bacterial strains associated with BSI by comparing the molecular characteristics of VREfm from patients with BSI to isolates from colonized patients who never developed infection.**Methods:** DNA was extracted from 38 blood stream isolates and 41 stool isolates followed by whole genome sequencing using NexteraXT library preparation and the MiSeq sequencing platform (Illumina). Read filtering, trimming and de novo assembly of contigs was completed with CLC Genomics Workbench. Assembled contigs were uploaded into the MLST, ResFinder 2.1, and CSI Phylogeny 1.1 pipelines available at the Center for Genomic Epidemiology for molecular characterization. A local BLAST database of Enterococcus virulence genes was generated and searched against assembled contigs in CLC Genomics Workbench.**Results:** Blood isolates were composed of 13 MLST strain types while 12 STs were found in stool colonies, however there was no ST statistically associated with either isolate type. Similarly there was no statistical difference found for any virulence factor or antibiotic resistance gene. In fact isolates showed a high homogeneity of virulence factors, with all isolates containing: *acm*, *ebp*A,B,C, *scm*, *fms*14-17-13, *fms*11-19-16, and *fms*21-20 while none contained *gel*E and only 1 contained *hyl*E.**Conclusions:** Our data indicate that patients on our hematology floor are colonized and infected by similar isolates, indicating that the risk of BSI in colonized patients is more indicative of patient characteristics than traits of the colonizing strain.

Author Disclosure Block:

M.A. Gazdik: None. **Z. Burr:** None. **J. Coombs:** None. **B. Lopansri:** None. **C. Ford:** None.

Poster Board Number:

FRIDAY-356

Publishing Title:

Changes in Antimicrobial Resistance and Population Structure of *Streptococcus pneumoniae* Colonizing Children after Four Years of Routine Conjugate Vaccines Use In Brazil

Author Block:

F. P. G. Neves[1], N. T. Cardoso[2], A. R. V. Souza[3], R. E. Snyder[4], M. M. Marlow[4], T. C. A. Pinto[3], S. Chochua[5], P. Hawkins[5], L. McGee[5], L. M. Teixeira[3], L. W. Riley[4]; [1]Univ. of California Berkeley, Berkeley, CA, [2]Univ.e Federal Fluminense, Niterói, Brazil, [3]Univ.e Federal do Rio de Janeiro, Rio de Janeiro, Brazil, [4]Univ. of California, Berkeley, CA, [5]CDC, Atlanta, GA

Abstract Body:

In 2010, the 10-valent pneumococcal conjugate vaccine (PCV10) was progressively implemented regionally and free of charge into the Brazilian childhood immunization schedule, whereas simultaneously the 13-valent vaccine (PCV13) was only offered in private clinics. As part of a study of the response of the pneumococcal population to conjugate vaccines, we report the findings from isolates collected immediately before and after four years of routine use of conjugate vaccines in Niterói, Brazil. We used multilocus sequence typing (MLST) to analyze 257 pneumococcal carriage isolates obtained from children (aged < 6 years) during 11/2009-08/2010 (125 isolates; pre-PCV10/13) and 09-12/2014 (132 isolates; post-PCV10/13). We determined minimum inhibitory concentrations (MIC) to penicillin (PEN), ceftriaxone (CTX) and erythromycin (ERY) with E-test method. MLST revealed 98 different sequence-types (ST), including 37 new ST. Thirty-three ST were only found pre-PCV10/13, 48 only post-PCV10/13 and 17 from both periods. PEN non-susceptible pneumococci (MIC = 0.12-8 µg/mL) made up 24% (30/125) and 37.9% (50/132) of the pre- and post-PCV10/13 isolates, respectively, encompassing 14 and 24 distinct ST in each period. The highest PEN and CTX MIC were observed for the serotype 14-ST156 complex isolates pre-PCV10/13 and for the serotype 19A-ST320 complex isolates post-PCV10/13. Pre-PCV10/13, ERY resistance (MIC = 2 and 4 µg/mL) was restricted to two (1.6%) serotype 6A-ST1190 isolates Post-PCV10/13, 36 (27.3%) isolates of 15 different ST were ERY-resistant (MIC from 1.5 to >256 µg/mL). Changes in ST distribution and increasing resistance to PEN and ERY were evident. Although sources of PEN resistance source remained polyclonal pre- and post-introduction, most resistant isolates belonged to a few clones, namely ST156 pre-PCV10/13 and ST386 post-PCV10/13. The increase in ERY resistance rates was also polyclonal, but largely explained by the emergence of the ST386.

Author Disclosure Block:

F.P.G. Neves: None. **N.T. Cardoso:** None. **A.R.V. Souza:** None. **R.E. Snyder:** None. **M.M. Marlow:** None. **T.C.A. Pinto:** None. **S. Chochua:** None. **P. Hawkins:** None. **L. McGee:** None. **L.M. Teixeira:** None. **L.W. Riley:** None.

Poster Board Number:

FRIDAY-357

Publishing Title:

Superantigen Gene Profile Diversity among Non-Pharyngeal Group A *Streptococcal* Isolates

Author Block:

E. Troche Gonzalez[1], **A. Inzunza Montiel**[1], R. Garza Velasco[2], L. M. Perea Mejia[1]; [1]Faculty of Med., UNAM, Mexico, Mexico, [2]Faculty of Chemestry, UNAM, Mexico, Mexico

Abstract Body:

Group A Streptococcus (GAS) is a strict human pathogen; clinical manifestation varies from noninvasive diseases, including tonsillitis and erysipelas, to invasive diseases, such as sepsis, streptococcal toxic shock syndrome (STSS), and necrotizing fasciitis. M-protein and superantigens are important virulence factors in GAS.To determine differences between *emm*-type and superantigens profile in GAS were analyzed 125 isolates from non-pharyngeal source. The *emm*-genotype and distribution of *speA, speB, speC, speG, speH, speI, speJ, speK, speL, speM, ssa, smeZ, prtF,* and *sic* genes to each isolate were determined using PCR and DNA sequencing.Twenty-four different *emm* types were determinate, of them *emm1, emm12, emm6, emm75* and *emm89* account >60% of isolates. *speB, speG* and *smeZ* genes were detected in >90% of all isolates in contast to *speA, speK, speL* and *ssa* genes detected in <25%. Nevertheless some genes were detected in association with certain *emm* types: *sic* (*emm1*); *speA*(*emm1* and *3*); *speC* (*emm2, 4, 11, 43, 59* and *89*); *speM* (*emm1, 12, 75* and *89*), *speL* (*emm75*); *speH* and *speI* (*emm2, 12* and *75*); *speK*(*emm3, 6, 49* and *60*); *ssa* (*emm4* and *22*).A high diversity in superantigen gene profiles both within and between *emm*-genotypes was found in this study indicating that superantigen gene profiling might be useful in tracking the spread of clones in the community and nosocomial outbreaks.

Author Disclosure Block:

E. Troche Gonzalez: None. **A. Inzunza Montiel:** None. **R. Garza Velasco:** None. **L.M. Perea Mejia:** None.

Poster Board Number:

FRIDAY-358

Publishing Title:

Decrease in Genotypic Penicillin Resistance Due to Serotype Replacement in Isolates from Pediatric Invasive Pneumococcal Diseases after PCV13 Introduction in Japan

Author Block:

S. Iwata, M. Morozumi, M. Takata, K. Ubukata; Keio Univ. Sch. of Med., Tokyo, Japan

Abstract Body:

Background: In Japan, pneumococcal vaccination of children was switched from 7-valent pneumococcal conjugate vaccine (PCV7) to PCV13 in November 2013. We aimed to clarify the capsular types and genotypes based on *pbp* gene alterations in *Streptococcus pneumoniae* (*Spn*) strains that were isolated from pediatric patients with invasive pneumococcal diseases (IPD) around the time of the change from PCV7 to PCV13.**Methods:** We collected *Spn* isolates (n=853) from IPD patients under 16 years of age between 2010 and 2015. We analyzed the capsular type using antisera from Statens Serum Institute (Denmark). Genotypic (g) penicillin resistance was classified into gPSSP, gPISP with one or two abnormal *pbp* genes, and gPRSP with the three abnormal *pbp* genes of *pbp1a*, *pbp2x*, and *pbp2b*, using real-time PCR methods for all *Spn*.**Results:** Introduction of PCV7 has significantly reduced vaccine-type IPD, especially meningitis, within 3 years. Whereas IPD caused by non-PCV7 types such as 19A, 15A, and 24 showed a relative increase, the resulting rate of PCV7 type isolates rapidly decreased from 73.3% in 2010 to 15.2% in 2012. Switching from PCV7 to PCV13 vaccination clearly resulted in a decrease in serotype 19A in 2015. The rate of PCV13 type isolates decreased from 89.0% in 2010 to 28.5% in 2013 and 10.4% in 2015, respectively. Yearly increases in non-PCV13 types were seen in serotypes 12, 22F, and 24. The statistical prevalence of gPRSP decreased from 54.7% in 2010 to 16.0% in 2015. In contrast, gPSSP gradually increased from 8.7% to34.0%.**Conclusions:** In highly populated Japan, epidemiological surveillance of vaccine-induced capsular type replacement of IPD isolates is necessary to continue on a national scale.

Author Disclosure Block:

S. Iwata: None. **M. Morozumi:** None. **M. Takata:** None. **K. Ubukata:** None.

Poster Board Number:

FRIDAY-359

Publishing Title:

***In Silico* Analyses of Antigenicity and Surface Structure Variation of an Emerging Pcv2b Mutant**

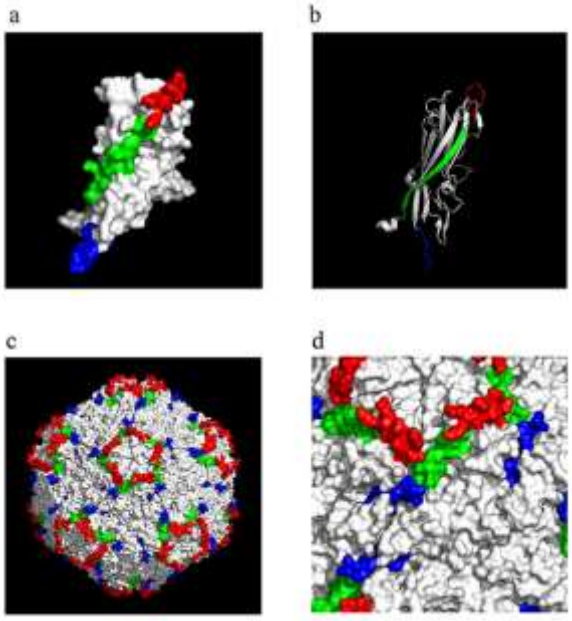
Author Block:

Y. Zhan; HUNAN AGRICULTURAL Univ., CHANGSHA, China

Abstract Body:

Background: Infection with PCV2 is a huge burden to swine industry worldwide. Currently there is no specific treatment available for PCV2 infection and PCVADs. Vaccination has proved to be the best way to prevent PCV2 infection in field. Thus, investigation of antigens and capsid structure variations of the virus is critical to improve diagnosis, new vaccine design and our understanding of PCV2 pathogenesis.**Methods:** In this study, 62 PCV2 isolates were identified from seven farms in southern China from 2013 to 2015, and phylogenetic trees were constructed based on whole-genome sequences or the *cap* gene. A novel putative recombination isolate was identified by recombination analysis software. The effects of the PCV2 capsid surface variation on antigenicity and surface structure were displayed by 3D structure simulation.**Results:** PCV2b was the main genotype in circulation throughout these farms, and an emerging mutant (PCV2b-1C), isolated from PCV2-vaccinated farms was the predominant strain prevalent on these farms. In addition, we isolated a new cluster that may represent evolution of the virus through recombination of PCV2b-1A/1B and PCV2b-1C. Finally, we discussed evidence that antigenicity and surface structure variation of the capsid resulted from mutation of the carboxyl terminus loop (Loop CT) of the PCV2b-1C Cap *in silico*.**Conclusions:** Our work characterized the prevalence, genetic variation and phylogenetic characteristics of PCV2 isolates in southern China over the past 3 years. In particular, alterations in antigenicity and surface structure of the capsid due to altered Cap amino acid, were evaluated and discussed. Therefore, ongoing evolution of PCV2b poses a future challenge regarding efficacy of PCV2a-based commercial vaccines.

Supplemental figure:



Author Disclosure Block:

Y. Zhan: None.

Poster Board Number:

FRIDAY-360

Publishing Title:

Molecular Characterization of *Mycobacterium orygis* Isolates from Animals of South Asia

Author Block:

J. Thapa, C Nakajima, Z Rahim, S Paudel, Y Shah, B Maharjan,A Poudel, A Sadula, GE Kaufman, D McCauley, KP Gairhe, Tsubota and Y Suzuki; Hokkaido Univ., Res. Ctr. for Zoonosis Control, Sapparo, Hokkaido, Japan

Abstract Body:

Background: *Mycobacterium orygis* has recently been categorized as a member of *M. tuberculosis* complex (MTC) and has been reported to cause tuberculosis (TB) in a variety of animals and humans. We have isolated this bacterium from different wild animals in Nepal and captured monkeys and dairy cattle in Bangladesh and have performed molecular characterization. **Methods:** MTC isolates from a captive spotted deer, a blue bull and a free ranging greater one-horned rhinoceros in Nepal and 18 isolates from a dairy cattle herd and 2 isolates from captured rhesus monkeys in Bangladesh were included. Most of these animals had TB suspected lung lesions. Spoligotyping, mycobacterial interspersed repetitive units-variable number of tandem repeats (MIRU-VNTR), region of difference analysis and multi locus sequence typing (MLST) of *gyrB, mmpL6*, TbD1, *PPE55* and Rv2042c genes were performed to ascertain species and molecular epidemiology. **Results:** All the isolates had a typical *M. orygis* spoligotype, SIT587. RD analysis and MLST typing of confirmed the isolates to be *M. orygis*. The MIRU-VNTR phylogenetic analysis of these isolates grouped into five clusters. These finding suggests wide distribution and long historical existence of *M. orygis* in the region. Also, most of the other reported isolates of *M. orygis* are from South Asia. Thus, these findings and observation provide evidence for endemic distribution of *M. orygis* in South Asia. **Conclusions:** *M. orygis* isolated from different animals in Nepal and Bangladesh were molecularly characterized. Findings suggested its endemic distribution in South Asia and presents new picture of tuberculosis in South Asia that may be helpful to understand the evolutionary history of MTC.

Author Disclosure Block:

J. Thapa: None.

Poster Board Number:

FRIDAY-361

Publishing Title:

Molecular Investigation of Circulating *Leptospira* spp. among Water Buffaloes in an Intensive Farming System in the Philippines

Author Block:

M. Villanueva[1], C. Mingala[2], C. Nakajima[1], N. Koizumi[3], Y. Suzuki[1]; [1]Res. Ctr. for Zoonosis Control, Hokkaido Univ., Sapporo, Japan, [2]Philippine Carabao Ctr. Natl. Headquarters and Gene Pool, Science City of Muñoz, Philippines, [3]Natl. Inst. of Infectious Diseases, Tokyo, Japan

Abstract Body:

Background: The water buffalo industry has earned more attention as an alternative source of milk and income for the rural farming communities in the Philippines. However, few serological studies showed the water buffalo as a potential reservoir host of *Leptospira* spp. causing leptospirosis which is also endemic in the country. Therefore, we investigated the circulating *Leptospira* spp. among water buffaloes in the Philippines using molecular techniques primarily in an intensive farm setting.**Methods:** We collected urine samples from field rats (*n*=21) and water buffaloes (*n*=170) from different groups and locations in one intensive-type water buffalo farm in the Philippines. Extracted DNA from urine was subjected to nested PCR amplifying *lipL32* and *flaB* genes as markers for pathogenic leptospires followed by amplification and analysis of *secY* gene for species identification.**Results:** Combined results of *lipL32* and *flaB* nested PCR found 29.4% (50/170) and 38% (8/21) pathogenic leptospires from buffaloes and rats, respectively. Sequence analysis of *secY* nested PCR-positive samples revealed its grouping into *L. borgpetersenii* and *L. kirschneri*. Moreover, clustering of sequences from both animals was found, which may indicate an on-going interspecies transmission. In addition, mixed *Leptospira* sequences were found from several positive animals, suggesting multiple infections. Finally, age and animal grouping was found to be associated with *Leptospira* infection.**Conclusions:** This study highlighted the role of water buffalo and the impact of management in the persistence of leptospirosis in an intensive farm setting in the Philippines. However, further investigation and appropriate control strategies are required to prevent leptospirosis from causing risks to public health and economic losses to the water buffalo farming industry.

Author Disclosure Block:

M. Villanueva: None. **C. Mingala:** None. **C. Nakajima:** None. **N. Koizumi:** None. **Y. Suzuki:** None.

Poster Board Number:

FRIDAY-362

Publishing Title:

Proteomic Analysis and Molecular Characterization of Acanthomoeba Spp. Isolates from Amoebic Keratitis and Meningo-Encephalitis from Northern India

Author Block:

G. Satpathy, H. S. Behera, T. Agarwal, R. Tandon; All India Inst. of Med. Sci., New Delhi, India

Abstract Body:

Background: Ocular infections caused by *Acanthomoeba spp*. are severe, sight threatening and are increasingly reported worldwide. This study aimed to determine genotypes of *Acanthomoeba spp*. isolates from corneal scrapings (n=20) and cerebrospinal fluid (CSF) (n=6) of meningo-encephalitis patients attending a tertiary care hospital and characterize trophozoite and cyst specific proteins from *Acanthomoeba spp.***Methods:** DF3 region (280bp) of 18S RNA gene was PCR amplified and amplicon sequences used for phylogenetic analysis using MEGA-6 programme for genotyping with DNA from axenic culture of 26 *Acanthomoeba spp.* isolates. The trophozoites and cyst proteins from an *Acanthomoeba spp*. eye isolate were purified from axenic culture and analyzed by 2D gel electrophoresis. Two unique, prominent protein spots each from trophozoites and cysts were characterized by MALDI TOF/TOF MS analysis and validated by reverse genomics using real time PCR.**Results:** Phylogenetically, 16 eye and 6 CSF isolates aligned with T4 genotypes of *Acanthomoeba spp*., 2 eye isolates aligned with T10 genotype and 2 were nontypable. Nontypable strains showed <90% sequence similarity with other isolates and reference genotypes from NCBI database. Both trophozoites and cysts showed proteins varying from 1kDa to >200 kDa. In mass spectroscopy, the trophozoite unique proteins were identified as; T1 (acidic) "hypothetical protein ACA1 of *A.castellani* (score: 331 threshold: 64) with mol. Mass: ~36kDa and pI: 4.78 and T2 (basic) "eukaryotic porin protein" (score: 93 threshold: 64), with mol. Mass: ~33 kDa and pI: 9.10 from NCBI *nr* database.The unique cyst proteins (acidic) were identified as chaperonin protein *DanK* (mol. Mass: ~69kDa, pI: 4.78) and chaperonin protein (score: 284, threshold: 49), mol. Mass: ~57kDa, pI: 5.08. In real time PCR, using primers derived from amino acid sequences of proteins, regions encoding trophozoite protein T1&T2 and cyst proteins C1&C2 showed 4-35&1388 fold and 15&12 fold increase in mRNA respectively.**Conclusions:** T4 genotype was dominant genotype in amoebic keratitis. The 2 trophozoite proteins may have a role in pathogenesis/growth, while the cyst protein chaperonins may have role in survival in adverse conditions.

Author Disclosure Block:

G. Satpathy: None. **H.S. Behera:** None. **T. Agarwal:** None. **R. Tandon:** None.

Poster Board Number:

FRIDAY-363

Publishing Title:

Molecular Characterization of Rotavirus and Astrovirus in Children with Gastroenteritis

Author Block:

G. González-Ochoa[1], G. D. J. Quintero Ochoa[2], P. M. Calleja-García[1], J. A. Rosas-Rodríguez[1], P. Tamez-Guerra[2]; [1]Univ. de Sonora, Navojoa, Mexico, [2]Univ. Autónoma de Nuevo León, San Nicolas de los Garza, Mexico

Abstract Body:

Background: Rotavirus and Astrovirus are viruses associated to diarrhea in children up to five years old. Human Astroviruses are detected in 5-20% of the cases of acute and middle gastroenteritis in children. The common Astroviruses reported worldwide are the classic type (HAstV-1). On the other hand, Rotavirus causes severe gastroenteritis in children with 453,000 children deaths each year. Most of the cases of rotavirus infections are associated to genotypes G1P[8], G2P[8], G4P[8], G2P[4] and G9P[8]. Several studies have shown the Rotavirus genotypes variability between different seasons or geographic areas. In this study, we analyzed 100 fecal samples of children with gastroenteritis testing for human Rotavirus and Astrovirus. **Methods:** The viral RNA was purified from the samples by Trizol® method, followed by the synthesis of cDNA and the amplification of VP4 and VP7 gene of Rotavirus and ORF1b or ORF2 of Astrovirus with specific primers. The PCR products were cloned in the pGEM-T vector and sequenced. **Results:** The results showed that 5% (5/100) samples were positive to Astrovirus and 14% (14/100) to Rotavirus. Twenty percent (1/5) of the Astrovirus strains sequences presented a 98% homology with human astroviruses type 2 (HAstV-2) and 20% (1/5) with HAstV-6. The rotavirus genotypes detected were G12P[8] in 28.6% (4/14) of the analyzed samples and G no typed P[8] in 14.3%(2/14). The phylogenetic analysis of VP7 genotype G12 showed that the sequences reported in this study clustered with rotavirus strains G12 lineage III. **Conclusions:** To the best of our knowledge, this study provides the first report of rotavirus G12 in México. These results together with the detection of HAstV-6, which is an uncommon genotype reported worldwide, indicates that studies of Rotavirus and Astrovirus surveillance in children with gastroenteritis are important for the analysis of the genotype variability.

Author Disclosure Block:

G. González-Ochoa: None. **G.D.J. Quintero Ochoa:** None. **P.M. Calleja-García:** None. **J.A. Rosas-Rodríguez:** None. **P. Tamez-Guerra:** None.

Poster Board Number:

FRIDAY-364

Publishing Title:

Molecular Epidemiology of Azole-Resistance in *Aspergillus fumigatus* Obtained from Patients with *Aspergillus* Diseases in Iran over the Last 5 Years

Author Block:

S. Hashemi[1], F. Mohammadi[1], J. Zoll[2], W. Melchers[2], H. van der Lee[3], P. Verweij[2], **S. Seyedmousavi**[4]; [1]Tehran Univ. of Med. Scinces, Tehran, Iran, Islamic Republic of, [2]Radboud Univ. Med. Ctr., Nijmegen, Netherlands, [3]Radboud Univ. Med. center, Nijmegen, Netherlands, [4]Erasmus Univ. Med. Ctr., Nijmegen, Netherlands

Abstract Body:

Background: Azole-resistance in *Aspergillus fumigatus* is a global and evolving public health threat, which translates into treatment failure. Surveillance studies indicate that azole-resistance is increasing, with the emergence of the "TR_{34}/L98H" and the "TR_{46}/Y121F/T289A" mutations in *A. fumigatus* in multiple European countries and in the Middle East, Asia, and Africa and more recently in United States. We investigated the prevalence rate of these mutations in clinical *Aspergillus fumigatus* isolates obtained from patients in Iran over the recent 5 years. **Methods:** One hundred seventy-two clinical *A. fumigatus* isolates were investigated for the antifungal activity of itraconazole, voriconazole and posaconazole using EUCAST broth microdilution method. For all isolates, the *Cyp*51A-gene and its promoter region was amplified and sequenced. **Results:** Of the 172 *A. fumigatus* isolates tested during 2010 to 2014, 6 (3.5%) had high MIC values of itraconazole (>16 mg/L) and voriconazole (≥4 mg/L). The DNA-sequencing of Cyp51A-gene showed the TR_{34}/L98H mutation in *Cyp*51A-gene of six isolates. No isolates harboring the TR_{46}/Y121F/T289A mutation were detected. All of the azole-resistant isolates were different in genotypes by microsatellite typing, from those previously isolates from Iran and from the European TR_{34}/L98H controls. **Conclusions:** In conclusion, there was not significant increase in the prevalence of azole-resistant *A. fumigatus* harboring the TR_{34}/L98H resistance mechanism over the recent 5 years in Iran.

Author Disclosure Block:

S. Hashemi: None. **F. Mohammadi:** None. **J. Zoll:** None. **W. Melchers:** None. **H. van der Lee:** None. **P. Verweij:** None. **S. Seyedmousavi:** None.

Poster Board Number:

FRIDAY-366

Publishing Title:

Colony Lift Immunoassay (Cli) as a Successful Technique to Identify Mixed *Helicobacter pylori (H.pylori)* Gastric Infections

Author Block:

D. F. Rojas-Rengifo[1], L. F. Jimenez-Soto[2], B. Mendoza de Molano[3], R. Haas[2], M. P. Delgado[1], J. F. Vera[3], J. Alvarez[3], P. A. Rodriguez-Urrego[3], C. A. Jaramillo Henao[1]; [1]Univ. de los Andes, Bogotá, Colombia, [2]Max von Pettenkofer Inst., München, Germany, [3]Fundación Santa Fe de Bogotá, Bogotá, Colombia

Abstract Body:

Background: *H. pylori* is a gram negative bacteria which efficiently colonizes the human gastric mucosa.There are two types of strains classified by genotype. Type I contains the gene for CagA toxin and type II do not have it. Relative risk to present a gastric pathology increases by CagA positive strains. Colony Lift Immunoassay is a method to analyze and characterize isolated bacterial colonies. We have successfully adapted the assay for the detection of the CagA toxin production by *H. pylori* human isolates[1]. The aim of this study was to determine the prevalence of multiple infections with the *H.pylori* in Colombian patients.**Methods:** Patients older than 18 years presenting digestive symptoms were studied and an upper endoscopy was performed. Biopsies of the gastric antrum and corpus were taken. A culture of antrum and corpus's biopsies was performed and up to 50 colonies from antrum and corpus were isolated and analyzed for CagA expression through a CLI with specific CagA antibodies, as recently published[1]. A paired t-test was used considering a p value <0.05 as significant **Results:** From 97 patients, 24 samples showed a colonization rate with *H. pylori* (24.7%), 4 patients (4/24) presented only type I strains while 20 (20/24) showed mixed infections with *H. pylori* Type I and Type II strains. From these mixed infections, 6 (6/20) patients showed strain specificity tropism for antrum or corpus colonization. There was not statistical significance of tropism for Type I strains (P=0.6) **Conclusions:** We have developed a method for the analysis of several isolated strains from a patient in a fast and reliable way allowing us to evaluate the presence of CagA in up to 100 strains from each patient. The data obtained using the CagA as marker for the type of strains present in the patients show a high prevalence of mixed infections in the population analyzed. Our data opens the possibility of a higher relevance and other implications of mixed infections in gastric pathology and should be considered not only in the evolution of pathological changes in the stomach associated with this bacteria, but as well in their possible relevance in the eradication therapies

Author Disclosure Block:

D.F. Rojas-Rengifo: None. **L.F. Jimenez-Soto:** None. **B. Mendoza de Molano:** None. **R. Haas:** None. **M.P. Delgado:** None. **J.F. Vera:** None. **J. Alvarez:** None. **P.A. Rodriguez-Urrego:** None. **C.A. Jaramillo Henao:** None.

Poster Board Number:

FRIDAY-367

Publishing Title:

Cluster Analysis of *Mycobacterium tuberculosis* Using Whole Genome Sequencing (Wgs): The Irish Mycobacteria Reference Laboratory (Imrl) Experience

Author Block:

E. Roycroft[1], M. M. Fitzgibbon[2], A. Gibertoni Cruz[3], M. O'Meara[4], P. Downes[4], R. F. O'Toole[1], J. Keane[1], T. Walker[3], T. Peto[3], D. Crook[3], T. R. Rogers[1]; [1]Sch. of Med., Trinity Coll., Dublin, Ireland, [2]Irish Mycobacteria Reference Lab., Dublin, Ireland, [3]Modernising Med. Microbiol. Group, Nuffield Dept. of Med., Oxford, United Kingdom, [4]Dept. of Publ. Hlth., Dublin, Ireland

Abstract Body:

Tuberculosis (TB) incidence in Ireland, although low at 7/100,000, remains a challenge to Public Health. Genotyping aims to disrupt transmission chains by flagging potential outbreaks. The IMRL performs 24-locus Mycobacterial-Interspersed-Repetitive-Unit Variable-Number-Tandem-Repeat (MIRU-VNTR) genotyping. However, WGS could be the ultimate genotyping tool as it surveys the entire genome. In this study, Illumina Next-Generation-Sequencing was used to further investigate MIRU-VNTR clusters to discover whether WGS confirms or outrules them as outbreaks. Ten clusters were chosen from the IMRL national collection over the period 2006-14. The largest of these was Cluster 10 from an institutional outbreak (n=25) followed by Cluster 1 (n=23). Cluster 2 contained two sub-clusters (a, n=21 and b, n=20) that differed by a single locus variation (SLV). Seven smaller clusters were also included. Isolates were grown in liquid culture followed by WGS, using published protocols. Fastq files were analysed using a pipeline developed by the Modernising Medical Microbiology Group (Stampy, BWA, Samtools and PhyML form part of a custom pipeline). Phylogenetic trees were visualised using freely available software. The Public Health Department collected linked epidemiological data. Cluster 10 sequences differed by less than 4 single nucleotide variations (SNVs) confirming a single strain outbreak and raising the possibility of a ‘super-spreader’. Cluster 2 was identified as a single strain outbreak (n=41). Cluster 6 isolates differed by at least 60 SNVs, which rules out recent transmission. All six patients in this cluster shared the same country of origin indicating that their isolates are probably from a common ancestor. Our results show that WGS can both rule-in and rule-out outbreaks with greater discrimination than MIRU-VNTR genotyping and can confirm recent transmission events, making it a valuable tool in the fight against TB.

Author Disclosure Block:

E. Roycroft: None. **M.M. Fitzgibbon:** None. **A. Gibertoni Cruz:** None. **M. O'Meara:** None. **P. Downes:** None. **R.F. O'Toole:** None. **J. Keane:** None. **T. Walker:** None. **T. Peto:** None. **D. Crook:** None. **T.R. Rogers:** None.

Poster Board Number:

FRIDAY-368

Publishing Title:

Molecular Evolution of Human Influenza A/H3n2 and A/H1n1 Viruses in MéXico from 2010 to 2015

Author Block:

P. Ramos Cervantes, V. Ibarra, J. Martínez, F. Ledesma-Barrientos, L. Garcia, L. Cervantes-Villar, L. Guerrero, A. Galindo-Fraga, G. Ruiz-Palacios; INCMNSZ, Mexico DF, Mexico

Abstract Body:

Background: The global surveillance of influenza has yielded a substantial amount of sequence and antigenic information covering the virus evolution. From 2010 to date, the Mexico Emerging Infectious Diseases Clinical Research Network (LaRed) implemented a hospital-based program to study the clinical and epidemiologic characteristics of influenza and other respiratory viruses. The aim of this study was to describe the molecular evolution of Influenza AH3 and AH1N1pdm09 from 2010 to 2015. **Methods:** Clinical samples were collected over 5 consecutive seasons, samples from patients with culture-confirmed infection were included, 55 with AH3N2 and 112 with AH1N1pdm09. Segment 4 (HA) gene was amplified and sequenced; phylogenetic analyses of the HA1 region were conducted and 3D protein modeling of cluster representative strains was performed. **Results:** Both types of Influenza circulated in alternate years; for AH3N2, the phylogenetic analyses showed two principal clusters, strains from 2009-2011 belonged to influenza genetic clades 1 and 5, with 9 aminoacid (aa) substitutions in 4 antigenic sites and 1 in a non-antigenic site with respect to APerth16 2009 vaccine strain, 7 of them conserved in all strains. 2012-2015 strains belonged to clade 3, divided in 4 subgroups, showing 10 aa substitutions in 4 antigenic sites and 6 in non-antigenic sites with respect to ATexas50 2012 vaccine strain. AH1N1pdm09 showed 5 clusters, related to influenza season; 2010-2011 cluster belonged to clade 1 and 2, 2012 to clade 5, 2013 to clade 7 and 2014-2015 to clade 6, with 2 subgroups. 2 aa substitution related with enhanced virulence have remained in all the seasons. Since 2013, all Mexican strains possesed K166Q substitution, related with an increment in the number of hospitalized patients. For both AH3N2 and AH1N1pdm09, 3D modeling showed that non-antigenic substitutions are near of receptor binding site. **Conclusions:** Genetically distinct lineages from AH3N2 and AH1N1pdm09 circulate simultaneously. AH3N2 presents a major genetic variation than AH1N1pdm09, with 3 antigenic drift in these 5 seasons. The role of non-antigenic substitution in the antigenic drift and immune response must be studied

Author Disclosure Block:

P. Ramos Cervantes: None. **V. Ibarra:** None. **J. Martínez:** None. **F. Ledesma-Barrientos:** None. **L. Garcia:** None. **L. Cervantes-Villar:** None. **L. Guerrero:** None. **A. Galindo-Fraga:** None. **G. Ruiz-Palacios:** None.

Poster Board Number:

FRIDAY-369

Publishing Title:

Molecular and Clinical Characterization of Human Rhinovirus in Mexico from 2010 to 2014

Author Block:

F. Ledesma B, P. Ramos-Cervantes, J. Ruiz-Quiñones, A. Galindo-Fraga, J. Beigel, G. Ruiz-Palacios; Inst. Natl. de Ciencias Médicas y Nutrición, Mexico DF, Mexico

Abstract Body:

Keywords: Molecular epidemiology, severe respiratory disease, Rhinovirus**Background:** Rhinoviruses (RVs), which are frequently found in the nasopharynx of patients with respiratory disease were identified some years ago and, although they have been widely studied, many aspects of their role in causing respiratory infections remain unclear. Recent studies have related the presence of RV in respiratory disease requiring hospitalization, viral load and severity of disease has been described in few studies.**Objective:** To describe clinical and molecular features of RV infection in the mexican population and determine viral load correlation with severity of disease.**Methods:** Clinical samples from 222 patients with positive infection for hRV/hEV were collected over 5 consecutive seasons (April 2010-2014). VP4 gene was amplified and sequenced and phylogenetic analyses was performed. Viral load was tested by Real Time RT-PCR. We used Fisher's exact test to estimate association between viral load and severity of disease.**Results:** VP4 typing was successful in 78% of samples. hRVA (45.5%) was predominat in all seasons, following by hRVC (19.8%), hRVB (9.9%). hEVD68 (2.8%) was detected only in 2011-2012. hRVA and hRVB strains could be grouped in 4 clusters in the phylogenetic analyses, respectively. hRVC strains shows a great diversity and couldn´t be clustered. Viral load was determined in 119 of a total 222 samples and we found no association between severity of disease and viral load with a p value of 0.8, we also found no association between viral load and ICU patients.**Conclusions:** Viral load did not correlate with severity in hRV infection in the Mexican population. Prevalence of subtypes of hRV was different between consecutive seasons.F. Ledesma-Barrientos fledesmab@outlook.comP. Ramos-Cervantes p_ramos_cervantes@hotmail.comJ.A. Ruiz-Quiñones chucho_ruiz@hotmail.comG.M. Ruiz. Palacios gmrps@unam.mxA. Galindo-Fraga galindofraga@yahoo.com

Author Disclosure Block:

F. Ledesma B: None. **P. Ramos-Cervantes:** None. **J. Ruiz-Quiñones:** None. **A. Galindo-Fraga:** None. **J. Beigel:** None. **G. Ruiz-Palacios:** None.

Poster Board Number:

FRIDAY-370

Publishing Title:

Maximum Release of DNA from Difficult-to-Lyse Bacteria for Nucleic Acid Testing Using A Novel Chemical Lysis Reagent

Author Block:

O. M. de Bruin, J. Niles, B. Ray, C. Kelly-Cirino; DNA Genotek Inc., Ottawa, ON, Canada

Abstract Body:

Background: Identification of microorganisms by nucleic acid testing (NAT) is a critical tool for the rapid diagnosis of infectious diseases and identification of bio-threat agents. The sensitivity and clinical utility of any NAT strategy relies on the availability of nucleic acids in a sample. Hardy bacteria such as *Bacillus* spores and *Mycobacterium tuberculosis* (MTB) are notoriously difficult to break open, and as such, the total nucleic acid available for NAT may limit the effectiveness of the assays. The development of a new chemical lysis method called spore•LYSE now enables larger amounts of high quality nucleic acids to be released in a safe manner from difficult-to-lyse microbes. **Methods:** Extraction of DNA by spore•LYSE was compared to bead-beating in phosphate-buffered saline or SDS buffer for release of DNA from *in vitro*-grown microbes, and MTB spiked into sputum. spore•LYSE was evaluated by comparing the amount of DNA released from bacteria to the amount of DNA present inside the bacteria prior to lysis treatment. This was done using acid-hydrolysis/HPLC, and by quantification of extracted DNA using qPCR.**Results:** spore•LYSE was able to release approximately 85% of DNA from *Mycobacterium smegmatis* and greater than 90% of DNA from surrogates of Category A bioterrorism agents, including *Bacillus* spores. Overall, qPCR results support that spore•LYSE releases an equal or greater amount of DNA than bead-beating from both gram positive and negative bacteria. Lysis of *Bacillus thuringiensis* spores with spore•LYSE resulted in qPCR Ct values of 18.3 +/- 0.3 (n=3), whereas C_t values of bead-beating extracts were 19.3 +/- 0.3 (n=3). spore•LYSE was also compared to standard-of-care for DNA extraction from MTB in sputum. When 7 x 10^4 cells of MTB were spiked into sputum, the C_t value of samples extracted with spore•LYSE was 23.7 +/- 2.8 (n=15) compared to 25.3 +/- 2.4 for bead-beating (n=15).**Conclusions:** spore•LYSE employs an easy-to-use liquid reagent that only requires incubation for 5-20 min at 70°C for release of large amounts of DNA from a variety of bacteria, including MTB and *Bacillus* spores. spore•LYSE eliminates the need for bead-beating and simplifies DNA extraction protocols, and can decrease the time and cost associated with diagnosis and identification of low-abundance or difficult-to-lyse bacteria.

Author Disclosure Block:

O.M. de Bruin: D. Employee; Self; DNA Genotek Inc. **J. Niles:** D. Employee; Self; DNA Genotek Inc. **B. Ray:** D. Employee; Self; DNA Genotek Inc. **C. Kelly-Cirino:** D. Employee; Self; DNA Genotek Inc..

Poster Board Number:

FRIDAY-371

Publishing Title:

Evaluation of the Siemens Tissue Preparation System for Extraction of Microbial Dna from Formalin-Fixed Paraffin-Embedded Tissue

Author Block:

S. P. Buckwalter, L. S. Sloan, B. J. Connelly, J. C. Berry, J. W. Sullivan, II, B. S. Pritt, N. L. Wengenack; Mayo Clinic, Rochester, MN

Abstract Body:

Background: The detection of pathogens by PCR in formalin-fixed paraffin embedded tissue is challenging due to the labor intensive methods required to prepare the tissue for extraction. In this study, we evaluated the performance of the MagNA Pure (Roche) with the Siemen's Tissue Preparation System (TPS). The Siemens platform extracts and purifies nucleic acid using magnetic particle-based isolation, is automated, and requires the use of smaller quantities of tissue than the MagNA Pure system**Methods:** 86 formalin-fixed, paraffin-embedded tissue blocks were sectioned, extracted and tested in parallel on the MagNA Pure and TPS systems; a variety of bacterial and fungal pathogens were tested using previously described real-time PCR assays.**Results:** Of the 86 paraffin blocks tested, 32 (37%) tissues were positive by both extraction systems while 42 (49%) tissues were negative by both methods. 12 (14%) tissues were positive when processed with the TPS, but negative when processed using the MagNA Pure.**Conclusions:** The TPS instrument performed better than the MagNA Pure method for extraction from formalin-fixed, paraffin-embedded tissue. In addition, the TPS is fully automated, faster, limits exposure to hazardous chemicals, and uses 80% (10µm vs 50µm) less tissue than the MagNA Pure system. However, the TPS system did have a higher rate of mechanical issues that led to invalid runs as compared with the MagNA Pure system.

Author Disclosure Block:

S.P. Buckwalter: None. **L.S. Sloan:** None. **B.J. Connelly:** None. **J.C. Berry:** None. **J.W. Sullivan:** None. **B.S. Pritt:** None. **N.L. Wengenack:** None.

Poster Board Number:

FRIDAY-372

Publishing Title:

Provincial Implementation of a New Hiv Diagnostic Algorithm

Author Block:

J. Fenton[1], J. Poelzer[1], **C. L. Charlton**[2]; [1]Provincial Lab. for Publ. Hlth., Edmonton, AB, Canada, [2]Univ. of Alberta, Edmonton, AB, Canada

Abstract Body:

Background: Guidelines for HIV diagnosis recommend replacement of Western blot (WB) as final HIV confirmation with Antibody Differentiation (AD) followed by NAT testing. AD was recently Health Canada approved, however an approved HIV NAT test for diagnosis of infection is currently not available in Canada. Here we report the validation of both AD and NAT testing, and outline the provincial roll out program for the new diagnostic algorithm. **Methods:** Only samples repeatedly reactive on the Architect 4th generation HIV Ag/Ab combo assay were used in this evaluation. 102 plasma and 102 matched serum samples were tested by the Abbott *m*2000 RealTime HIV-1 PCR assay, and 105 serum samples were tested by the Bio-Rad Geenius HIV-1/2 Confirmatory Assay (AD) in parallel with WB, according to manufacturer's protocol.**Results:** 70 known HIV-1 positive and 32 negative samples were tested by NAT. Specificity, sensitivity, positive and negative predictive values were calculated compared to true clinical diagnosis (TCD; determined by clinical symptoms, serology and NAT) for plasma (99, 100, 100, 97%) and serum samples (97, 100, 100, 94%) respectively. 42 known HIV-1, 10 known HIV-2 and 53 NEG specimens were tested by AD (92, 100, 100, 93%) and WB (90, 92, 92, 91%) and compared to TCD. AD resolved 94% of all indeterminate (IND) WB samples, and identified HIV earlier in infection than WB. The performance of AD and plasma samples for NAT testing was deemed acceptable for use as a diagnostic tool in our patient population, and a new HIV diagnostic algorithm was developed. A provincial task force recommended a dedicated sample be sent for NAT testing following IND or NEG AD results; active patient follow-up by Public Health teams was therefore required to ensure appropriate specimen collection. An automated system was created to alert physicians and Public Health teams to samples pending for NAT $\geq$10 days. Direct patient contact by Public Health teams ensured required samples were obtained for testing.**Conclusion:** The Bio-Rad Geenius HIV 1/2 assay out performed WB for diagnosis of HIV infection. Implementation of the new HIV algorithm is predicted to decrease the number of IND cases requiring follow-up, and to diagnose HIV earlier in infection. Ultimately, both laboratory and public health collaboration were needed to ensure appropriate specimen collection for acutely infected patients.

Author Disclosure Block:

J. Fenton: None. **J. Poelzer:** None. **C.L. Charlton:** None.

Poster Board Number:

FRIDAY-373

Publishing Title:

A Real-time Quantitative Pcr for Specific Detection of *A. baumannii* in Water and Blood

Author Block:

D. P. Karumathil, **M. Surendran Nair**, K. Venkitanarayanan; Univ. of Connecticut, Storrs, CT

Abstract Body:

Background: Multi-drug resistant (MDR) *Acinetobacter baumannii* is a major nosocomial pathogen causing a wide array of clinical conditions with significant mortality rates. Identification of *A. baumannii* by traditional cultural methods requires at least 48 to 72 h for obtaining results. In addition, biochemical methods for the confirmation of *A. baumannii* from other *Acinetobacter* species are not completely accurate. Thus, there is a need for rapid methods for specific identification of *A. baumannii*.**Methods:** The objective of this study was to develop a real-time polymerase chain reaction (RT-PCR) for specifically detecting *A. baumannii* in water and blood using TaqMan primer/probe set targeting a highly conserved 102-bp DNA sequence in *adeT*, an efflux pump found in *A. baumannii*. For testing the limit of detection, RT-PCR was done directly with 10-fold dilutions of *A. baumannii* suspension in blood or water (10^6 to 10^1 CFU) and various concentrations of genomic DNA. Further, the sensitivity of the RT-PCR was tested after enrichment of *A. baumannii* in tryptic soy broth.**Results and Conclusion:** The results revealed that all *A. baumannii* isolates yielded a 102-bp PCR product, however, none of the tested negative control isolates, including other *Acinetobacter* species produced any amplification. The sensitivity of PCR for detecting *A. baumannii* in blood and water was 3 $\log_{10}$ CFU or 0.1 ng of DNA. However, upon enrichment, the PCR was able to detect 2 $\log_{10}$ and 1 $\log_{10}$ CFU/ml of *A. baumanni* in water after 6 h and 14 h of incubation at 37^{o}C, respectively. The RT-PCR developed in this study could be used for the rapid detection of *A. baumanni* in environmental and clinical samples.

Author Disclosure Block:

D.P. Karumathil: None. **M. Surendran Nair:** None. **K. Venkitanarayanan:** None.

Poster Board Number:

FRIDAY-374

Publishing Title:

The Utility of Rrna Gene Sequencing in Patient Specimens with No Growth in Traditional Culture

Author Block:

K. Lindsey, S. Edappallath, F. Moore; Baystate Hlth., holyoke, MA

Abstract Body:

Background: Broad primer PCR followed by sequencing of rRNA genes can be very valuable in the detection of organisms in patients that have received antimicrobial therapy and may have no growth in traditional culture. Thus there may be no other means of identifying the pathogen. Guidelines for this diagnostic modality are still in the process of being adopted and the impact of this test in clinical decision making is unclear. In this study we summarize data from 52 samples sent to a reference laboratory for 16s gene sequencing. We report on our overall recovery rate and the effects of the results on antibiotic utilization.**Methods: We tested only if the following criteria were met: 1) The test was ordered by an infectious disease specialist 2) the patient received recent antimicrobial therapy 3) there was a minimum of 48 hours of no growth on the traditional aerobic culture and 4) the specimen type was obtained using sterile technique. Specimens were sent to a reference laboratory where DNA was isolated and amplified by conventional PCR using universal primers. Amplified products are sequenced and the organism(s) identified on the basis of sequence data. Results:** Patient ages ranged from 4 months old to 78 years old with the average age of 44. Overall 22 of 52 specimens (43%) were positive for an organism. We recovered 26 different organisms with *Streptococcus* species being the most common (34%), and *Fusobacterium nucleatum* being the second most common (15%), with various other organisms comprising the remainder. Aspirates (n=7) yielded the highest recovery rate at 57%. Fluids (n=23) yielded a 52% recovery and tissues (n=22) 36% recovery. 77% of patients with positive results had their antibiotics changed to a more targeted therapy, while the remaining 23% had no change in therapy. In those with negative results, antibiotic therapy was changed in 37% of patients.**Conclusions:** The relatively high rate of organism recovery in this study is likely due to the restricted ordering criteria. Our results show that 16s RNA gene testing can improve antibiotic stewardship since patients with positive results were more likely to be deescalated from broad spectrum antibiotics to a more targeted therapy. The clinical impact (eg. length of stay, cost of care, mortality)of these results on patient outcome is currently being investigated.

Author Disclosure Block:

K. Lindsey: None. **S. Edappallath:** None. **F. Moore:** None.

Poster Board Number:

FRIDAY-375

Publishing Title:

Comparison of Ddpcr and Qpcr Assays for Quantifying Nucleic Acid Targets from Arthropod and Ffpe Samples

Author Block:

G. A. Dasch, J. R. Hensley, A. M. Denison, V. N. Loparev; CDC, Atlanta, GA

Abstract Body:

The accurate molecular detection of any infectious disease agent in a sample depends upon the quality and quantity of the target molecules derived from the clinical and environmental source. Formalin-fixed paraffin-embedded (FFPE) samples for pathology are challenging as formalin and embedding lead to degradation and chemical modification of RNA and DNA. Archived ethanol-preserved environmental samples like museum arthropod specimens can also be difficult, especially with hematophagous ectoparasites containing blood. Droplet digital PCR (ddPCR) technologies from BioRad Laboratories QX100 (BR) and RainDance Technologies Rain Droplet (RDT) offer potential for improved agent detection and assessment of sample quality and quantity as the target is partitioned into thousands (BR) or millions (RDT) of individual assays, thus diluting inhibitors and allowing quantitation without a reference standard. We compared TaqMan qPCR assays and derived ddPCR assays with both FFPE and tick samples to assess the enhanced value of ddPCR.Initially, 18 commercial kits (8 RNA, 6 DNA, 4 both) and a conventional phenol-chloroform method were evaluated on 2 non-infected FFPE blocks from human lung, and subsequent extractions were done on 7 additional human lung blocks with the best kits. Sections infected with parvovirus B19, hantavirus, *Mycobacterium tuberculosis*, and influenza A/H3 were analyzed by qPCR and ddPCR. The recovery and quality of DNA and RNA were measured by Qubit fluorometer and Agilent BioAnalyzer, respectively, and compared with housekeeping qPCR and ddPCR assays (RNase P for DNA and beta-2-microglobulin for RNA). Qiagen and Promega kits were used to extract DNA from ticks, and it was assayed for the amount of *Rickettsia*, *Coxiella* and tick DNA by qPCR and derived ddPCR.Nucleic acid yields with different extraction methods varied widely. Quality samples based on BioAnalyzer analysis generally worked well by both qPCR and ddPCR. Although the BR and RDT instruments both performed satisfactorily with some ddPCR assays, direct adaptation of TaqMan assays from qPCR to ddPCR required optimization. The higher throughput of the BR with automated droplet maker made it easier to compare samples but the graphics of the RDT and number of smaller droplets it creates offered some advantages in quantitative analysis, even if it is more expensive per sample.

Author Disclosure Block:

G.A. Dasch: None. **J.R. Hensley:** None. **A.M. Denison:** None. **V.N. Loparev:** None.

Poster Board Number:

FRIDAY-376

Publishing Title:

Comparison of a Molecular Multiplex Panel to Api® Rapid Strips for Bacterial Identification

Author Block:

M. Stonebraker, P. Dawson, D. Hockman, D. Stalons, L. Malone, E. Grigorenko; Diatherix Lab., LLC, Huntsville, AL

Abstract Body:

Background: Conventional microbiological methods are the gold standard for bacterial identification (ID) in clinical laboratories. While accurate, these phenotypic and biochemical tests are laborious and time-consuming. Molecular methods of ID are as sensitive and specific as conventional methods and take hours instead of days for result reporting. Rapid diagnosis is increasingly important for patient therapy where laboratory results can alter treatment. In this study, we compare the performance of Target Enriched Multiplex PCR (*TEM-PCR*™) to gold-standard API® RapID 20E strips (BioMérieux, Marcy-l'Étoile, France) for bacterial ID. **Methods:** Twenty-six well-characterized, blinded isolates were obtained from IHMA, Inc. Isolates were cultured on non-selective media, and 0.5 McFarland standards were prepared. API® RapID 20E strips were inoculated for ID of Enterobacteriaceae. Simultaneously, nucleic acids were extracted using a KingFisher™ Flex instrument (Thermo Fisher Scientific, Waltham, MA) and amplified with TEM-PCR™, utilizing a panel of fourteen gram-negative and gram-positive pathogen targets. Amplified products were hybridized to target probes on microarray plates and detected using a fluorescence imaging reader. Results were reported as "Detected" or "Not Detected".**Results:** Bacterial ID of each isolate was confirmed with IHMA upon completion of TEM-PCR™ and API testing. Results obtained with TEM-PCR™ had 100% correlation with API testing for targets present in the TEM-PCR™ panel. A gene target for *Providencia rettgeri* was not included in the TEM-PCR™ panel, therefore was not detected, yielding an overall correlation of 96% between the two methods.**Conclusions**: Molecular and microbiological techniques are both accurate means for determining bacterial ID, but molecular multiplex methods have added benefits over traditional gold standard testing. TEM-PCR™ is performed directly from the patient specimen and can detect multiple pathogens in a single reaction. Bacterial ID from API® strips takes at least two days, while molecular tests can provide results in less than eight hours, eliminating empirical antibiotic therapy and resulting in faster, more accurate patient treatment.

Author Disclosure Block:

M. Stonebraker: D. Employee; Self; Diatherix Laboratories. **P. Dawson:** D. Employee; Self; Diatherix Laboratories. **D. Hockman:** D. Employee; Self; Diatherix Laboratories. **D. Stalons:** D.

Employee; Self; Diatherix Laboratories. **L. Malone:** D. Employee; Self; Diatherix Laboratories. **E. Grigorenko:** D. Employee; Self; Diatherix Laboratories.

Poster Board Number:

FRIDAY-378

Publishing Title:

Highly Sensitive Dual Amplicon-Based Ngs Approach for Bacterial Identification in Primary Clinical Specimens

Author Block:

P. Khil, J-H. Youn, J. Ho, J. P. Dekker, K. M. Frank; NIH Clinical Ctr., Bethesda, MD

Abstract Body:

Next-generation sequencing (NGS) coupled with traditional amplicon-based methods holds promise for diagnostic characterization of pathogens in primary, uncultured, clinical specimens. Here we report the development of a highly sensitive, pan-bacterial diagnostic assay based on nested PCR amplification of the V1-V2 and V3-V4 regions of the 16S rRNA gene followed by sequencing using an Illumina MiSeq. Reads are analyzed with a newly designed modular pipeline that performs data pre-processing, OTU clustering and taxonomic assignment, species abundance estimation, and diagnostic report generation. Three improvements distinguish our assay from previously reported 16S-based approaches for use in clinical diagnostics: (1) nested PCR amplification combined with NGS, (2) semi-quantitative target concentration estimation using barcoded internal controls, and (3) analysis of four 16S hypervariable regions. For OTU load estimation, heptanucleotide barcodes were inserted into cloned *Actinomadura nitritigenes* 16S rDNA and these engineered targets were spiked at different concentrations. Unlike previous single-amplicon methods, our strategy integrates data from two targets allowing improved species-level resolution of certain important human pathogens. Initial analysis of the method including LoD studies using defined DNA mixes and isolate dilutions demonstrated reliable detection of < 100 copies of pathogen in the presence of 1000-fold excess of human cells. Initial clinical evaluation was performed with 24 primary uncultured urine samples representing a mixture of no growth, cultured urinary pathogens, and background commensal flora as reported using routine clinical culture. The method identified 110 organisms corresponding to commensal flora and all 12/12 urinary pathogens reported from culture in this set (100% sensitivity). In addition, we detected *E. faecalis* and *Ureaplasma parvum* that were not reported in culture. Discrepancy analysis was performed using unbiased shotgun sequencing, which detected genomes of both organisms, and repeat culture of specimen identified *E. faecalis*, confirming these results. Blinded validation of the method using 250 specimens is currently in progress under an NIH Clinical Center IRB protocol.

Author Disclosure Block:

P. Khil: None. **J. Youn:** None. **J. Ho:** None. **J.P. Dekker:** None. **K.M. Frank:** None.

Poster Board Number:

FRIDAY-379

Publishing Title:

Diagnosis of Culture-Negative Infections Using a Novel Plasma-Based Next-Generation Sequencing Assay

Author Block:

J. Kim[1], L. Shieh[2], **D. K. Hong**[3]; [1]Stanford Univ. Sch. of Med., Palo Alto, CA, [2]Stanford Univ. Sch. of Med., Stanford, CA, [3]Karius, Inc, Menlo Park, CA

Abstract Body:

Background: Many infectious diseases can be difficult to diagnose with conventional culturing methods either due to the need for invasive biopsies for deep infections or the inability of standard cultures to readily isolate viruses, fungi, or fastidious bacteria. Furthermore, single or multiplex PCR assays, while more sensitive, require the physician to target specific organisms. Next-generation sequencing (NGS)-based assays have high sensitivity and have the additional advantage of being completely open-ended, thus allowing for hypothesis-free diagnosis. **Methods:** We developed a minimally invasive NGS-based infectious disease assay that takes advantage of circulating cell-free plasma DNA originating from invasive pathogens. After filtering human sequences, remaining sequences are aligned to a pathogen reference sequence database and relative abundance is assigned. Patients who had negative blood cultures and who had diagnostic biopsies for infectious disease testing had a single blood sample drawn prior to biopsy for NGS analysis. **Results:** We report two patients in whom the etiologic diagnosis for their febrile illness was confirmed with this assay. The first patient had a *Staphylococcus aureus* implantable cardiac defibrillator infection. Initial blood cultures were negative but culture of the explanted device yielded *S. aureus. S. aureus* was identified by NGS from a plasma sample obtained prior to the device being removed. The second patient had extensive cutaneous disease, which on biopsy was thought to be consistent with leprosy. NGS of plasma, however, revealed high levels of Epstein-Barr virus (EBV) which was confirmed by quantitative EBV PCR. The patient was eventually diagnosed with EBV-positive diffuse large B-cell lymphoma (DLBCL) of the elderly. **Conclusions:** We demonstrate the potential utility of a novel plasma-based NGS infectious disease assay. This assay has high sensitivity and can aid in the open-ended diagnosis of culture-negative infections.

Author Disclosure Block:

J. Kim: None. **L. Shieh:** None. **D.K. Hong:** D. Employee; Self; Karius, Inc..

Poster Board Number:

FRIDAY-380

Publishing Title:

Validation Panel for the Implementation of Clia-Compliant Whole Genome Sequencing (WGS) in Public Health Laboratory

Author Block:

V. Kozyreva, C-L. Truong, A. Greninger, V. Chaturvedi; California Dept. of Publ. Hlth., Richmond, CA

Abstract Body:

Background: Public health microbiology (PHL) laboratories use WGS for outbreak investigation, pathogen identification (ID), and antibiotic resistance (ABR) detection. Different platforms, chemistries and analytical tools complicate standardization across the field. We aimed to develop a validation panel of bacterial isolates for multi-laboratory comparisons.**Methods:** WGS validation panel comprised of 34 bacterial samples: 10 *Enterobacteriaceae* samples, 5 gram-positive cocci, 5 gram-negative non-fermenting species, 9 *Mycobacterium tuberculosis*, and 5 miscellaneous bacteria representative of typical workflow in PHL. WGS was done using Nextera XT library prep with 2x300 bp sequencing chemistry on Illumina MiSeq Sequencer with min coverage 30x, min base quality 200. Different operators sequenced bacteria in triplicate within single run, and on three different days. *De novo* assembly was done on CLCbio GW 8.0.2. Genomes were annotated with Prokka v1.1. Accuracy, reproducibility, sensitivity, and specificity was deduced from genotyping based on genome-wide SNP calling, *in silico* multi-locus sequence typing (MLST), 16S rRNA species ID, and ABR genes detection. Accuracy of bioinformatics pipeline was assessed by phylogenetic analysis of raw sequencing reads from previously published outbreaks.**Results:** Genome sizes of validation organisms varied from 1.8 to 4.7kb, with wide range of GC content 32.1-66.1%. Average depth of genome coverage of 78.4x (30.3-216.4x) was achieved. Accuracy of MiSeq platform for individual base calling in bacterial genomes was >99.9%. Accuracy of phylogenetic analysis performed on bioinformatics pipeline was 100%. Both reproducibility and repeatability of genome-wide base calling were >99.9%, while this metrics for selected tests were 100%. Specificity and sensitivity inferred from MLST and genotyping tests were 100%. A customized test report was developed for end users with and without specialized knowledge of WGS.**Conclusions:** We have validated a test panel of bacterial isolates for WGS on MiSeq according to CLIA requirements for laboratory developed test (LDT). The test panel, sequencing analytics and raw sequences are available as valuable tools for future multi-laboratory comparisons of WGS in PHL.

Author Disclosure Block:

V. Kozyreva: None. **C. Truong:** None. **A. Greninger:** None. **V. Chaturvedi:** None.

Poster Board Number:

FRIDAY-382

Publishing Title:

Classification of *Staphylococcus schleiferi* Subspecies Using Whole Genome Comparisons

Author Block:

B. Duim, A. L. Zomer, J. de Laat, J. A. Wagenaar, E. M. Broens; Utrecht Univ., Faculty of veterinary medicine, Utrecht, Netherlands

Abstract Body:

Background: The species *Staphylococcus schleiferi*, includes both a coagulase-negative subspecies (*S. schleiferi* subsp. *schleiferi*) and a coagulase-positive subsp. (*S. schleiferi* subsp. coagulans). Coagulase negative staphylococci are considered less clinical relevant and the species is often not identified in routine diagnostics. Traditional diagnostics using clotting of plasma by extracellular free staphylococcus coagulase has been shown to be variable in *S. schleiferi*. In this study we questioned the phenotypic differentiation of *S. schleiferi* subspecies by identifying the staphylococcal coagulases and subspecies specific characteristics, using whole genome sequencing. **Methods:** The genomes of 12 *S. schleiferi* strains (including 3 Type strains) were obtained from Illumina MiSeq reads. The genomes were annotated using Prokka and the protein orthology was determined using Roary. Phenotypes were determined using slide and tube coagulase tests, Maldi-TOF analysis, and API ID 32 STAPH (bioMérieux, France).**Results:** Maldi-TOF and API tests failed to distinguish coagulase positive strains from coagulase negative strains. Furthermore, coagulase production was not consistently associated with subspecies designation. In the genomes, 9 genes were identified whose presence was putatively associated with coagulase production. These consisted of genes encoding specific variants of Coa coagulase, Von Willebrand factor-binding proteins and variants of the fibrinogen-binding protein Efb. Although in all cases the genomes encoded a coagulase, different variants were present, which may explain the observed phenotypic variable coagulase activity.**Conclusion:** Phenotypic characteristics and the presence of different genes encoding staphylococcal coagulases, questions the reliability of coagulase detection for distinction of *S. schleiferi* subspecies.

Author Disclosure Block:

B. Duim: None. **A.L. Zomer:** None. **J. de Laat:** None. **J.A. Wagenaar:** None. **E.M. Broens:** None.

Poster Board Number:

FRIDAY-383

Publishing Title:

Validation of the Modified Abbott Real*time* Assays for Residual Viremia Detection

Author Block:

J. E. McKinnon[1], J. I. Bernardino[2], J. Y. Zhou[1], F. Pulido[3], R. Delgado Vasquez[3], D. Lucic[4], J. R. Arribas[2]; [1]Henry Ford Hosp., Detroit, MI, [2]Hosp. La Paz. IdiPAZ, Madrid, Spain, [3]Hosp. Univ.rio 12 de Octubre, i+12, Madrid, Spain, [4]Abbott Molecular, Des Plaines, IL

Abstract Body:

Background: The study of residual HIV-1 viremia (RV) for clinical trials and cure strategies requires reproducible and practical assays. We modified the Abbott Real*Tim*e HIV-1 (MA) assay to examine RV using low plasma volumes using either an ultracentrifugation (UMA) or tabletop centrifugation (TMA), and tested the feasibility on samples from the KRETA trial, a study of HIV patients with lipodystrophy. **Methods:** HIV stock virus was obtained from the Rush University VQA laboratory. HIV spiked viral controls from 5000 to 0 c/mL were prepared. The UMA assay uses 3 mL of HIV positive plasma added to a 10 mL centrifuge tube with 7mL of tris-buffered saline, then spun at 170,000xg for 30 minutes. Viral pellets were re-suspended in PBS and transferred to the *m*2000sp for testing. The TMA assay uses a new extraction protocol for the *m*2000sp for processing 3mL of plasma and a 2hr- 21,000xg centrifugation step. Plasma samples from the KRETA study were obtained for viremia (VL) testing.**Results:** 129 HIV spiked control samples were used to validate both assays. The MA assays had 100% detection of samples with expected VL >12.5 c/mL. RV detection by UMA and TMA was at 12.5 c/mL (100%/83.3%), 6.25 c/mL (100%/100%), 3.125 c/mL (75%/50%) and 1.56 c/mL (50%/33.3%). A 5 c/mL control was used for testing and validation with >90% detection. UMA and TMA assays viremias correlated well to the expected VL levels, $p<0.000$ & $p=0.026$. At VL 5 c/mL & above, HIV RNA control levels were significantly different from each other, in both assays ($p<= 0.026$). Controls with VL <=5c/mL were not statistically different. For clinical validation, 71 patients from the KRETA study were tested. Viremia was detected in at least one sample in 51 patients (mean 35 c/mL), 20 patients had undetectable samples by MA assays but only 2 had any detectable viremia during the study.**Conclusions:** Ultracentrifuge MA assay is able to detect HIV-1 RNA of ~3 c/mL in 3mL of plasma, allowing for the study of residual viremia. A second simpler TMA assay with new extraction protocol allows for comparable quantification at 6 c/mL as the UMA protocol. These assays provide reproducible methods for HIV RV quantification using clinically attainable plasma volumes.

Author Disclosure Block:

J.E. McKinnon: E. Grant Investigator; Self; Abbott Molecular. J. Scientific Advisor (Review Panel or Advisory Committee); Self; Gilead Sciences. L. Speaker's Bureau; Self; Abbott

Molecular. **J.I. Bernardino:** None. **J.Y. Zhou:** None. **F. Pulido:** None. **R. Delgado Vasquez:** E. Grant Investigator; Self; Abbott, Beckman Coulter, Gilead Sciences, Roche, ViiV Healthcare. **D. Lucic:** D. Employee; Self; Abbott Molecular. **J.R. Arribas:** None.

Poster Board Number:

FRIDAY-384

Publishing Title:

Evaluation of the Abbott Realtime Cmv Investigational Use Only (IUO) Assay for the Quantification of Cytomegalovirus (CMV) DNA in Plasma

Author Block:

F. Nolte, A. M. Kegl; Med. Univ. of South Carolina, Charleston, SC

Abstract Body:

Background: Quantification of CMV DNA in the blood has become standard of care for surveillance and diagnosis of active CMV infection and for monitoring response to therapy in bone marrow and organ transplant recipients. The Abbott RealTime CMV assay is available in the US as an investigational use only (IUO) assay. **Methods:** We compared the performance characteristics of the above IUO assay with that of a laboratory-developed assay (LDA) using analyte-specific reagents manufactured by Qiagen. Both the IUO and the LDA were run on the Abbott m2000 sp/rt platform. The IOU assay targeted the UL80.5 and UL34 genes and included calibrators which were processed through extraction as patient samples. Calibrators were also standardized to the 1st WHO International Standard. The LDA targeted a region of the MIE gene and calibrators were prepared from quantified CMV AD 169 DNA (Advanced Biotechnologies). Clinical plasma samples, linearity panels and positive controls from Exact Diagnostics, AcroMetrix, and ZeptoMetrix were included in the study. Agreement, accuracy, measurement range and reproducibility of the IOU and LDA were compared.**Results:** Valid results were obtained in both assays for 70 plasma specimens from 54 patients. CMV DNA was detected by both assays in 37, by the IOU alone in 14, by the LDA alone in none of the specimens. CMV was not detected by both assays in 19 specimens ($p=0.0005$). Those detected by the IUO alone had low viral loads ranging from <1.3 to 2.5 log copies (c)/ml. The quantitative results were highly correlated ($p<0.0001$), with $R^2=0.911$. However, the mean difference between the IOU and LDA values was 1.3 log c/ml (95% limits of agreement 0.6 to 2 log). The IUO measured values were on average 0.12 log c/ml lower and 0.12 log c/ml higher than the labelled values for CMV linearity panels obtained from Exact Diagnostics and AcroMetrix, respectively. The IUO inter-run coefficients of variation were 2.8% and 0.7% with low and high external controls, respectively. We confirmed that the IUO reliably quantitated low levels of CMV DNA (≤1.6 log c/ml).**Conclusions:** The results of the IUO and LDA were highly correlated, but the IUO detected and quantified significantly more CMV-positive samples and the results were consistently more than one log greater than the LDA. The IUO appeared to provide a better estimate of the actual CMV viral load than the LDA.

Author Disclosure Block:

F. Nolte: E. Grant Investigator; Self; Abbott Molecular. **A.M. Kegl:** None.

Poster Board Number:

FRIDAY-385

Publishing Title:

Salvage Microbiology - Detection of Pathogens by Pcr & Electrospray Ionization Mass Spectrometry of Dna Extracts from Archived Formalin-fixed, Paraffin-embedded Tissue Sections in Patients with Suspected Infection

Author Block:

J. J. Farrell[1], R. Sampath[2], R. A. Bonomo[3]; [1]Univ. of Illinois Coll. of Med., Peoria, IL, [2]Ibis BioSci.s, Carlsbad, CA, [3]Case Western Reserve Univ. Sch. of Med., Cleveland, OH

Abstract Body:

Background: Formalin-fixed, paraffin-embedded (FFPE) tissue blocks are typically archived indefinitely. These archived tissue specimens represent a potential resource for recognition of pathogens in patients with suspected infectious diseases but negative cultures. PCR detection of infectious organisms from FFPE tissue is a well described diagnostic technique occasionally employed when microscopic histopathology findings from tissue biopsies suggest a specific underlying infectious pathogen. But when tissue inflammation is only suspicious for infection, without suggesting a specific pathogen, and cultures are negative (or where not requested), conventional PCR is not an option. **Methods:** IRB approval was obtained for examination of archived FFPE tissue sections for potential infectious pathogens by polymerase chain reaction combined with electrospray ionization mass spectrometry (PCR/ESI-MS). A No. 11 blade disposable surgical scalpel (AD Surgical, Sunnyvale, CA) was used to remove tissue samples from FFPE tissue blocks from two patients with suspected infection, but negative cultures. We employed a commercial DNA extraction kit (QIAprep miniprep kit [Qiagen, Inc.]) for DNA extraction of the sample of FFPE tissue. Both DNA extracts were submitted for PCR/ESI-MS (Ibis Biosciences Inc, Carlsbad, CA).**Results:** *Streptococcus intermedius* was detected by PCR/ESI-MS from a FFPE mediastinal lymph node tissue block from a 29 year old man with pulmonary infitrates and metastatic central nervous system lesions. PCR/ESI-MS detected *Ajellomyces capsulatus* (anamorphic name *Histoplasma capsulatum*) from a FFPE block of tissue from an oral ulcer taken from a 34 year old with congenital Hemophilia A, and well controlled HIV-1/AIDS.**Conclusions:** This IRB approved pilot study suggests that PCR/ESI-MS may have utility for identification of infectious pathogen(s) directly from DNA extracts of FFPE tissue blocks when histopathology is suspicious for a non-specific infectious etiology. A prospective study and validation with FFPE tissue from patients with culture confirmed infection is planned.

Author Disclosure Block:

J.J. Farrell: None. **R. Sampath:** D. Employee; Self; Ibis Biosciences, an Abbott Company. **R.A. Bonomo:** None.

Poster Board Number:

FRIDAY-386

Publishing Title:

Clinical Correlates of Adenovirus Detection Using the Focus Diagnostics qPCR Assay

Author Block:

C. Jacquot, A. Plourde, Z. Nagymanyoki, S. Miller; Univ. of California, San Francisco, San Francisco, CA

Abstract Body:

Background: Adenovirus infection is increasingly recognized as a cause of morbidity and mortality in immunosuppressed and transplant patients. It may involve any organ system and can be fatal. Viral detection in blood has been associated with a higher incidence of symptomatic disease. Viral titers may be predictive of disease outcome and could be used to monitor response to antiviral therapy. **Methods:** The Focus Diagnostics Adenovirus Assay is a real-time polymerase chain reaction assay that quantitatively detects adenovirus DNA. Following extraction from plasma, DNA is amplified and detected by fluorescent probe-primers targeting the adenovirus hexon gene. Results from the Focus assay were compared to those from a reference lab (Viracor). Patient chart review was performed to determine laboratory and clinical markers associated with adenovirus blood titers.**Results:** The reportable linear range of the assay was 1x10^3 to 1x10^10 copies/mL (cp/mL); the limit of detection was 812 cp/mL. Serotypes 1, 2, 5, 7a, and 40 were detected at a dilution level of 1x10^3 cp/mL; serotype 3 was detected at a dilution level of 1x10^4 cp/mL. The assay showed no cross-reactivity with other viral and bacterial organisms, and was 100% specific for adenovirus.Assay sensitivity was 94% (50 of 53 samples tested adenovirus positive) and specificity was 98% (62 of 63 samples tested adenovirus negative) as compared to reference lab. Copy numbers for the Focus assay tended to be higher, with an average difference of 1.05 log cp/mL.Chart review of 25 UCSF patients positive for adenovirus infection showed that a number of factors (e.g. immunosuppression, co-infection, age) affected clinical management following virus detection. Changes in viral load over time appeared to correlate with symptoms; however, no consistent correlation was found with other laboratory markers of infection (WBC count) or organ damage (transaminases, serum creatinine).**Conclusions:** The Focus adenovirus qPCR assay showed good analytical sensitivity and specificity. Tested patients had multiple co-morbidities, which may have obscured a correlation between adenovirus viral loads and other laboratory markers of infection or organ damage. Further studies are needed to establish clinical correlation for adenovirus titers and optimal patient management strategies.

Author Disclosure Block:

C. Jacquot: None. **A. Plourde:** None. **Z. Nagymanyoki:** None. **S. Miller:** None.

Poster Board Number:

FRIDAY-387

Publishing Title:

Altona Diagnostics Realstar™ Varicella-Zoster Virus Real-Time Quantitative and In-House Qualitative Polymerase Chain Reaction Assays Comparative Analysis

Author Block:

S. Grandjean Lapierre, K. Boissinot, C. Renaud; Ctr. Hosp.ier Univ.ire Sainte-Justine, Montreal, QC, Canada

Abstract Body:

Background: *Varicella Zoster Virus* (VZV) can be responsible for cerebral, cutaneous and congenital infections. Since effective antiviral treatment and prophylaxis are available, rapid and accurate laboratory diagnosis is mandatory. However, no commercial assay has yet been approved by the FDA. The objective of this study was to compare Altona Diagnostics RealStar™ VZV Kit 1.0 commercial real time quantitative polymerase chain reaction (PCR) assay with in house qualitative PCR assay. **Methods:** This study was performed on 145 clinical specimens submitted for routine VZV PCR testing including cerebrospinal fluid (CSF) (43%), cutaneous (43%) and other specimen types (14%) such as serum, ocular and respiratory secretions. Specimens were selected on the basis of their result on the in house assay. To account for this potential selection bias, a variety of positive and negative specimens for each specimen types were included. DNA extraction was performed using Promega extraction kit on a Maxwell instrument. RealStar™ PCR assay was performed according to manufacturer's instructions on the ABI 7500. In house PCR targeting the UL21 gene and producing a 647-bp amplicon for detection by agarose electrophoresis was performed on the ABI 9700.**Results:** Total, positive and negative concordance percentages between both assays were respectively 98.6% (95% CI 94.8-99.9), 98.4% (95% CI 90.7-99.9) and 98.8% (95% CI 92.8-99.9). Kappa statistic value was 0.97 (95% CI 0.93-1.00) indicating excellent agreement beyond chance. One CSF specimen was only positive on RealStar™ PCR with 392 copies/mL at a Ct of 30. One corneal scrapping specimen was only positive on in house PCR. Positive specimens on RealStar™ PCR were so at an average Ct of 23.98 (SD 6.18) and quantitative viral loads varied from $1.85x10^2$ and $6.67x10^7$ copies/mL. Average quantitative viral loads in CSF and cutaneous specimens were respectively $4.4x10^5$ and $1.1x10^7$ copies/mL, a difference found to be statistically significant (p=0,004).**Conclusions:** RealStar™ VZV Kit 1.0 real time quantitative and in house conventional qualitative VZV PCR assays have excellent concordance in CSF, cutaneous and other specimen types. As expected, RealStar™ PCR found quantitative viral loads to be significantly higher in cutaneous than in CSF specimens.

Author Disclosure Block:

S. Grandjean Lapierre: None. **K. Boissinot:** None. **C. Renaud:** None.

Poster Board Number:

FRIDAY-388

Publishing Title:

Ultra-Sensitive Detection of *Borrelia S*pecies Using Droplet Digital PCR

Author Block:

A. Bemis; Digital Biology Ctr., Bio-Rad Lab., Pleasanton, CA

Abstract Body:

Lyme disease (LD), or Lyme borreliosis (LB), is a zoonotic, tick borne illness responsible for an estimated 385,000 combined cases in the United States and Europe annually (1,2). Underestimates in the number of confirmed LD cases have been described (3,4), and are partially attributed to the limited specificity and sensitivity of commercially available tests (3,4). Detection of clinically-relevant antibody titers may take several weeks from the time of infection, when utilizing these traditional serologic testing methods (5). Here we describe a novel PCR method, Droplet Digital PCR (ddPCR), to interrogate multiple *Borrelia* species. *Borrelia garinii* gDNA was titrated in a constant human DNA background to yield a dynamic range of more than 5 log, with the limit of detection falling below one DNA molecule per microliter (Fig. 1). *Borrelia garinii, B. afzelii, and B. burgdoferi* were subsequently multiplexed in a single well, in order to demonstrate the efficacy of screening for multiple *Borrelia* strains in parallel. Given these findings, ddPCR may provide additional resolution to traditional serologic testing methods.

Author Disclosure Block:

A. Bemis: D. Employee; Self; Bio-Rad Laboratories.

Poster Board Number:

FRIDAY-390

Publishing Title:

Epidemiology, Clinical Characteristics and Outcome of Deep Sternal Wound Infections in a Tertiary Cardiac Surgery Centre in the United Kingdom

Author Block:

A. L. Chue, I. Das, A. Ranasinghe, R. Warner; Univ. Hosp. Birmingham NHS Trust, Birmingham, United Kingdom

Abstract Body:

Background: Deep sternal wound infection is a life threatening complication following median sternotomy. The optimal management remains unclear, with no recent epidemiology from the UK. Over 40,000 cardiac operations are carried out over a 5 year period in the UK, with 3.3% resulting in surgical site infection.**Aim:** To review incidence and epidemiology of deep sternal wound infections.**Method:** A retrospective review of microbiologically confirmed deep sternal wound infections from 1 November 2011 to 1 November 2015 through electronic patient and laboratory records. We defined deep sternal wound infection as infections requiring debridement surgery with microbiologically positive results.**Results:** Forty-eight patients with deep sternal wound infections, a rate of 1.6%, were identified. The following risk factors were common: diabetes (23%), immunosuppression (29%), obesity (35%). Infections mostly first manifested in the early post-operative period with the earliest occurring 16 days after surgery, and the latest 7 years after initial surgery. Two hundred and twenty-six deep samples from 78 patients were received by the Microbiology laboratory, of which 128 from 48 patients were culture positive (57%). Coagulase negative staphylococci were the leading organisms (32%), followed by Gram-negative organisms (26%) and *Staphylococcus aureus* (11%). Forty-six percent of patients (22/48) were found to have polymicrobial infections and only three patients (6%) had a sole fungal aetiology, predominantly a *Candida sp.*. Thirteen patients (27%) were managed with debridement (often multiple, range 1 to 15) alone, 15 patients (31%) were managed with vacuum dressings and 18 patients (38%) were managed with flap reconstructions. The majority of patients received tailored antibiotic regimes according to the aetiological organisms. The antibiotic course ranged from five days to 6 months either through continuous or interrupted courses. Sixty-nine percent (33/48) were cured. In-hospital mortality was low (15%) but associated morbidity with frequent admissions and surgical procedure was high.**Conclusion:** A multidisciplinary approach with meticulous long clinical and microbiological review is important in the management of this infection.

Author Disclosure Block:

A.L. Chue: None. **I. Das:** None. **A. Ranasinghe:** None. **R. Warner:** None.

Poster Board Number:

FRIDAY-391

Publishing Title:

Diversity of Spa Types in *Staphylococcus aureus* Isolates from Three Regions in Kenya

Author Block:

L. Musila[1], V. Oundo[1], D. Matano[1], S. Muriithi[1], W. Sang[2]; [1]USAMRD-K, Village Market, Kenya, [2]KEMRI, Nairobi, Kenya

Abstract Body:

Staphylococcus aureus is a pathogen of global clinical significance with abundant genetic diversity within and between human populations. However there are notable gaps in strain typing data from sub-Saharan Africa. An ongoing surveillance study is being conducted to examine the strain diversity of *S. aureus* from geographically diverse regions in Kenya to provide information on the distribution of methicillin-sensitive and -resistant *S. aureus* (MSSA, MRSA) strains in Kenya. The study is being conducted in 3 hospitals located in the western lake, the western highland and central regions of Kenya. Bacterial isolates were cultured from skin, wound and urine infections. Thirty one *S. aureus* isolates were identified phenotypically by gram stain, catalase, coagulase and API tests. Two MRSA were identified based on the cefoxitin disc screen as per CLSI guidelines. DNA was extracted from overnight cultures of S. aureus and PCR performed using published primers to amplify the highly variable X repeat region of the spa gene. PCR products were purified and sequenced by Sanger sequencing. Spa types were detected using the Ridom SpaServer and compared between MSSA and MRSA isolates in the three geographical regions. From these isolates, 23 different spa types were identified. The predominant spa types were t335 (5), t064 (3), t2029 (2) and t005 (2). 10 isolates had unique spa types (t084, t002, t1886, t223, t10499, t318, t7499, t941, t7258, t037) while 9 isolates had novel unassigned spa types. Isolates of the Spa type t064 and t2029 and 7 unique and distinct spa types were confined to the lake region. The western highlands and central sites both had isolates with spa types t335 and t005, with each site also having their own unique and distinct spa types. The only MRSA strains detected were from the lake region and had spa types t2029 and t002, suggesting emergence from endemic strains. The spa type t037 which is recognized as a globally distributed ST239 strain type was detected in this study and has been reported previously in Kenya. The significant diversity, unique spa types and geographical differences in the distribution of spa types of *S. aureus* in Kenya underscores the need for systematic surveillance to appreciate the genetic geodiversity and identify emergent strain types.

Author Disclosure Block:

L. Musila: None. **V. Oundo:** None. **D. Matano:** None. **S. Muriithi:** None. **W. Sang:** None.

Poster Board Number:

FRIDAY-392

Publishing Title:

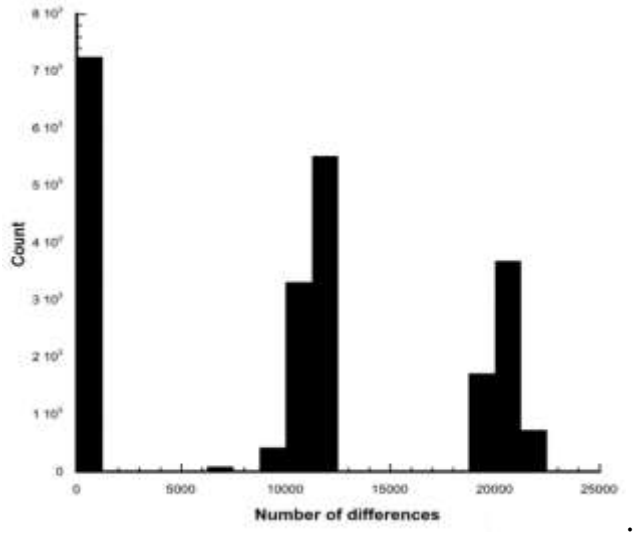
Molecular Analysis of Methicillin Resistant S*taphylococcus aureus* in a Cohort of Cystic Fibrosis Patients

Author Block:

A. Ankrum; Cincinnati Children's Hosp. Med. Ctr., Cincinnati, OH

Abstract Body:

Avoiding chronic lung infection with pathogenic bacteria is critically important to maintaining lung health in Cystic Fibrosis patients. Strict Infection Control measures are implemented in clinical settings in order to prevent patient to patient transmission of these pathogens. We analyzed MRSA isolates from our CF patients to determine strain relatedness in order to validate our infection control practices.From April 2014 to March 2015, 69 MRSA isolates were retained from respiratory cultures on unique CF patients, including 7 pairs of siblings, with 30 isolates representing new acquisition within the past year. Strain relatedness was determined by Whole Genome Sequencing utilizing phylogenetic analysis comparing Single Nucleotide Polymorphism differences between isolates.Three main clusters were identified with Group 1 containing 5 isolates, Group 2 with 36 isolates and Group 3 with 28 isolates (Fig 1) .



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33 isolates contained a SCCmec IV cassette, while 36 isolates contained SCCmec II. Using a SNP cutoff value of 40 differences, 5 pairs of siblings were the only MRSA isolates that displayed relatedness (Fig 2).

Fig 1. Clusters of MRSA isolates**Fig 2. SNP differences between a sibling pair**We can definitively conclude that none of the non-sibling CF patients with recent acquisition acquired MRSA from other CF patients in this study. These results support our infection control practices by providing evidence that MRSA acquisition is not occurring between CF patients in our clinical setting.

Author Disclosure Block:

A. Ankrum: None.

Poster Board Number:

FRIDAY-393

Publishing Title:

Changes in Gene Expression of *Staphylococcus aureus* in the Presence of Zinc Oxide Nanoparticles

Author Block:

U. Kadiyala, J. VanEpps, N. Kotov; Univ. of Michigan, Ann Arbor, MI

Abstract Body:

Background: Annually over 1 million healthcare associated infections are related to implanted medical devices. *Staphylococcus aureus* is one of the most common causative organisms. We have shown that layer-by-layer coatings of zinc oxide nanoparticles (ZnO-NPs) reduce staphylococcal biofilm growth by 3 logs. Furthermore, ZnO-NP mediated growth inhibition is not likely the result of oxidative stress as previously thought. In addition, we have shown that ZnO-NPs crystalized into hexagonal pyramids inhibit β-galactosidase and horseradish peroxidase activity in a biomimetic fashion similar to natural substrate inhibitors. To better understand how this enzyme inhibition function may contribute to the antimicrobial properties, we performed a DNA microarray analysis to determine changes in gene expression for ZnO-NP-exposed *S. aureus*.**Methods:** ZnO-NPs were synthesized into 15-20nm hexagonal pyramids as previously described. *S. aureus* (COL) was grown to mid-log in the presence of sub-lethal but growth inhibitory concentrations of ZnO-NPs. RNA was extracted and transcriptome analysis was performed using Affymetrix Microarray GeneChip®.**Results:** A total of 815 genes were significantly up/down regulated: 333 upregulated and 482 down regulated. Genes related to oxidative stress and redox homeostasis were minimally changed or down regulated. This supports our previous results demonstrating that oxidative stress is not the primary mechanism of action for ZnO-NPs. The genes most highly upregulated by ZnO-NPs relate to uridine monophosphate (UMP) biosynthesis, pyrimidine metabolism, and translation. Genes that were down regulated primarily relate to purine metabolism, amino acid biosynthesis, sugar phosphate transporters, and pathogenesis.**Conclusion**: UMP biosynthesis pathway is highly upregulated in the presence of ZnO-NPs. It is commonly upregulated in anaerobic conditions and is involved in accelerated cell wall synthesis. Notable gene expression changes are currently being confirmed by RT-PCR. Future experiments will be performed on isogenic mutants to better understand the role of the UMP biosynthesis pathway in ZnO-NP mediated growth inhibition. This work sets the foundation to better identify ZnO-NP's mechanism of action against *S. aureus*. Such insight is necessary to optimize coating strategies to prevent medical device infection.

Author Disclosure Block:

U. Kadiyala: None. **J. VanEpps:** None. **N. Kotov:** None.

Poster Board Number:

FRIDAY-394

Publishing Title:

Molecular Typing for Methicillin-resistant *Staphylococcus aureus*: Comparison Between Pcr-Based Open Reading Frame Typing, Multilocus Sequence Typing (Mlst), and *Staphylococcus* Protein A Gene (*spa*) Typing

Author Block:

S. Ogihara[1], R. Saito[2], Y. Kitamura[1], E. Sawabe[1], T. Kozakai[1], M. Shima[1], Y. Nukui[1], M. Hagihara[1], S. Tohda[1]; [1]Tokyo Med. and Dental Univ. Med. Hosp., Tokyo, Japan, [2]Tokyo Med. and Dental Univ., Tokyo, Japan

Abstract Body:

Background: Nosocomial outbreaks of methicillin-resistant *Staphylococcus aureus* (MRSA) infection increase the mortality rate as well as the cost for healthcare. Molecular characterization of MRSA clones is an indispensable prerequisite for investigations into such outbreaks and epidemiological studies. The most widely used techniques for MRSA typing are pulsed-field gel electrophoresis, multilocus sequence typing (MLST) and *Staphylococcus* protein A gene (*spa*) typing. PCR-based open reading frame typing (POT), a multiplex-PCR based typing technique, has been recently developed as a clinical diagnostic tool. In this study, we evaluated the performance of POT for molecular typing of MRSA clinical isolates, and compared it with that of MLST and *spa* typing. **Methods:** A total of 37 non-repetitive MRSA isolates that were suspected to be responsible for a nosocomial outbreak were collected between July 2012 and May 2015 from Tokyo Medical and Dental University hospital of Medicine in Japan. We performed MLST, *spa* typing, and POT, and evaluated their discriminatory power in terms of the Simpson's index. **Results:** The 37 MRSA isolates were classified into 11 types by MLST, 18 types by *spa* typing, and 33 types by POT. The predominant strains identified by MLST, *spa* typing, and POT were ST8 and ST764 (9 strains), t002 (13 strains), and 93-191-127 (3 strains), respectively. The discriminatory power as estimated by Simpson's index was 0.853, 0.875, and 0.992 for MLST, *spa* typing, and POT, respectively. **Conclusion:** Our results show that POT had greater discriminatory power than MLST and *spa* typing. Moreover, while MLST and *spa* typing took more than 2 days to yield results, the turn around time for POT could be shortened to 5 h. POT is easy and rapid to perform, simple to interpret, and is highly discriminatory; we therefore recommend it as a molecular typing tool to monitor nosocomial transmission of MRSA.

Author Disclosure Block:

S. Ogihara: None. **R. Saito:** None. **Y. Kitamura:** None. **E. Sawabe:** None. **T. Kozakai:** None. **M. Shima:** None. **Y. Nukui:** None. **M. Hagihara:** None. **S. Tohda:** None.

Poster Board Number:

FRIDAY-395

Publishing Title:

***Staphylococcus aureus* Nasal Carriage Rate of Preclinical Nursing Students**

Author Block:

A. Kanayama, I. Kanesaka, H. Takahashi, I. Kobayashi; Toho Univ., Tokyo, Japan

Abstract Body:

Background: Colonization of the nasal cavity with *Staphylococcus aureus* can lead to transmission to others by contaminated fingers. In particular, the high prevalence of MRSA in healthcare settings and its spread within hospitals is a concern. In this study, we report on our 7 year monitoring of *S. aureus* and MRSA nasal cavity carriage rates in preclinical nursing students.**Methods:** During the period 2009 to 2015, nostrils of over 100 preclinical nursing students were sampled each year using sterile swabs and cultured on mannitol salt agar for the presence of *S. aureus*. Colonies with typical *S. aureus* morphology were gram stained. Gram-positive cocci were tested for coagulase agglutination to identify as *S. aureus. S. aureus* isolates were screened for the presence of MRSA using the CLSI cefoxitin disk diffusion method.**Results:** During the period of the study, the overall *S. aureus* nasal cavity colonization rate of nurses was 30.2% (235/779). Colonization rates from 2009 to 2015 were 34%, 37%, 28%, 37%, 28%, 23% and 25%, respectively, showing a *S. aureus* carriage rate in approximately one-third of the nursing students. Of the *S. aureus* carriers, 1 MRSA carrier was detected in 2011 and 2015.**Conclusions:** While the MRSA carriage rate was low of preclinical nursing students, the rate of colonization with *S. aureus* was consistent over the study period which dictates a need to enforce compliance with hand hygiene to prevent transmission within the hospital.

Author Disclosure Block:

A. Kanayama: None. **I. Kanesaka:** None. **H. Takahashi:** None. **I. Kobayashi:** None.

Poster Board Number:

FRIDAY-396

Publishing Title:

Clinical Outcomes In Vancomycin-Resistant *enterococcus* (Vre)/Methicillin-Resistant *staphylococcus Aureus* (Mrsa) Bloodstream Coinfection Treated With Daptomycin Or Linezolid

Author Block:

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Abstract Body:

Background: Previous research has demonstrated improved outcomes associated with daptomycin (DAP) treatment for vancomycin-resistant enterococcal bloodstream infection (VRE-BSI) in comparison to linezolid (LZD). However, optimal treatment of bloodstream coinfection with VRE and MRSA is unclear. **Methods:** This was a retrospective cohort study of hospitalized Veterans Affairs (VA) patients (2004-2014) with ≥ 1 blood culture positive for VRE (*E. faecium* or *E.faecalis*) and vancomycin-susceptible MRSA treated with LZD or DAP. Exclusion criteria included: i) treatment < 48 hours; ii) DAP/LZD combination or sequential treatment; iii) concomitant treatment with another active anti-VRE or anti-MRSA agent; and iv) infection caused by a DAP-nonsusceptible or LZD-resistant isolate. The primary outcome was 30-day mortality. Secondary outcomes were hospital mortality, persistent BSI ≥ 7 days (among those with at least one follow-up blood culture), and time to overall microbiologic clearance. A multivariable logistic regression model of factors associated with 30-day mortality was derived using a backward stepwise approach.**Results:** A total of 3,023 patients with VRE-BSI treated with LZD or DAP were identified. Of these, 228 (7.5%) had a concomitant MRSA BSI. Overall mortality was 32.0% (*n*=73/228). Clinical outcomes were compared between DAP and LZD (Table 1). In multivariable regression, factors significantly associated with 30-day mortality were age, *E. faecium*, unknown source, thrombocytopenia, and intensive care unit status.**Conclusions:** DAP was associated with improved time to overall microbiologic clearance and decreased risk of persistent BSI versus LZD in a cohort of VRE/MRSA bloodstream coinfection, with no difference in associated mortality.

Table 1. Comparison of clinical outcomes by treatment for VRE/MRSA bloodstream coinfection

Outcome (*N*=228)	**DAP (*n*=80)**	**LZD (*n*=148)**	***P*-value**
30-day mortality, *n* (%)	24 (30.0)	49 (33.1)	0.631
Hospital mortality, *n* (%)	30 (37.5)	57 (38.5)	0.880
Persistent BSI, *n* (%)	5 (8.2)	20 (19.8)	0.048

Time to overall microbiologic clearance, mean (SD)	2.8 (2.2)	5.4 (10.4)	0.017

Author Disclosure Block:

N.S. Britt: None. **E.M. Potter:** None. **N. Patel:** None. **M.E. Steed:** None.

Poster Board Number:

FRIDAY-397

Publishing Title:

Compatibility of Five Sample Collection Devices with the Cobas® Mrsa/Sa Test on the Cobas® 4800 System

Author Block:

S. Cagas, O. Peraud, R. Hein, S. Moseley; Roche Diagnostics Corp., Indianapolis, IN

Abstract Body:

Background: *Staphylococcus aureus* (SA) and methicillin-resistant *Staphylococcus aureus* (MRSA) strains are associated with significant healthcare burdens and cost. Guidelines recommend active screening and isolation and/or decolonization of patients to control the spread of MRSA and SA. The sensitivity of assay and time to result are key factors in successful screening and treatment strategies. Conventional culture-based methods require several days for results to become apparent and do not allow rapid implementation of specific infection control measures. Challenges for testing include turn around time and media collection types. This study was conducted to determine the compatibility of different sample collection media for assessment of contrived samples with a new PCR based molecular test performed on the cobas® 4800 system. **Methods:** Quantified MRSA and SA material was introduced to MSwab (COPAN Italia, Brescia Italy), ESwab (COPAN Italia, Brescia Italy), CultureSwab Plus Amies gel with and without charcoal (BD, Franklin Lakes, New Jersey) and CultureSwab Liquid Stuart (BD, Franklin Lakes, New Jersey), at 5X and 50X limit of detection (LoD) with the cobas® MRSA/SA Test and evaluated in triplicate and mean Ct values calculated.**Results:** The cobas® MRSA/SA Test detected MSwab, ESwab, CultureSwab Plus Amies gel without charcoal collection media at 5x and 50x LoD. Results obtained with ESwab and CultureSwab Plus Amies gel without charcoal were within 1cycle of the approved MSwab media. CultureSwab Liquid Stuart MRSA and SA specimens were detected at 50X LoD, only one of the three 5X LoD replicates for MRSA and SA were detected. CultureSwab Plus Amies gel with charcoal MRSA and SA specimens were detected at 50X LoD, neither MRSA or SA were detected at 5X LoD.**Conclusions:** Preliminary results show compatibility of MSwab, ESwab, CultureSwab Plus Amies gel with and without charcoal and CultureSwab Liquid Stuart collection devices with the cobas® MRSA/SA Test, performed on the fully automated cobas® 4800 system. Further feasibility studies with clinical specimens are necessary to determine full compatibility. PCR technology has potential for media type flexibility and fast turn around time.

Author Disclosure Block:

S. Cagas: D. Employee; Self; Roche Diagnostics Corporation. **O. Peraud:** D. Employee; Self; Roche Diagnostics Corporation. **R. Hein:** D. Employee; Self; Roche Diagnostics Corporation. **S. Moseley:** D. Employee; Self; Roche Diagnostics Corporation.

Poster Board Number:

FRIDAY-398

Publishing Title:

Prospective Study of *Staphylococcus aureus* and *Streptococcus pyogenes C*arriage and Infections in Royal Marines Commando Recruits

Author Block:

L. Lamb[1], E. Jaunelkaite[1], T. Davey[2], J. Fallowfield[2], N. Thorpe[2], T. Ferguson[1], M. Pyzio[1], M. Mosavie[1], A. Allsopp[2], M. O'Shea[3], D. Wilson[3], S. Batumbu[3], B. Pichon[4], M. Morgan[5], A. Kearns[4], S. Sriskandan[1]; [1]Imperial Coll., London, United Kingdom, [2]Inst. of Naval Med., Gosport, United Kingdom, [3]Royal Ctr. for Defence Med., Birmingham, United Kingdom, [4]Publ. Hlth.England, Colindale, United Kingdom, [5]Royal Devon and Exeter Hosp., Exeter, United Kingdom

Abstract Body:

Introduction: Skin and soft tissue infections (SSTI) represent a significant clinical burden in recruits, causing significant morbidity and economic burden. Recently clindamycin has been advocated for use due to concerns regarding PVL *S. aureus*. We sought to determine risk factors for acquisition of organisms associated with SSTI and the relevance of PVL. **Methods:** The anterior nares and throats of consenting recruits were swabbed at weeks 1, 6, 15 and 32 of training. Swabs were cultured to detect *S.aureus* and *S. pyogenes* and isolates were subjected to antimicrobial susceptibility testing, *spa* typing or *emm* typing as appropriate. Demographic data and clinical history of SSTI were collected and linked to microbiological data.**Results:** 1015 male recruits consented (92%) and disease data were collected from 991 recruits (98%). 351 recruits (35%) had an SSTI of which cellultis was the most common. 49% of recruits with a SSTI required admission to the medical centre and 8% to hospital. Carriage of *S.aureus* in week 1 was 41% and showed seasonal variation. Most colonizing *S.aureus* strains were susceptible to standard antibiotics (77%), inducible clindamycin resistance detected in 16% and MRSA in less than 1%. Antimicrobial resistance (AMR) increased during training (16 to 33%, $p<0.001$). The commonest Clonal complexes were 30, 15, 5 and 8. Preliminary work showed PVL and GAS carriage rate to be low (<1%) and no association between carriage of *S.aureus* and smoking status.**Conclusion:** This work aimed to evaluate relationships between carriage and acquisition of SSTI, focussing on risk factors for *S.aureus* that may be amenable to intervention. Of particular note in this study was the low carriage rate of *S.pyogenes.* Incision and drainage was not advised as there was a low rate of purulent infections (<2%). Preliminary analysis shows a rise in AMR during training suggesting the use of clindamycin as first line treatment for SSTI in this military group may need to be re-considered.

Author Disclosure Block:

L. Lamb: None. **E. Jaunelkaite:** None. **T. Davey:** None. **J. Fallowfield:** None. **N. Thorpe:** None. **T. Ferguson:** None. **M. Pyzio:** None. **M. Mosavie:** None. **A. Allsopp:** None. **M. O'Shea:** None. **D. Wilson:** None. **S. Batumbu:** None. **B. Pichon:** None. **M. Morgan:** None. **A. Kearns:** None. **S. Sriskandan:** None.

Poster Board Number:

FRIDAY-399

Publishing Title:

Prevalence and Molecular Epidemiology of *Staphylococcus aureus* Among Bhutanese Refugees in Nepal and in Northeast Ohio

Author Block:

J. Kadariya[1], D. Thapaliya[1], R. Mahatara[2], S. Bhatta[1], N. Dhakal[3], T. Smith[1]; [1]Kent State Univ., Kent, OH, [2]Intl. Organization for Migration, Jhapa, Nepal, [3]AMDA Hosp., Jhapa, Nepal

Abstract Body:

Every year the United States resettles thousands of refugees from around the world. Although studies have shown that human migration is one of the risk factors for the spread of drug-resistant organisms such as methicillin-resistant *Staphylococcus aureus* (MRSA), there has not been any study conducted regarding the prevalence of MRSA among refugee populations in the U.S. This study aimed to assess the prevalence and molecular characteristics of *S. aureus* among Bhutanese refugees living in Nepal and in Northeast Ohio (NEO). One hundred adult Bhutanese refugees from each location were enrolled between August 2015 and January 2016. The participants were interviewed to collect the demographic information and exposures to potential risk factors for carriage. Nasal and throat swabs were collected from each individual and processed within 24 hours according to the study protocol. All *S. aureus* isolates were typed by *spa* typing and multi-locus sequence typing (MLST). The presence of the Panton-Valentine Leukocidin (PVL) and *mec*A genes were detected via PCR. The Vitek-2 System was used to test the antibiotic susceptibility. Of the 100 participants enrolled in Nepal, the median age was 35 years (mean 36.75; standard deviation, 13.28; range, 57). Twenty-nine percent and 71% of the participants were male and female, respectively. The overall prevalence of *S. aureus* was 45% (45/100). The prevalence of MRSA was 2% (2/100). The overall prevalence of PVL genes among *S. aureus* isolates was 25% (13/52). A total of 31 *spa* types were detected from 52 *S. aureus* isolates. The most common *spa* type was t345(9.6%). One isolate was t008 (USA300), a common community-associated strain. One isolate was found to be t002, a common healthcare-aasociated strain (USA100). Twenty-one isolates (40.4%) were multi-drug resistant. Analyses of data from NEO are ongoing. The findings of this study indicate that Bhutanese refugees living in Nepal had high prevalence of *S. aureus* and high prevalence of multi-drug resistant *S. aureus.*

Author Disclosure Block:

J. Kadariya: None. **D. Thapaliya:** None. **R. Mahatara:** None. **S. Bhatta:** None. **N. Dhakal:** None. **T. Smith:** None.

Poster Board Number:

FRIDAY-400

Publishing Title:

Environmental Sampling of *Staphylococcus aureus* at a Large Midwest University: A Connection with Health Care Students

Author Block:

M. Taha[1], D. Thapaliya[2], **M. R. Dalman**[2], J. Kadariya[2], T. Smith[2]; [1]Iraqi Ministry of Sci. and Technology, Baghdad, Iraq, [2]Kent State Univ., Kent, OH

Abstract Body:

Staphylococcus aureus is a commonly found bacterium that colonizes a variety of animal species, including humans. Additionally, it is also found on fomites and has the potential to be transferred from person to person via contaminated surfaces. Methicillin-resistant *S. aureus* strains (MRSA) first emerged in hospital settings (HA-MRSA), but are also found in the community (CA-MRSA). With over 11,000 deaths from MRSA and the ability to survive on surfaces for several months, we characterized environmental contamination of both healthcare and non-healthcare associated college student buildings in order to determine molecular type and antibiotic susceptibility profiles of *S. aureus* isolates. Environmental swabs (n=152) were collected from healthcare (n=2) and nonhealthcare- associated buildings (n=2) at a state university. A total of 38 samples were collected per building from high hand-touch areas (door handles, sink handles, etc.) and processed within 24 hours using typical bacteriological methods. Five colonies per positive sample were then subjected to antibiotic susceptibility testing and molecular characterization (multilocus sequence typing, PVL and *mec*A PCR, and *spa* typing). A total of 34 of 152 (22.4%) samples were contaminated with *S. aureus* and 9 (5.9%) were positive for MRSA. Three sites were positive from multidrug resistant (MDR) strains. Within healthcare and non-healthcare facilities, 5/75 (6.75%) and 4/75 (5.26%) were MRSA positive, respectively. The most frequently contaminated surface for MRSA in buildings frequented by healthcare-associated students was the main door, whereas in non-healthcare buildings it was the classroom. All isolates were resistant to penicillin, with methicillin resistant (26.5%) and MDR (8.8%) present. The most common *spa* types found were t1149, t068, t216, t008, and t334. These results point to the ease of *S. aureus* contamination on fomite surfaces and the presence of t008 (a community-associated strain type) in healthcare-associated student buildings, along with MDR *S. aureus* indicates a need for increased awareness of the potential for environmental surfaces, even outside of the hospital setting, to act as reservoirs for *S. aureus*.

Author Disclosure Block:

M. Taha: None. **D. Thapaliya:** None. **M.R. Dalman:** None. **J. Kadariya:** None. **T. Smith:** None.

Poster Board Number:

FRIDAY-401

Publishing Title:

Predicting Severity or Duration of Infection Based on the Characteristics of *Staphylococcus aureus* Strains Causing Bovine Mastitis

Author Block:

S. Pichette-Jolette[1], G. Millette[1], E. Demontier[1], D. Bran-Barrera[1], M. Cyrenne[1], C. Ster[1], F. Malouin[1], G. Keefe[2], D. Haine[3], J-P. Roy[3]; [1]Univ. de Sherbrooke, Sherbrooke, QC, Canada, [2]Univ. of Prince Edward Island, Charlottetown, PE, Canada, [3]Univ. de Montreal, Saint-Hyacinthe, QC, Canada

Abstract Body:

Background: Bovine mastitis is one of the most frequent infections observed in dairy cattle. *Staphylococcus aureus* is the leading pathogen causing mastitis. The infection is transmitted to healthy cows by the milking instruments and farm workers. The severity of the disease caused by the presence of *S. aureus* can be qualified as subclinical (no visible sign of inflammation) or clinical. Antibiotic treatment often fails and infections can extend for a long period of time. In such cases, more drastic measures must be taken like culling of the infected animals. Here, we compared the genotypes and phenotypes of *S. aureus* isolates collected from subclinical or clinical mastitis cases of varying duration.**Method:** The study includes more than 340 unique quarter isolates from documented mastitis cases and quarter milk samples collected from 64 farms across Canada (Canadian Bovine Mastitis and Milk Quality Research Network (CBMMQRN), Mastitis Pathogen Culture Collection). Variable number of tandem repeats typing was used to assess the persistence of the same *S. aureus* strain in consecutive quarter milk samples and to estimate the duration of infection (up to 500 days in some cases). Strains were compared based on the extent of biofilm production, *spa* typing and presence of virulence genes (*seg*, *tst*, *lukM*).**Results:** The major *spa* types found were t529 (n=167), t267 (n=87), t359 (n=46) and t605 (n=16). The *spa* type significantly influenced the production of biofilm and the severity of infection. The presence of virulence genes was also associated to *spa* types and some virulence genes correlated with the severity of the disease. For example, t605 produced more biofilm and was more likely to lack *lukM* while t529 was most often *seg+* and associated with clinical mastitis. There was no statistical difference between the *spa* type and the duration of the infection.**Conclusion:** Mastitis caused by *S. aureus* is difficult to eliminate from dairy herds. Predicting the severity or duration of infection based on strain characteristics would be extremely useful in herd management practices. We reported here some attributes linked to problematic strains.

Author Disclosure Block:

S. Pichette-Jolette: None. **G. Millette:** None. **E. Demontier:** None. **D. Bran-Barrera:** None. **M. Cyrenne:** None. **C. Ster:** None. **F. Malouin:** None. **G. Keefe:** None. **D. Haine:** None. **J. Roy:** None.

Poster Board Number:

FRIDAY-402

Publishing Title:

Multiresistant Methicillin-resistant *Staphylococcus aureus* St5-Scc*mec IV* and St30-Scc*mec IV* Recovered from Cystic Fibrosis Patients

Author Block:

S. Haim[1], P. Pena Amaya[2], **S. Di Gregorio**[1], S. Fernández[1], A. Teper[2], M. Vázquez[2], S. Lubovich[2], L. Galanternik[2], M. Mollerach[1]; [1]Univ. de Buenos Aires, CABA, Argentina, [2]Hosp de Niños R. Gutiérrez, CABA, Argentina

Abstract Body:

Background: *Staphylococcus aureus* is an early observed pathogen in Cystic Fibrosis (CF) that can chronically infect the lungs of these patients. Methicillin-resistant *S. aureus* (MRSA) colonization in CF patients attending the Hospital de Niños R. Gutiérrez is an increasing problem climbing from 23% in 1995 up to 32% in 2011. However, the information about its epidemiological features is limited. The aim of this study was to analyze the diversity of MRSA isolates recovered from respiratory samples of CF patients. **Methods:** Antimicrobial susceptibility testing (Vitek or disk diffusion method on Mueller Hinton agar + 5% sheep blood) was performed to all *S. aureus* isolates recovered from 41 out of 120 CF patients from June 2012 to May 2013. For each patient, only the first MRSA isolate was included, except when isolates with a different resistance pattern were recovered. *luk*-SF/PV and *mec*A genes were studied by PCR. Strains were genotyped by SCC*mec* typing, PFGE, *spa* typing and MLST. **Results:** 57 isolates from 41 CF patients were included. All were *mec*A positive and 32%, PVL positive. 41/57 (72%) isolates harbored SSC*mec* IV. Among these, 24 (42%) belonged to the main community-acquired MRSA (CA-MRSA) clone complexes described in Argentina: Pulsotype A (PA) ST5-SCC*mec* IVa (16/57) and Pulsotype C (PC) ST30-SCC*mec* IVc (8/57). The antibiotic resistance rates were: gentamicin (63%-57%), erythromycin (44%-43%), clindamycin (44%-14%), ciprofloxacin (25%-57%) and rifampicin (31%-0%), PA and PC respectively. Multiresistance was detected in 11/24 (46%) MRSA strains, 8 corresponding to PA and 3 to PC. For those isolates related to PA, 56% were *spa* type t002 and 44%, t311. Among those with PC, the most frequent *spa* type was t019 (75%). **Conclusions**: The ST5-SCC*mec* IVa-PA and ST30-SCC*mec* IVc-PC clones were the most frequent CA-MRSA clones recovered in this study. Nonetheless, the rate of multiresistance showed by these clones was higher compared to what is reported in non cystic fibrosis patients in our country. These clones could represent an emerging health threat not only for cystic fibrosis patients but also for the general community, as they seem to have originated from CA-MRSA lineages, which are highly transmissible.

Author Disclosure Block:

S. Haim: None. **P. Pena Amaya:** None. **S. Di Gregorio:** None. **S. Fernández:** None. **A. Teper:** None. **M. Vázquez:** None. **S. Lubovich:** None. **L. Galanternik:** None. **M. Mollerach:** None.

Poster Board Number:

FRIDAY-403

Publishing Title:

Prevalence and Characterization of *Staphylococcus aureus* on Public Recreational Beaches in Northeast Ohio

Author Block:

D. Thapaliya, E. Hellwig, J. Kadariya, M. Dalman, K. Kennedy, M. DiPerna, A. Orihill, D. Grenier, M. Taha, T. Smith; Kent State Univ., Kent, OH

Abstract Body:

Staphylococcus aureus is a major public health concern due to the emergence of virulent and drug-resistant strains such as methicillin-resistant *Staphylococcus aureus* (MRSA), and due to its ever-changing epidemiology. Although MRSA has been isolated from marine water and intertidal beach sand, only few studies have conducted to assess the prevalence of *S. aureus* in freshwater recreational beaches. This study aimed to determine the prevalence and molecular characteristics of *S. aureus* at freshwater recreational beaches in Northeast Ohio. A total of 280 beach sand and water samples were collected from 10 public fresh water beaches in Northeast Ohio, USA between June 24, 2014 and April 30, 2015. Samples were analyzed using established microbiology methods, and resulting *S. aureus* isolates were typed by *spa* typing and multi-locus sequence typing (MLST). PCR was used to detect the presence of the Panton-Valentine Leukocidin (PVL) and *mec*A genes. Antibiotic susceptibility was tested via the Vitek-2 System. The overall prevalence of *S. aureus* in sand and water samples was 22.9% (64/280). The prevalence of MRSA was 7.9% (22/280). The highest prevalence was observed in summer (45.8%; 55/120) compared to fall (4.2%; 5/120), and spring (10%; 4/40). The prevalence of PVL genes among *S. aureus* isolates was 23.4% (15/64). A total of 26 *spa* types were detected from 64 *S. aureus* isolates. Overall, t008 was the most common *spa* type.Two isolates were t002, a common human-associated strain. One isolate was t571 (ST398), a livestock-associated strain. Twenty-eight (43.8%) isolates were multi-drug resistant.The results of this study indicate that beach sand and fresh water of Northeast Ohio was contaminated with *S. aureus* and MRSA. The high prevalence of *S. aureus* in summer months and presence of human –associated strains may indicate the possible role of human beachgoers in *S. aureus* contamination of beach water and sand.

Author Disclosure Block:

D. Thapaliya: None. **E. Hellwig:** None. **J. Kadariya:** None. **M. Dalman:** None. **K. Kennedy:** None. **M. DiPerna:** None. **A. Orihill:** None. **D. Grenier:** None. **M. Taha:** None. **T. Smith:** None.

Poster Board Number:

FRIDAY-404

Publishing Title:

Analysis of Morphologically Similar *Staphylococcus aureus* Colonies to Assess Phenotypic and Genotypic Correlation

Author Block:

M. Totten, T. Ross, A. Voskertchian, A. Milstone, K. Carroll; Johns Hopkins Med. Inst.s, Baltimore, MD

Abstract Body:

Background: When performing surveillance cultures from non-sterile sites for *Staphylococcus aureus* (*S. aureus*), a single bacterial isolate is selected as representative of the cultured strain. Standard practice presumes that morphologically similar colonies constitute the same strain and are thus phenotypically and genetically identical. The objective of this study was to determine how frequently colonies with the same morphology are phenotypically and genetically diverse. **Methods**: As part of a larger study examining parent to infant transmission of *S aureus*, swabs were collected and cultured from multiple body sites. Cultures were inoculated into Tsoy broth with 6.5% NaCl (BD Diagnostics, Inc. Sparks, MD), incubated overnight and subcultured onto 5% sheep blood agar (BD) and SASelect™ chromogenic media (Bio-rad, Hercules, CA). For positive cultures, a set of five separate isolates for each unique colony morphology was characterized using pulsed field gel electrophoresis (PFGE). Additionally, the antimicrobial susceptibility (AST) profile and identification of each isolate was determined using the Phoenix PMIC/ID-105 panel (BD). **Results:** Fourteen neonate and adult participants had positive *S aureus* cultures that yielded 205 isolates (5 isolates tested for each of the 41 observed morphologies). Of the 205 isolates, 204 (99.5%) had the same PFGE pattern as other isolates recovered from the same group of patients. One isolate had a one band difference from the others in its group. AST testing showed that 195 of the 205 isolates tested had the same antibiogram to all 16 antibiotics tested. **Conclusion:** Results from both PFGE and AST support the common assumption that *S aureus* isolates from the same culture with similar morphologies are of the same strain. Thus, saving a single bacterial isolate from a cultured *S. aureus* sample is an appropriate standard practice when archiving isolates for future susceptibility testing and epidemiological studies.

Author Disclosure Block:

M. Totten: None. **T. Ross:** None. **A. Voskertchian:** None. **A. Milstone:** None. **K. Carroll:** None.

Poster Board Number:

FRIDAY-405

Publishing Title:

Community-Associated *Staphylococcus aureus* (CA-MRSA) is Associated with Low Level Resistance against Oxacillin, Cefoxitin, and Vancomycin

Author Block:

W-Y. Wang[1], S-M. Tsao[2]; [1]Feng-Yuan Hosp., Taichung, Taiwan, [2]Chung Shan Med. Univ. Hosp., Taichung, Taiwan

Abstract Body:

Background: *Staphylococcus aureus*, including methicillin-susceptible (MSSA) and methicillin-resistant *S. aureus* (MRSA), is a leading pathogen for clinical infections with increasing resistance. *mecA*+ MRSA with oxacillin (OX) and cefoxitin (FOX) susceptibility has been reported elsewhere. We aim at delineating the molecular distribution and prevalence of low level resistance to OX and FOX, and vancomycin (VA) susceptibility pattern among *S. aureus*. **Material/methods:** *S. aureus* were collected from clinical specimens of one medical center in central Taiwan between July, 2011 and June, 2014, and they were identified according to CLSI. MRSA was confirmed with *mecA* existence by PCR. The minimal inhibitory concentrations (MIC) of isolates against OX, FOX, and VA were determined with agar dilution. The staphylococcal cassette chromosome *mec* (SCC*mec*) types were determined by PCR. **Results:** Totally 543 non-duplicate *S. aureus*, including 226 MSSA and 317 MRSA, were collected. Most were cultivated from pus (233, 42.9%), sputum (139, 25.6%), blood (117, 21.5%), and urine (32, 5.9%). Six SCC*mec* types, including I (1, 0.3%), II (36, 11.3%), III (96, 30.3%), IV (95, 30.0%), V (24, 7.6%), and V_T (50, 15.8%), were determined. Also 15 MRSA were not determined by SCC*mec* typing. High sensitivity and specificity were noted for OX (89.6%/99.6%) and FOX (92.4%/99.6%). Mean VA MIC for *S. aureus*, MSSA, and MRSA were 1.24 ± 0.57, 1.16 ± 0.51, and 1.29 ± 0.61 mg/L, respectively ($p = 0.006$). Higher mean VA MIC was seen in SCC*mec* II (1.45 mg/L) and III (1.25 mg/L) than in SCC*mec* IV (0.93 mg/L), V (1.16 mg/L), and V_T (1.03 mg/L) ($p < 0.01$). Mean VA MIC of healthcare-associated MRSA (HA-MRSA, SCC*mec* II and III) was higher than community-associated MRSA (CA-MRSA, SCC*mec* IV, V, and V_T) (1.44 ± 0.60 *vs*. 1.13 ± 0.54 mg/L, $p < 0.001$). Higher mean VA MIC was seen in *S. aureus* and MRSA from blood (1.90/1.91 mg/L) and sputum (1.18/1.26 mg/L) than those from pus (0.99/0.98 mg/L) ($p < 0.001$). Thirty-three MRSA (10.4%) and 24 (7.6%) with OX and FOX susceptibility were found, which most (18 for OX and 9 for FOX) were CA-MRSA. **Conclusions:** Low level resistance againt oxacillin, cefoxitin, and vancomycin are found among CA-MRSA. MRSA harbors increasing vancomycin resistance. Molecular and specimen types can be used as predictors of vancomycin resistance.

Author Disclosure Block:

W. Wang: None. **S. Tsao:** None.

Poster Board Number:

FRIDAY-406

Publishing Title:

Prevalence and Characterization of *Staphylococcus aureus* on Geese Feces Collected from 10 State Parks in Northeast Ohio

Author Block:

D. Grenier, D. Thapiliya, K. Little, V. Mansell, J. Kadariya, M. Dalman, M. Taha, T. Smith; Kent State Univ., Kent, OH

Abstract Body:

Staphylococcus aureus can colonize wide range of hosts including domestic as well as wild animals. Migratory birds colonized with *S. aureus* may spread drug-resistant strains of *S. aureus* such as methicillin-resistant *S. aureus* (MRSA). Although numerous studies have isolated other pathogenic bacteria from wild birds; very limited data are available examining the presence of *S. aureus* among these species. The objective of this study was to determine the prevalence and molecular characteristics of *S. aureus* in geese fecal samples collected from 10 state parks across Northeast Ohio (NEO). A total of 182 fecal samples were collected between April 22, 2015 and April 29, 2015. The samples were processed within 24 hours according to the established microbiology methods. Multi-locus sequence typing (MLST) and *spa* typing were carried out on all isolates. PCR was conducted to detect the presence of the Panton-Valentine Leukocidin (PVL), *mec*A, and *scn* genes. The overall prevalence of *S. aureus* in the fecal samples was 7.1% (13/182). The prevalence of *mec*A gene among the *S. aureus* isolates was 53.8% (7/13), while the prevalence of PVL was 7.7% (1/13). All the isolates harbored the *scn* gene. A total of 8 different *spa* types were observed among 13 isolates. Overall, t008 (USA-300, ST-8), a common community-associated (CA) strain was the most common (46.1%, 6/13) *spa* type. One isolate was t002 (USA-100, ST-5), a common hospital-associated (HA) strain. One livestock-associated (LA) strain (t571, ST-398) was also found. All isolates were resistant to penicillin. Eight isolates (61.5%) were resistant to oxacillin. Nine isolates (69.2%) were multi-drug resistant. The results of this study indicate that the feces of geese collected at various state parks in NEO harbored *S. aureus* and MRSA. The presence of HA, LA, and CA strains of *S. aureus* in freshly voided geese fecal samples suggests that geese in NEO may be subject to bidirectional spread of these pathogens, and may play a role in the transmission of *S. aureus* in the environment.

Author Disclosure Block:

D. Grenier: None. **D. Thapiliya:** None. **K. Little:** None. **V. Mansell:** None. **J. Kadariya:** None. **M. Dalman:** None. **M. Taha:** None. **T. Smith:** None.

Poster Board Number:

FRIDAY-407

Publishing Title:

Retrospective Analysis of Adverse Events (Aes) between Nafcillin Vs. Cefazolin for Complicated Methicillin Susceptible *Staphylcoccus aureus* (Mssa) Infections

Author Block:

L. Chan[1], A. J. Guarascio[2], J. Como[1], N. H. Chan-Tompkins[1]; [1]Allegheny Gen. Hosp., Pittsburgh, PA, [2]Duquesne Univ., Mylan Sch. of Pharmacy, Pittsburgh, PA

Abstract Body:

Background: Nafcillin or cefazolin are frequently prescribed for MSSA infections. Adverse events such as neutropenia, thrombocytopenia, serum creatinine elevation (Scr), and serum transaminase elevation have been reported. Acute kidney injury (AKI), defined as increase in Scr of >0.5 mg/dL or 50% increase from baseline, has previously been described. AKI incidence based on RIFLE (Risk, Injury, Failure, Loss of kidney function, and End-stage kidney disease) criteria has not been studied with nafcillin or cefazolin. Primary objective: To quantify the incidence and time to AKI based on RIFLE criteria for inpatients (pts) who received nafcillin vs. cefazolin therapy. Secondary objectives: To measure the incidence and time to event for neutropenia, thrombocytopenia, elevated transaminases, and *Clostridium difficile* infection (CDI) in this group. **Methods:** IRB approval was obtained for this retrospective cohort study. Inclusion criteria: age ≥ 18 years old, hospitalization from 7/1/2013-6/30/15, and receipt of IV nafcillin or cefazolin for ≥ 72 hours to treat MSSA infection.**Results:** Of 358 pts screened, 96(26.8%) met inclusion criteria: 46(47.9%) nafcillin vs. 50(52.1%) cefazolin. Ten (21.7%) pts developed AEs (all types) with nafcillin vs. no AEs (0%) with cefazolin. Median days of therapy to event was 9 (range, 3-16). Nine (19.6%) nafcillin pts developed AKI. Median baseline Scr was 1.2 mg/dL (range, 0.4-1.9).For patients receiving nafcillin, thrombocytopenia occurred in 1(2.2%)[median time to event = 2 days] and AST elevation occurred in 2(4.3%)[median time = 5 days]. No pts developed neutropenia, CDI, or ALT elevation. The median Naranjo score was 3(range, 0-6).**Conclusions:** AEs were more common with nafcillin vs. cefazolin in the inpatient setting. AKI, the predominant nafcillin-related AE, occurred most commonly in patients with elevated risk and/or subsequent injury leading to renal dysfunction.

Table 1. Incidence and Time to AKI Based on RIFLE Criteria

Adverse Event	Nafcillin (N=46)		Cefazolin (N=50)
	N (%)	Median (range) Time to Event (days)	N(%)
AKI			
Risk	3 (6.5%)	8 (2-13)	0 (0%)
Injury	4 (8.7%)	4.5 (1-14)	0 (0%)

Failure	4 (2.2%)	12	0 (0%)
Loss	1 (2.2%)	4	0 (0%)
ESRD	0 (0%)	--	0 (0%)

Author Disclosure Block:

L. Chan: None. **A.J. Guarascio:** None. **J. Como:** None. **N.H. Chan-Tompkins:** None.

Poster Board Number:

FRIDAY-408

Publishing Title:

Bolaamphiphile Nanocomplex Delivery of Antisense Molecules as a Treatment for *C. difficile*

Author Block:

J. P. Hegarty, J. Krzeminski, A. Sharma, D. B. Stewart; Penn State Univ. Coll. of Med., Hershey, PA

Abstract Body:

Background: Antisense molecules can specifically inhibit the expression of bacterial genes through blocking of RNA translation. As a potential alternative to conventional antibiotics, a critical issue is achieving efficient cellular delivery. We have previously used cell penetrating peptide conjugation to deliver antisense morpholinos into *C. difficile*. Vesicles of bolaamphiphile compounds (e.g. dequalinium) are known to condense and deliver DNA in the form of nanocomplexes into cardiolipin-rich mitochondria. As cardiolipin is also a component of bacterial membranes, we investigated the potential use of dequalinium analogs as nanocarriers of bacterial antisense cargo. **Methods:** Phosphorothioate oligonucleotide sequences were designed *in silico* to target the expression of essential *C. difficile* genes and then quantitatively assessed for their ability to block gene translation using plasmid expression constructs in a cell-free system. Analogs of dequalinium chloride containing polar head groups modified for lower toxicity and with varying alkyl chain lengths were synthesized, HPLC purified, and structures confirmed by NMR spectroscopy. Stable vesicles (~100 nm) of compounds were assembled through probe-sonication in salt-free buffer. The concentrations of cationic delivery vesicles required for nanocomplexation of anionic 12- to 25-mer phosphorothioates were determined using fluorescent dye-exclusion assays. Log phase BHIS cultures of ribotypes 027 and 012 were treated in triplicate with serial doses of nanocomplexes and matched empty vesicles, followed by anaerobic incubation for 24 hours. Cell growth and MIC values were determined by culture absorbance and viable colony plate counts.**Results:** Compared to our previously tested cell penetrating peptides, bolaamphiphile antisense nanocomplexes resulted in a 10-fold decrease in MIC (40 nM). No significant growth inhibition was observed from treatment with matched empty delivery vesicles.**Conclusion:** Bolaamphiphile nanocomplexes show promise as a carrier for antisense molecules into bacteria, with a favorable toxicity profile and with MICs near that of conventional antibiotics. Nanocomplexes may provide a future microbiome-sparing therapy for *C. difficile* infection.

Author Disclosure Block:

J.P. Hegarty: None. **J. Krzeminski:** None. **A. Sharma:** None. **D.B. Stewart:** None.

Poster Board Number:

FRIDAY-409

Publishing Title:

Tobramycin and Bicarbonate Synergize to Kill Planktonic *Pseudomonas aeruginosa*, but Antagonize to Promote Biofilm Survival

Author Block:

K. S. Kaushik, J. Stolhandske, O. Shindell, H. Smyth, V. Gordon; Univ. of Texas at Austin, Austin, TX

Abstract Body:

Rising antibiotic resistance and declining development and approval of new antibiotics motivates approaches to increase the efficacy of existing antibiotics. For the opportunistic human pathogen *Pseudomonas aeruginosa*, we previously observed that alkalis including sodium bicarbonate enhance the bactericidal effect of aminoglycoside antibiotics against antibiotic-resistant mutants. Here, we examine the possibility of using bicarbonate in conjunction with aminoglycosides to enhance the efficacy of antibiotic therapy against *P. aeruginosa* infections.The aminoglycoside tobramycin is standard-of-care for many types of *P. aeruginosa* infections, including acute infections, often caused by planktonic bacteria, and chronic, biofilm infections, such as those found in lungs of cystic fibrosis (CF) patients. Inhaled bicarbonate is being evaluated as a therapy to improve antimicrobial activity of the airway surface liquid and decrease the viscosity of CF mucus. Using checkerboard microdilution assays for planktonic cells and *in vitro* biofilms, we measure the effect of combining tobramycin and bicarbonate against *P. aeruginosa*, for laboratory and clinical CF strains. Bicarbonate synergizes with tobramycin to enhance the killing of planktonic bacteria. In contrast, bicarbonate antagonizes with tobramycin to promote better biofilm growth. This suggests caution when evaluating bicarbonate as a therapy for CF lungs infected with *P. aeruginosa* biofilms.We analyze the above drug interactions using an interpolated dose-response surface. This allows more accurate estimation of therapeutic combinations than do standard isobolograms. Using predictions based on Loewe additivity, we can consolidate information on a wide range of combinations across the dose-response surface into a net effect, enabling rapid initial estimation of the potential benefit or harm of a therapeutic combination.The mechanism(s) underlying antagonism of tobramycin and bicarbonate in biofilm treatment is unknown, but one possibility is binding of tobramycin to the extracellular biofilm matrix. We are currently testing the effect of combining bicarbonate with a chemically modified form of tobramycin (conjugated to polyethylene glycol), which has reduced affinity for matrix polymers and greater efficacy against biofilms.

Author Disclosure Block:

K.S. Kaushik: None. **J. Stolhandske:** None. **O. Shindell:** None. **H. Smyth:** None. **V. Gordon:** None.

Poster Board Number:

FRIDAY-410

Publishing Title:

Antibiofilm Effect of Selenium on Uropathogenic *Escherichia coli*

Author Block:

A. Narayanan, M. Surendran Nair, M. S. Muyyarikkandy, M. A. Amalaradjou, K. Venkitanarayanan; Univ. of Connecticut, Storrs, CT

Abstract Body:

Background: Urinary tract infections (UTI) are the most common hospital-acquired infections in humans, caused primarily by uropathogenic *Escherichia coli* (UPEC). Indwelling urinary catheters for bladder drainage in humans become colonized with UPEC biofilms that are resistant to common antibiotics, resulting in chronic infections. Therefore it is important to control UPEC biofilms on catheters.Se is a natural essential trace mineral which acts as a co-factor for many critical metabolic enzymes in human cells. It plays a significant role in many biological processes in the body, such as immunity and antioxidant defense. This study investigated the efficacy of Se in inhibiting and inactivating UPEC biofilms on urinary catheters.**Materials and methods:** Urinary catheters were inoculated with UPEC (~5 log CFU) and treated with 0%, 0.6% and 0.75% Se at 37°C. In addition, catheters with pre-formed mature UPEC biofilms were treated with a lock solution containing Se (0%, 1.5%, or 2%), and incubated at 37°C. Biofilm-associated UPEC counts on control and Se treated catheters were enumerated on days 0, 1, 3 and 5 of incubation. The study was replicated twice with duplicate samples.**Results:** All Se concentrations were effective in preventing UPEC biofilm formation on catheters compared to controls ($P<0.05$). Biofilm-associated UPEC was below detectable limits (< 1.0 log CFU) on catheters treated with 0.75% Se, while ~5.5 log CFU of UPEC was recovered from control samples after 5 days of incubation. Furthermore, Se as a catheter lock solution ingredient decreased UPEC biofilm populations by > 5 log on catheters.**Conclusion:** Results suggest that Se could be applied as a coating on catheter surface or as an ingredient in catheter lock solution to potentially control UTI in humans.

Author Disclosure Block:

A. Narayanan: None. **M. Surendran Nair:** None. **M.S. Muyyarikkandy:** None. **M.A. Amalaradjou:** None. **K. Venkitanarayanan:** None.

Poster Board Number:

FRIDAY-411

Publishing Title:

HT-07 — A Bis-Catechol cefaclor Targeted Sideromycin is a Potent Agent against Both *Acinetobacter baumannii* and *E. coli*

Author Block:

Y-M. Lin[1], P. Miller[2], D. L. Shinabarger[3], **M. J. Miller**[2]; [1]Hsiri Therapeutics, South Bend, IN, [2]Univ. of Notre Dame, Notre Dame, IN, [3]Micromyx, Inc, Kalamazoo, MI

Abstract Body:

Background: Our rationally designed sideromycin HT-07 has previously been shown to be active *in vivo* in a mouse peritoneal sepsis model, with no evidence of bacterial adaptation. HT-07 is targeted using a bis-catechol siderophore as a specific iron chelator, which is recognized by the outer membrane receptors (OMR) of Gram-negative bacteria to enable the cellular uptake of HT-07 through the active transport mechanism initiated by the OMR. **Methods:** Under careful iron deficient conditions, we evaluated the MICs of HT-07 against 40 strains of *Acinetobacter baumannii*, and 40 strains of *E. coli* in the presence or absence of Sulbactam (Su) (*Acinetobacter*) and Su, Tazobactam (Tz), and Clavulanate (Cl) (*E. coli*). MHII Broth and otherwise standard CLSC methods were used in 96 microwell plates.**Results:**Table I. *In vitro* Activity against 40 *Acinetobacter baumannii* strains

Ampicillin (alone) *MIC (μg/mL)*	**HT-07** (alone)	**HT-07+Sulbactam** (1.2 *μg/mL)*	**Ampicillin+ Su** (1.2 *μg/mL)*
MIC < 0.5: 1/40	MIC < 0.5: 21/40	MIC < 0.5: 30/40	MIC< 0.5: 1/40
MIC=16–64: 22/40	MIC =0.5: 2/40	MIC=8: 1/40	MIC=8: 1/40
MIC >64: 17/40	MIC >64: 17/40	MIC>64: 9/40	MIC=2-4:19/40 MIC=4-8: 7/40 MIC>32: 13/40

Table II.*In vitro* Activity against 40 *E. coli* strains

HT-07 (alone) MIC *(μg/mL)*	**HT-07+Su** *(1.2 μg/mL)*	**HT-07+Cl** *(10 μg/mL)*	**HT-07+ Tz** *(10 μg/mL)*
MIC<2: 22/40	MIC<2: 28/40	MIC<2: 35/40	MIC<2: 37/40
MIC>2: 18/40	MIC>2: 12/40	MIC>2: 5/40	MIC>2: 3/40

Meropenem (alone)	**Ampicillin+Su** (2:1)	**Ampicillin+Cl** (2:1)	**Piperacillin+Tz**
MIC<2: 38/40	MIC<2: 4/40	MIC<2: 3/40	MIC<2: 22/40
MIC>2: 2/40	MIC>2: 36/40	MIC>2: 37/40	MIC>2: 18/40

Conclusions: HT-07, when supplemented with Su, Tz, or Cl, was active against the majority of strains of *Acinetobacter baumannii* and had comparable results to meropenem against *E. coli*. Although beta-lactamase inactivation is a major obstacle for beta-lactam containing antibiotics, HT-07 is active against most Gram-negative bacteria with a variety of beta-lactamase enzymes.

Author Disclosure Block:

Y. Lin: None. **P. Miller:** None. **D.L. Shinabarger:** None. **M.J. Miller:** None.

Poster Board Number:

FRIDAY-412

Publishing Title:

The Antibacterial Effects of Silver Polyvinyl Pyrrolidone (Ag-Pvp) against *P aeruginosa* and *S epidermidis*

Author Block:

D. Ashmore, A. A. Chaudhari, S. R. Singh, V. A. Dennis, S. R. Pillai; Alabama State Univ., Montgomery, AL

Abstract Body:

Silver-Polyvinyl Pyrrolidone (Ag-PVP) is not only biocompatible but has been shown to possess antimicrobial activity. Currently, resistance to commonly used antibiotics has increased the need for novel antimicrobials. Ag-PVP has been demonstrated to be a good candidate to inhibit the growth of several Gram-positive and Gram-negative bacteria. In this study, we tested the antimicrobial activity of Ag-PVP against Gram-negative *Pseudomonas aeruginosa* and Gram-positive *Staphylococcus epidermidis. Pseudomonas aeruginosa* is the most frequent Gram-negative pathogen associated with high mortality of patients with ventilator associated pneumonia (VAP) in intensive care units and affects immuno-compromised individuals. *Staphylococcus epidermidis* is a Gram-positive pathogen that causes human disease such as skin and soft tissue infections (SSTI) and invasive disease that lead to bacteremia, sepsis, endocarditis, or pneumonia. Both organisms are known to develop resistance to commonly used antibiotics.The antimicrobial activity of Ag-PVP was examined against Gram-negative *Pseudomonas aeruginosa* and Gram-positive *Staphylococcus epidermidis* using minimum inhibition concentration (MIC), minimum bactericidal concentration (MBC) and time-kill studies. Our preliminary data revealed some antimicrobial activity of Ag-PVP against other various strains. We determined that the MIC for *P. aeruginosa* was 125 µg/mL and that the MBC was 250 µg/mL. The MIC for S. epidermidis was determined to be >500 µg/mL. The Time Kill study for *P. aeruginosa* showed cell death at concentrations of 500 µg/mL and 250 µg/mL by 8hrs. Future studies will focus upon the possible mechanism of action and by testing a pool of genes essential for viability using quantitative real-time polymerase chain reaction and sequencing after exposure to Ag-PVP at sub-MIC levels. Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) will also be used to evaluate cellular change in both *P. aeruginosa* and *S. epidermidis*.

Author Disclosure Block:

D. Ashmore: None. **A.A. Chaudhari:** None. **S.R. Singh:** None. **V.A. Dennis:** None. **S.R. Pillai:** None.

Poster Board Number:

FRIDAY-413

Publishing Title:

Peptide-Conjugated Phosphorodiamidate Morpholino Oligomers (PPMOS) for Burkholderia Cepacia Complex Retain Activity in Sputum and During Aerosolization

Author Block:

J. J. Howard[1], K. Marshall-Batty[2], F. C. Afolabi[1], M. Wong[3], B. L. Geller[4], D. E. Greenberg[1]; [1]UT Southwestern Med. Ctr., Dallas, TX, [2]Frederick Natl. Lab. for Cancer Res., Frederick, MD, [3]Sarepta Therapeutics, Cambridge, MA, [4]Oregon State Univ., Corvallis, OR

Abstract Body:

Burkholderia cepacia complex (Bcc) cause significant morbidity and mortality in patients with chronic granulomatous disease (CGD) and cystic fibrosis (CF). Bcc are frequently antibiotic-resistant, making treatment difficult. We have been using antisense molecules (PPMOs) to inhibit pathogens in a sequence-specific manner. We have shown efficacy *in vitro* and *in vivo* when targeting the essential gene *acpP* encoding an acyl carrier protein. Studies were performed to assess the efficacy of PPMOs in the sputum environment as well as determine the activity of PPMOs when delivered via aerosol.*B. cenocepacia* K56-2 was grown in artificial CF sputum. AcpP PPMO (20 μM, 5570 g/mol) added to culture at 0, 2, 8, and 12 hours resulted in time-dependent killing with a ~3-log decrease in colony-forming units (cfu) at 24 hours (p=0.00004 ANOVA). A scrambled sequence control (Scr PPMO) had no effect on growth. Aerosol delivery of PPMOs was tested in a CGD mouse model. Groups of mice were infected intranasally (IN) with $6x10^5$ cfu of *B. multivorans* (SH-2) and then treated with either AcpP (300 μg or 30 μg; ~1-10 mg/kg) or Scr PPMO (300 μg) via an Aeroneb® Lab nebulizer 6 hours post-infection. All mice were euthanized at 24 hours and lung burden was quantified. Compared to the SCR PPMO group, the AcpP PPMO resulted in a 34% and 93% reduction in cfu/gram of lung (30 μg vs 300 μg, respectively, $p<0.005$) Mice infected IN with K56-2 received aerosolized AcpP PPMO (300 μg) conjugated to the fluorophore Cy3. Fluorescent microscopy was used to analyze the degree of co-localization of PPMO with K56-2. At 24 hours post-infection, 60% of PPMO had co-localized with K56-2 (Mander's coefficient of 0.608; Imaris® 7).The AcpP PPMO demonstrates *in vitro* activity in settings likely to be encountered in the lungs of patients. In addition, we demonstrate for the first time that PPMOs targeted against bacterial pathogens can be successfully delivered via the aerosol route. PPMOs represent a novel approach for the development of antibacterials in multidrug-resistant pathogens such as the Bcc.

Author Disclosure Block:

J.J. Howard: None. **K. Marshall-Batty:** None. **F.C. Afolabi:** None. **M. Wong:** D. Employee; Self; Senior Medical Director Sarepta Therapeutics. **B.L. Geller:** C. Consultant; Self; Sarepta Therapeutics. N. Other; Self; Inventor and Patents Pending, Sarepta Therapeutics. **D.E.**

Greenberg: E. Grant Investigator; Self; Research Funding, Sarepta Therapeutics. N. Other; Self; Inventor and Patents Pending, Sarepta Therapeutics.

Poster Board Number:

FRIDAY-414

Publishing Title:

Antimicrobial Activity of Halogenated Biaryl Compound against Gram-Positive Pathogens

Author Block:

K. N. Shah[1], P. N. Shah[1], H. Gao[2], O. Ogun[1], M. Yabe-Gill[3], L. Kurti[2], C. Cannon[1]; [1]Texas A & M Hlth.Sci. Ctr., College Station, TX, [2]Rice Univ., Houston, TX, [3]UT Southwestern Med. Ctr., Dallas, TX

Abstract Body:

Fatal infections caused by multi-drug resistant Gram-positive pathogens are increasing at an alarming rate, yet the antimicrobial pipeline is rapidly shrinking. Resistance against several standard-of-care antimicrobials including vancomycin has been reported. MC21-A (C58), a natural brominated biaryl, is a strong candidate for development as a clinically relevant antimicrobial due to its ability to eradicate methicillin-resistant *Staphylococcus aureus* (MRSA). However, its progress has been mired by cumbersome biosynthesis and purification. We have developed a unique scheme that allows large-scale synthesis of C58 and its analogues. From a library of 33 synthesized compounds, two non-toxic candidates, C58 and C59, exhibit superior antimicrobial activity over standard-of-care antibiotics against bacteria growing in planktonic, stationary, and biofilm modes. These compounds inhibit and eradicate 90% of 37 test MRSA strains (MIC_{90}, MBC_{90}) at lower concentrations compared with vancomycin and linezolid. Similar results were obtained with *S. epidermidis* and *Streptococcus pneumoniae.* An MRSA strain grown to stationary phase exhibits dose dependent eradication at a higher rate with C59 treatment compared with vancomycin. Similarly, C58 treatment (16 μg/mL) results in complete eradication of established MRSA biofilms after 6 hours. Incubation of MRSA strain USA300 with sub-MIC concentrations of vancomycin, but not C59, results in development of spontaneous resistance. Additionally, these biaryls are non-toxic to on- and off-target mammalian cells at concentrations that exert antimicrobial activity; the lethal dose at median cell viability (LD_{50}) is at least five fold higher than the MBC_{90}. Finally, we have verified that the biaryl mechanism of action involves membrane permeabilization. Our results demonstrate the potential of biaryls as non-toxic, next-generation antimicrobials that can be structurally optimized to yield superior, pathogen-specific efficacy over current antimicrobials.

Author Disclosure Block:

K.N. Shah: None. **P.N. Shah:** None. **H. Gao:** None. **O. Ogun:** None. **M. Yabe-Gill:** None. **L. Kurti:** None. **C. Cannon:** None.

Poster Board Number:

FRIDAY-415

Publishing Title:

Comparative Evaluation of Oritavancin, Dalbavancin, and Tedizolid *In Vitro* Antimicrobial Activity Against Clinical Isolates of *Staphylococcus aureus* at a Tertiary Care Hospital

Author Block:

F. Foolad, C. Young, D. Newton, A. Smith, T. S. Patel; Univ. of Michigan Hlth.System, Ann Arbor, MI

Abstract Body:

Background: *Staphylococcus aureus* continues to be a problematic pathogen causing high rates of morbidity and mortality. New antimicrobial agents, oritavancin, dalbavancin, and tedizolid, have been added to the arsenal of agents used to treat infections caused by *S. aureus*. However, there are minimal data comparing the in vitro activities of these new antimicrobials for clinical isolates from a variety of sources using a custom TREK Sensititre panel.**Methods:** All cultures from patient infections with isolated *S. aureus* from February 2015 to November 2015 were identified at the University of Michigan Health System. Organisms were identified by MALDI-TOF mass spectrometry using the Bruker Microflex instrument (Bruker Daltonik, Bremen, Germany). Minimum inhibitory concentrations (MICs) for all antimicrobials were determined using broth microdilution methods (TREK Diagnostic Systems, Cleveland, Ohio). The percentage of isolates within the susceptible range was determined using Clinical and Laboratory Standards Institute (CLSI) and US Food and Drug Administration (FDA) breakpoint criteria.**Results**: A total of 2,579 *S. aureus* isolates from clinical specimens were included. Similar MIC_{50} and MIC_{90} were determined for methicillin-sensitive *S. aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA, table). Using published interpretive criteria, 98.8%, 100%, and 99.8% of all *S. aureus* isolates were considered susceptible to oritavancin, dalbavancin, and tedizolid, respectively as compared to 100% and 99.7% susceptible to vancomycin and daptomycin.**Conclusions**: Oritavancin, dalbavancin, and tedizolid demonstrated good in vitro activity against *S. aureus* isolated from a variety of clinical infections with no major differences observed between MRSA and MSSA.

	Range (µg/ml)	**MIC_{50} (µg/ml)**	**MIC_{90} (µg/ml)**
MSSA (n= 1550)			
Oritavancin	≤0.004 - >0.5	0.03	0.06
Dalbavancin	≤0.06 - >0.25	≤0.06	≤0.06
Tedizolid	≤0.12 - 1	0.25	0.5

MRSA (n=1029)			
Oritavancin	≤0.004 - >0.5	0.03	0.06
Dalbavancin	≤0.06 – 0.12	≤0.06	≤0.06
Tedizolid	≤0.12 - 2	0.25	0.5

Author Disclosure Block:

F. Foolad: None. **C. Young:** None. **D. Newton:** None. **A. Smith:** None. **T.S. Patel:** None.

Poster Board Number:

FRIDAY-416

Publishing Title:

Effectiveness of Oritavancin to Treat Acute Bacterial Skin and Skin Structure Infections in a Physician Owned Infusion Center

Author Block:

P. J. Anastasio[1], P. Wolthoff[2], **W. Fan**[3]; [1]Emerald Coast Infusion Ctr., Fort Walton Beach, FL, [2]Med. Data Analytics, Parsippany, NJ, [3]The Med.s Company, Parsippany, NJ

Abstract Body:

Background: Acute bacterial skin and skin structure infections (ABSSSI) can be treated in the outpatient setting, but most IV antibiotics require multiple days of treatment. Single-dose oritavancin has been demonstrated to be non-inferior to 7-10 days of vancomycin in two randomized controlled trials, but little is known about its effectiveness in the real world. The objective of this analysis was to assess the effectiveness of oritavancin in the real world. **Methods:** A retrospective, chart-review was conducted at the Emerald Coast Infusion Center of consecutive patients who received at least one dose of oritavancin for treatment of ABSSSI from August 6, 2014 to June 30, 2015. Patients were matched by age, gender, insurance status, type of ABSSSI, diabetes, BMI and fever to standard of care (SOC) patients who received either vancomycin, daptomycin, dalbavancin or other antibiotics. Clinical response was assessed between 5 to 30 days post treatment. Success was defined as either cured or improved at follow-up visit. Clinical and resource utilization data were extracted from patient records. **Results:** Fifty-nine (59) oritavancin patients and 59 SoC patients were matched. The average age was 64.6 years, with 57.6% female. 57.6% cellulitis, 23.7% abscess and 18.6 % wound infection. Eighteen patients had confirmed MRSA. 41 oritavancin patients and 31 standard of care patients could be assessed for clinical cure. Oritavancin had a 90% success rate (cured or improved) compared to 77% in SOC, with 73% vs. 48% considered cured, respectively. Similar success rates can be seen in clinically-relevant subgroups such as elderly, female, diabetes, and infection type. The average antibiotic treatment length was 7.2 days for SOC vs. 1 day for oritavancin. No oritavancin patients visited the ED following index treatment compared to 2 (3%) in the SOC group, and no oritavancin patients were admitted to a hospital following index treatment, compared to 3 (5%) in the SOC group. **Conclusions:** Data from this analysis of ABSSSI treatment in an outpatient infusion center indicate that oritavancin is effective in real world use. Cure rates were higher than the standard of care, while use of follow-up resources was lower.

Author Disclosure Block:

P.J. Anastasio: F. Investigator; Self; The Medicines Company. **P. Wolthoff:** H. Research Contractor; Self; The Medicines Company. **W. Fan:** D. Employee; Self; The Medicines Company.

Poster Board Number:

FRIDAY-417

Publishing Title:

***In Vitro* Activity of Oritavancin against Gram-positive Pathogens Isolated in Canadian Hospitals from 2011 to 2015**

Author Block:

J. Karlowsky[1], H. Adam[1], M. Baxter[2], K. Nichol[1], B. Weshnoweski[1], R. Vashisht[2], D. Hoban[1], G. G. ZHANEL[2], CARA; [1]Diagnostic Services Manitoba, Winnipeg, MB, Canada, [2]Univ. of Manitoba, Winnipeg, MB, Canada

Abstract Body:

Background: Oritavancin (ORI) is a semisynthetic lipoglycopeptide that was approved by the US FDA in 2014 for the single dose (1200 mg) intravenous treatment of adult patients with acute bacterial skin and skin structure infections caused by susceptible isolates of designated gram-positive bacteria, including methicillin-susceptible and methicillin-resistant *Staphylococcus aureus* (MSSA and MRSA). This surveillance study assessed the susceptibility of recent clinical isolates to ORI and comparators.**Methods:** From January 2011 to October 2015, 12-15 sentinel Canadian hospital laboratories were requested to submit consecutive bacterial pathogens, 1 per patient, from blood (*n* = 100), respiratory (*n* = 100), urine (*n* = 25), and wound (*n* = 25) infections each year to the CANWARD Surveillance Program (Winnipeg, MB, Canada). Antimicrobial susceptibilities were determined for 4,480 gram-positive isolates using the CLSI broth microdilution standard method (M07-A10, 2015). MICs for ORI were determined using validated frozen broth microdilution panels provided by The Medicines Company (St. Laurent, PQ, Canada). MICs were interpreted using CLSI M100-S25 (2015) breakpoints against indicated species, where available; ORI MICs were interpreted using US FDA breakpoints.**Results:** MIC and % susceptibility data for selected organisms and antimicrobial agents are presented in the Table.

	MIC_{90} (µg/mL)/% Susceptible					
Organism (n)	**ORI**	**VAN**	**DAP**	**LZD**	**CIP**	**SXT**
MSSA (2084)	0.12/99.8	1/100	0.25/100	2/100	4/87.0	≤0.12/99.5
MRSA (588)	0.12/99.8	1/100	0.5/99.8	4/100	>16/20.9	≤0.12/97.0
MSSE (234)	0.12/NA	2/100	0.25/NA	1/100	>16/53.1	8/66.5
MRSE (32)	0.12/NA	2/100	0.25/NA	1/100	>16/3.1	8/12.5
S. pyogenes (166)	0.25/99.4	0.5/100	0.12/100	2/100	1/NA	0.25/NA
S. agalactiae (217)	0.12/100	0.5/100	0.25/100	2/100	1/NA	0.25/NA

PS *S. pneumoniae* (490)	0.008/NA	0.25/100	0.12/NA	1/100	2/98.5	0.5/91.8
PNS *S. pneumoniae* (83)	0.008/NA	0.25/100	0.25/NA	1/100	2/95.5	>8/43.8
VS *E. faecium* (138)	0.015/NA	1/100	2/100	4/88.7	>16/9.5	>8/NA
VR *E. faecium* (33)	0.12/NA	>32/0	2/100	4/83.0	>16/0	>8/NA
VS *E. faecalis* (415)	0.06/99.0	2/100	2/100	2/93.2	>16/70.6	0.25/NA

Abbreviations: MSSE, methicillin-susceptible *Staphylococcus epidermidis*; MRSE, methicillin-resistant *S. epidermidis*; VAN, vancomycin; DAP, daptomycin; LZD, linezolid; CIP, ciprofloxacin; SXT, trimethoprim-sulfamethoxazole; PS, penicillin-susceptible (MIC ≤0.06 µg/ml); PNS, penicillin non-susceptible; VS, vancomycin-susceptible; VR, vancomycin-resistant; NA, not available.

Conclusions: ORI demonstrated in vitro activity (MIC_{90} and/or % susceptibility) that was equivalent to, or more potent than, VAN, DAP, LZD, CIP, and SXT against a recent Canadian collection of frequently-isolated gram-positive pathogens (MSSA, MRSA, MSSE, MRSE, β-hemolytic streptococci, penicillin-resistant *Streptococcus pneumoniae*, and vancomycin-susceptible and -resistant *Enterococcus* spp.).

Author Disclosure Block:

J. Karlowsky: None. **H. Adam:** None. **M. Baxter:** None. **K. Nichol:** None. **B. Weshnoweski:** None. **R. Vashisht:** None. **D. Hoban:** None. **G.G. Zhanel:** I. Research Relationship; Self; Abbott, Astellas, Cubist, Galderma, Merck, Pharmascience, Sunovion, The Medicines Co, Tetraphase, Zoetis.

Poster Board Number:

FRIDAY-418

Publishing Title:

Oritavancin Activity Against *Enterococcus faecalis In vitro* and in a Murine Thigh Infection Model

Author Block:

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Abstract Body:

Background: Oritavancin (ORI) is a lipoglycopeptide that has in vitro activity against enterococci. We evaluated the pharmacodynamics of a single dose of ORI against *Enterococcus faecalis* (EF) isolates in a neutropenic murine thigh infection (TI) model and in vitro time-kill (TK) assays.**Methods:** 3 vancomycin-susceptible EF isolates were tested: ATCC 29212, 23241 and 33186. MIC determinations followed CLSI M7-A10 guidelines while MBC and TK assays followed M26-A guidelines. TK assays were conducted at both standard and high inoculum density (~10^6 and ~10^8 colony forming units (CFU)/ml, respectively) using ORI at concentrations approximating its mean free peak (*f*C_{max}, 16 µg/mL) in plasma based on a single 1200 mg dose. TI was established in neutropenic mice (n = 2/group) with an inoculum of 10^6 CFU in each thigh. ORI and vancomycin (VAN) were dosed 40 mg/kg once (IV) and 120 mg/kg BID (SQ), respectively, which produce *f*AUCs equivalent to human exposures. Efficacy was evaluated by determining mean log CFU/thigh changes from baseline after 24 h treatment.**Results:** ORI MICs ranged from 0.015 - 0.03 µg/mL against the 3 EF isolates and ORI MBC/MIC ratios ranged from 4 - >16. In TK assays, ORI was bactericidal (>= 3 log kill by 24h) against all isolates at both inoculum densities. Results in the TI model are shown in the table below.

Strain	**Compound**	**Total Daily Dose (mg/kg)**	**MIC (µg/mL)**	**Change in Log CFU/thigh at 24h**
*E. faecalis A*TCC 29212	ORI	40	0.016	-1.24
	VAN	240	2	-0.51

E. faecalis ATCC 23241	ORI	40	0.016	-1.50
	VAN	240	4	-1.24
E. faecalis ATCC 33186	ORI	40	0.03	-0.76
	VAN	240	1	-0.86

Conclusion: ORI was bactericidal at fC_{max} concentrations in TK assays. In the TI model, ORI activity was similar to VAN and both drugs produced bacterial killing at human equivalent exposures. Further studies are warranted.

Author Disclosure Block:

M. Sabet: D. Employee; Self; The Medicines Company. **Z. Tarazi:** D. Employee; Self; The Medicines Company. **D. Lalonde Seguin:** D. Employee; Self; The Medicines Company. **F.F. Arhin:** D. Employee; Self; The Medicines Company. **G. Moeck:** D. Employee; Self; The Medicines Company. **D.C. Griffith:** D. Employee; Self; The Medicines Company.

Poster Board Number:

FRIDAY-419

Publishing Title:

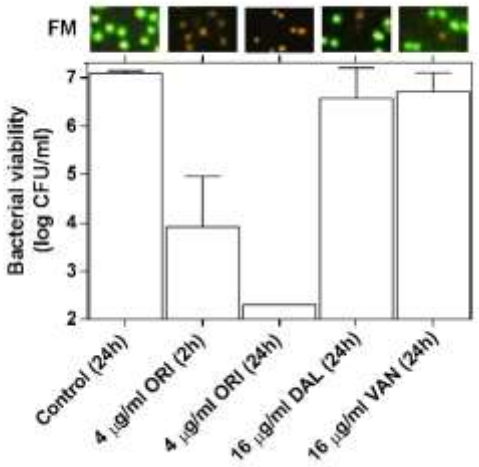
Assessment of Antibacterial Activity (AA) of Oritavancin (ORI), Dalbavancin (DAL) andVancomycin (VAN) against Non-Dividing (ND) Methicillin-Resistant *Staphylococcus aureus* (MRSA) Using Fluorescence Microscopy (FM)

Author Block:

A. Belley, D. Lalonde-Seguin, F. Arhin, G. Moeck; The Med.s Company, St-Laurent, QC, Canada

Abstract Body:

Background: Antibacterial agents that kill ND bacteria may be of utility in treating persistent infections. ORI and DAL are bactericidal lipoglycopeptides approved for acute bacterial skin and skin structure infections. Using FM, we assessed the AA of ORI, DAL and VAN against MRSA isolates maintained in a ND state in vitro.**Methods:** Stationary-phase cultures of MRSA ATCC 43300 and ATCC 700698 were resuspended in 5 mM HEPES pH 7.2 containing 0.1% dextrose and 0.002% polysorbate-80 (Buffer) at 10^7 CFU/ml and exposed to 4 µg/ml of ORI or 16 µg/ml of either DAL or VAN for 24h at 37ºC. Bacterial viability (BV) was assessed by serial dilution plating and by FM using the Live/Dead *Bac*light reagent containing the green fluorophore SYTO 9 (live cells appear green) and the red fluorophore propidium iodide (dead cells appear red).**Results:** Control (unexposed) cultures of MRSA ATCC 43300 in a ND state remained viable in Buffer over 24h with the majority of bacteria observed appearing green by FM (Figure, FM panel). ND ATCC 43300 exposed to 4 µg/ml ORI for 2h and 24h mostly appeared red by FM, consistent with concomitant decreases in BV of 3.2 ± 1.1-log and 4.8 ± 0.0-log CFU/ml, respectively. In contrast, the majority of bacteria exposed to 16 µg/ml of either DAL or VAN for 24h appeared green by FM, consistent with observed minor decreases in BV of 0.5 ± 0.6-log and 0.4 ± 0.4-log CFU/ml, respectively. Similar trends in FM and BV were observed for MRSA ATCC 700698 in a ND state exposed to each agent (data not shown). **Conclusion:** The AA of ORI against ND MRSA, as confirmed by FM and BV assessment, may prove to be of benefit against persistent infections.



Author Disclosure Block:

A. Belley: D. Employee; Self; The Medicines Company. **D. Lalonde-Seguin:** H. Research Contractor; Self; The Medicines Company. **F. Arhin:** D. Employee; Self; The Medicines Company. **G. Moeck:** D. Employee; Self; The Medicines Company.

Poster Board Number:

FRIDAY-420

Publishing Title:

Evaluation of the Development of Resistance in a Novel Class of Antimicrobial Agents

Author Block:

J. REMY, A. Marra, E. Duffy; Melinta Therapeutics, Inc., New Haven, CT

Abstract Body:

Background: The pyrrolocytosines have been designed and optimized for broad-spectrum activity, focusing on isolates resistant to commercial antibiotics. As novel protein synthesis inhibitors interacting principally with ribosomal RNA in the large subunit, they are not expected to exhibit a high frequency of resistance. The spontaneous development of resistance for this novel class was evaluated. **Methods:** Pyrrolocytosine exemplars were evaluated for spontaneous resistance. In this study, *Escherichia coli* W3110 was studied as it allowed for sequencing of any resistant mutants. *E. coli* W3110 was inoculated onto plates containing compounds at 2X, 4X, and 8X MIC. After 48 hrs the resistance frequency (RF) for each compound was determined. Colonies were chosen for further evaluation. Mutant MICs for the pyrrolocytosines and commercial agents were determined by broth microdilution from plates containing the pyrrolocytosine selecting compound at day 1 and following 10 days of drug-free passaging. Cross-resistance (XR) to an antibiotic was defined as being >4X the parent MIC. Growth curves were performed on representative mutants to determine fitness cost compared to the parent. DNA was isolated from nine mutants selected on plates containing RX-P873, and whole genome sequencing was performed.**Results:** RFs depended on the drug concentration and the inoculum (~10^{-5} to ~10^{-10}). Fewer than half of the pyrrolocytosines generated mutants with elevated MICs to the selecting agent. XR was observed for the aminoglycosides tested and some beta-lactams, to differing extents. A fitness cost was observed for most of the mutants, with log phase growth rates of up to 180 minutes. For RX-P873, whole-genome sequencing revealed there was no single mutation common to all mutants and no mutations were identified in the ribosomal target.**Conclusions:** Although the RFs were higher than expected for this novel class, there are three main findings: mutants with elevated MICs to the selecting agent were uncommon; most mutants demonstrated a profound fitness cost and target-based resistance was not observed. These support the continued investigation of the pyrrolocytosines against multidrug- and extremely-drug resistant pathogens.

Author Disclosure Block:

J. Remy: D. Employee; Self; Employee. **A. Marra:** D. Employee; Self; Employee. **E. Duffy:** D. Employee; Self; Employee.

Poster Board Number:

FRIDAY-421

Publishing Title:

A Risk Assessment Framework for Antimicrobial Resistance Development Associated with the Use of Microbicides in Home and Personal Care Products

Author Block:

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Abstract Body:

Background: Responsible evaluation and development of new microbicidal systems need to consider the risk of microbial resistance developing during their use, particularly where clinically-relevant cross-resistance might emerge. There are currently no risk assessment frameworks available for this purpose, resulting in lack of consistency of data interpretation from susceptibility studies and ultimately on decisions on safety. We have therefore developed a risk assessment framework and validated it with six different case studies.**Methods:** The framework consists of three tiers of increasing experimental data demands. The starting point (Tier 0) is an evidence-weighted model for assessment of candidate microbicides considering realistic exposure (consumer use) conditions; this progresses to Tier 1, which is an *in vitro* exposure test on a broad range of bacteria in various physiological states (planktonic, biofilm, and desiccated cells); and, Tier 2, which consists of mechanism of resistance studies for favoured systems. The end-point of the framework is the probability of resistance development (i.e. microbicide resistance and cross-resistance to clinically-relevant antimicrobials) following use of home and personal care (HPC) products containing microbicides. Validation was conducted with the following products (microbicides in brackets): hand soap bar (thymol, terpineol, silver), liquid hand wash (thymol, terpineol, triclosan), mouthwash (zinc sulphate, sodium benzoate), eye make-up remover (chlorhexidine), general purpose cleaner (benzalkonium chloride), and hand dish wash liquid (sodium salicylate).**Results:** Enough evidence was available for the hand soap bar to conclude on negligible risk of resistance development at Tier 0. The other formulations were taken through to Tier 1, resulting in no significant changes in microbicide susceptibility and no development of clinical resistance to any of the antibiotics tested (using EUCAST breakpoints). As Tier 0 and Tier 1 provided sufficient data/information to conclude on safety, Tier 2 data were not needed for these risk assessments.**Conclusions:** The risk assessment framework is evidence and realism-based. It provides a systematic approach to assure HPC products are safe by design.

Author Disclosure Block:

A. Amezquita: None. **M. Parker:** None. **L. Knapp:** None. **D. Tang:** None.

Poster Board Number:

FRIDAY-422

Publishing Title:

Treatment of Acute Bacterial Skin and Skin Structure Infection (ABSSSI) with Single Dose Dalbavancin in the Intravenous Drug User (IVDU) Population

Author Block:

P. L. Gonzalez, U. Rappo, K. Akinapelli, S. Puttagunta; Allergan, PLC, Jersey City, NJ

Abstract Body:

Background: Medical care for patients with a history of IVDU is complicated by medical, social and economic issues that affect adherence to treatment. We describe the demographics and outcomes for patients with and without a history of IVDU in a phase 3 clinical trial evaluating the efficacy of a single 1500 mg dose of dalbavancin vs a 2-dose regimen for treatment of ABSSSI. **Methods:** In a double-blind, single dummy Phase 3 trial, adult patients with ABSSSI were randomized to dalbavancin 1500 mg as a single IV infusion over 30 minutes *or* 1000 mg IV on Day 1 and 500 mg IV on Day 8. The primary endpoint was ≥20% reduction in erythema 48-72 hours after start of treatment. Clinical success assessed on Days 14 and 28 was based on a composite of clinical measures. P values were obtained using Fisher's exact test for categorical variables and the Wilcoxon rank sum test for continuous variables.**Results: Table 1 Baseline Characteristics**

Characteristic	**IVDU (n=212)**	**Without IVDU (n=486)**	**p-value**
Age, y, mean	44.9	49.6	<0.0001
Male (%)	67.0	54.5	0.003
Hispanic or Latino (%)	41.0	4.9	<0.001
BMI, kg/m^2, median	25.1	28.2	<0.0001
Major abscess (%) Traumatic wound/surgical site infection (%) Cellulitis (%)	42.9 40.1 17.0	18.1 21.2 60.7	<0.001
SIRS (%)	28.3	50.0	<0.0001
Bandemia ≥ 10% (%)	4.9	26.7	<0.0001

Table 2 Clinical Outcomes

Timing	Outcome (Population)	IVDU		Without IVDU	
		Dalbavancin Single-Dose n/N (%)	**Dalbavancin 2-dose n/N (%)**	**Dalbavancin Single-Dose n/N (%)**	**Dalbavancin 2-dose n/N (%)**
48-72 hours	Treatment response (ITT)	94/105 (89.5)	92/107 (86.0)	190/244 (77.9)	202/242 (83.5)
	95% CI	3.5 (-5.6, 12.7)		-5.6 (-12.7, 1.4)	
Day 14	Clinical success (CE)	78/87 (89.7)	78/85 (91.8)	189/215 (87.9)	192/217 (88.5)
	95% CI	-2.1 (-11.5, 7.1)		-0.6 (-6.8, 5.6)	
Day 28	Clinical success (CE)	80/85 (94.1)	72/75 (96.0)	170/186 (91.4)	175/192 (91.1)
	95% CI	-1.9 (9.7, 6.0)		1.3 (-5.7, 6.1)	

Adverse events were similar between the IVDU and non-IVDU, and between the single and 2-dose regimens.**Conclusion:** Patients with a history of IVDU had similar success rates for treatment of ABSSSI at all time-points as those without IVDU, with either regimen. A single, convenient 30-minute IV infusion and lack of a need for indwelling IV access may optimize adherence without increasing the potential for abuse in the IVDU.

Author Disclosure Block:

P.L. Gonzalez: None. **U. Rappo:** None. **K. Akinapelli:** None. **S. Puttagunta:** None.

Poster Board Number:

FRIDAY-423

Publishing Title:

Control of *Staphylococcus aureus* and *Staphylococcus epidermidis* Skin Strains Virulence by Substance P and Cutaneous Defense Peptides

Author Block:

A. R. Ndiaye, L. MiJouin, M. G. J. Feuilloley; Lab. of Microbiol., Signals and Microenvironment, Evreux, France

Abstract Body:

Background: Skin is the largest human neuroendocrine organ and hosts the second most numerous microbial population, but little is known about the bacteria/skin interactions. In 2013, Mijouin *et al*. demonstrated that Substance P (SP) increases *Bacillus cereus* virulence, an occasional member of the cutaneous microflora. Hence, s SP can likely have the same effect on other members of the cutaneous microflora such as *Staphylococcus aureus (SA) and Staphylococcus epidermidis (SE).***Methods:** The effect of SP and two antimicrobial peptides were tested on bacterial cytotoxicity, its biofilm activity. Virulence factors were identified via 2D gel. The bacteria virulence was also observed on Reconstitued Epidermidis genome via a qRT-PCR of 64 genes.**Results:** SP promotes *SA* and *SE* virulence and enhances their adhesion of on keratinocytes. However, whereas *SE* reacts to SP by an increase in biofilm formation activity, *S. aureus* shows a raise in Staphylococcal Enterotoxin C2 (SEC2) production. The ribosomal elongation factor Ef-Tu was identified as the SP binding site in *S. aureus* and *S. epidermidis.* Tested at non-antibacterial concentrations, human B-defensin 2 (HBD2) and the cathelicidin LL37 alter the biofilm formation activity and cytotoxicity of *SE* and *SA*. *SA* and *SE* treated with SP showed some effects on the 64 genes selected for qRT-PCR.**Conclusion:** *Staphylococci* are the principal human skin commensal bacteria but they have also high infectious potential. As SP strongly increases during inflammation, stress or depression, this could mediate an increase in Staphylococci virulence. This process should explain the link between some skin inflammatory syndromes involving the skin microbiote but where dysbiosis are rarely observed, such as atopic dermatitis, and the mental status of individuals.

Author Disclosure Block:

A.R. Ndiaye: None. **L. MiJouin:** None. **M.G.J. Feuilloley:** None.

Poster Board Number:

FRIDAY-424

Publishing Title:

Exploring *Escherichia coli* Clinical Isolate Collateral Sensitivity Networks

Author Block:

N. L. Podnecky[1], E. G. A. Fredheim[1], J. M. Kloos[1], A. L. G. Utnes[1], R. Primicerio[1], Ø. Samuelsen[2], P. J. Johnsen[1]; [1]UiT – The Arctic Univ. of Norway, Tromsø, Norway, [2]Univ. Hosp. of North Norway, Tromsø, Norway

Abstract Body:

Background: Recent studies on collateral sensitivity (CS) suggest the use of suppressive drug pairs and drug cycling may reduce antimicrobial resistance (AMR) development and ensure future treatment options. CS patterns and the underlying mechanism(s) have been investigated in a few laboratory and clinical strains. The purpose of our study is to further map CS networks across diverse clinical isolates and determine if and to what extent genetic diversity and resistance mechanism contribute to CS. In this study we focus on *Escherichia coli* and antimicrobials commonly used for urinary tract infections.**Methods:** AMR mutants were selected for resistance to single antimicrobials (ciprofloxacin, CIP; mecillinam, MEC; nitrofurantoin, NIT; and trimethoprim, TMP) in 10 genetically diverse pan-susceptible *E. coli* strains from the ECO-SENS collection of clinical urinary tract infection isolates to achieve clinically significant resistance. Minimal inhibitory concentration (MIC) testing for 16 antimicrobials was determined by gradient strip diffusion assays. The overarching collateral effects were modeled and assessed by multivariate statistical approaches.**Results:** MIC analysis of 40 AMR mutants revealed frequent and diverse collateral effects both within and across drug classes. A principal component analysis of the log MIC values showed CIP and MEC mutants display clear patterns of cross-resistance and CS, respectively, in most of the strains. CS and cross-resistance in TMP and NIT mutants were overall less pronounced. In our study, the selection drug had a greater impact on CS networks than strain background.**Conclusions:** From these experiments we can begin to understand cross-resistance and CS networks in a broader context within *E. coli*. MEC is the preferred treatment as it increases susceptibilities to many clinically relevant antimicrobials, while TMP would maintain pre-existing susceptibilities. Conversely, CIP resistance will greatly limit downstream treatment options. Broader knowledge of CS networks will provide important insight for future treatment recommendations.

Author Disclosure Block:

N.L. Podnecky: None. **E.G.A. Fredheim:** None. **J.M. Kloos:** None. **A.L.G. Utnes:** None. **R. Primicerio:** None. **Ø. Samuelsen:** None. **P.J. Johnsen:** None.

Poster Board Number:

FRIDAY-425

Publishing Title:

Evaluation of Extended- Versus Traditional-infusion Cefepime in Gram-negative Pneumonia and Bacteremia

Author Block:

M. Guido[1], S. Liao[1], **A. Miller**[2]; [1]Univ. of Cincinnati Med. Ctr., Cincinnati, OH, [2]Univ. of Kansas Hosp., Kansas City, KS

Abstract Body:

Based on the challenges of treating multi-drug resistant Gram-negative infections, several Monte-Carlo simulations have demonstrated higher probability of target attainment when administering cefepime by extended (4 hour) infusion (EI) compared to traditional (30 minute) infusion (TI). Studies showing conflicting results question whether EI cefepime is necessary for all indications. This study was designed to evaluate the clinical response with EI cefepime compared to TI and identify unique population that would benefit from EI cefepime.This single center, retrospective study evaluated subjects receiving at least 48 hours of cefepime for treatment of pneumonia or bacteremia. Subjects with a positive blood or respiratory culture for a Gram-negative pathogen were included. Subjects were excluded if they received cefepime at a dose not appropriate for renal function. The primary outcome was clinical response, defined for each included indication, which was evaluated at the point of antibiotic de-escalation or end of therapy.A total of 110 subjects met criteria for inclusion during the study period. Baseline characteristics were similar except for an older population in TI group (65.7 vs 57.3 years, p=0.01). Approximately 49% of subjects were treated for *Enterbacteriaceae* infection and 51% were treated for infection caused by *Pseudomonas* (PSA) or *Acinetobacter spp*. Overall, there was no difference in clinical response between the EI and TI group (78% vs 81.7%, p=0.811). No difference was found between EI and TI cefepime based on treatment indication or isolated pathogen. A multi-variable logistic regression analysis showed no significant increase in odds ratio to predict clinical improvement for independent variables including age, presence of critical illness, pneumonia, and SOFA score. Isolate MIC greater than or equal to 8 mg/L, while not statistically significant, had the most suggestive effect on clinical outcome (OR 0.592, CI=0.192-1.921).Implementation of EI cefepime did not improve clinical outcome when compared to traditional infusion. The effect of extended infusion for pathogens with an MIC of greater than or equal to 8 mg/L is a trend that should be further explored, as it is possible that EI cefepime may have the greatest impact on clinical outcome in this population.

Author Disclosure Block:

M. Guido: None. **S. Liao:** None. **A. Miller:** None.

Poster Board Number:

FRIDAY-426

Publishing Title:

Evaluation of Patients with Complicated Intra-abdominal Infections (cIAI) and Concomitant Bacteremia (CB) from IGNITE1: A Phase 3 Study to Evaluate the Efficacy and Safety of Eravacycline (ERV) versus Ertapenem (ETP) in Complicated Intra-Abdominal Infections (cIAI)

Author Block:

M. Michaud[1], **H. Hoffman-Roberts**[1], A. Marsh[1], P. Horn[1], J. Solomkin[2]; [1]Tetraphase Pharmaceuticals, Watertown, MA, [2]Univ. of Cincinnati, Cincinnati, OH

Abstract Body:

BACKGROUND: Empiric treatment of cIAI represents a clinical challenge because of the polymicrobial etiology and the emergence of antibiotic resistance. There is no consensus on the best way to treat patients with cIAI and CB. We report results in a subgroup of patients in IGNITE1 with cIAI and CB.**METHODS:** In this randomized, double-blind, non-inferiority phase 3 trial, patients with documented cIAI were randomized (1:1) to either ERV (1.0 mg/kg IV q12h) or ETP (1g IV daily) for up to 14 days. Clinical outcome at the test of cure (TOC) visit, approximately 28 days after randomization, was the primary efficacy endpoint in the microbiological intent-to-treat (micro-ITT) population (≥ 1 pathogen consistent with cIAI in baseline cultures).**RESULTS:** 541 patients were randomized and 446 were included in the micro-ITT population. The study met criteria for non-inferiority (Δ=-0.8, 95% CI=-7.1 to 5.5). 40 patients had positive blood cultures of which 5 cultures on post-hoc analysis were determined to be contaminants. Among the 35 remaining patients, (ERV 18, ETP 17), there were 44 baseline pathogens identified in the blood. The most common pathogens isolated were *Streptococcus* sp. 12 (27.3%), *E. coli* 10 (22.7%), and *Bacteroides* sp. 8 (18.2%). 24/35 (68.6%) patients had at least one organism present in the blood duplicated in the IAI culture. Patients with CB received an average 1.2 days longer duration of antibiotic therapy.

	Combined (ERV + ETP)		**Eravacycline**		**Ertapenem**	
	CB (n=35)	**No CB (n=411)**	**CB (n=18)**	**No CB (n=202)**	**CB (n=17)**	**No CB (n=209)**
Average duration of therapy (days)	8.3	7.1	8.0	7.2	8.5	7.0
Diagnosis Complicated appendicitis	8 (22.9%)	124 (30.2%)	4 (22.3%)	61 (30.2%)	4 (23.5%)	63 (30.1%)

Other cIAI	27 (77.1%)	287 (69.8%)	14 (77.7%)	141 (69.8%)	13 (76.5%)	146 (69.9%)
Clinical Response Cure	29 (82.9%)	360 (87.6%)	17 (94.4%)	174 (86.1%)	12 (70.6%)	186 (89.0%)
Failure	1 (2.8%)	29 (7.1%)	0 (0.0%)	19 (9.4%)	1 (5.9%)	10 (4.8%)
Indeterminant	5 (14.3%)	22 (5.3%)	1 (5.6%)	9 (4.5%)	4 (23.5%)	13 (6.2%)

CONCLUSIONS: These data suggest that the presence of CB in cIAI does not increase treatment failure or require prolonged antimicrobial therapy duration. Treatment outcomes among patients with cIAI and CB were similar with ERV compared to those without CB. High success rates in this study suggest ERV may be a treatment option for patients with cIAI and CB.

Author Disclosure Block:

M. Michaud: D. Employee; Self; Tetraphase Pharmaceuticals. K. Shareholder (excluding diversitied mutual funds); Self; Tetraphase Pharmaceuticals. **H. Hoffman-Roberts:** D. Employee; Self; Tetraphase Pharmaceuticals. K. Shareholder (excluding diversitied mutual funds); Self; Tetraphase Pharmaceuticals. **A. Marsh:** D. Employee; Self; Tetraphase Pharmaceuticals. K. Shareholder (excluding diversitied mutual funds); Self; Tetraphase Pharmaceuticals. **P. Horn:** D. Employee; Self; Tetraphase Pharmaceuticals. K. Shareholder (excluding diversitied mutual funds); Self; Tetraphase Pharmaceuticals. **J. Solomkin:** C. Consultant; Self; Tetraphase Pharmaceuticals. J. Scientific Advisor (Review Panel or Advisory Committee); Self; Tetraphase Pharmaceuticals. M. Independent Contractor; Self; Tetraphase Pharmaceuticals.

Poster Board Number:

FRIDAY-427

Publishing Title:

Piperacillin-Tazobactam versus Carbapenem for the Treatment of Blood Stream Infections Caused by Extended-spectrum Beta-Lactamase Producing *Enterobacteriaceae*

Author Block:

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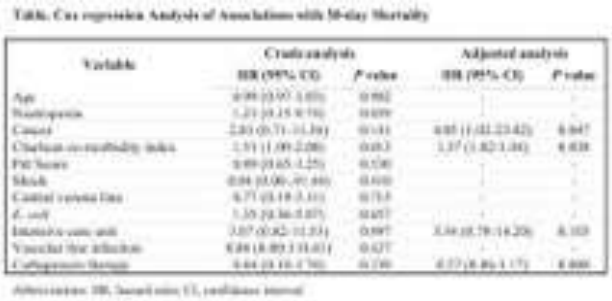
Abstract Body:

Background: The role of piperacillin-tazobactam (PTZ) in the treatment of blood stream infections (BSI) caused by extended-spectrum beta-lactamase producing *Enterobacteriacea*e (ESBL-PE) remains unconfirmed. We investigated clinical outcomes in patients with ESBL-PE BSI who received empiric therapy with PTZ or a carbapenem (CAR). **Methods:** In a retrospective, cohort, single-center study, we included adult patients who received empiric PTZ or CAR therapy within 48 hours of isolation of an ESBL-PE from a blood culture. The primary endpoint was 30-day mortality. Chi square, t-test, Kaplan-Meier and Cox Regression were used for the analysis.**Results:** The study included 93 patients (43% males), mean age 53.8 years (SD 19.9). The most frequent source of BSI was the urinary tract (45.2%). All BSI were caused by *E. coli* (66.7%) or *K. pneumoniae* (33.3%). Patients received CAR (48.4%) or PTZ (51.6%). Baseline characteristic were similar except underlying diagnosis of cancer (PTZ 54.2% *vs.* CAR 26.7%; *P* 0.007) and the presence of central venous lines (PTZ 25.0% *vs.* CAR 46.7%; *P* 0.029). 30-day mortality was not significantly different between the two groups (PTZ 6.3% *vs.* CAR 13.3%; *P* 0.225 (log rank test). In adjusted analysis, cancer and Charlson co-morbidity index were associated with 30-day mortality, but not CAR therapy (HR 0.27, 95% confidence interval 0.06 to1.17, *P* 0.08) (Table).**Conclusion:** PTZ is not associated with increased mortality in patients with ESBL-PE BSI. Further evaluation in randomized clinical trials is required to confirm these findings.



Author Disclosure Block:

N.N. Mukhtar: None. **L.A. Akkielah:** None. **M.M. Ayas:** None. **L.N. Albalawi:** None. **M.M. Shoukri:** None. **S.I. Althawadi:** None. **A.A. Alrajhi:** None. **A.S. Omrani:** None.

Poster Board Number:

FRIDAY-428

Publishing Title:

Clinical Outcomes with Ceftazidime-Avibactam in Patients with Carbapenem-Resistant *Enterobacteriaceae* (Cre) Infections

Author Block:

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Abstract Body:

Background: Ceftazidime-avibactam is a cephalosporin-beta-lactamase inhibitor combination that is active against *Enterobacteriaceae* and *Pseudomonas aeruginosa* that is resistant to other agents, including carbapenems and late-generation cephalosporins. This purpose of this study is to describe the outcomes of patients receiving ceftazidime-avibactam for CRE infections.**Methods**: A retrospective chart review was completed from March 2014 through December 2015 at Temple University Hospital for adult patients who received ceftazidime-avibactam for a CRE infection, defined as having an MIC>4 to imipenem and meropenem. Patients were included if they received ceftazidime-avibactam for at least 24 hours. Primary outcomes were 30-day and in-hospital mortality. Microbiologic and clinical outcomes were also evaluated. Microbiological success required a negative culture at the end of therapy. Clinical success was judged by improved symptoms and defervesence.**Results:**

Characteristic	**Results (N=9)**
Male gender (n,%))	6 (67%)
Age (median, range)	70 (26-82)
Charlson Comorbidity Index (median, range)	4 (2-11)
Pitt Bacteremia Score (median, range)	4 (0-7)
ICU (n,%)	7 (78%)
Organ transplant (n,%)	6 (67%)
Organism (n, %)) *Klebsiella pneumoniae* *Enterobacter aerogenes*	 8 1
Primary Infection (n,%) Pneumonia Bacteremia Urinary tract Intra-abdominal	 5 1 2 1

30-day mortality (n,%)	2 (22%)
Microbiologic Cure (n,%)	5 (55%)
Clinical Success (n,%)	5 (55%)

Conclusion: In our series, 5/9 (55%) of patients had both clinical and microbiological success, and 7/9 (78%) were alive 30-days after therapy. Ceftazidime-avibactam is a potential option for patients with multi-drug resistant organisms causing *Enterobacteriaceae* infections, including those in intensive care.

Author Disclosure Block:

M. King: None. **J. Gallagher:** C. Consultant; Self; Allergan, Inc. J. Scientific Advisor (Review Panel or Advisory Committee); Self; Allergan, Inc. L. Speaker's Bureau; Self; Allergan, Inc.

Poster Board Number:

FRIDAY-429

Publishing Title:

Colistin Nephrotoxicity: Not as High as We Fear! -A Study from South India

Author Block:

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Abstract Body:

Background: Colistin is extensively used in tertiary care hospitals in countries with high prevalence of carbapenem resistant Gram-negative bacteria(CRGNB). Though nephrotoxicity is a well know side effect, there is limited published data from India, a country with very high CRGNB rate and high colistin utilization. Aim of our study was to analyse nephrotoxicity data in patients who received colistin for the treatment of CR GNB bacteremic infections**Methods:** We conducted a retrospective observational study of patients with CRGNB blood stream infections treated with colistin,in our oncology and bone marrow transplant centre. RIFLE score was used to detect nephrotoxicity while on colistin therapy. Concomitant administration of other nephrotoxic agents was looked into. **Results:** Over a 3-year study period (2011-14), we could identify 91 patients satisfying study criteria. Out of 91 cases of CRGNB bacteremia treated with colistin,10 had pre-existing renal failure and so excluded. Mean colistin dose in the study group was 8.6± 1.02 million units/day. Of the 81 patients included in the study,9(11.11%) developed renal impairment during colistin therapy ,as per RIFLE criteria (3 risk, 6 failure). Of these, 2 received concomitant diuretics, 3 Liposomal amphotericin B, 2 aminoglycosides, 4 inotropes, and 7 out of 9 received 2 nephrotoxic agents in addition to colistin. **Conclusions:** Colistin nephrotoxicity rate in our study was low and most patients who developed renal impairment received other concomitant nephrotoxic agents.

Author Disclosure Block:

A. Ghafur: None. **V. Lakshmi:** None. **V. P:** None.

Poster Board Number:

FRIDAY-430

Publishing Title:

Analysis of Diabetes Patients with Complicated Intra-abdominal Infection or Complicated Urinary Tract Infection in Phase 3 Trials of Ceftolozane/Tazobactam

Author Block:

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Abstract Body:

Diabetes mellitus (DM) increases susceptibility to bacterial infections and is often associated with poor outcome. Ceftolozane/tazobactam (C/T) is an IV antibacterial with activity against Gram-negative pathogens approved for treatment of complicated intra-abdominal infection (cIAI), in combination with metronidazole (MTZ), and complicated urinary tract infection (cUTI). Patient (pt) characteristics and outcomes were evaluated post hoc in DM (65 cIAI; 133 cUTI) and non-DM pts (741 cIAI; 667 cUTI) in phase 3 trials of C/T. cIAI pts received C/T (1.5 g q8h) + MTZ (500 mg q8h) or meropenem (1 g q8h); cUTI pts received C/T (1.5 g q8h) or levofloxacin (750 mg qd). DM pts were older, had higher body mass index, and had a higher incidence of renal impairment than non-DM pts. In cIAI, a higher percentage of DM pts had APACHE II scores ≥10. Biliary and colonic infections were more common in DM pts; appendiceal infections were more common in non-DM pts (**Table**). In cUTI, pyelonephritis rates were similar in DM and non-DM pts. Bacteriology across all groups was similar; *E coli* was the most common pathogen. In general, DM pts had lower cure rates than non-DM pts; but, cure rates were similar between treatments in both indications (**Table**). Compared with non-DM pts, DM pts had a higher rate of adverse events (AEs; 49.0% vs 37.3%) and serious AEs (10.6% vs 4.6%). However, rates of treatment-related AEs were similar between DM and non-DM pts (8.2% vs 10.1%), suggesting comorbidities were responsible for differences in AE rates. AE rates were similar between treatments in both indications. This analysis showed that C/T was effective in treating cIAI and cUTI in DM pts despite a higher incidence of complicating factors.

Table. Patient Characteristics and Clinical Outcomes in DM and Non-DM Patients with cIAI and cUTI

Patient Characteristics	cIAI[1] (N=806)		cUTI[2] (N=800)	
	DM n=65	Non-DM n=741	DM n=133	Non-DM n=667
Mean age, years (SD)	61.7 (12.6)	49.6 (17.6)	59.1 (14.4)	46.5 (20.2)
Mean BMI, kg/m^2 (SD)	29.3 (6.6)	26.7 (5.2)	29.2 (5.4)	25.2 (5.5)
Mild to moderate renal impairment,[3] n (%)	32 (49.2)	211 (28.5)	63 (47.4)	211 (31.6)
APACHE II score ≥10, n (%)	22 (33.8)	126 (17.0)	–	–
Pyelonephritis, n (%)	–	–	106 (79.7)	550 (82.5)
Infection site, n (%)				
Biliary (cholecystitis)	21 (32.3)	121 (16.3)	–	–
Colon	14 (21.5)	104 (14.0)	–	–
Appendix	14 (21.5)	370 (49.9)	–	–
Clinical/composite[4] cure at TOC visit, m/n (%)				
C/T + MTZ	23/32 (71.9)	300/357 (84.0)	–	–
MEM	26/33 (78.8)	338/384 (88.0)	–	–
C/T	–	–	43/67 (64.2)	263/331 (79.5)
LVX	–	–	40/66 (60.6)	235/336 (69.9)

APACHE II, Acute Physiology and Chronic Health Evaluation II; BMI, body mass index; cIAI, complicated intra-abdominal infection; C/T, ceftolozane/tazobactam; cUTI, complicated urinary tract infection; DM, diabetes mellitus; LVX, levofloxacin; m, number of clinical cures in each category; MEM, meropenem; MTZ, metronidazole; N, number of patients in each group; n, number of patients in each category; "–", not applicable; TOC, test of cure.

[1]In the microbiological intent-to-treat population (all randomized patients with at least 1 baseline intra-abdominal pathogen regardless of susceptibility to study drug).

[2]In the microbiological modified intent-to-treat population (all randomized patients with at least 1 dose of study drug and at least 1 uropathogen at baseline).

[3]Creatinine clearance ≥30 to <80 mL/min.

[4]Clinical cure rates are presented for cIAI patients and composite (composite microbiological eradication and clinical cure) rates are presented for cUTI patients.

Author Disclosure Block:

M.W. Popejoy: D. Employee; Self; Cubist/Merck. **B. Miller:** D. Employee; Self; Ben Miller. **J.A. Huntington:** D. Employee; Self; Jennifer Huntington. **E. Hershberger:** D. Employee; Self; Elham Hershberger. **J. Long:** D. Employee; Self; Jianmin Long.

Poster Board Number:

FRIDAY-431

Publishing Title:

Efficacy of Fosfomycin for the Treatment of Cystitis in Abdominal Transplant Recipients

Author Block:

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Abstract Body:

Purpose: Increased use of antimicrobial prophylaxis following abdominal solid organ transplant (aSOT) has led to a rise the emergence of multidrug resistant organisms (MDRs) in this population. Fosfomycin (FOS) has broad-spectrum activity and is FDA approved for the treatment of uncomplicated urinary tract infections (UTIs) in women. Literature supports in vitro efficacy against MDR pathogens including vancomycin resistant enterococci (VRE). However, there is limited data supporting the clinical efficacy of FOS in aSOT. The aim of this study is to investigate the efficacy of FOS for the treatment of cystitis in the aSOT population.**Methods:** Retrospective chart review of all adult aSOT recipients treated with FOS from 1/1/2007-1/1/2013.**Results:** A total of 76 courses of FOS were identified in 64 patients, with all treatment courses documented as targeted therapy. Of the total courses, 66% were in woman. Patients with a history of renal transplant alone or in combination accounted for 74% of courses. Approximately 40% of courses were initiated in patients who had urinary foreign body placement, of which 20% were ureteral stents. The overall rate of treatment success was 85.5%. Enterococcus was isolated in 59% of courses, 72.2% of these were VRE. In 22% of courses FOS was used for the treatment of gram-negative organisms. Concomitant systemic antibiotics for other indications were present in 36.8% of treatment courses. These antibiotics were not targeted therapy for the urinary pathogens however, synergy with FOS could be possible. In the 11 treatment courses that failed, 90% were targeted against enterococcus, of which 82% were VRE. Six of these failures were then successfully retreated with nonsystemic therapy (FOS or nitrofurantoin). A subgroup analysis was conducted comparing single dose therapy (n=36) against multidose therapy (n=40) in a matched cohort of courses. There was no difference in the success rate of single dose therapy versus multidose therapy (80.6% vs 90%, p=0.24). However, concomitant systemic antimicrobial therapy was more common in the multidose group (70% vs 47.2%, p 0.04), possibly suggesting selection bias toward multidose for more subjectively ill individuals.**Conclusion:** FOS appears to be efficacious for the treatment of cystis in aSOT recipients and in this study single dose was non-inferior to multidose therapy.

Author Disclosure Block:

A. Loethen: None. **M. Jorgenson:** None. **J. Fose:** None. **S. Emanuele:** None. **J. Smith:** None.

Poster Board Number:

FRIDAY-432

Publishing Title:

Faropenem and Fosfomycin Combination Treatment for Complicated Urinary Tract Infections Due to Extended-Spectrum Beta-Lactamase Producing Enterobacteriaceae

Author Block:

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Abstract Body:

Background: There are few oral agents that are active against Extended-spectrum beta-lactamase producing Enterobacteriaceae (ESBL-E). Fosfomycin (FOM) has been used for lower urinary tract infections (UTIs), but increasing resistance is a problem. Faropenem (FRPM) is a penem-class oral beta-lactam agent and active against ESBL-E. We evaluated the susceptibility of urinary isolates positive for ESBL-E to FRPM and FOM, and the clinical efficacy of the combination treatment with FRPM and FOM for complicated UTIs due to ESBL-E. **Methods:** 1. We examined the susceptibility to FRPM and FOM of all urinary isolates positive for ESBL-E using K-B disc method from Nov 2012 to Feb 2015 at St. Luke's Intl Hosp (530beds acute care hosp in Tokyo).2. We also retrospectively investigated all the pts who were diagnosed as complicated UTIs due to ESBL-E, and were treated with combination of FRPM 1200mg/day and FOM 3.0g/day for minimum 7 days. Clinical success was defined as resolution of symptoms at 2 weeks after starting the treatment and 4 weeks after the completion of therapy.**Results:** 1**.** In total 220 ESBL-E isolated from urine cultures, the percentage and the susceptibility were; *E.coli* (89%): FRPM97.3%, FOM89.4%, *Klebsiella pneumoniae* (5.9%): FRPM69%, FOM23%, *K.oxytoca* (3.6%): FRPM88%, FOM13%, and *P. mirabilis* (3.6%): FRPM75%, FOM13%. 2. Total 37 pts (aged 71.3 ± 14.1, range 34-95; 23 women) with complicated UTIs due to ESBL-E were treated with FRPM and FOM. 3. Ten of the 37 pts were treated with only the combination without IV agents. Nine pts (90%) were successfully treated with the combination therapy. In only one pt the therapy was stopped because of diarrhea. 4. Twenty-seven pts were initially treated with Meropenem (88.8%) or PIPC/TAZ (11.1%) for median 14 days (7-34) and as a sequential therapy the pts received FRPM and FOM for median 14 days (6-75). 26 pts (96%) were successfully treated with no clinical recurrence for 4 weeks after the therapy. Side effect was diarrhea in 7 pts (18.9%).**Conclusion:** Combination of FRPM and FOM could be a good oral treatment of choice for complicated UTIs due to ESBL-E as a sequential therapy after IV carbapenem or as an initial outpatient therapy in mild infection.

Author Disclosure Block:

A. Sakurai: None. **K. Furukawa:** None.

Poster Board Number:

FRIDAY-433

Publishing Title:

Multicenter Evaluation of Clinical Outcomes Among Cefepime-treated Patients with Gram-negative Bloodstream Infections (Gnbsi) According to S, Sdd, or R Category

Author Block:

N. J. Rhodes[1], S. N. Rao[2], S. K. Wang[1], J. Liu[1], B. J. Lee[3], B. G. Chiu[4], J. Gener[1], E. Hang[1], K. Singh[5], M. H. Scheetz[1]; [1]Midwestern Univ., Downers Grove, IL, [2]Actavis, PLC, Gurnee, IL, [3]UC Irvine Hlth., Orange, CA, [4]Swedish Covenent, Chicago, IL, [5]Rush Univ. Med. Ctr., Chicago, IL

Abstract Body:

Background: Increased rates of clinical failure at cefepime (FEP) MICs within the susceptible range have been seen. Current Enterobacteriaceae (EB) breakpoints are S:≤2mg/L, SDD:4-8mg/L, R:≥16mg/L, yet few studies have reported mortality rates according to SDD and organism type.**Methods:** We conducted a retrospective, multicenter, cohort study of FEP-treated patients with GNBSI. Clinical variables: demographics, comorbidities, modified APACHE II (A2) score, ICU status, and concurrent neutropenia. Microbiologic variables: pathogen genera, MIC category, and EB status. Susceptibility testing: Vitek2 and E-test. Day 0 was considered the first culture-positive day of GNBSI. Primary outcome: all-cause, in-hospital, mortality stratified by MIC category. Secondary outcomes: A2-adjusted in-hospital mortality probability stratified by MIC category and organism subgroup (i.e., EB v. non-EB).**Results:** A total of 209 patients were included. The majority were male (56.9%) with a mean age of 57.2 years. The majority of patients (65.6%) received FEP as their first GNBSI therapy. A total of 45 patients (21.5%) were in the ICU on day 0. All cause in-hospital mortality occurred in 31 (14.8%) patients with death occurring in 15 (9.7%) S, 11 (33.3%) SDD, and 5 (23.8%) R (P=0.001) infections. A2 [mean (SD)] scores on day 0 were higher among those who died v. those who survived [16.4 (5.0) v. 14.2 (4.6); P=0.02]. Logistic regression of the MIC category, adjusted for A2, revealed that the probability of death within the cohort was significantly higher (P=0.002) at SDD v. S MICs [9.2% (S), 30.7% (SDD), 22.7% (R)]. Adjusted probabilities of death were higher (P=0.03) for SDD v. S MICs in non-EB [8.8%(S), 35.1%(SDD), 29.6%(R)] and numerically higher (P=0.06) in EB [9.0%(S), 26.6%(SDD), 16.7%(R)].**Conclusions:** Among FEP-treated GNBSI, SDD MICs carry an excess risk of mortality which was more similar to R MICs.

Author Disclosure Block:

N.J. Rhodes: None. **S.N. Rao:** None. **S.K. Wang:** None. **J. Liu:** None. **B.J. Lee:** None. **B.G. Chiu:** None. **J. Gener:** None. **E. Hang:** None. **K. Singh:** None. **M.H. Scheetz:** None.

Poster Board Number:

FRIDAY-434

Publishing Title:

The Role of Intravenous Colistin for Carbapenem-Resistant *Acinetobacter baumannii* Bacteremia Treatment

Author Block:

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Abstract Body:

Backgrounds: Colistimethate sodium (Colistin) has been used as one of the last options for carbapenem-resistant *Acinetobacter baumannii* (CRAB) bacteremia. However, the efficacy of colistin has not been fully investigated yet.**Methods:** All patients aged ≥18 years with a CRAB-positive blood culture were enrolled retrospectively at five teaching hospitals in South Korea. Risk factors for 14-day mortality were evaluated by a multivariate analysis.**Results:** A total of 332 patients with CRAB bacteremia were identified. After excluding 11 patients who transferred out, 14-day mortality was 53.0% (170/321). Of them, excluding 125 (37.7%) patients who died before the result of blood culture was reported, 196 patients enrolled in the analysis (45 died vs. 151 survived). All isolates from enrolled patients were susceptible to colistin. Patients receiving colistin monotherapy had highest 14-day mortality (12/37, 32.4%)(Table). Interestingly, 18 (20.7%) of patients with CRAB bacteremia survived, even they did not receive appropriate antibiotics. SOFA score > 8 (adjusted odds ratio [aOR] 4.73, 95% confidence interval [CI] 2.19 - 10.21, P < 0.001) and colistin monotherapy (aOR 2.53, 95% CI 1.02 - 6.29, P = 0.045) were independently associated with 14-day mortality.**Conclusions:** Our data suggest the intravenous colistin therapy may not alter the outcome of patients with CRAB bacteremia. Colistin monotherapy should be avoided for CRAB bacteremia treatment.**Table. Comparison of clinical characteristics between carbapenem-resistant *Acinetobacter baumannii* (CRAB) bacteremic patients with and without 14-day mortality.**

Clinical characteristics	Survived (n = 151)	Died (n = 45)	P-value
Age, median (IQR)	69 (55 - 77)	71 (56 - 77)	0.31
Gender, male	92 (60.9)	30 (66.7)	0.6
Underlying medical conditions			
MaCabe classification			

Non-fatal	96 (63.6)	19 (42.2)	0.02
Fatal	53 (35.8)	22 (55.6)	0.12
Neurologic disease	50 (38.4)	13 (28.9)	0.29
Diabetes mellitus	40 (26.5)	18 (40.0)	0.10
Malignancy	34 (22.5)	12 (26.7)	0.55
Chronic lung disease	19 (12.6)	11 (24.4)	0.06
Chronic renal failure	17 (11.2)	6 (13.3)	0.79
Liver cirrhosis	10 (6.3)	3 (6.7)	> 0.99
Previous surgery	35 (23.2)	8 (17.8)	0.54
Previous hospital stay, median (IQR)	13 (7 - 39)	13 (7 - 22)	0.007
Previous ICU admission	89 (58.9)	38 (84.4)	0.001
Severity			
SOFA score, median (IQR)	6 (4 - 9)	10 (6 - 12)	< 0.001
Mechanical ventilation	58 (34.4)	30 (66.7)	< 0.001
Source of bacteremia			
Pneumonia	52 (34.4)	23 (51.1)	0.06
Catheter-related infection	21 (13.9)	3 (6.7)	0.30
Intra-abdominal infection	12 (7.9)	1 (2.2)	0.31
Unknown	54 (35.8)	16 (35.6)	> 0.99
Treatment			
Colistin monotherapy	25 (16.6)	12 (26.7)	0.13
Colistin combination therapy	40 (26.5)	11 (24.4)	0.85
Appropriate antibiotics	17 (11.3)	4 (8.9)	0.79

Data are numbers (%) of patientsSOFA = sequential organ failure assessment, IQR = interquartile range

Author Disclosure Block:

S. Park: None. **E. Lee:** None. **T. Kim:** None. **S. You:** None. **K. Park:** None. **M. Lee:** None. **M. Jeon:** None. **E. Choo:** None. **T. Kim:** None.

Poster Board Number:

FRIDAY-435

Publishing Title:

Carbapenem-resistant *Enterobacteriaceae* (Cre) Bloodstream Infection (Bsi) Treatment Strategies and Corresponding Outcomes

Author Block:

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Abstract Body:

Background: Infections caused by CRE are difficult to treat and associated with poor outcomes. Combination therapy including a carbapenem (CBP) is generally recommended for severe CRE infections. We sought to identify preferred regimens for CRE BSI from an outcomes perspective. **Methods:** All patients with a blood culture growing a CRE isolate (*E. coli* or *K. pneumoniae*) between 3/1/2010 - 6/30/2014 were included, only counting the first isolate per patient per admission. Patients were assessed for antibiotics received for at least 48 hours within 72 hours of the culture date (or within 7 days of colistin or tigecycline). Treatment outcomes evaluated included: all-cause inpatient mortality (ACM), total length of stay (LOS), LOS following culture result, and time to documented blood culture clearance (TTDBCC). **Results:** A total of 29 isolates were included. *K. pneumoniae* was the most common organism (n=28). The CBP MIC was >=16 for 20 isolates. Among 26 patients who received antibiotic therapy, ACM was 38% (n=11), LOS 43.9 days, LOS following culture result 26.6 days, and TTDBCC 3.5 days. The treatment regimens and associated outcomes are shown in Table 1, differences in outcomes were non-statistically significant. Available antibiotic susceptibilities (VITEK-2® or e-test) are as follows: GENT (n=29, 76%); TIG (n=24, 71%), TIGEUCAST (n=24, 21%), COL (n=27, 70%); AMIK (n=28, 71%); TOBRA (n=29; 3%). **Conclusions:** While the sample size precludes definitive conclusions, we have identified that treatment outcomes may not be affected by the number of additional agents and may not be improved when a CBP is part of a combination regimen for CRE BSI. The lack of added benefit of a CBP may be related to high CBP MIC >=16 as most isolates had in this data-set.

TABLE 1: Outcomes based on antimicrobial regimen

Regimen	Specific Agents	ACM (%)	LOS (days, mean)	TTDBCC (days, mean)	LOS following culture result (days, mean)	Total Duration antibiotics (days, mean)
CBP Given (n=11)	CBP + GENT (n=3) CBP + COL (n=4) CBP + TIG + COL (n=1) CBP + COL + GENT (n=2) CBP + TIG + COL + AMIK (n=1)	54	34.2	4.4	20.4	23.7
No CBP Given (n=15)	TIG + COL (n=3) TIG + COL + GENT (n=2) TIG + GENT (n=3) TIG + COL + AMIK (n=1) COL + GENT (n=1) GENT + CIPR (n=1) AMIK alone (n=1) COL alone (n=3)	33	44.9	3.5	23.4	22.8
CBP + 1 Antibiotic (n=7)	CBP + GENT (n=3) CBP + COL (n=4)	43	26.1	2.3	17.3	26.7
CBP + 2 -3 Antibiotics (n=4)	CBP + TIG + COL (n=1) CBP + COL + GENT (n=2) CBP + TIG + COL + AMIK (n=1)	75	48.2	9.5*	26	35

CBP: meropenem or imipenem; TIG: tigecycline, COL: colistin; GENT: gentamicin; AMIK: amikacin; CIPR: ciprofloxacin.
* TTBCC only evaluable in 3 of 4 patients, 1 patient expired prior to documentation of blood culture clearance
Note: All differences in outcomes between groups were non-statistically significant (using Fisher's exact for categorical variables, Mann-Whitney U for continuous variables)

Author Disclosure Block:

N.N. Pettit: None. **Z. Han:** None. **J. Pisano:** None. **A. Charnot-katsikas:** None.

Poster Board Number:

FRIDAY-436

Publishing Title:

Clinical Outcomes of Patients with Infections Due to Carbapenem-Resistant Enterobacteriaceae (CRE) Treated with Ceftazidime-Avibactam (C/A)

Author Block:

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Abstract Body:

Background: C/A is a new agent with *in vitro* activity against Enterobacteriaceae, including extended-spectrum β lactamases (ESBL) and *K. pneumoniae* carbapenemase (KPC) producers. Real-world experience with C/A against CRE infections is limited.**Methods:** We performed a retrospective review of patients (pts) with CRE infection treated with C/A from April to Nov 2015. Pts with CRE colonization were excluded. C/A susceptibility testing was performed by broth microdilution. Presence of KPC and other ESBLs were detected by PCR.**Results:** 32 pts with 33 episodes of CRE infection were identified. Mean age was 59 years. Solid organ and stem cell transplant recipients were 28% and 6%, respectively. Median SAPS II and SOFA scores were 39 (range 12-72) and 7 (range 0-18), respectively. Infection types were pneumonia (11, 4 were ventilator-associated), bacteremia (9), complicated urinary tract or pyelonephritis (4), intra-abdominal infection (2), wound infection (2), and 1 each of subdural empyema and meningitis, mediastinitis, osteomyelitis, septic arthritis, and disseminated infection. 32 CRE isolates from unique pts were available for β-lactamase testing: KPC and ESBL- (23 *K. pneumoniae*, 1 *E. cloacae*, 1 *K. oxytoca*), and ESBL- producing (3 *K. pneumoniae*, 3 *E. coli* and 1 *E. cloacae).* Among non-KPC producers, all were CTX-M group 1; 3 SHV, and 1 TEM variants. All were susceptible to C/A *in vitro* with median MIC of 1 µg/mL (range 0.25 to 4). Median duration of C/A therapy was 11 days (range 7-72). C/A was used as part of combination therapy in 47% (mostly aminoglycosides). Therapeutic success at 14 days (clinical improvement and survival) was achieved in 69%. Both 14- and 30-day mortality was 29%. Among 22 survivors at 30 days, 9% had relapse at the same site, and 1 pt had relapsing CRE infection at a different site. One pt had 2 bacteremia relapses at 34 and 129 days from the initial episode; the KPC isolates remained susceptible to C/A.**Conclusions: Outcomes of pts with CRE infections treated with C/A were promising, and emergence of resistance was not observed. The absence of resistant isolates is encouraging particularly when empiric coverage against CRE is warranted. C/A tolerance assays and whole genome sequencing of isolates may provide insight into treatment failures.**

Author Disclosure Block:

B.A. Potoski: None. **R.K. Shields:** None. **B. Hao:** None. **G. Haidar:** None. **Y. Doi:** None. **C.J. Clancy:** None. **M.H. Nguyen:** None.

Poster Board Number:

FRIDAY-437

Publishing Title:

Utility of Combination Antimicrobial Therapy for Bloodstream Infections Due to *Enterobacteriaceae* and Non-Fermenting Gram-Negative Bacilli

Author Block:

C. Troficanto[1], J. Kohn[2], J. A. Justo[3], P. B. Bookstaver[3], H. Albrecht[4], M. N. Al-Hasan[4]; [1]Palmetto Hlth.Baptist, Columbia, SC, [2]Palmetto Hlth.Richland, Columbia, SC, [3]SC Coll. of Pharmacy, Univ. of South Carolina, Columbia, SC, [4]Univ. of South Carolina Sch. of Med., Columbia, SC

Abstract Body:

Background: The role of empiric combination antimicrobial therapy for gram-negative bloodstream infections (GN BSI) remains uncertain. This study examines potential benefit of combination therapy on adequacy of empiric antimicrobial regimen in GN BSI patients.**Methods:** Hospitalized adults with GN BSI from January 2010 to June 2015 at Palmetto Health Hospitals in Columbia, SC, USA were identified. The effect of adding a combination agent on antimicrobial susceptibility rates within the study cohort was estimated using matched pair mean differences. The background regimen was a third-generation cephalosporin (3GC), i.e. ceftriaxone for *Enterobacteriaceae*, ceftazidime for non-fermenting gram-negative bacilli (NFGNs).**Results:** Among *Enterobacteriaceae*, 996/1063 (94%) isolates were susceptible to 3GC with marginal change in susceptibilities by adding either ciprofloxacin or gentamicin (mean difference +2.4% [95% CI: 1.5, 3.4] and +3.0% [95% CI: 2.0, 4.0], respectively). In a subset of patients with *Enterobacteriaceae* BSI and prior beta-lactam use, 168/200 (84%) isolates were susceptible to 3GC with mean improvement in susceptibilities of +4.0% (95% CI: 1.3, 6.8) and +7.6% (95% CI: 3.9, 11.3) after adding ciprofloxacin and gentamicin, respectively. Regarding NFGNs, 83/105 (79%) bloodstream isolates were susceptible to 3GC overall and 29/39 (74%) in patients with prior beta-lactam exposure. Adding ciprofloxacin or gentamicin contributed to mean difference in susceptibilities of +15.2% (95% CI: 8.3, 22.2) and +17.1 % (95% CI: 9.8, 24.5), respectively, overall and +23.1% (95% CI: 9.2, 36.9) for both combinations in patients with prior beta-lactam use.**Conclusions:** Empiric combination antimicrobial therapy has limited utility in *Enterobacteriaceae* BSI. However, combination regimens provided significant additional antimicrobial coverage to 3GC for NFGNs. Identification of patients with NFGN BSI using clinical risk factors or rapid diagnostics may aid the decision to utilize empiric combination therapy.

Author Disclosure Block:

C. Troficanto: None. **J. Kohn:** None. **J.A. Justo:** J. Scientific Advisor (Review Panel or Advisory Committee); Self; Advisory board member for Cempra Pharmaceuticals. **P.B.**

Bookstaver: J. Scientific Advisor (Review Panel or Advisory Committee); Self; Actavis Pharmaceuticals (now Allergan). **H. Albrecht:** None. **M.N. Al-Hasan:** None.

Poster Board Number:

FRIDAY-439

Publishing Title:

Medical vs. Interventional Treatment of Intra-Abdominal Abscess in Patients with Crohn's Disease

Author Block:

E. Graham, S. Cinti, K. Rao; Univ. of Michigan, Ann Arbor, MI

Abstract Body:

Background: Few studies exist to guide the treatment approach to intra-abdominal abscesses in Crohn's disease, which can include antimicrobials alone or in conjunction with percutaneous drainage or surgery. The aims of this study are to compare outcomes among medical and interventional approaches and to evaluate the impact of infectious disease (ID) consultant involvement in management.**Methods:** Medical records were reviewed for patients admitted to the University of Michigan healthcare system from January 2010 to September 2014 with a diagnosis of Crohn's disease and radiographically confirmed intra-abdominal abscess. Treatment groups were defined as medical (antimicrobials alone without aspiration or drainage) and interventional (antimicrobials plus percutaneous drainage or surgery). Treatment failure was defined as abscess recurrence or non-resolution within six months. ID consultant involvement was also recorded and compared between treatment success and treatment failure groups. The X^2 test was used to test for statistical significance.**Results**: Of the 33 patients included, 13 were in the medical treatment group and 20 were in the interventional treatment group (**Table**). Abscess recurrence/non-resolution occurred in 31% of patients in the medical group and 25% of patients in the interventional group ($P = .7$). ID consultants were involved in managing 33% of patients with abscess resolution and 33% of patients with abscess recurrence/non-resolution. There was no difference in outcome between patients with and without ID consults ($P > .99$).**Conclusions:** In this study, there was no significant difference in outcome between medical and interventional therapy for intra-abdominal abscess in Crohn's disease. ID consultant involvement did not affect treatment outcome.**Outcome of medical vs interventional treatment for intra-abdominal abscess**

Variable	Abscess recurrence or non-resolution, n (%)	Abscess resolution, n (%)	*P*
Medical	4 (31%)	9 (69%)	.7
Interventional Percutaneous Surgical	5 (25%) 3 (23%) 2 (29%)	15 (75%) 10 (77%) 5 (71%)	
ID consult	3 (33%)	8 (33%)	> 0.99
No ID consult	6 (67%)	16 (67%)	

Author Disclosure Block:

E. Graham: None. **S. Cinti:** None. **K. Rao:** None.

Poster Board Number:

FRIDAY-440

Publishing Title:

Targeting Myd88/Arf6 Pathway As A Novel Treatment For Multidrug-Resistant *acinetobacter* infection

Author Block:

L. Lin[1], T. Gebremariam[1], A. Mueller[2], D. Li[2], **A. S. Ibrahim**[1]; [1]Los Angeles BioMed. Res. Inst. at Harbor-UCLA Med. Ctr., Torrance, CA, [2]Navigen Pharmaceuticals, Salt Lake City, UT

Abstract Body:

Background: Multi-drug resistant (MDR) organisms due to Gram negative bacilli (GNB), including those related to *Acinetobacter baumannii* (*AB*) have become endemic in healthcare systems throughout the world. Thus, novel methods to treat these infections are needed. Septicemia triggered by lipopolysaccharide (LPS) is a common manifestation of GNB infections. LPS triggers an inflammatory immune response via the MyD88/NF-κB cascade. However, LPS also induces vascular leak which leads to tissue edema resulting in organ failure and death. Vascular leak is caused by the GTPase Arf6 which falls in the MyD88/Arf6 pathway and functions independently from the MyD88/NF-κB cascade. We hypothesize that the pharmacological inhibition of Arf6 will be a novel treatment for *AB* infection.**Methods:** We used immunoprecipitation (IP) and trans-well permeability assays to study the role of small molecules on inhibiting GTPase Arf6 formation in human umbilical vein endothelial cells (HUVECs) infected with *AB*. Immunofluorescence was used to evaluate the effect of Arf6 inhibitors on VE-Cadherin expression in HUVECs infected with *AB*. Inhibitors were evaluated for their protective effect in neutropenic mice (cyclophosphamide [200 mg/kg] and cortisone acetate [500 mg/kg] on day -2, +3, relative to infection) with *AB* pneumonia. Treatment with 30 mg/kg inhibitors started 2 h post infection and continued through day +7.**Results:** *AB* infection resulted in activation of Arf6 (Arf6·GTP by IP assay) and the inhibitors significantly reduced this activation. The inhibitors reduced trans-membrane HUVECs leakage caused by *AB* by ~85% ($P<0.05$). Further, *AB* disrupted cell-to-cell junction of HUVECs via reduction of surface expression of VE-Cadherin and the inhibitors reversed this effect. Finally, two inhibitors (NAV2729, and NAV4424) significantly improved survival of mice (n=20 per arm) when compared to placebo (21 day survival of 5% for placebo, 85% for NAV2729, and 50% for NAV4424, $P<0.05$) with surviving mice appearing healthy.**Conclusions:** Inhibition of GTPase Arf6 formation reduces vascular leak and enhances survival of mice infected with lethal *AB*. Continued investigations of Arf6 inhibitors as a novel treatment of MDR-GNB is warranted.

Author Disclosure Block:

L. Lin: None. **T. Gebremariam:** None. **A. Mueller:** D. Employee; Self; Navigen. **D. Li:** A. Board Member; Self; Navigen. D. Employee; Self; Navigen. **A.S. Ibrahim:** C. Consultant; Self; Navigen. F. Investigator; Self; Navigen.

Poster Board Number:

FRIDAY-441

Publishing Title:

Arf6 Inhibitors for Treatment of Acute Lung Injury

Author Block:

A. L. Mueller[1], K. Ostanin[1], C. Dunn[1], Z. Tong[1], D. Li[2]; [1]Navigen, Inc., Salt Lake City, UT, [2]Univ. of Utah, Salt Lake City, UT

Abstract Body:

Background: Acute lung injury (ALI) and the more severe acute respiratory distress syndrome (ARDS) result from a common pathogenic process: pulmonary injury or infection triggers an overwhelming inflammatory response ("cytokine storm") that results in increased endothelial and epithelial permeability and efflux of inflammatory cells, protein, and water from the vascular system into the alveolar space. The laboratory of Dr. Dean Li and Navigen scientists have discovered a critical role of the GTPase ARF6 in control of vascular barrier integrity in the face of cytokine storm. Navigen has discovered a first-in-class chemical series of direct small molecule ARF6 inhibitors that show robust efficacy in a mouse model of LPS-induced ALI.**Methods:** LPS was instilled into the trachea of anesthetized mice. ARF6 inhibitor was injected IP at 30 or 60 mg/kg at 0 (T=0) or 3 h (T=3) after LPS. BAL fluid was collected 24 h after LPS; cell count and protein were measured. **Results:** LPS-induced marked increases in BALF cell count that were inhibited significantly by NAV2729 (49% at 30 mg/kg T=0; 61% at 60 mg/kg T=0; 26% at 30 mg/kg T=3; 64% at 60 mg/kg T=3) and NAV4424 (57% at 30 mg/kg T=0; 95% at 60 mg/kg T=0; 55% at 30 mg/kg T=3; 91% at 60 mg/kg T=3). Cytospin analysis confirmed that >90% of the cells in BALF after LPS-induced injury were neutrophils, and that treatment with ARF6 inhibitor reduced neutrophil infiltration. LPS-induced increases in BALF protein were inhibited significantly only at 60 mg/kg of either compound (NAV2729: 61% at 60 mg/kg T=0, 60% at 60 mg/kg T=3; NAV4424: 46% at 60 mg/kg T=0, 55% at 60 mg/kg T=3). Importantly, efficacy is observed when treatment with ARF6 inhibitor is delayed by 3 h after LPS.**Conclusions:** The small GTPase ARF6 plays a critical role in vascular leak elicited by a variety of inflammatory mediators. Small molecule inhibitors of ARF6 provide significant protection in an LPS-induced ALI model in mice. These inhibitors have potential as first-in-class therapeutics for treatment of ALI/ARDS.

Author Disclosure Block:

A.L. Mueller: None. **K. Ostanin:** None. **C. Dunn:** None. **Z. Tong:** None. **D. Li:** None.

Poster Board Number:

FRIDAY-442

Publishing Title:

Complex Defined Bacteriotherapy Inhibits Acute Colitis in Mice

Author Block:

T. Fofanova[1], F. Ihekweazu[1], D. Nagy-Szakal[2], K. Hulten[1], A. Opekun[3], J. Petrosino[1], D. Graham[1], R. Kellermayer[1]; [1]Baylor Coll. of Med., Houston, TX, [2]Columbia Univ., New York, NY, [3]BCM, Houston, TX

Abstract Body:

Inflammatory bowel disease (IBD) is thought to develop secondary to an uncontrolled immune response against the gut microbiome in the intestinal mucosa. Therefore, dysbiosis may be an important element in disease development, and restoration of normal microbiota structure via fecal microbiota transplantation (FMT) may be therapeutic for IBD. The fecal microbiome is a complex and dynamic community, which complicates dissection of the therapeutic contributions of FMT. Therefore, the identification of stable, defined bacterial communities that carry the therapeutic effects of FMT could result in a more consistent bacteriotherapy for IBD. Such a treatment has been developed for *C. difficile* colitis, where a 10 species preparation has been efficacious in treating disease. Observing that many of the species in this community belong to the *Bacteroides* genus, we compared the anti-colitic effects of a triple-*Bacteroides* (tri*Bac*) bacteriotherapy against FMT in a murine model of acute colitis. Experimental colitis was induced in 8-12 week old C57BL/6 mice using 3% dextran sulfate sodium for 5 days. Mice were simultaneously treated by oral gavage for 9 days with the tri*Bac* combination, individual *Bacteroides* strains, FMT using stool from healthy donor mice (FMT group), or autologous FMT (control group). Survival was significantly lower in the control group than in the FMT and tri*Bac* groups (40%, 73%, and 100% respectively). Importantly, mice receiving tri*Bac* therapy lost significantly less body weight than both the control and FMT groups (3.5%, 17.4%, 9.1% respectively). Histopathologic and gene expression analyses are pending. Microbiome analysis (16s rDNA profiling) of stool and mucosal samples is near completion. All 3 strains, individually and in combination, resulted in significantly increased survival and decreased weight loss compared to controls. However, treatment with *B. ovatus* alone resulted in the least loss of body weight. Our tri*Bac* approach was significantly more effective than FMT in reducing weight loss during acute colitis in mice. We are currently validating our results in an alternative model of murine colitis and are co-culturing these strains with human, colon-derived enteroid monolayers to investigate their effects on the barrier integrity, cytokine profile, and gene expression of the human intestinal epithelium.

Author Disclosure Block:

T. Fofanova: None. **F. Ihekweazu:** None. **D. Nagy-Szakal:** None. **K. Hulten:** None. **A. Opekun:** None. **J. Petrosino:** None. **D. Graham:** None. **R. Kellermayer:** None.

Poster Board Number:

FRIDAY-443

Publishing Title:

Recombinant Human Trefoil Factor 2: Expression and Impact on Protection of Neonatal Rats against *Escherichia coli* K1 Systemic Infection

Author Block:

A. J. McCarthy[1], D. Negus[1], F. Dalgakiran[1], N. Crespo Tapia[2], I. Lopes Periera[1], K. Malec[1], G. M. H. Birchenough[3], P. W. Taylor[1]; [1]Univ. Coll. London, London, United Kingdom, [2]London Sch. of Hygiene & Tropical Med., London, United Kingdom, [3]Univ. of Gothenburg, Gothenburg, Sweden

Abstract Body:

Background: The newborn infant is highly susceptible to systemic bacterial infection during the first four weeks of life, and morbidity and mortality associated with neonatal bacterial meningitis and associated sepsis remains high. A major causative agent is *Escherichia coli* K1. During neonatal development, the gastrointestinal (GI) defences mature and thereby prevent translocation of colonizing bacteria from the GI tract to the blood and subsequent systemic infection. Trefoil factor 2 (TFF2) is proposed to have a key role in the stabilization of mucin structure and maintenance of mucosal health. Notably, *Tff2* expression is dysregulated in two-day-old (P2) but not nine-day-old (P9) rat pups colonised with the major neonatal pathogen *Escherichia coli* K1. **Objective:** To construct an *E. coli* SHuffle strain for the production of recombinant human TFF2 (r-hTFF2), in order to examine the capacity of r-hTFF2 to provide protection for P2 rat pups against systemic infection by *E. coli* K1 A192PP. **Methods:** We amplified and cloned a human *Tff2* transcript into vector pET26b. The pET26b.h*Tff2* vector was transferred into *E. coli* SHuffle, an expression strain able to express correctly folded, disulphide bonded proteins. The production of recombinant proteins by *E. coli* SHuffle + pET26b.h*Tff2* under various growth conditions was quantified using IMAC columns. Rat pups were fed r-hTFF2 or vehicle control at P2 (and every 12 h) and challenged by the oral route with *E. coli* K1 A192PP at P3; the health of the animals was monitored up to P10. **Results:** We confirmed the expression of r-hTFF2 by SDS-PAGE (12kDAa protein) and Western Blotting (anti-human TFF2), optimised expression within the *E. coli* SHuffle strain, and assessed functional activity of r-hTFF2 with *in vitro* assays. Survival of rat pups fed r-hTFF2 was ~30%, whereas survival of pups fed vehicle alone was 10%. **Conclusions:** We have generated and validated *E. coli* Shuffle expressing bioactive r-hTFF2; oral administration of the protein to neonatal rats susceptible to *E. coli* K1 infection protects against lethal systemic disease.

Author Disclosure Block:

A.J. McCarthy: None. **D. Negus:** None. **F. Dalgakiran:** None. **N. Crespo Tapia:** None. **I. Lopes Periera:** None. **K. Malec:** None. **G.M.H. Birchenough:** None. **P.W. Taylor:** None.

Poster Board Number:

FRIDAY-444

Publishing Title:

Control of Korean Sacbrood Virus Using Dsrna in Apis Cerana

Author Block:

M-S. Yoo, H-J. Seo, H-N. Jung, J-S. Lee, W. Bae, H-S. Lee, Y. Cho; Animal and Plant Quarantine Agency, Anyangsi, Gyeonggido, Korea, Republic of

Abstract Body:

Background: Sacbrood virus (SBV), a causative pathogen of larval death in honeybees, is one of the most devastating diseases in bee industry throughout the world. Since 2010, the Korean Sacbrood virus (KSBV) caused great losses in Korean honeybee (*Apis cerana*) colonies. However, there is no treatment for honeybee viruses including SBV. RNA interference (RNAi) is a gene-silencing technology by which small double-stranded RNAs are used to target the degradation of RNA with complementary sequence.**Methods:** In this study, we report on prevention of SBV infection by feeding with double-stranded RNA both in vitro and in vivo. SBV sequences corresponding to a segment of structural protein VP1 gene for dsVP1 and a segment of the structural polyprotein open reading frame for dsSBV1 were used for cloning. Small scale of dsRNA synthesis was carried out according to the protocol of the mMESSAGE mMACHINE T7 kit and large scale of dsRNA was synthesized by Genelution Inc. It was treated with food.**Results:** The mortality and viral load were observed every day. Experiments were carried out to examine whether ingestion of dsRNAs of SBV sequences would protect bees from SBV infection. The survivability of larvae was increased upto 40% in vitro test. The viral load of laevae and bee colonies were significantly decreased to a level similar to pre-infection in vivo test. The result indicated that two SBV-derived dsRNAs (dsSBV1 and dsVP1) protected bee larvae from subsequent SBV infection.**Conclusions:** dsRNA of SBV will be used as an efficient and feasible way of controlling other bee viral diseases as well as SBV.

Author Disclosure Block:

M. Yoo: None. **H. Seo:** None. **H. Jung:** None. **J. Lee:** None. **W. Bae:** None. **H. Lee:** None. **Y. Cho:** None.

Poster Board Number:

FRIDAY-445

Publishing Title:

Effects of Nspef on *In-Vitro* Viability of HPV-16 Infected Cervical Carcinoma Cell Line Caski

Author Block:

H. A. Ryan, S. J. Beebe; Old Dominion Univ., Norfolk, VA

Abstract Body:

Background: Human papillomavirus 16 (HPV-16) is a double-stranded DNA virus known to associate highly with human cervical cancer. Nanosecond pulsed electric fields (nsPEF) are ultra-short pulses with high electric field intensity (kV/cm) and high power (MW), but low energy density (mJ/cc). Recent studies suggest that nsPEFs can ablate tumors, alter gene expression and interfere with protein synthesis, but studies relating nsPEF and viral gene expression and the efficacy of nsPEF as an anti-viral biotechnology are few or unknown. This study examined the in-vitro anti-proliferative effect of nsPEF in human epithelial cervical carcinoma cell line CaSki infected with HPV-16.**Methods:** Adherent CaSki cells were detached from culture and treated with nsPEF in a 0.1 cm gapped electrode cuvette at different electric field strengths (0, 20, 40, 50 and 60 kV/cm) and pulse numbers (0, 1, 5, 10, 50 and 100 pulses). 1 × 10^5 cells from each sample were transferred into a 96-well plate and incubated for 4-6 and/or 24 hours, after which time cell viability was determined using a colorimetric cell proliferation assay (MTS) at an absorbance wavelength of 490 nm. Samples were tested in triplicate, and each set of experiments was replicated at least three times. Viability is reported using median values as percent of untreated cells. Statistical significance was evaluated by Student's t-test ($p<0.05$).**Results:** The MTS assay showed that nsPEF induced a distinct electric field strength- and pulse number-dependent reduction of cell proliferation. The EC50 under the various electric field and pulsing conditions occurred within the first 6 hours after treatment for: nsPEF of 50 kV/cm (50 and 100 pulses) and 60 kV/cm (10 pulses). The conditions 50 kV/cm and 10 pulses at 24 hours post-treatment achieved the most significant decrease in cell viability. NsPEF induced cell death was not shown to be statistically significant after 4 and 24 hours for nsPEF intensities of 20, 40 for 1, 5, and 10 pulses, and 60 kV/cm for 1 and 5 pulses.**Conclusions:** NsPEF inhibits cell proliferation in human papillomavirus-infected tumor cell line CaSki. Anti-proliferative effects of nsPEF may contribute to reducing cancer metastasis, and possibly play a direct antiviral role in clearing virus-associated metastatic cells.

Author Disclosure Block:

H.A. Ryan: None. **S.J. Beebe:** None.

Poster Board Number:

FRIDAY-446

Publishing Title:

Development of Orally-Delivered Therapeutics to Protect the Gut Microbiome from Antibiotic-Mediated Damage

Author Block:

S. Connelly[1], C. Freguia[1], T. Parsley[2], N. Hasan[3], P. Subramanian[3], M. Kaleko[1]; [1]Synthetic Biologics, Inc., Rockville, MD, [2]SynPhaGen, LLC., Rockville, MD, [3]CosmosID, Inc., Rockville, MD

Abstract Body:

Beta-lactam antibiotics (abx) are excreted in bile and can damage the colonic microflora leading to serious adventitious infections. SYN-004 is an orally-delivered beta-lactamase intended to degrade certain intravenous (IV) abx in the gut to protect the microbiome. A Phase 2b clinical study is in progress to assess SYN-004-mediated prevention of *C. difficile* infection (CDI) and abx-associated diarrhea (AAD). SYN-004 degrades penicillins and cephalosporins, but not carbapenems. We identified SYN-006, a potent carbapenemase, to expand this prophylactic approach to all beta-lactam abx classes.SYN-006, a metallo-beta-lactamase derived from *B. cereus*, was produced in *E. coli* with yields of ~600 mg/L at 95% purity. Using a bacterial growth assay as a readout for abx degradation, SYN-006 displayed a broad degradation profile that included carbapenems, penicillins, and cephalosporins, and was resistant to beta-lactamase inhibitors. SYN-006 retained biological activity for at least 6 hrs in human chyme. The inactivation of the carbapenem, meropenem (30 mg/kg, IV), in the GI tract was evaluated in fistulated dogs (n=6) using SYN-006 formulated in PBS, pH 7.5 (1 mg/kg). While SYN-006 jejunal levels were variable, most likely due to its sensitivity to low pH, at SYN-006 concentrations of $\geq$ 0.5 U/ml, meropenem was undetectable. SYN-006 did not affect serum meropenem levels, verifying it functioned solely in the GI tract. SYN-006 is currently being formulated into enteric-coated pellets that release at pHs >5.5. To evaluate the effect of carbapenems on the gut microbiome, a piglet model was developed. Animals (20 kg; n=5) were treated with ertapenem (30 mg/kg, QD, IV) for 7 days. Analysis of microbiome data from fecal DNA whole genome shotgun sequencing demonstrated that ertapenem caused dysbiosis, including loss of species diversity and changes in species abundance (Likelihood Ratio Test, $p=7.0 \times 10^{-16}$). Pig efficacy studies using the enteric-coated SYN-006 are being planned.These data demonstrate that SYN-006 displays manufacturability and sufficient potency to continue to be developed into an oral therapeutic. SYN-006 has the potential to protect the microbiome from all classes of beta-lactam abx and to provide prophylaxis for CDI and AAD.

Author Disclosure Block:

S. Connelly: D. Employee; Self; Synthetic Biologics, Inc.. K. Shareholder (excluding diversitied mutual funds); Self; Synthetic Biologics, Inc. **C. Freguia:** D. Employee; Self; Synthetic Biologics, Inc.. K. Shareholder (excluding diversitied mutual funds); Self; Synthetic Biologics, Inc. **T. Parsley:** D. Employee; Self; SynPhaGen, LLC. **N. Hasan:** D. Employee; Self; CosmosID, Inc. **P. Subramanian:** D. Employee; Self; ComosID, Inc. **M. Kaleko:** D. Employee; Self; Synthetic Biologics, Inc..

Poster Board Number:

FRIDAY-447

Publishing Title:

Lipid Nanoparticles for Reviving Antibiotics: Efficacy of a Gel of Daptomycin in a Methicillin-Resistant *Staphylococcus aureus* Rabbit Osteomyelitis Model

Author Block:

C. Jacqueline, A. Reghal, K. Asehnoune, G. Potel, J. Caillon; ATLANGRAM, Nantes, France

Abstract Body:

Background: Daptomycin (DAP) is a bactericidal antibiotic with activity against Gram-positive organisms, including methicillin-resistant *Staphylococcus aureus* (MRSA) isolates, but its administration is exclusively by IV route. Lipid Nano-Capsules (LNCs) are known to vehicle medicines and could offer new therapeutic options. The efficacy of LNC-daptomycin (LNC-DAP) formulated in a gel was compared with that of other antistaphylococcal drugs in a MRSA osteomyelitis rabbit model. **Methods:** Femoral trepanation of rabbits was performed, followed by injection of 10^8 CFU *S. aureus* suspension into the knee cavity. A surgical debridement of the infected tissues was performed 3 days later and animals were randomly assigned to: no treatment (controls), LNC-DAP [one application of 50 mg (Low Dose) or 200 mg (High Dose)], linezolid (LZO, simulating a human-equivalent dose of 10 mg/kg/12h), or vancomycin (VAN, constant IV infusion to reach a 20xMIC serum steady-state concentration), or daptomycin (DAP, simulating a human-equivalent dose of 6 mg/kg). Surviving bacteria were counted in bone marrow (BM) and bone (BO) at day 3 and at the end of 4-days treatment (day 7). **Results**: The nanotechnology manufacturing process of daptomycin was demonstrated with an entrapment efficiency of 100%. The minimum inhibitory and bactericidal concentrations of the free antibiotic DAP and the LNC-encapsulated DAP (LNC-DAP) against the MRSA strain were equivalent (0.5 mg/L). One topic dose of the gel formulation of LNC-DAP versus four days of IV standard antibiotics (LZO, DAP, VAN) showed significant decrease of the bacterial burdens in both BO and BM compartments as compared to IV antibiotic regimens.

Treatment (no. of animals)	Mean ± SD $\log_{10}$ CFU/g of tissue (day 7 – day 3)	
	BM	BO
Controls (6)	0.19 ± 0.68	0.10 ± 0.87
VAN (11)	-0.65 ± 1.76	-0.61 ± 0.72
LZO (8)	-2.68 ± 1.91[a]	-2.27 ± 1.52[a]
DAP (10)	-0.56 ± 0.72	-0.67 ± 1.12
LNC-DAP LD (12)	-4.90 ± 1.48[b]	-5.27 ± 0.85[b]
LNC-DAP HD (6)	-5.76 ± 0.45[b]	-5.60 ± 0.31[b]

[a] *P*<0.05 vs controls, VAN, and DAP; [b] P <0.001 vs controls, VAN, LZO, and DAP. LD, Low Dose; HD, High Dose

Conclusions: In this model, a gel of lipid nano-encapsulated daptomycin (LNC-DAP) showed significant *in vivo* activity after ONE topic application in comparison with 4 days of IV anti-staphylococcal drugs. The use of LNCs for local delivery of antibiotics is a promising approach to revive old antibiotics or to develop new antibacterial agents.

Author Disclosure Block:

C. Jacqueline: None.

Poster Board Number:

FRIDAY-448

Publishing Title:

Double β-Lactam Combination Is Synergistic Against Daptomycin-Resistant *Enterococcus faecium* harboring YycFG Substitutions and an Intact LiaFSR System

Author Block:

T. T. Tran, C. A. Arias; Univ Texas Med Schl, Houston, TX

Abstract Body:

Background: Previous studies have shown that the daptomycin (DAP) and β-lactam combination is synergistic against DAP-resistant (R) isolates that harbor mutations in the *liaFSR* encoding a three-component regulatory system that control cell envelop homeostasis. However, a subset of DAP-R *E. faecium* isolates lack LiaFSR substitutions and the DAP-β-lactam combination seems to be ineffective against these organisms. A high proportion of these *E. faecium* harbor mutation in the *yycFG* system (and accessory proteins), an essential two-component system predicted to regulate cell wall synthesis. In this work, we aim to determine the activity of double β-lactams, alone and in combination with DAP, against a DAP-R *E. faecium* strain (R446) harboring 8 mutations, including one in YycG. **Methods:** MICs of ampicillin (AMP), ceftaroline (CPT), ceftriaxone (CRO) and DAP against R446 were determined by Etest. Time-kill assays were performed with initial bacteria inoculum of 10^7 CFU/ml in Mueller-Hinton broth supplemented with calcium (50 mg/L). Antibiotics (AMP 64 μg/ml, CPT 10 μg/ml, CRO 10 μg/ml, and DAP 13 μg/ml) were used independently and in combination (AMP + CPT, AMP + CRO, AMP + CPT + DAP, and AMP + CRO + DAP) for time-kill studies. Bacteria were enumerated at 0, 6, and 24 h.**Results:** *E. faecium* R446 was resistant to all antibiotics tested (MICs of 128 μg/ml, > 32 μg/ml, ≥ 256 μg/ml, and 16 μg/ml for AMP, CPT, CRO and DAP, respectively). None of the antibiotic tested were effective in killing *E. faecium* R446 alone. AMP + CPT and AMP + CPT + DAP decreased bacterial loads by 3.01 ± 0.951 and 3.38 ± 0.09 $\log_{10}$ CFU at 24 h, respectively. AMP + CRO modestly decreased bacterial load by 0.88 ± 1.057 $\log_{10}$ CFU/ml. The addition of DAP to the CRO + AMP combination effectively decreased bacterial loads by 3.56 $\log_{10}$ CFU/ml.**Conclusions:** Double β-lactam AMP + CPT without DAP was bactericidal against DAP-R *E. faecium* R446. The addition of AMP + CRO restored the bactericidal activity of DAP. These results suggest the utility of double β-lactam against DAP-R *E. faecium* which may not respond to the combination of DAP and AMP.

Author Disclosure Block:

T.T. Tran: None. **C.A. Arias:** C. Consultant; Self; Theravance, Cubist, Bayer. E. Grant Investigator; Self; Theravance. F. Investigator; Self; Forest. J. Scientific Advisor (Review Panel

or Advisory Committee); Self; Cubist, Bayer. L. Speaker's Bureau; Self; Forest, Theravance, Pfizer, Astra-Zeneca, the Medicines Company, Cubist, Novartis.

Poster Board Number:

FRIDAY-449

Publishing Title:

Synergistic Killing of Kpc-Producing *Klebsiella pneumoniae* (Kpc-Kp) by Fosfomycin (Fos) Plus Colistin (Col) in a Static Time-Kill Model

Author Block:

M. Zhao[1], Z. P. Bulman[2], J. R. Lenhard[2], M. J. Satlin[3], B. N. Kreiswirth[4], T. J. Walsh[3], V. Petraitis[3], P. N. Holden[2], P. B. Bergen[5], R. L. Nation[5], J. Li[5], J. Zhang[1], B. T. Tsuji[2]; [1]Huashan Hosp., Fudan Univ., Shanghai, China, [2]Sch. of Pharmacy and Pharmaceutical Sci., Univ. at Buffalo, Buffalo, NY, [3]Weill Cornell Med., New York, NY, [4]Publ. Hlth.Res. Inst., Newark, NJ, [5]Monash Univ., Parkville, Australia

Abstract Body:

Background: FOS and COL are often of the few remaining agents that remain susceptible to KPC-Kp. However, FOS and COL resistance develops rapidly in monotherapy requiring the need for combinations. We therefore evaluated the *in vitro* pharmacodynamics of FOS and COL alone and in combination against KPC-Kp.**Methods:** Static time-kill studies (~10^6 CFU/mL inoculum) over 48 h were conducted using two KPC-2 expressing KPC-Kp clinical isolates. One isolate was COL-susceptible (COL^S, MIC_{COL} 0.25 mg/L & MIC_{FOS} 32 mg/L) while the second isolate was COL-resistant (COL^R, MIC_{Col} 64 mg/L & MIC_{FOS} >128 mg/L). Three concentrations of COL (0.25, 1 & 4 mg/L) and seven concentrations of FOS (8, 16, 32, 64, 128, 256 & 512 mg/L) based on free plasma concentrations were studied alone and in combination. Synergy was defined as a ≥2-log_{10} CFU/mL between the combination and the most active single agent.**Results:** FOS & COL alone produced extensive initial killing (~2 to 5 log10 CFU/ml) against the COL^S strain followed by regrowth. Synergy was evident at 4 h with all combinations resulting in complete eradication, except for combinations with a low FOS concentrations of 8 mg/L, which regrew by 24 h. The combination time-kill data was modeled as FOS concentration versus 48 h log ratio area and fit excellently to a Hill function (R^2>0.999). In combination experiments, in the presence of COL of 0.25, 1 & 4 mg/L, FOS EC_{50} values were 9.90, 8.75 & 9.94 mg/L, whereas E_{max} values were -5.05, -5.06 & -5.05, respectively. Against the COL^R strain, synergy was evident in only one combination (COL 4 mg/L + FOS 512 mg/L at 48 h); combination time-kill data were fit linearly with slopes of -0.0036 ,-0.0035 & -0.0085 for FOS in the presence of COL 0.25, 1 & 4 mg/L, respectively.**Conclusions:** All FOS and COL combinations showed synergistic killing and complete eradication of COLS KPC-Kp; against COLR KPC-Kp, this was evident only at high FOS and COL concentrations. These data highlight the potential utility of FOS and COL combinations against COLS KPC-Kp in the clinic.

Author Disclosure Block:

M. Zhao: None. **Z.P. Bulman:** None. **J.R. Lenhard:** None. **M.J. Satlin:** None. **B.N. Kreiswirth:** None. **T.J. Walsh:** None. **V. Petraitis:** None. **P.N. Holden:** None. **P.B. Bergen:** None. **R.L. Nation:** None. **J. Li:** None. **J. Zhang:** None. **B.T. Tsuji:** None.

Poster Board Number:

FRIDAY-450

Publishing Title:

Synergistic Activity of Isavuconazole (Isa) and Micafungin (Mfg): Dosage Optimization in Treatment of Experimental Invasive Pulmonary Aspergillosis (Ipa)

Author Block:

R. Petraitiene[1], V. Petraitis[1], B. W. Maung[1], N. Shaikh[1], N. K. Sekhon[1], W. W. Hope[2], T. J. Walsh[1]; [1]Weill Cornell Med. of Cornell Univ, New York, NY, [2]Univ. of Liverpool, Liverpool, United Kingdom

Abstract Body:

Background: Despite the advances in antifungal therapy with single agents, mortality associated with IPA remains high. We hypothesize that simultaneous inhibition of biosynthesis of fungal cell membrane and cell wall by an antifungal triazole and echinocandin combination may result in synergistic interaction *in vivo* with more rapid elimination of organisms and prevention of organism-mediated pulmonary injury. We therefore studied ISA and MFG in combination therapy of experimental IPA in persistently neutropenic rabbits. **Methods:** Treatment (up to 12 days) started 24 h after endotracheal inoculation of $1x10^8$ *A. fumigatus* conidia. Treatment groups consisted of rabbits receiving orally active moiety of ISA (BAL4815, ISA) at 20 (ISA20), 40 (ISA40), and 60 (ISA60) mg/kg/day, MFG at 2 mg/kg/day IV (MFG2), combinations of (ISA20+MFG2), (ISA40+MFG2), (ISA60+MFG2), and untreated controls (UC). Blood samples for serum galactomannan (GM) antigen and (1,3)-β-D-glucan (BG) levels were obtained QOD.**Results:** GM index (GMI) increased on day 1 postinoculation in all groups. While GMI continued to increase in ISA20, ISA40, and MFG2 treated rabbits, GMI was lower in ISA20+MFG2 ($p \leq 0.05$) and ISA40+MFG2 ($p \leq 0.01$) treated in comparison to monotherapies. There also were lower serum BG levels and prolonged survival in ISA20+MFG2 ($p \leq 0.05$) and ISA40+MFG2 ($p \leq 0.01$) vs. monotherapies. These results correlated with a significantly lower organism-mediated pulmonary injury (measured by lung weights and pulmonary infarct scores) in ISA20+MFG2 ($p \leq 0.05$), ISA40+MFG2 treated ($p \leq 0.01$) animals in comparison to ISA20, ISA40, and MFG2 treated alone. Residual fungal burden (CFU/g) in ISA20, ISA40, ISA60, ISA20+MFG2, ISA40+MFG2, and ISA60+MFG2 treated rabbits also was reduced vs that of MFG2 treated or UC ($p < 0.01$).**Conclusion:** Rabbits treated with ISA20+MFG2 and ISA40+MFG2 demonstrated synergistic interaction resulting in significantly lower serum GMI, serum BG levels, and mortality in comparison of that of monotherapy. Synergistic interaction of combinations of ISA20+MFG2 and ISA40+MFG2 prevented organism-mediated pulmonary injury, resulting in significantly lower lung weights and pulmonary infarct scores.

Author Disclosure Block:

R. Petraitiene: None. **V. Petraitis:** None. **B.W. Maung:** None. **N. Shaikh:** None. **N.K. Sekhon:** None. **W.W. Hope:** E. Grant Investigator; Self; Astellas Pharma, Pfizer, F2G, Pulmocide, Basilea, Gilead. **T.J. Walsh:** C. Consultant; Self; Astellas, ContraFect, Cubist, Drais, iCo, Novartis, Pfizer, Methylgene, SigmaTau, and Trius. E. Grant Investigator; Self; Save Our Sick Kids Foundation,Astellas, ContraFect, Merk, Pfizer, Cubist, Medicines Company, Actavis, Theravancin.

Poster Board Number:

FRIDAY-451

Publishing Title:

Efficacy of Telavancin (Tlv) and Daptomycin (Dap) in an Experimental Endocarditis (Ie) Model Due to Dap-resistant Methicillin-resistant *Staphylococcus aureus* (Mrsa)

Author Block:

W. Abdelhady, A. Bayer, R. Gonzales, L. Li, **Y. Q. Xiong**; LABiomed at Harbor-UCLA Med. Ctr., Torrance, CA

Abstract Body:

Background: TLV is a semi-synthetic bactericidal lipoglycopeptide active against many pathogenic Gram-Positive bacteria, including MRSA. The bactericidal activity is produced by a dual mechanism of action: inhibition of bacterial cell wall synthesis; and disruption of bacterial membranes by depolarization. The drug was FDA-approved in 2009 for complicated skin and skin structure infections, and in 2013 for hospital-acquired and ventilator-associated pneumonia caused by *S. aureus*. The current studies compared the efficacy of TLV and DAP in an experimental IE model using DAP-resistant (DAP-R) MRSA strains.**Methods:** A well-characterized catheter-induced rabbit model of aortic IE was used to compare efficacy of TLV against two clinically-derived DAP-R MRSA strains from patients who failed DAP therapy ($B_{2.0}$ and SA684). The MICs of TLV and DAP were 0.12 μg/ml and 4.0 μg/ml for the both strains, respectively. At 24 hr post-induction of IE, animals were randomized to receive: i) no therapy; ii) TLV 30 mg/kg iv bid which simulates PK/PD of the recommended clinical dose (10 mg/kg iv once daily); or iii) DAP 12 or 18 mg/kg iv once daily which mimics human PK of DAP regimens at standard (6 mg/kg iv once daily) or high (10 mg/kg iv once daily) doses. Treatments were administered 3 days. Control and antibiotic-treated animals were sacrificed at 24 hr post-infection or 24 hr after the last antibiotic dose, respectively. At sacrifice, cardiac vegetations, kidney and spleen were quantitatively cultured. The mean $\log_{10}$ CFU/g of tissue (±SD) was calculated and statistical analyzed via Student t test.**Results:** Only TLV significantly reduced MRSA densities in all three target tissues in the IE model compared to those in the untreated controls and DAP-treated groups (P<0.000001). Importantly, TLV-treated rabbits had 71-100% culture-negative target tissues, while DAP did not sterilize any target tissues. In addition, there was no mortality observed in the TLV and DAP high dose groups as compared to 29% mortality observed in the standard DAP treatment group (12 mg/kg).**Conclusions:** TLV has potent *in vivo* efficacy against DAP-R MRSA isolates in this rigorous IE model, and should be evaluated further in endovascular infections in humans.

Author Disclosure Block:

W. Abdelhady: None. **A. Bayer:** E. Grant Investigator; Spouse/Life Partner; Self. **R. Gonzales:** None. **L. Li:** None. **Y.Q. Xiong:** E. Grant Investigator; Spouse/Life Partner; Self.

Poster Board Number:

FRIDAY-452

Publishing Title:

In-Vitro* Characterization of Amikacin and Polymyxin B Therapy in Combination with Meropenem for Carbapenem-resistant *Enterobacter cloacae

Author Block:

W. C. Rutter, D. S. Burgess; Univ. of Kentucky, Lexington, KY

Abstract Body:

Background: Carbapenem-resistant (CR) *Enterobacter cloacae* (EC) typically contain multiple resistance mechanisms for many alternative agents. Polymyxin B (PB) remains reliably effective against these organisms, but resistance develops rapidly with monotherapy, indicating that combination therapy is needed. It is unclear which combination regimen is most effective.**Methods:** Four CR EC isolates were chosen based on meropenem (MEM) MIC. Inocula of 10^6 CFU/mL were exposed to amikacin (AMK) 8 and 16 µg/mL, PB 0.25 and 1 µg/mL, and MEM 4 and 16 µg/mL alone and in combination of AMK or PB with MEM. Samples were obtained at 0, 1, 2, 4, 8, 24, and 48 hours, plated via an automated spiral plater, and subsequently read by colony counting software.**Results:** AMK and PB monotherapy was characterized by initial bactericidal activity and rapid regrowth at 24h in all strains. MEM 4 was not bactericidal at any point and all strains regrew. MEM 16 was bactericidal for isolate 19 at 4h and 24h regrowth was limited to isolates 10 and 17. The combination of MEM 16 and PB 0.25 was bactericidal at 2h for all isolates with no regrowth after 48h of incubation. MEM 4 and PB 0.25 or 1 were rapidly bactericidal but significant regrowth occurred after 24h in isolates 10 and 17. MEM/AMK combinations were less rapidly bactericidal compared to the MEM/PB combinations; however, MEM/AMK maintained bacterial levels below the lower limits of detection after 24h in all isolates.**Conclusions:** Combination therapy with MEM and AMK at lower concentrations were able to prevent regrowth of resistant subpopulations at 48h in AMK susceptible CR EC strains. In contrast, the lower concentration MEM/PB combinations were unable to prevent regrowth at 48h in 50% of strains despite achieving bactericidal activity quicker. AMK based combinations may be a valid treatment option in CR EC isolates.

Properties of selected organisms

Isolate	Resistance mechanism	MEM MIC (µg/mL)	PB MIC (µg/mL)	AMK MIC (µg/mL)
10	KPC2, VIM1	32	0.06	1
17	KPC2	8	0.125	16
19	KPC2	2	0.06	2
40	VIM1	16	0.125	4

Author Disclosure Block:

W.C. Rutter: None. **D.S. Burgess:** None.

Poster Board Number:

FRIDAY-453

Publishing Title:

Evaluation of Synergistic Activities & Efficacy of Combination Drug Therapy for Antibiotic-Resistant Inhalation Anthrax in a Murine Model

Author Block:

H. S. HEINE[1], S. Shadomy[2], A. Boyer[2], L. Chuvala[1], R. Riggins[1], J. Myrick[1], J. Craig[1], G. Drusano[1], M. Candela[2], J. Barr[2], K. Hendricks[2], W. Bower[2], H. Walke[2]; [1]Univ. of Florida, Orlando, FL, [2]CDC, Atlanta, GA

Abstract Body:

Background: Efficacy was tested using the murine model of inhalational anthrax to evaluate the therapeutic benefit and synergistic activity of various antimicrobial (AM) agent combinations for the treatment of inhalation anthrax resulting from a *Bacillus anthracis* (BA) strain resistant to the first-line AM treatment agent ciprofloxacin (C).**Methods:** Aerosolized spores from a C-resistant derivative of the Ames strain of BA were delivered to 10 groups of 46 female BALB/c mice. At 36h postexposure (PE), intraperitoneal therapy was initiated with combinations of two AM plus C. AMs combined with C included: linezolid (L), rifampin (R), meropenem (M), doxycycline (D), clindamycin (Cl) and penicillin (P). Controls received saline or C alone. Ten mice per group were treated for 14 days and observed until day 28 PE. The remainder were serially euthanized by threes at 6-12h intervals; from these, heparinized blood was collected, and lungs and spleens were removed, weighed, homogenized, and plated to determine bacterial load. 3x MIC AM-containing plates were also included to determine emergence of resistance. Plasma lethal factor (LF) levels were determined by MALDI-TOF mass spectrometry.**Results:** All AM combinations tested were significantly different from the saline control ($p < 0.0004$), and also from the C control, M/L ($p=0.004$), M/Cl ($p=0.005$), M/R ($p=0.012$), M/D ($p=0.032$), P/L ($p=0.026$), P/D ($p=0.012$), R/L ($p=0.001$), and R/Cl ($p=0.032$). There were no significant differences in survival between any of the AM combination groups. By 66h PE, plasma LF fell to low levels or below the limit of detection (LOD) for all AM combination groups with AM combinations containing L showing greatest reductions. No additional AM resistance was detected.**Conclusions:** Multi-drug therapy including a protein synthesis inhibitor can overcome a single first-line AM-resistant infection and also decrease circulating toxin levels. The data suggest that combinations with L had the greatest inhibitory effect on LF, and that combinations including L, Cl, or D, in addition to M or P, reduced LF to below LOD by 36h after initiation of therapy, validating their key role in treatment recommendations.

Author Disclosure Block:

H.S. Heine: None. **S. Shadomy:** None. **A. Boyer:** None. **L. Chuvala:** None. **R. Riggins:** None. **J. Myrick:** None. **J. Craig:** None. **G. Drusano:** None. **M. Candela:** None. **J. Barr:** None. **K. Hendricks:** None. **W. Bower:** None. **H. Walke:** None.

Poster Board Number:

FRIDAY-454

Publishing Title:

Maltodextrin Enhances the Effectiveness of Electrochemical Saffold Generated H_2o_2 Against Biofilms

Author Block:

S. T. Sultana, D. R. Call, H. Beyenal; Washington State Univ., PULLMAN, WA

Abstract Body:

Electrochemical scaffolds (e-scaffolds) reduce oxygen to low but constant concentrations of H_2O_2 that damage existing biofilms without damaging host tissue. Nevertheless, decomposition of H_2O_2 combined with retarded diffusion into mature and thicker biofilms can limit the efficacy of this potential clinical tool. Exposure to a hyperosmotic agent has the potential to mitigate this limitation by improving H_2O_2 diffusion into biofilms and by activating H_2O_2 membrane transportation channels in bacterial cells. Consequently, we hypothesized that e-scaffolds would be more effective against *Acinetobacter baumannii, Pseudomonas aeruginosa* and *Staphylococcus aureus* biofilms in the presence of maltodextrin, a hyperosmotic agent. Carbon fabric E-scaffolds were overlaid onto existing biofilms in medium containing different concentrations of maltodextrin. The e-scaffold alone decreased viable cell density of *A. baumannii, P. aeruginosa* and *S. aureus* biofilms by log (3.92 ± 0.15) (n=3, $P<0.001$), log (4.80 ± 0.22) (n=3, $P<0.001$) and log (2.31 ± 0.12) (n=3, $P<0.001$), respectively. Compared to treatment with e-scaffold alone, combining 10 mM maltodextrin with e-scaffold treatment achieved a further reduction in viable cells from *A. baumannii* biofilms log (4.35 ± 0.16) (n=3, $P<0.001$) and *P. aeruginosa* biofilms by log (1.0 ± 0.38) (n=3, $P<0.001$). A further reduction in *S. aureus* biofilm cell density by log (2.40 ± 0.17) (n=3, $P<0.001$) was observed in presence of 30 mM maltodextrin. Thus, compared to untreated biofilms, a combination of e-scaffold and maltodextrin achieved an overall reduction of log (8.20 ± 0.05) (n=3, $P<0.001$), log (5.7 ± 0.38) (n=3, $P<0.001$) and log (4.67 ± 0.12) (n=3, $P<0.001$) in *A. baumannii*, *P. aeruginosa* and *S. aureus* biofilms, respectively. The study demonstrates a simple means to enhance the effectiveness of e-scaffold treatment of biofilm infections in wounds.

Author Disclosure Block:

S.T. Sultana: None. **D.R. Call:** None. **H. Beyenal:** None.

Poster Board Number:

FRIDAY-455

Publishing Title:

***In Vitro* Antibacterial Effects and Time-kill Assessment of Amoxicillin Combined with Nalidixic Acid Against Bacteria of Clinical Importance**

Author Block:

O. O. Olajuyigbe[1], R. M. Coopoosamy[2]; [1]Bsbcock Univ., Ilisan Remo, Ogun State, Nigeria, [2]Mangosuthu Univ. of Technology, Durban, South Africa

Abstract Body:

Background: Sequel to the emergence of multidrug-resistant bacteria and lack of funds for development of novel anti-infective agents in third world countries, combining the existing antibiotics may be an alternative to combat the menace of bacteria. This study evaluates the *in vitro* effects of combining amoxicillin with nalidixic acid against bacterial isolates by agar diffusion, checkerboard and time-kill assays.**Methods:** Inhibition zones and MICs of antibiotics and their combinations were determined by agar diffusion and macrobroth dilution assays while checkerboard assay determined types of interactions between them.**Results:** Zones of inhibition ranged between 19.00 and 37.00 ± 0.00 mm for amoxicillin, 6.00 - 29.0 ± 0.58 mm for nalidixic acid and 21.0 - 39.33 ± 0.58 mm for the combined antibiotics at their highest concentrations. The MICs for amoxicillin alone ranged between 0.06 and 125.0 µg/ml. Those of nalidixic acid alone ranged between 1.953 and 62.5 µg/ml. The fractional inhibitory concentrations (FICs) of amoxicillin were between 0.0078 and 16.3, nalidixic acid alone between 0.0157 and 0.502 and those of the combinations ranged between 0.0703 and 16.316. Except for antagonistic interaction with FICIs ranging between 4.031 and 16.316 against *Pr. vulgaris* CSIR 0030 and *M. luteus* and indifference interaction with FICI equal to 0.517 against *Pr. vulgaris* ATCC 6830, the combination of these antibiotics resulted in synergistic interactions with FICIs ranging between 0.0468 and 0.345 against the *A. calcaoceuticus anitratus* CSIR, *B. cereus* ATCC 10702, *S. faecalis* ATCC 29212, *S. aureus* ATCC 6538, *S. aureus* OK_{2a}, *S. flexneri* KZN and *E. coli* ATCC 8739. The time kill assay showed that both ½ x MIC and MIC of the combined antibiotics eliminated the bacterial isolates from the cultures within 24 h. The post antibiotic effects after 48 h showed that all the broth cultures were sterile.**Conclusions:** Synergistic interactions between amoxicillin and nalidixic acid, rather than individual antibiotic usage, could result to improved therapy of bacterial infections in clinical settings.

Author Disclosure Block:

O.O. Olajuyigbe: None. **R.M. Coopoosamy:** None.

Poster Board Number:

FRIDAY-456

Publishing Title:

Syn-004, an Oral β-Lactamase to Prevent *Clostridium difficile*, Degrades Ceftriaxone Excreted in the Human Intestine in Phase 2a Trials

Author Block:

J. F. Kokai-Kun[1], T. Roberts[1], E. Sicard[2], M. Rufiange[2], R. Fedorak[3], C. Carter[3], O. Coughlin[1], H. Whalen[1], K. Gottlieb[1], J. Sliman[1]; [1]Synthetic Biologics, Inc., Rockville, MD, [2]Algorithme Pharma, Laval, QC, Canada, [3]Univ. of Alberta/Alberta Hlth.Service, Edmonton, AB, Canada

Abstract Body:

SYN-004 is a novel, orally-administered recombinant β-lactamase given during treatment with IV β-lactam antibiotics (ABX) for prevention of the adverse effects of the ABX in the intestine. SYN-004 is released in the small intestine when the pH > 5.5 to degrade β-lactam ABX excreted into the intestine via the bile. This is expected to protect the gut microbiome from disruption by residual ABX, preventing opportunistic infections like *C. difficile*. For SYN-004 to be effective, it must be capable of degrading β-lactam ABX in the intestine, without affecting systemic ABX concentrations. Phase 1 clinical trials of oral SYN-004 have demonstrated a good tolerability with no systemic absorption in healthy volunteers. Two phase 2a studies have now been conducted in subjects with ileostomies to allow sampling of intestinal chyme which provides a unique *in vivo* approach to support the mechanism of action of SYN-004. In the first study, subjects were administered 1 g of IV ceftriaxone alone or in combination with two dose strengths of oral SYN-004. Serial plasma and intestinal chyme samples were then analyzed for their concentrations of ceftriaxone and SYN-004. In the second study, ceftriaxone plus SYN-004 was administered in the presence or absence of a proton pump inhibitor to determine the effect of pH change on SYN-004 function. These studies demonstrate that, 1) SYN-004 is well tolerated when given with IV ceftriaxone, 2) SYN-004 effectively catabolizes excreted ceftriaxone in the small intestine reducing the concentration to below detection when both substances are present, 3) the plasma concentration of ceftriaxone is unchanged upon oral SYN-004 administration, 4) SYN-004 is not detected in the plasma and 5) the use of proton pump inhibitors does not reduce SYN-004 efficacy. These two studies provide evidence of SYN-004's ability to degrade certain β-lactam ABX in the human intestine and support progression into a currently enrolling phase 2b clinical trial to examine its capacity to prevent *C. difficile*-associated disease and antibiotic-associated diarrhea in patients being treated with IV ceftriaxone for lower respiratory tract infection.

Author Disclosure Block:

J.F. Kokai-Kun: D. Employee; Self; Synthetic Biologics, Inc. **T. Roberts:** D. Employee; Self; Synthetic Biologics. **E. Sicard:** F. Investigator; Self; Synthetic Biologics, Inc. **M. Rufiange:** F.

Investigator; Self; Synthetic Biologics, Inc. **R. Fedorak:** F. Investigator; Self; Synthetic Biologics, Inc. **C. Carter:** F. Investigator; Self; Synthetic Biologcs, Inc. **O. Coughlin:** D. Employee; Self; Synthetic Biologics, Inc. **H. Whalen:** D. Employee; Self; Synthetic Biologics, Inc. **K. Gottlieb:** D. Employee; Self; Synthetic Biologics, Inc. **J. Sliman:** D. Employee; Self; Synthetic Biologics, Inc..

Poster Board Number:

FRIDAY-457

Publishing Title:

***In Vitro* Antimicrobial Activity of Surma,Kajal, Antibiotics Alone and Synergistic Combination of Cefazolin Sodium andOfloxacin against Opportunistic Biofilm Formers on Contact Lens**

Author Block:

J. Zaheer, Z. Zahid, **N. H. Ali**; Dow Univ. of Hlth.Sci., Karachi, Pakistan

Abstract Body:

Background: In contact lens wearer rate of bacterial keratitis due to biofilm forming species of opportunistic *Pseudomonas*, *Serratia* and *Staphylococcus* has increased. In many cases antibiotic therapy fails if single antibiotic prescribed. This study relates biofilm formers present on hand may adhere to lens during its handling, routine cleansing of lens with its solution partially removes bacterial load, results in formation of biofilm on lens. These microbes then gets attached to surface that interacts with contaminated lens. Besides antimicrobial susceptibility profile of eye cosmetics Kajal, Surma and antibiotic evaluated. Combination of Cefazolin Sodium and Ofloxacin tested for its synergistic activity for isolated bacteria. So that new regime of antibiotics be developed. **Methods:** Biofilm forming species (30 each) of *P.aeruginosa*, *S.marcescans* and *S.aureus* isolated from hands. Assessment of Biofilm formation is done by Crystal violet binding assay. Sterile lens immersed for 3hrs in nutrient broth culture (turbidity 0.5 McFarland) of individual species, washed with lens solution stored overnight in case of (SILCO multi-acting disinfectant) sterile lens solution, incubated in broth for 24 hrs. Bacteria recovered identified by micro broth dilution and checkerboard titration, MIC and FICI evaluated.**Results:** Bacterial species in which lens immersed were re-isolated from solution. MIC for organisms isolated from Surma and Kajal 16 – 125 µg/ml. MIC Alone cefazolin and ofloxain 125-500, 64-500 µg/ml. Fractional Inhibitory Index (FICI) of combination is 0.03- 0.4 for 80%. No antagonism observed. Synergy is defined as FICI $\leq$ 0.5. MIC Cefoxitin 250-500µg/ml, Ceftadizime 125-500 µg/ml, Vancomycin 31.25-500 µg/ml, Erythromycin 31.25-500 µg/ml.**Conclusion:** Presence of identified microbes in lens solution reflects cornea of eye wearing contact lens may get infected with microbes present on it. After antibiotic therapy vision loss, a complication of keratitis frequently reported. Antibiotic prescribed for treatment of keratitis has high MIC value against bacteria studied. Kajal and Surma, synergistic combination of cefazolin and ofloxacin are potential candidates to develop antibiotics that can combat against biofilm formers and drug resistant bacteria.

Author Disclosure Block:

J. Zaheer: None. **Z. Zahid:** None. **N.H. Ali:** None.

Poster Board Number:

FRIDAY-458

Publishing Title:

Depletion of Neutrophils Attenuates *Paracoccidioides brasiliensis* Induced Pulmonary Fibrosis in Mice Might Be Meditated by Decreasing of TGF-β1, TNF-α, Il-17, MMP-8 and TIMP-2

Author Block:

J. Puerta[1], P. Pino[1], J. Arango[1], A. Gonzalez[2]; [1]CIB-UdeA-UPB, medellin, Colombia, [2]Univ. de Antioquia, medellin, Colombia

Abstract Body:

Introduction: Chronic stages of paracoccidioidomycosis are characterized by granulomatous lesions, which promote the development of pulmonary fibrosis with loss of respiratory function in 50% of the patients. Recently, we demonstrated that depletion of neutrophils is associated with a better control of infection and a decrease of both the inflammatory response and the fibrotic process; however the mechanism, in which neutrophils participate, is not completely understood. In the present study, we evaluated some of the mechanisms that could be involved in the development of fibrosis in *P. brasiliensis* infected and neutrophils-depleted mice during the chronic stages of infection.**Methodology:** Isogenic BALB/c male mice were inoculated i.n. with $1.5x10^6$ *P. brasiliensis* yeasts. Groups of mice were untreated or treated with a monoclonal antibody (mAb) specific to neutrophils or an isotype control. The treatment was administered at week 4 post-infection (PI) followed by additional doses every 48h for two weeks. The animals were sacrificed at week 12 PI in order to determine levels of cytokines and collagen, expression of TGF, matrix metalloproteinases (MMP) and tissue inhibitor metalloproteinases (TIMP), besides histopathological aspects.**Results:** We observed that depletion of neutrophils during the chronic stages of *P. brasiliensis* infection was associated with a significant decrease of TGFb1 expression, TNF and IL17 levels, the latter being linked to additional results suggesting that neutrophil was one of the most important sources of IL17 in this fungal infection. Furthermore, recovery of lung architecture was also associated with reduced levels of collagen and decreased expression of MMP8 and TIMP2.**Conclusions:** Depletion of neutrophils might attenuate lung fibrosis and inflammation through down-regulating TGFb1, TNF, IL17, MMP8 and TIMP2. These data suggest that neutrophil could be considered as a therapeutic target in pulmonary fibrosis induced by *P. brasiliensis*.Study supported by Colciencias (project No.183-2010).

Author Disclosure Block:

J. Puerta: None. **P. Pino:** None. **J. Arango:** None. **A. Gonzalez:** None.

Poster Board Number:

FRIDAY-459

Publishing Title:

Monoclonal Antibodies Targeting *staphylococcus Aureus* Capsular Polysaccharides Elicit Protection In A Murine Bacteremia Model

Author Block:

B. Liu, S. Park, X. Li, C. Thompson, J. C. Lee; Brigham and Women's Hosp. and Harvard Med. Sch., Boston, MA

Abstract Body:

The capsular polysaccharide (CP) produced by *Staphylococcus aureus* is a virulence factor that allows the organism to evade uptake and killing by host neutrophils. Passive immunotherapy using CP antibodies could be a novel therapeutic approach to prevent and/or treat invasive antibiotic-resistant *S. aureus* infections. We showed previously that polyclonal antibodies to the serotype 5 (CP5) and type 8 (CP8) capsular polysaccharides are opsonic and protect mice against experimental bacteremia provoked by highly encapsulated staphylococci. In this study, we generated monoclonal antibodies (mAbs) against *S. aureus* CP5 or CP8. Backbone specific mAbs reacted with native and O-deacetylated CPs, whereas O-acetyl specific mAbs reacted only with native CPs. Reference strains of *S. aureus* and a selection of clinical isolates reacted by colony immunoblot with the CP5 and CP8 mAbs in a serotype-specific manner. The mAbs mediated CP type-specific opsonophagocytic killing of *S. aureus* strains, but the CP5 O-acetyl specific mAb was not opsonic for a *S. aureus* mutant that expresses O-deacetylated CP5. Mice passively immunized with mAbs specific for CP5 were protected against infection in a mouse model of *S. aureus* bacteremia. CP8 mAbs were protective against *S. aureus* Reynolds (CP8)-induced bacteremia, but not against bacteremia provoked by clinical isolates that readily released CP8 into culture supernatants. mAbs to *S. aureus* CPs, in combination with mAbs targeting other virulence factors, may be a promising therapeutic candidate for prophylaxis against or therapeutic treatment of staphylococcal infections.

Author Disclosure Block:

B. Liu: None. **S. Park:** None. **X. Li:** None. **C. Thompson:** None. **J.C. Lee:** C. Consultant; Self; Sanofi Pasteur and Crucell.

Poster Board Number:

FRIDAY-460

Publishing Title:

An Ebpa Antibody-Based Therapeutic Against A Broad Range Of Enterococcal Catheter Associated Urinary Tract Infections

Author Block:

A. L. Flores-Mireles, M. G. Caparon, S. J. Hultgren; Washington Univ. Sch. of Med., St. Louis, MO

Abstract Body:

Urinary catheterization is a very common intervention in hospitalized patients, estimating that 20%-50% of the hospitalized patients are catheterized. The duration of a catheter increases the risk of developing a catheter-associated urinary tract infection (CAUTI) 3%-10% per day and can lead to serious complications such as bloodstream infections and death. *Enterococcus faecalis* is a leading cause of CAUTI and it is becoming increasingly resistant to multiple antibiotics, including last line of defense antibiotics such as the vancomycin, leaving few or no treatment options. Biofilm formation on the catheter is critical for CAUTI pathogenesis as it enables *E. faecalis* persistence in the bladder and dissemination to the kidneys. **In this study, we identified and targeted a critical step in biofilm formation to develop a therapy that prevents and resolves enterococcal CAUTI.** We found that host fibrinogen (Fg) is released and deposited on catheters following their introduction into the bladders of mice and/or humans. *E. faecalis* expresses a Fg-binding adhesin, EbpA, that tips the endocarditis and biofilm-associated (Ebp) pilus, which is critical for the formation of catheter-associated biofilms in a CAUTI murine model. The Fg-binding activity resides in the N-terminal domain of EbpA ($EbpA^{NTD}$) to promote catheter colonization and biofilm formation. We found that *E. faecalis* requires Fg for catheter colonization in humans and that vaccination with the EbpA and $EbpA^{NTD}$, but not its C-terminal domain or other Ebp subunits, inhibited biofilm formation *in vivo* and significantly protected against CAUTI in a murine model. Moreover, passive transfer of $EbpA^{NTD}$ antibodies prevented CAUTI and reduced bacterial titers of an existing *E. faecalis* infection. This therapy was also tested against a broad range of clinical strains, which included *E. faecalis, E. faecium*, VRE, and urinary tract, blood, and gut isolates. We found that the bacterial titers were reduced when mice were treated with anti-$EbpA^{NTD}$ antibodies. This suggests that this approach could be use against infections caused by abroad range of enterococcal and may provide a new strategy for the prevention of CAUTI and for other enterococcal infections where Fg is present such as infective endocarditis, bacteremia, and intra-abdominal and surgical-site infections.

Author Disclosure Block:

A.L. Flores-Mireles: None. **M.G. Caparon:** None. **S.J. Hultgren:** A. Board Member; Self; Fimbrion Therapeutics.

Poster Board Number:

FRIDAY-461

Publishing Title:

Efficacy of Therapeutic Monoclonal Antibodies against Multi-Drug Resistant *Escherichia coli* St131

Author Block:

V. Szijarto, L. Guachalla, K. Hartl, Z. Visram, E. Nagy, **G. Nagy**; Arsanis BioSci.s, Vienna, Austria

Abstract Body:

Background: The globally spread multi-drug resistant *E. coli* clone ST131-O25b:H4 is responsible for a significant proportion of resistant extra-intestinal infections. By definition, isolates of this clone express the O25b antigen, whose unique structure was resolved recently. We aimed to develop humanized monoclonal mAbs against the O25b antigen that may provide an alternative adjunct or stand-alone therapeutic option against this *E. coli* lineage. **Methods**: Murine mAbs against the O25b carbohydrate epitopes were generated by standard hybridoma technique following repeated immunizations with live bacteria. Selected murine mAbs were subjected to humanization. Specificity and binding characteristics were assessed by immunoblots and biolayer interferometry using purified antigens. Surface staining of *E. coli* was measured by flow cytometry. Protective efficacy of passive immunization with the mAbs was investigated in murine and rat models of bacteremia and endotoxemia. Mode of action for protection was assessed in serum and whole blood bactericidal tests as well as in an endotoxin neutralization assay. Experiments were performed with bacteria grown in standard culture medium as well as in heat-inactivated pooled human serum in order to mimic *in vivo*-like conditions. **Results:** Specific humanized mAbs with high affinity to the O25b antigen were discovered. These antibodies showed strong binding to ST131-O25b strains irrespective of the capsular type. Upon binding to the bacterial surface, mAbs triggered a complement mediated bactericidal activity in the presence of human serum. An additional opsonophagocytotic activity was confirmed in the whole blood assay. Prophylactic immunization with the mAbs elicited significant protection against lethal systemic infection in mice and rats. Surprisingly, the mAbs efficiently neutralized endotoxic activity of purified O25b LPS or bacterial lysates. Protection in a murine endotoxemia model was also confirmed. **Conclusions:** Humanized mAbs against the *E. coli* O25b antigen exhibited protection in various animal models via different modes of action. Prophylactic passive immunization of colonized individuals or adjunct therapy of infected patients by mAbs may replace/complement antibiotic therapy against this drug resistant clone.

Author Disclosure Block:

V. Szijarto: D. Employee; Self; Arsanis GmbH. K. Shareholder (excluding diversitied mutual funds); Self; Arsanis GmbH. **L. Guachalla:** D. Employee; Self; Arsanis GmbH. K. Shareholder

(excluding diversitied mutual funds); Self; Arsanis GmbH. **K. Hartl:** D. Employee; Self; Arsanis GmbH. K. Shareholder (excluding diversitied mutual funds); Self; Arsanis GmbH. **Z. Visram:** D. Employee; Self; Arsanis GmbH. K. Shareholder (excluding diversitied mutual funds); Self; Arsanis GmbH. **E. Nagy:** A. Board Member; Self; Arsanis GmbH. D. Employee; Self; Arsanis GmbH. K. Shareholder (excluding diversitied mutual funds); Self; Arsanis GmbH. **G. Nagy:** D. Employee; Self; Arsanis GmbH. K. Shareholder (excluding diversitied mutual funds); Self; Arsanis GmbH.

Poster Board Number:

FRIDAY-462

Publishing Title:

Enhanced Activity of Medi3902 with Adjunctive Antibiotic Treatment Against Resistant *P. aeruginosa* is Driven by Anti-Psl Activity

Author Block:

J. Quetz, A. Keller, C. K. Stover, A. DiGiandomenico; MedImmune, Gaithersburg, MD

Abstract Body:

Background: *P. aeruginosa* is a leading cause of morbidity and mortality in hospital infections. Because of its ability to develop resistance to antibiotics, there is a need for novel strategies to combat these infections. Recently described anti-Pseudomonal monoclonal antibodies (Mabs) that target the exopolysaccharide (EPS) Psl, the type 3 secretion (T3S) protein PcrV, and a bispecific construct that targets both Psl and PcrV (clinical candidate, MEDI3902), have shown promising preclinical activity. MEDI3902 mediates enhanced activity against resistant *P. aeruginosa* when combined with tobramycin (TOB). We sought to define the underlying mechanism(s) behind this enhanced activity with TOB. **Methods:** Anti-Psl/Anti-PcrV mAbs and TOB were evaluated in an acute pneumonia (AP) model. The role of Psl EPS in mediating antibiotic resistance and in potentiating anti-Psl mAb activity was evaluated with a Psl-inducible strain. We also determined the effect of sub-inhibitory concentrations (sub-MICs) of TOB and other standard of care (SOC) antibiotics on: membrane-associated Psl accessibility (flow cytometry), *pslA* expression (qPCR), and opsonophagocytic killing (OPK) activity. **Results:** In the AP model, TOB administration with anti-Psl (p=0.0014), but not anti-PcrV (p=0.31), resulted in a significant increase in survival when compared to animals treated with MAbs or TOB alone. In addition, Psl overexpression with a Psl-inducible strain correlated with enhanced resistance to TOB and greater anti-Psl Mab mediated OPK activity. Furthermore, exposure of *P. aeruginosa* to sub-MICs of TOB increased *psl* transcript levels 3-fold, enhanced binding of anti-Psl mAbs (41.3% *vs.* 27.9%; p=0.006), and increased anti-Psl OPK activity (61% *vs.* 42%; p=0.014) compared to non-TOB-treated controls. Similar results were observed with other SOC antibiotics. **Conclusion:** Our results suggest that the enhanced activity of MEDI3902 administered in combination with TOB against TOB-resistant *P. aeruginosa* is driven by the anti-Psl arm of the bispecific molecule. In addition, we provide evidence that sub-MICs of antibiotics enhance *in vitro* Psl expression and anti-Psl mAb mediated activity. Taken together these data demonstrate the potential of targeting Psl to mitigate clinical *P. aeruginosa* infections where chemotherapeutic pressure is a reality.

Author Disclosure Block:

J. Quetz: None. **A. Keller:** None. **C.K. Stover:** None. **A. DiGiandomenico:** None.

Poster Board Number:

FRIDAY-463

Publishing Title:

Monoclonal Antibodies Targeting the Conserved Lps O-Antigen of *Klebsiella pneumoniae* St258 Elicit Protection

Author Block:

V. Szijarto, L. Guachalla, K. Hartl, Z. Visram, E. Nagy, **G. Nagy**; Arsanis BioSci.s, Vienna, Austria

Abstract Body:

Background: ST258 is an epidemic carbapenem resistant *K. pneumoniae* clone. Within ST258 two different clades are distinguished that express at least 2 different capsular types. In contrast, we showed recently, that the LPS O-antigen (D-galactan III) is conserved within the lineage. Binding, function, and protective capacity of mAbs against this carbohydrate antigen were investigated in the present study.**Methods**: Selected D-galactan-III specific murine mAbs generated by hybridoma technology were humanized by CDR grafting. Surface staining of representative strains from both ST258 clades was measured by flow cytometry. Complement mediated killing was investigated with or without specific mAbs in a serum bactericidal assay. Neutralization of endotoxin was assessed in a cell based TLR4 reporter assay. Protective efficacy of passive immunization with the mAbs was tested in murine and rabbit models of systemic infection.**Results:** Humanized mAbs with high specificity and affinity to D-galactan-III antigen were discovered. Antibodies showed strong binding to both clades of ST258 strains irrespective of the capsular polysaccharide expressed. Surprisingly, most ST258 isolates showed high susceptibility to normal human serum in vitro, and rapid lysis in mouse blood in vivo. Still, in extensively depleted serum samples that allowed bacterial survival, antibody dependent complement mediated killing could have been shown. Furthermore, mAbs elicited in vitro endotoxin neutralization activity of purified LPS or bacterial lysates. The efficacy of neutralization exceeded that of polymixin B by several magnitudes. Importantly, high level of protection in a murine model of endotoxemia model was observed. Protection in the rabbit model of bacteremia, i.e. in a host naturally susceptible to endotoxin was confirmed.**Conclusions:** Monoclonal antibodies to D-galactan-III elicit significant protection against *K. pneumoniae* ST258 isolates in animal models of systemic infection. Given the low survival of ST258 in serum the protection observed is considered to originate mainly from the excellent endotoxin neutralizing potency exhibited by these mAbs. Passive immunization with bactericidal and endotoxin-neutralizing mAbs may complement antimicrobial therapy by last resort antibiotics and reduce disease severity and mortality.

Author Disclosure Block:

V. Szijarto: D. Employee; Self; Arsanis GmbH. K. Shareholder (excluding diversitied mutual funds); Self; Arsanis GmbH. **L. Guachalla:** D. Employee; Self; Arsanis GmbH. K. Shareholder (excluding diversitied mutual funds); Self; Arsanis GmbH. **K. Hartl:** D. Employee; Self; Arsanis GmbH. K. Shareholder (excluding diversitied mutual funds); Self; Arsanis GmbH. **Z. Visram:** D. Employee; Self; Arsanis GmbH. K. Shareholder (excluding diversitied mutual funds); Self; Arsanis GmbH. **E. Nagy:** A. Board Member; Self; Arsanis Biosciences. D. Employee; Self; Arsanis GmbH. K. Shareholder (excluding diversitied mutual funds); Self; Arsanis GmbH. **G. Nagy:** D. Employee; Self; Arsanis GmbH. K. Shareholder (excluding diversitied mutual funds); Self; Arsanis GmbH.

Poster Board Number:

FRIDAY-464

Publishing Title:

The *candida* Hyr1p And Anti-Hyr1p Antibodies Protect Against Multidrug-Resistant *acinetobacter* infections

Author Block:

L. Lin, T. Gebremariam, G. Luo, S. Soliman, M. Yeaman, J. Edwards, Jr., **A. S. Ibrahim**; Los Angeles BioMed. Res. Inst. at Habor-UCLA Med. Ctr., Torrance, CA

Abstract Body:

Background: Infections due to multi-drug resistant (MDR) Gram-negative bacilli (GNB) such as *Acinetobacter baumannii* (*AB*) are now endemic in healthcare. New methods to prevent or treat these infections are needed. We used computational molecular modeling and bioinformatics to discover antigen candidates for immunotherapy targeting distinct pathogens. *Candida* cell surface Hyr1p shares 3D and sequence homology with the putative hemagglutinin/hemolysin and siderophore-interacting proteins of *AB* and other GNB, respectively. We hypothesized that active and passive immunization targeting Hyr1p can protect against *AB* and other GNB.**Methods:** Cross reactivities of rabbit anti-Hyr1p IgG with MDR *AB*, carbapenemase producing *Klebsiella pneumoniae* (*KP*), or *Pseudomonas aeruginosa* (*PA*) were studied by flow cytometry and Western blotting. Recombinant Hyr1p (30 μg; 0.1% alum) was used to vaccinate diabetic CD1 mice on D0, boosted on D21 prior to infecting them i.v. with a lethal dose of MDR *AB* on D35. Further, cyclophosphamide + cortisone acetate-treated mice were infected via inhalation with MDR *AB* and treated 24 h post infection with antiHyr1p IgG (30 μg) or isotype control IgG. Terminal morbidity served as a primary endpoint. The ability of Hyr1p IgG to kill MDR *Ab, KPC,* or *PA* was also studied *in vitro*. **Results:** AntiHyr1p IgG specifically bound *AB,* but not *PA,* and identified an *AB* siderophore-interacting protein as a major cross reacting antigen. The Hyr1p vaccine protected mice from hematogenously disseminated *AB* (60% survival in the vaccinated mice at D20 post infection vs. 0% for controls, P=0.005 by Log Rank test). Further, immunosuppressed mice with *AB* pneumonia were extensively protected by antiHyr1p IgG (90% survival at D20 post infection vs. 0% for isotype control IgG, P=0.002). Finally, Hyr1p IgG exposure killed *AB,* and *KPC,* but not *PA.* The killing effect was reversed by administration of iron.**Conclusions:** AntiHyr1p IgG cross-reacts to siderophore-binding protein and protects mice from *AB* infection, potentially by a cidal mechanism. These results affirm our convergent immunity strategy to target cross-kingdom antigens for innovative anti-infectives. Further development of immunotherapies exploiting *Candida* Hyr1p homology in MDR *AB* and other GNB is warranted.

Author Disclosure Block:

L. Lin: None. **T. Gebremariam:** None. **G. Luo:** None. **S. Soliman:** None. **M. Yeaman:** None. **J. Edwards, Jr.:** None. **A.S. Ibrahim:** None.

Poster Board Number:

FRIDAY-465

Publishing Title:

Interleukin-10 Encapsulated Within Poly (Lactic Acid)-B-Poly (Ethylene Glycol) Nanoparticles Is Functional By Down-Regulating Cytokines And Inducing Suppressor Of Cytokine Signaling 1 And 3 In Mouse Macrophages Exposed To Chlamydia Trachomatis

Author Block:

S. A. Duncan, S. Dixit, R. Sahu, E. Nyairo, S. R. Singh, V. A. Dennis; Alabama State Univ., Montgomery, AL

Abstract Body:

Inflammation which is induced by the presence of cytokines and chemokines is an integral part of *Chlamydia trachomatis* (CT) infection. Nevertheless, it can be regulated using effective anti-inflammatory molecules. We recently reported that the anti-inflammatory cytokine, IL-10 can effectively regulate CT inflammation by inhibiting its associated-induced inflammatory mediators in mouse J774 macrophages. A major problem with IL-10 however, is its short biological half-life thus requiring frequent applications at high dosages for biomedical applications. Our goal in this study was to encapsulate IL-10 within the biodegradable polymer, PLA-PEG (Poly (lactic acid)-b-Poly (ethylene glycol) nanoparticles in an attempt to prolong its half-life. IL-10 was encapsulated in PLA-PEG by the double emulsion method, followed by physiochemical characterizations and functional studies. Results from Ultra Violet (UV) visible and Fourier Transform-Infrared Spectroscopy (FT-IR) revealed the successful encapsulation of IL-10 within PLA-PEG. Encapsulated IL-10 had an average size of ~ 100 to 200 nm, with an encapsulation efficiency > 90 %. Temperature stability of encapsulated IL-10 was up to 89C as shown by differential scanning calorimetry analysis. The anti-inflammatory effect of encapsulated IL-10 was tested using various concentrations (1-1000 ng/mL) over a 24-72 hour time-point in mouse J774 macrophages exposed to the recombinant major outer membrane protein of CT. Cytokine specific ELISAs showed that encapsulated IL-10 reduced the levels of both IL-6 and IL-12 in macrophages in a time- and concentration-dependent fashion, correlating with its stability and slow release capacity. By Taqman qRT-PCR, we demonstrate that encapsulated IL-10 induced higher levels of SOCS3 as compared to SOCS1, independent of the encapsulated IL-10 concentration, suggesting their probable role in the encapsulated IL-10 molecular mechanism of inhibition. Conclusively, our data shows successful encapsulation of IL-10 and that PLA PEG can prolong the half-life of IL-10. Moreover, encapsulated IL-10 is functional by down-regulating cytokines and inducing SOCS1 and 3 in macrophages exposed to CT at low dosages.

Author Disclosure Block:

S.A. Duncan: None. **S. Dixit:** None. **R. Sahu:** None. **E. Nyairo:** None. **S.R. Singh:** None. **V.A. Dennis:** None.

Poster Board Number:

FRIDAY-466

Publishing Title:

Oral Immunization with Non-Toxic *C. difficile* Strains Expressing Chimeric Fragments of TCDA and TCDB Elicit Protective Immunity against *C. difficile* Infection in Both Mice and Hamsters

Author Block:

Y. Wang[1], L. Bouillaut[2], X. Ju[3], Y. Wang[1], A. L. Sonenshein[2], X. Sun[1]; [1]Morsani Sch. of Med., Univ. of South Florida, Tampa, FL, [2]Tufts Univ. Sch. of Med., Boston, MA, [3]Tufts Univ. Cummings Sch. of Vet. Med., Grafton, MA

Abstract Body:

The symptoms of *Clostridium difficile* infection (CDI) are attributed largely to two *C. difficile* toxins, TcdA and TcdB. Significant efforts have been devoted to developing vaccines trargeting both toxins through parenteral immunization routes. However, accumulating evidence shows that anti-TcdA IgG, but not IgA, dramatically enhances TcdA-mediated cytotoxicity *in vitro* and disease *in vivo*, raising safety concerns with parenteral immunization. In addition, *C. difficile* is an enteric pathogen, and mucosal/oral immunization would be particularly useful to protect the host against CDI considering that the gut is the main site of disease onset and progression. Moreover, vaccines directed only against toxins do not target the cells and spores that transmit the disease. Previously, we constructed a chimeric vaccine candidate mTcd138, comprised of the glucosyltransferase and cysteine proteinase domains of TcdB and the receptor binding domain of TcdA. Purified mTcd138 induced protective immunity against *C.difficile* challenge in mice and hamsters. In the current study, we exressed mTcd138 in a non-toxigenic *C.difficile* strain with the goal of developing an oral vaccine that targets both *C.difficile* toxins and colonization/adhesion factors. mTcd138 expression in the non-toxigenic *C.difficile* strain was verified by Western-blot analysis. Oral immunization with spores of the non-toxigenic strain expressing mTcd138 provided mice with full protection against the clinically relevant *C.difficile* strain UK1 (ribotype 027). The protective strength and efficacy of the mTcd138-expressing non-toxigenic *C.difficile* strain were further tested in the acute hamster model of CDI. Oral immunization with spores of the mTcd138-expressing non-toxigenic strain also provided hamsters significant protection against infection with 10^4 spores of UK1, a dose100-fold higher than the lethal dose of UK1 in hamsters. In conclusion, the genetically modified, non-toxigenic *C.difficile* strain expressing mTcd138 represents a novel mucosal vaccine candidate against CDI.

Author Disclosure Block:

Y. Wang: None. **L. Bouillaut:** None. **X. Ju:** None. **Y. Wang:** None. **A.L. Sonenshein:** None. **X. Sun:** None.

Poster Board Number:

FRIDAY-467

Publishing Title:

Bone Marrow-Derived Mesenchymal Stem Cells Transplantation Exacerbates the Chronic Inflammatory Response and Pulmonary Fibrosis in Mice Infected with *Paracoccidioides brasiliensis*

Author Block:

J. Arango[1], J. D. Puerta-Arias[1], P. A. Pino[1], D. Arboleda[2], A. Gonzalez[2]; [1]CIB-UdeA-UPB, Medellin, Colombia, [2]Univ. de Antioquia, Medellin, Colombia

Abstract Body:

Introduction: Paracoccidioidomycosis (PCM) is an endemic and systemic mycosis caused by *Paracoccidioides* spp. PCM is characterized as a chronic progressive disease, which leads to pulmonary fibrosis (PF) in 50% of the patients. Currently, the treatment of PF, developed during PCM, uses immunosuppressive drugs; however, they have not been completely successful. Recently, the usage of bone marrow-derived mesenchymal stem cells (BMSCs) transplantation, as alternative therapy for PF induced by chemicals, has demonstrated to be beneficial. In the present study, we used BMSCs in an experimental model of PF induced by the fungus *P. brasiliensis* in order to determine their immunoregulatory and regenerative capabilities.**Methodology:** BMSCs were isolated from femur of BALB/c mice using Cell Depletion Kit and cultured in a low glucose medium. BALB/c male mice were inoculated i.n. with $1.5x10^6$ *P. brasiliensis* yeasts. BMSCs ($1x10^6$ cells) were administered i.v. eight weeks after infection in a single dose and animals were sacrificed four weeks later in order to determine: cellularity, fungal burden and histopathological aspects.**Results:** BMSCs-treated mice showed a significant increase of: i) fungal burden in the lungs and spleen, and ii) M2 macrophages, eosinophils and Th1 lymphocytes in comparison with control animals. M1 Macrophages, neutrophils, T-reg cells, Th17 and Th9 lymphocytes were decreased in number in those animals. Furthermore, histopathological analysis of the lungs from BMSCs-treated mice showed an increase in the number and size of granulomas as well as in the deposition of collagen fibers.**Conclusion:** Contrary to previous reports on chemical-induced pulmonary fibrosis, our results showed a deleterious effect of the BMSCs therapy on the *P. brasiliensis* infected animals. We hypothesize that in our animal model, BMSCs induced an immunosuppressive effect on the immune response and do not participate in the reparation of the tissue damaged during PCM.Study supported by Colciencias (project No. 358-2011).

Author Disclosure Block:

J. Arango: None. **J.D. Puerta-Arias:** None. **P.A. Pino:** None. **D. Arboleda:** None. **A. Gonzalez:** None.

Poster Board Number:

FRIDAY-468

Publishing Title:

***Candida albicans* and *Acinetobacter baumannii* Interactions in a Mixed Species Biofilm**

Author Block:

S. Alshehri, H. Shah, J. Edwards, A. Ibrahim, **P. Uppuluri**; LA BioMed. Res. Inst., Torrance, CA

Abstract Body:

Background: *Candida albicans* (CA) and *Acinetobacter baumannii* (AB) share common niches in healthcare settings, and can co-develop biofilms on vascular and urinary catheters, and patient wounds. Owing to the drug resistant nature of biofilms, novel methods to combat co-habiting organisms are needed. We have investigated the nature of interaction between CA and AB in a biofilm setting. Our bioinformatics studies have predicted significant homology between CA hyphal surface protein Hyr1p and a siderophore-interacting protein of AB. We have tested the efficacy of anti-Hyr1p antibodies to disrupt the stubborn mixed species biofilm and make the biofilms more susceptible to antimicrobials. **Methods:** Biofilms of CA and AB were grown individually or together *in vitro* for 24 h. Interaction between the two organisms was monitored microscopically and viability of both populations was quantified by cfu. Conditioned medium of each organism was obtained after stationary growth phase and its impact on single as well as mixed spp. biofilm formation was studied. The ability of anti-Hyr1 antibodies to inhibit biofilms, as well as disrupt a mature mixed spp. biofilm was investigated *in vitro*. **Results:** In a co-culture**,** AB did not inhibit CA germination. However once filaments developed, the bacterium interacted only with, and killed > 60% of the hyphae overnight which lead to predomination of the bacterium in the mixed spp. biofilm. Interestingly, the conditioned medium of AB biofilms promoted CA filamentation and biofilm formation in conditions non-permissive for hyphenation and biofilm growth (at 30^{o}C). In contrast, conditioned medium from CA yeast, but not filaments, inhibited growth of AB. Anti-Hyr1 antibodies were lethal to AB cells but not to CA in the mixed spp. biofilm. However, in the presence of antibodies, the otherwise drug resistant CA demonstrated significant susceptibility to the antifungal drug fluconazole. **Conclusions:** Our results depict a tug-of-war that exists between two species of organisms in an intimate setting such as a biofilm, indicating that the predominance of one over the other is highly dependent on the environmental conditions that they co-inhabit. Investigations targeting the CA Hyr1p as novel therapeutics for drug resistant AB and CA biofilms is currently being tested by us *in vivo*.

Author Disclosure Block:

S. Alshehri: None. **H. Shah:** None. **J. Edwards:** None. **A. Ibrahim:** None. **P. Uppuluri:** None.

Poster Board Number:

FRIDAY-469

Publishing Title:

Allogeneic Bone Marrow Transplantation in Patients with Active Invasive Fungal Disease the Role of Prophylactic Granulocyte Transfusions

Author Block:

F. Carlesse[1], A. Mendonça[1], A. S. S. Ibanez[1], C. Monteiro[1], O. M. W. O. Felix[1], R. F. C. Guimarães[1], V. Ginani[2], V. G. Zecchin[1], R. Gouveia[2], A. Seber[2]; [1]Oncology Paediatric Inst. São Paulo Federal Univ., Sao Paulo, Brazil, [2]Hosp. Samaritano, Sao Paulo, Brazil

Abstract Body:

Background: Active IFD defer bone marrow transplantation (BMT). **Objective:** analyze the impact of granulocyte transfusions in patients undergoing allogeneic BMT with active IFD diagnosed immediately prior to the procedure. **Methods:** Adult ABO compatible donors received G-CSF 10 µg/kg , SQ, 12-18h before continuous flow leukapheresis in a COBE Spectra 7.0 LRS. We processed 7.5L with Htc 5%-7%, <65 ml/min and a 1:13 blood:ACD ratio and continuous infusion of 500 mL of hydroxyethyl starch 6% (Halexlstar) as an RBC-sedimenting agent. The leukapheresis products were irradiated with 40 Gy and divided in 3 bags with <1x109 granulocytes/kg, kept at 4°C and leukapheresis products were infunsed daily to keep neutrophils over 1000 cells/mm3 . **Results:** Between 2009- 2015, 5 children with proven active IFD were transplanted using antifungals and granulocyte transfusions to prevent neutropenia. 1) 5-yo girl with immunodeficiency and invasive pulmonary aspergillosis (IPA). She had a related BMT 2) 18-yo male with refractory AML developed disseminated fusariosis . He had double unrelated cord blood (UCB) transplant; 3) 8-yo male with AML in 2nd remission with *Fusarium* osteomyelits and skin infection, undergoing UCB 4) 10-yo male with relapsed AML, with IPA who had UCB and haploidentical graft 5) 11-yo female with AML in remission, with IPA , undergoing related BMT. Only one patient died due to bacterial sepsis. **Conclusion:** The use of granulocyte transfusions associated to conventional antifungals may allow the patient to proceed to BMT despite the fungal infection. No patient had worsening of the fungal infection

Author Disclosure Block:

F. Carlesse: None. **A. Mendonça:** None. **A.S.S. Ibanez:** None. **C. Monteiro:** None. **O.M.W.O. Felix:** None. **R.F.C. Guimarães:** None. **V. Ginani:** None. **V.G. Zecchin:** None. **R. Gouveia:** None. **A. Seber:** None.

Poster Board Number:

FRIDAY-470

Publishing Title:

Human Monoclonal Antibodies That Neutralize Vaccine and Wild Type Poliovirus Strains

Author Block:

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Abstract Body:

Background: Eradication of poliomyelitis requires elimination of reservoirs of immunodeficiency-related vaccine-derived poliovirus (iVDPV), chronically excreted by individuals with immune dysfunction and reintroduced into general population. MAbs specific for poliovirus may contribute to clearance of iVDPV in chronic carriers by neutralizing the virus directly and reducing the outgrowth of PV strains resistant to anti viral drugs. Human mAbs, specifically cross-neutralizing mAbs are ideal for anti-PV therapeutics. Here, we describe a panel of cross neutralizing human mAbs with potent neutralizing activity against Sabin and wild type strains.**Methods:** We isolated mAbs from OPV-vaccinated, IPV-boosted subjects using a novel cell fusion method and measured the neutralization against Sabin and wild type PV strains. We generated escape mutant PV strains using selected cross-neutralizing mAbs (1E4, 12F8) and mono-neutralizing mAbs, mapped the mutated amino acids to virion structure. We compared these mAbs to neutralize each other's escape mutant strains and strains resistant to a previously described mAb, A12, that reduces viral excretion in animal studies. We tested 1E4 and 12F8 to neutralize a panel of patient-derived, type 1 PVs including WT strains, iVDPVs and aVDPV, also strains resistant to anti-PV small molecule drug V-073.**Results:** We identified a panel of mAbs with potent neutralization activities against PV wild type and Sabin strains. Escape mutant studies showed that mono-specific mAbs 7E2 and 8F9 interact with residues in antigenic site 1, whereas cross-specific mAbs, 1E4 and 12F8 bind to residues in the canyon domain, which interacts with the PV cellular receptor, CD155. Cross specific mAbs, 12F8 and 1E4 neutralized A12 escape mutant PV strains. They also neutralized a panel of patient-derived type 1 PVs including WT strains, iVDPVs and aVDPV. The virus mutants resistant to the anti-poliovirus drug V-073 were also neutralized by the human mAbs.**Conclusions:** The isolated cross-neutralizing human mAbs may be used as a PV therapeutic for immunodeficient subjects. These mAbs were able to neutralize A12 escape mutant strains, suggesting that they could be given along with A12 to reduce the outgrowth of mAb-resistant PV strains. The cross specific mAbs 12F8 and 1E4 may be suitable to clear iVDPVs in chronically infected individuals, particularly in combination with V-073.

Author Disclosure Block:

R. Puligedda: None. **S. Dessain:** None.

Poster Board Number:

FRIDAY-471

Publishing Title:

Mechanisms of Neutralization of *Clostridium difficile* Toxins by the Antitoxin Antibodies Actoxumab and Bezlotoxumab

Author Block:

L. D. Hernandez[1], H. K. Kroh[2], M. Beaumont[3], P. R. Sheth[1], X. Yang[1], E. DiNunzio[1], S. A. Rutherford[2], M. D. Ohi[2], N. J. Murgolo[1], L. Xiao[1], F. Racine[1], E. Hsieh[1], G. Ermakov[3], D. B. Lacy[2], **A. G. Therien**[1]; [1]Merck & Co., Inc., Kenilworth, NJ, [2]Vanderbilt Sch. of Med., Nashville, TN, [3]Merck & Co., Inc., Palo Alto, CA

Abstract Body:

Clostridium difficile is a spore forming anaerobic bacterium that infects the intestinal tract. The organism expresses two exotoxins, TcdA and TcdB, that target the epithelial cells of the gut and cause damage and inflammation leading to symptoms ranging in severity from mild diarrhea to colonic rupture and death. The toxins are thought to bind to specific cell-surface receptors in part via C-terminal domains known as combined repetitive oligopeptides (CROP). The neutralizing antitoxin antibodies actoxumab and bezlotoxumab, specific for TcdA and TcdB respectively, are protective in animal models of CDI when administered in combination. Furthermore bezlotoxumab was recently shown to reduce the rate of recurrent CDI in phase 3 clinical trials. In this study, we characterize the binding of actoxumab and bezlotoxumab to their respective toxins and propose mechanisms of neutralization for the two antibodies. The epitopes of each antibody were identified using a combination of Western blotting, surface plasmon resonance, hydrogen-deuterium exchange - mass spectrometry, and negative stain electron microscopy. The nature of the immune complexes formed between the antibodies and toxins was investigated using size exclusion chromatography coupled with multi-angle laser light scattering. Overall, our data show that actoxumab and bezlotoxumab bind to two homologous but distinct epitopes within the CROP domains of TcdA and TcdB, respectively. Binding of actoxumab to TcdA causes formation of large immune complexes whose sizes increase at higher antibody:toxin ratios, whereas binding of bezlotoxumab to TcdB does not. Despite these differences, the mechanisms of toxin neutralization are similar; each antibody prevents binding of their respective toxin to host cells, thereby blocking the intoxication cascade at its first step. These mechanisms of neutralization are presumed to underlie the efficacy of the antibodies in pre-clinical models of CDI and of bezlotoxumab alone in prevention of recurrent CDI in patients.

Author Disclosure Block:

L.D. Hernandez: D. Employee; Self; Merck & Co., Inc.. **H.K. Kroh:** None. **M. Beaumont:** D. Employee; Self; Merck & Co., Inc. **P.R. Sheth:** D. Employee; Self; Merck & Co., Inc. **X. Yang:** D. Employee; Self; Merck & Co., Inc. **E. DiNunzio:** D. Employee; Self; Merck & Co., Inc. **S.A.**

Rutherford: None. **M.D. Ohi:** None. **N.J. Murgolo:** D. Employee; Self; Merck & Co., Inc. **L. Xiao:** D. Employee; Self; Merck & Co., Inc. **F. Racine:** D. Employee; Self; Merck & Co., Inc. **E. Hsieh:** D. Employee; Self; Merck & Co., Inc. **G. Ermakov:** D. Employee; Self; Merck & Co., Inc. **D.B. Lacy:** E. Grant Investigator; Self; Merck & Co., Inc., Medimmune. **A.G. Therien:** D. Employee; Self; Merck & Co., Inc.. K. Shareholder (excluding diversitied mutual funds); Self; Merck & Co., Inc..

Poster Board Number:

FRIDAY-472

Publishing Title:

Utilizing the Antimicrobial and Immunomodulatory Properties of Platelets to Combat Infectious Arthritis *In Vitro*

Author Block:

J. Gilbertie, T. Schaer; Univ. of Pennsylvania, Kennett Square, PA

Abstract Body:

Bacterial colonization of synovial structures can cause infections that are difficult to treat and is a significant clinical problem in both veterinary and human medicine. It has recently been shown that antimicrobial recalcitrance in human synovial fluid could be due to bacterial clumping or the formation of biofloats. These biofloats have similar gene expression to bacteria within biofilms and are equally difficult to treat exhibiting profound resistance to antimicrobials *in vitro.* Data from our lab has shown that bacteria form biofloats within equine synovial fluid as well. Recent reports show that platelet-rich plasma (PRP) has bacteriostatic effects. Platelets are powerful immunomodulators and key players in innate immunity. Upon activation, they release bioactive factors that can be directly bactericidal. We hypothesized that the bioactive factors derived from the platelet-rich plasma will decrease bacterial load in synovial fluid and increase immune cell function *in vitro*. PRP was isolated from 16 healthy horses; platelet-poor plasma (PPP) was also isolated as a negative control. The PRP and PPP underwent five consecutive freeze-thaw cycles to lyse the platelets. Equine synovial fluid was inoculated with two clinical isolates from musculoskeletal infections, *Staphylococcus aureus* and *Escherichia coli*. Two hours after inoculation the synovial fluid was treated with PPP lysate (PPP-L), PRP plasma lysate (PRP-L), vancomycin or left untreated. PRP-L resulted in a 5 log reduction in bacterial load while PPP-L resulted in an 2 log reduction compared to the untreated control. Vancomycin has negligible effect on bacterial load; however, when PRP-L and vancomycin were used together no bacterial growth was observed. Neutrophil functionality was assessed by measuring respiratory burst and phagocytosis. Neutrophils stimulated with PRP-L had significantly higher levels of respiratory burst and phagocytosis compared to those stimulated with PPP-L or the untreated control. Taken together PRP-L was both antimicrobial and immunomodulatory. This biologic is easily accessible and could have a significant impact on cases of infectious arthritis when used in combination with current antimicrobials such as Vancomycin.

Author Disclosure Block:

J. Gilbertie: None. **T. Schaer:** None.

Poster Board Number:

FRIDAY-473

Publishing Title:

Progress Toward a Multivalent, Monoclonal Antibody Cocktail to Prevent *Acinetobacter baumannii* Infections

Author Block:

S. Singh, J. Luka, A. Jacobs, I. Soojhawon, Y. Alamneh, M. G. Thompson, R. Abu-Taleb, S. Noble, S. D. Tyner, **D. V. Zurawski**; Walter Reed Army Inst. of Res., Silver Spring, MD

Abstract Body:

The lack of new antibiotics currently being pursued against Gram-negative, ESKAPE pathogens is a concern given the increase of infections and antibiotic resistance. While some monotherapy treatments are in the pipeline, small molecules are susceptible to rapid resistance; and therefore, alternative approaches should be considered. One such approach could be monoclonal antibodies (mAb) as serum was used with success before the "Golden Age" of antibiotics. A key to successful mAb development is choosing the appropriate antigen(s). Targets have to be required for virulence at the various stages of infection. To this end, we have identified proteins on the surface of *Acinetobacter baumannii* required for pathogenicity. Once we found an appropriate model strain (AB5075) and developed tools to manipulate the genome, we hypothesized that the Type Six Secretion System (T6SS), and in particular, the Hcp needle, was a virulence factor that could be exploited for therapy. First, we identified a transposon mutant *hcp*::Tn*5* from our mutant library that was avirulent in a murine pulmonary model when compared to wild-type infected mice. Then, we cloned, overexpressed, and purified a 6XHis-tagged Hcp protein that was injected into mice to generate mAb. The mAb 13F7 was selected because of its high affinity for Hcp. We confirmed that 13F7 could recognize native Hcp on the surface of *A. baumannii* using ELISA and immunofluorescence. Then, we confirmed that antibody did not recognize Hcp in the *hcp*::Tn5 background. Subsequently, 13F7 was evaluated in two murine models of *A. baumannii* infection where 13F7 was prophylactically delivered up to 100 µg (5 mg/kg) via intraperitoneal injection. In the pulmonary infection model, 13F7 provided >60% protection from infection in a dose-dependent manner when compared to a mock-treated control. In a wound infection model, wound area and inflammation were significantly reduced in animals that received 13F7 as compared to a mock-treated control ($P \leq 0.01$). Since protection was not 100%, we have identified additional surface targets and this strategy will be applied. Our goal is to combine all of these antibodies into a cocktail that could be a prophylactic treatment to prevent *A. baumannii* infection or treat infected patients in combination with antibiotics.

Author Disclosure Block:

S. Singh: None. **J. Luka:** None. **A. Jacobs:** None. **I. Soojhawon:** None. **Y. Alamneh:** None. **M.G. Thompson:** None. **R. Abu-Taleb:** None. **S. Noble:** None. **S.D. Tyner:** None. **D.V. Zurawski:** None.

Poster Board Number:

FRIDAY-474

Publishing Title:

Active Hexose Correlated Compound Oral Feeding Enhances Immune Function and *Chlamydia trachomatis* Clearance in a Stress Mouse Model

Author Block:

T. Belay, K. Brown; Bluefield State Coll., Bluefield, WV

Abstract Body:

Background: *Chlamydia trachomatis* genital infection is a serious public-health problem that disproportionately affects populations of low socio-economic status. Stress is a risk factor to infection that has a significant impact in public health and is generally greater in populations of lower socioeconomic status. We have shown that cold-induced stress results in suppression of the immune response and increased intensity of chlamydia genital infection in a mouse model. Here we evaluated the potential therapeutic value of active hexose correlated compound (AHCC) against chlamydia genital infection in cold water-induced stressed mice. AHCC is a extract of a mushroom commonly used as a dietary supplement known to boost the immune system.**Methods:** Mice were infected intravaginally with *Chlamydia trachomatis* after a 24-day cold water-stress series. Oral administration of AHCC to stressed or non-stressed mice was carried out seven days before and during the course of infection along with cervicovaginal swabbing. Splenic T cells from both animal groups were co-cultured with mouse monocyte J774.2 cell line or cultured by addition of supernatants of AHCC-treated J774.2 cell line for 24 hours. Cytokine production by peritoneal and splenic T cells isolated from mice was measured by ELISA.**Results:** Stressed mice suffered loss of weight before feeding, however, improvement in body weight gain was shown in AHCC-fed mice. A significant reduction in the shedding of *Chlamydia trachomatis* from the genital tract of AHCC-fed mice on day 18 after infection was observed. Increased levels of tumor necrosis factor-alpha (TNF-α), interleukin 6 (IL-6) in peritoneal cells of stressed mice receiving AHCC compared to control mice receiving. Decreased production of interferon gamma (IFN-γ) and interleukin 2 (IL-2) in the AHCC group was observed.**Conclusions:** Results obtained in this study show that AHCC improves the function of immune cells as indicated by the restoration of levels of cytokines production that were suppressed under cold induced-stress conditions. This is the first report showing that oral administration of AHCC enhances the function of the immune system, which could result in increased resistance of the host to chlamydia genital infection.

Author Disclosure Block:

T. Belay: None. **K. Brown:** None.

Poster Board Number:

FRIDAY-475

Publishing Title:

Immunomodulation Potential of Seeds of *Bixa orellana*

Author Block:

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Abstract Body:

Objective: The main purpose of study is to check different extracts of dried seeds of *B. orellana* for their anti-inflammatory activity.**Background:**Inflammatory diseases are important cause of mortality and morbidity. The concept of strengthening the host immune system to combat diseases is not new, plants has been used for immunomodulation since ages. *Bixa orellana* belongs to family Bixaceae, popular in treatment of various diseases. Therefore, extracts of seeds of *B. orelana* were choosen to check their anti-inflammatory potential.**Methods***Bixa* seeds were extracted with methanol (M), petroleum ether (PE) and acetone (Ace) for 48 hours. The solvents were evaporated to give respective residues, Bixa P-1, P-2, M-1, M-2, Ace 1 and Acc-.2.The immunomodulatory activity of the extracts were evaluated on whole human blood phagocytes, isolated neutrophils and J774.2 macrophages cell lines for ROS (reactive oxygen species) production using luminol- and lucigenin-based chemiluminescence tests.**Results:**Bixa P-1, P-2, M-1, M-2 exhibited active inhibition of ROS production on whole blood phagocytes IC 50 values (10.6±0.21 to 14.7±0.5 µg/ml), whereas, Bixa P-1, P-2, Ace-1, Ace-2 showed moderately active inhibition with IC 50 values (24.6±0.11 to 34.7±0.23 µg/ml) compared with control Ibuprufen (11.2 ± 0.1µg/ml). All extracts performed remarkable inhibition on intracellular and extracellular production of neutrophils ROS with an IC50 values ((2.5±0.32 to < 1) compared with controls Ibuprufen (2.5±<1) and Diphenylene iodonium (0.56±0.04). Similarly profound inhibition on the intracellular and extracellular macrophage ROS production (1.60±0.561 to <1) was observed.**Conclusion**Extracts of seeds of *B.orellana* showed significant inhibition of ROS production. Studies are carried out for exploration of active component and evaluation for its molecular mechanism of action in order to use them as anti-inflammatory agent in future.

Author Disclosure Block:

F.Z. Khan: None. **N. Dastagir:** None. **M.A. Mesaik:** None. **S. Faizi:** None. **S.U. Kazmi:** None.

Poster Board Number:

FRIDAY-476

Publishing Title:

***In Vitro* Activity of WCK 5999 against *Acinetobacter baumannii* and *Pseudomonas aeruginosa* Isolates from a Worldwide Surveillance Program (2015)**

Author Block:

M. D. Huband, D. J. Farrell, R. K. Flamm, R. N. Jones, H. S. Sader; JMI Lab., North Liberty, IA

Abstract Body:

Background: WCK 5999 is a novel carbapenem/β-lactamase inhibitor combination comprising meropenem (MEM) and WCK 4234, a broader-spectrum β-lactamase inhibitor with enhanced activity against Class D carbapenemases. The *in vitro* activity of MEM-WCK 4234 using both fixed 4 (F4) and fixed 8 (F8) µg/mL of WCK 4234 was evaluated against *A. baumannii* (ACB) and *P.* aeruginosa (PSA) isolates (n=490) collected during 2015 from the SENTRY worldwide surveillance program and also included PSA resistant isolate subgroups.**Methods:** MIC values for MEM-WCK 4234 (F4 and F8) and comparator compounds were determined using reference broth microdilution methodology. Breakpoint interpretative criteria followed CLSI guidelines.**Results:** MEM-WCK 4234 (F4 and F8) was active against ACB isolates (MIC_{50}/MIC_{90} values of 2/16 and 1/8 µg/mL, respectively; Table). The addition of WCK 4234 to MEM significantly increased the % of ACB isolates with MEM-WCK 4234 MICs ≤4 µg/mL (based on 2g TID MEM dose) from 35.8% (MEM alone) to 78.8% (F4) and 87.6 (F8). Against PSA, the % of isolates with MEM-WCK 4234 MICs ≤4 µg/mL increased from 73.7% (MEM alone) to 83.6% (F4) and 84.1% (F8). MEM-WCK 4234 MIC_{50} values (F4 and F8) against PSA were also two-fold lower than MEM. MEM-WCK 4234 (F4 and F8) combinations demonstrated enhanced activity against resistant PSA subgroups including ceftazidime (CAZ) non-S (43.9-47.4% S), MDR (33.3-36.8% S), and XDR (21.4-23.8% S) isolates whereas comparator agent S ranged 0.0-21.1%.**Conclusions:** WCK 5999 was very active against ACB isolates and demonstrated enhanced activity over MEM alone against PSA, most notably against CAZ NS, MDR, and XDR PSA isolates. These data support the continued development of this promising antibacterial combination.

	MIC_{50}/MIC_{90} µg/mL (%Susceptible[a])				
Organism / Phenotype (n)	MEM-WCK 4234 (F4)	MEM-WCK 4234 (F8)	CAZ	MEM	P/T
ACB (137)	2/16 (78.8%)[b]	1/8 (87.6%)[b]	>32/>32 (29.2%)	32/>32 (35.8%)	>64/>64 (27.2%)

PSA (353)	0.25/16 (83.6%)[b]	0.25/16 (84.1%)[b]	2/32 (83.9%)	0.5/16 (73.7%)	4/64 (83.3%)
PSA / CAZ NS (57)	8/>32 (43.9%)[b]	8/>32 (47.4%)[b]	32/>32 (0.0%)	16/>32 (21.1%)	64/>64 (12.3%)
PSA / MDR (57)	8/>32 (33.3%)[b]	8/>32 (36.8%)[b]	32/>32 (19.3%)	16/>32 (5.3%)	64/>64 (17.5%)
PSA / XDR (42)	16/>32 (21.4%)[b]	16/>32 (23.8%)[b]	32/>32 (4.8%)	16/>32 (2.4%)	64/>64 (11.9%)
a. According to CLSI breakpoints; b. % inhibited at ≤ 4 µg/mL based on 2 g TID MEM dose					

Author Disclosure Block:

M.D. Huband: H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Wockhardt. **D.J. Farrell:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Wockhardt. **R.K. Flamm:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Wockhardt. **R.N. Jones:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Wockhardt. **H.S. Sader:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Wockhardt.

Poster Board Number:

FRIDAY-477

Publishing Title:

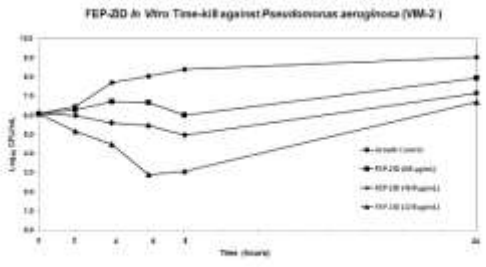
Wck 5222 (Cefepime-Zidebactam) *In Vitro* Time-kill Studies Against *Pseudomonas aeruginosa* (Psa) and *Acinetobacter baumannii* (Acb) Isolates with Defined β-Lactamases

Author Block:

M. D. Huband, M. Castanheira, R. N. Jones, P. R. Rhomberg, A. A. Watters, H. S. Sader; JMI Lab., North Liberty, IA

Abstract Body:

Background: Zidebactam (ZID) is a new bicyclo-acyl hydrazide with a dual mechanism of action including selective binding to Gram-negative PBP2 and β-lactamase inhibition for Class A and C but not of Class B. We evaluated the activity of cefepime (FEP) and ZID alone and combined against 3 ACB and 7 PSA isolates expressing Class A (KPC), B (IMP or VIM), C (AmpC) or D (OXA) β-lactamases by MIC and time-kill studies.**Methods**: Broth microdilution MIC values for FEP, ZID and FEP-ZID (1:1 ratio and ZID at fixed 4, 8 and 16 µg/mL) were determined according to CLSI guidelines. Isolates were selected for time-kill based on β-lactamase content and MIC values. Time-kill testing employed sub-inhibitory concentrations of FEP and ZID as well as MIC multiples. Time-kill studies were sampled for colony counts at T_0, T_2, T_4, T_6, T_8 and T_{24}.**Results**: Time-kill studies showed that ≤32 µg/mL of FEP or ≤16 µg/mL of ZID tested alone were not bactericidal against PSA (VIM-2). However, FEP-ZID combinations (32/8 [Figure] and 8-32/16 µg/mL) were bactericidal against VIM-2 producing PSA by T_8. Other FEP-ZID combinations were bactericidal against PSA producing IMP-13 (32/8 and 8-32/16 µg/mL) and AmpC (8-32/8 µg/mL) by T_8 and T_{24}, respectively. Two $\log_{10}$ CFU/mL colony count reductions were observed for FEP-ZID combinations against OXA-23 (16-32/8 µg/mL) and OXA-24 (32/8 µg/mL) producing ACB by T_8.**Conclusion**: FEP-ZID combinations showed potent activity in time-kill studies against PSA and ACB isolates expressing clinically relevant β-lactamases including AmpC, IMP, KPC, OXA, and VIM enzymes for which limited treatment options may be available. These results support further clinical development studies



with FEP-ZID (WCK 5222).

Author Disclosure Block:

M.D. Huband: H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Wockhardt. **M. Castanheira:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Wockhardt. **R.N. Jones:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Wockhardt. **P.R. Rhomberg:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Wockhardt. **A.A. Watters:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Wockhardt. **H.S. Sader:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Wockhardt.

Poster Board Number:

FRIDAY-478

Publishing Title:

Antimicrobial Activity of WCK 5222 (Cefepime-Zidebactam) Tested against Clinical Isolates of *Pseudomonas aeruginosa* and *Acinetobacter* spp. Collected Worldwide (2015)

Author Block:

H. S. Sader, D. J. Farrell, R. K. Flamm, R. N. Jones; JMI Lab., Inc., North Liberty, IA

Abstract Body:

Background: WCK 5222 consists of cefepime (FEP) combined with zidebactam (ZID), a bicyclo-acyl hydrazide with a dual mechanism of action involving selective and high binding affinity to Gram-negative PBP2 and β-lactamase inhibition.**Methods:** 353 *P. aeruginosa* (PSA) and 137 *Acinetobacter* spp. (ASP) isolates were collected from 68 medical centers (21 countries) in 2015 by the SENTRY Antimicrobial Surveillance Program and susceptibility (S) tested by a reference broth microdilution method against FEP-ZID (1:1 and 2:1 ratios) and comparator agents.**Results:** FEP-ZID was very active against PSA with $MIC_{50/90}$ of 1/4 μg/mL and 99.7% of isolates inhibited at ≤8 μg/mL (1:1 ratio; Table). FEP-ZID (1:1 ratio) MIC values were generally 2-fold lower than those for FEP ($MIC_{50/90}$, 2/16 μg/mL) and ZID alone ($MIC_{50/90}$, 2/8 μg/mL). Colistin (COL; $MIC_{50/90}$ of ≤0.5/1 μg/mL; 100.0% S) and amikacin (AMK; $MIC_{50/90}$, 4/16 μg/mL; 92.4% S) were also active against PSA. FEP-ZID exhibited consistent activity against PSA from all continents (99.0-100.0% inhibited at ≤8 μg/mL) and retained potent activity against ceftazidime (CAZ)-non-S and meropenem (MEM)-non-S PSA (98.2-98.9% inhibited at ≤8 μg/mL). FEP-ZID 1:1 and 2:1 ratios ($MIC_{50/90}$, 16/32 μg/mL for both) were 4-fold more active than FEP against ASP. The most active compounds tested against ASP were COL ($MIC_{50/90}$, ≤0.5/1 μg/mL; 94.9% S) and AMK ($MIC_{50/90}$, 16/>32 μg/mL; 50.4% S).**Conclusion:** FEP-ZID (WCK 5222) demonstrated potent in vitro activity against this worldwide collection of PSA, including isolates resistant to CAZ and/or MEM, and moderate activity against ASP. These in vitro results support further development of WCK 5222 for treatment of systemic PSA and ASP infections.

	MIC_{50}/MIC_{90} (% susceptible[a])			
	P. aeruginosa			**Acinetobacter spp.**
Antimicrobial agent	**All (353)**	**CAZ-NS (57)**	**MEM-NS (87)**	**(137)**
FEP-ZID (1:1)	1/4 (99.7)[b]	4/8 (98.2)[b]	4/8 (98.9)[b]	16/32 (43.8)[b]
Cefepime	2/16 (82.7)	16/64 (17.5)	16/64 (47.1)	64/>64 (26.3)
Zidebactam	2/8 (96.3)[b]	8/16 (86.0)[b]	8/16 (87.4)[b]	>64/>64 (0.0)[b]
Ceftazidime	2/32 (83.9)	32/>32 (0.0)	16/>32 (49.4)	>32/>32 (32.8)
PIP-TAZ[c]	4/64 (83.3)	64/>64 (12.3)	32/>64 (48.3)	>64/>64 (27.2)

Meropenem	0.5/16 (73.7)	16/>32 (21.1)	16/>32 (0.0)	32/>32 (35.8)
a. According to CLSI breakpoints; b. % inhibited at ≤8 μg/mL; c. PIP-TAZ = piperacillin-tazobactam.				

Author Disclosure Block:

H.S. Sader: H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Wockhardt. **D.J. Farrell:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Wockhardt. **R.K. Flamm:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Wockhardt. **R.N. Jones:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Wockhardt.

Poster Board Number:

FRIDAY-479

Publishing Title:

WCK 5107 (Zidebactam, Zid): Structure Activity Relationship (Sar) of Novel Bicyclo Acyl Hydrazide (Bch) Pharmacophore Active against Gram-Negatives Including P*seudomonas* (Pa)

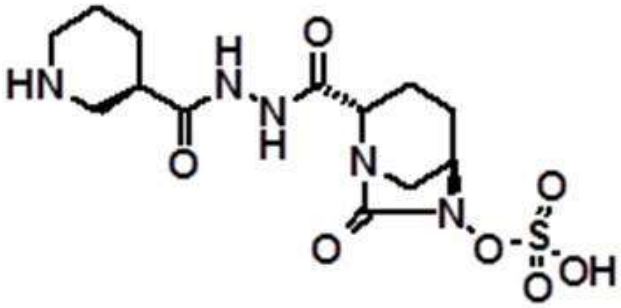
Author Block:

P. K. Deshpande, S. B. Bhavsar, S. N. Joshi, S. S. Pawar, R. P. Kale, A. Mishra, S. B. Jadhav, L. S. Pavase, S. V. Gupta, R. D. Yeole, V. P. Rane, V. K. Ahirrao, S. S. Bhagwat, M. V. Patel; Wockhardt Res. Ctr., Aurangabad, India

Abstract Body:

Background: β-lactam-β-lactamase inhibitor (BL-BLI) combinations lack coverage of all 4 classes of β-lactamases. Here, we report the first pan Gram-negative BL enhancer (ZID) with high specificity and affinity for PBP2 and overcomes resistance in combination with cefepime.**Methods:** Extensive modifications of diazabicyclo octane (DBO) core were made to obtain novel series of BCHs with intrinsic antibacterial activity. BCH NCEs were prepared by coupling acid hydrazide side chains with DBO core followed by hydrogenolysis, sulfation, and de-protection. ZID MICs (as a measure of PBP2 affinity) were determined as per CLSI guidelines for MDR enterics (ENT) and PA strains. In *Acinetobacter* (AB) expressing Class D β-lactamases, microscopy was performed to confirm minimum spheroplasting concentration (MSC) required for spheroplastation, to judge PBP2 binding efficiency.**Results:** BCH pharmacophore with amine substitutions on hydrazide moiety with C_1-C_4 carbon distance rendered activity against ENT and PA (MIC 0.25-8 and 8->32 µg/mL respectively). Further, amine function and its position from the point of attachment and in particular two carbon distance between amine function and BCH core provided both PA and AB activity (PA MIC 2-8 µg/mL, AB MSC 0.25-1 µg/mL). Terminal amine function and hydrazide moiety determined superior activity over classical DBO, while 'R' stereochemistry in the terminal heterocycles led to improved activity.**Conclusion:** SAR and preclinical studies led to clinical candidate WCK 5107 with potent PBP2 binding activity against MDR ENT, PA and AB strains.

WCK 5107



Author Disclosure Block:

P.K. Deshpande: D. Employee; Self; Wockhardt Research Center. K. Shareholder (excluding diversitied mutual funds); Self; Wockhardt Ltd.,. **S.B. Bhavsar:** D. Employee; Self; Wockhardt Research Center. **S.N. Joshi:** D. Employee; Self; Wockhardt Research Center. **S.S. Pawar:** D. Employee; Self; Wockhardt Research Center. **R.P. Kale:** D. Employee; Self; Wockhardt Research Center. **A. Mishra:** D. Employee; Self; Wockhardt Research Center. **S.B. Jadhav:** D. Employee; Self; Wockhardt Research Center. **L.S. Pavase:** D. Employee; Self; Wockhardt Research Center. **S.V. Gupta:** D. Employee; Self; Wockhardt Research Center. **R.D. Yeole:** D. Employee; Self; Wockhardt Research Center. K. Shareholder (excluding diversitied mutual funds); Self; Wockhardt Ltd.,. **V.P. Rane:** D. Employee; Self; Wockhardt Research Center. **V.K. Ahirrao:** D. Employee; Self; Wockhardt Research Center. **S.S. Bhagwat:** D. Employee; Self; Wockhardt Research Center. K. Shareholder (excluding diversitied mutual funds); Self; Wockhardt Ltd.,. **M.V. Patel:** D. Employee; Self; Wockhardt Research Center. K. Shareholder (excluding diversitied mutual funds); Self; Wockhardt Ltd.,.

Poster Board Number:

FRIDAY-480

Publishing Title:

***In Vitro* Susceptibility of a Collection of Carbapenem-resistant *Enterobacteriaceae* and *Pseudomonas aeruginosa* to Ceftazidime/Avibactam and Ceftolozane/Tazobactam**

Author Block:

A. Clowes, M. Lam, C. Hamula, **T. Dingle**; Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract Body:

Background: The frequency of isolation of pan-resistant Gram-negative organisms has been steadily increasing. Ceftazidime/avibactam (C/A) and ceftolozane/tazobactam (C/T) are two new cephalosporin β-lactamase inhibitor combination antibiotics targeting Gram-negative organisms. Notably, C/A and C/T have been shown to retain activity against some carbapenem-resistant *Enterobacteriaceae* and *P. aeruginosa*, respectively. The goal of this study was to test the susceptibility of a collection of carbapenem-resistant *Enterobacteriaceae* and *P. aeruginosa* isolates collected in New York City against C/A and C/T. **Methods:** All clinical isolates were collected in the Mount Sinai Clinical Microbiology Laboratory. The collection consisted of 57 *Enterobacteriaceae*, resistant to all β-lactams including ertapenem and imipenem, and 20 *Pseudomonas aeruginosa* isolates resistant to ceftazidime, cefepime, imipenem and meropenem. The *Enterobacteriaceae* isolates were tested against C/A and the *P. aeruginosa* isolates were tested against both C/A and C/T. All isolates were tested by standard E-test procedures and susceptibility interpretations were recorded per the breakpoints found in the package insert for each drug.**Results:** Of the 57 multi-drug resistant *Enterobacteriaceae* isolates, 55 (96.5%) were susceptible *in vitro* to C/A. The two resistant isolates, an *Escherichia coli* and *Klebsiella pneumoniae*, were pan-resistant to all drugs tested other than tigecycline. Of the multi-drug resistant *P. aeruginosa* isolates tested, 8/18 (44.4%) were susceptible to C/A and 13/20 (65%) were susceptible to C/T. Of note, both C/A susceptible-C/T resistant and C/A-resistant-C/T susceptible *P. aeruginosa* isolates were observed.**Conclusion:** Whereas C/A showed good activity against the carbapenem-resistant *Enterobacteriaceae*, decreased susceptibility was observed for the *P. aeruginosa* isolates. When comparing the activity of each drug against the *P. aeruginosa* isolates, C/T showed improved activity over C/A. Determination of the genotypic mechanism of resistance for this collection of carbapenem-resistant isolates is currently underway and will provide further associations between resistance mechanism and susceptibility patterns of C/A and C/T.

Author Disclosure Block:

A. Clowes: None. **M. Lam:** None. **C. Hamula:** None. **T. Dingle:** None.

Poster Board Number:

FRIDAY-481

Publishing Title:

A Challenging Case of Cystic Fibrosis (Cf) Child Infected with Two Multidrug Resistant (Mdr) *Pseudomonas aeruginosa* (Pa) Isolates: Effect of Ceftolozane/Tazobactam (C/T) and Pharmacokinetics (Pk) of Simulated Doses

Author Block:

J. Y. Ang[1], N. Abdel-Haq[1], F. Zhu[1], A. K. Thabit[2], D. P. Nicolau[2]; [1]Child. Hosp. of Michigan/ Wayne State Univ., Detroit, MI, [2]Hartford Hosp. Ctr. of Anti-Infective Res. and Dev., Hartford, CT

Abstract Body:

Background: Antibiotic options for treatment of CF exacerbations due to MDR PA are limited. C/T is a novel cephalosporin/β-lactamase inhibitor with potent activity against MDR PA. C/T use in children has not been described.**Methods:** A 14 year old CF girl with history of cefepime allergy was infected with 2 strains of MDR PA that was susceptible to C/T (MIC: 0.05 and 1 ug/mL). She underwent C/T desensitization until 1.5 gm q8h (30 mg/kg/day of C component).C/T concentration and PK modeling was performed using HPLC. PK samples were collected after the 4th dose. PK parameters were modeled using one-compartment first-order input and elimination, by nonlinear least-squares techniques. PK parameters were also used to simulate a variety of C/T dosing regimens against various MICs.**Results:** PK estimates are shown in Table 1. The percentage of dosing interval in which free drug concentrations remain above the MIC (%*f*T>MIC) for each simulated doses against tested MIC range are shown in Table 2. All regimens exceeded the target *f*T>MIC of 40% against the tested MICs. There was rapid clinical improvement over the following 5 days. After a 14-day treatment course her respiratory status returned to baseline.**Conclusions:** C/T may be considered to treat CF patients infected with MDR PA with MIC of up to 8 µg/ml when given at doses of 1.5 g q8h over 1h or higher. Further studies assessing the PK of C/T in CF patients are warranted.**Table 1.** Modeled PK parameters of C/T at steady state based on total plasma concentrations

Drug	**C_{max} (µg/ml)**	**C_{min} (µg/ml)**	**AUC_{0-8} (µg·h/ml)**	**V_d (L)**	**K_e (h^{-1})**	**$T_{½}$ (h)**	**Cl (L/h)**
Ceftolozane	94.1	1.2	201.1	7.9	0.6	1.1	5.0
Tazobactam	12.1	0.04	21.4	28.1	0.8	0.8	23.3

Table 2. %*f*T>MIC values of simulated C/T dosing regimens against various MICs (µg/ml)

Dose	0.5	1	2	4	8
1.5 g q8h (1h)	100%	97.5%	83.8%	70%	56.3%
1.5 g q8h (3h)	100%	100%	98.8%	84.6%	69.6%
1.5 g q6h (1h)	100%	100%	100%	94.6%	76.3%
1.5 g q6h (3h)	100%	100%	100%	99.6%	95.4%
3 g q8h (1h)	100%	100%	100%	92.5%	78.8%
3 g q8h (3h)	100%	100%	100%	100%	93.8%

Author Disclosure Block:

J.Y. Ang: E. Grant Investigator; Self; Astellas. H. Research Contractor; Self; Cubist. **N. Abdel-Haq:** None. **F. Zhu:** None. **A.K. Thabit:** None. **D.P. Nicolau:** None.

Poster Board Number:

FRIDAY-482

Publishing Title:

A Novel Kynureninase Inhibitor Reduces Quorum Sensing and Virulence Factor Expression in *Pseudomonas aeruginosa*

Author Block:

S. H. Kasper[1], R. P. Bonocora[2], J. T. Wade[2], R. A. Musah[3], **N. C. Cady**[1]; [1]SUNY Polytechnic Inst., Albany, NY, [2]New York State Dept. of Hlth., Albany, NY, [3]Univ. at Albany, SUNY, Albany, NY

Abstract Body:

Pseudomonas aeruginosa is an opportunistic pathogen that utilizes multiple quorum sensing (QS) systems to regulate virulence factor expression. In a high-throughput, phenotypic screen, we have previously identified a small molecule that does not affect bacterial viability, but is capable of interfering with *P. aeruginosa* biofilm formation as well as QS in a *las*-based reporter. This inhibitor, *S*-phenyl-L-cysteine sulfoxide, lacks structural similarity to any of the known *P. aeruginosa* autoinducers. Therefore, we sought a deeper understanding of the mechanism of action and the effects of this compound. In this work we performed a global transcriptomic analysis of *P. aeruginosa* in response to *S*-phenyl-L-cysteine sulfoxide using RNA-seq. This analysis revealed that the inhibitor down-regulates expression of several QS-regulated virulence operons. Of the genes that were differentially regulated, many are interrelated through the metabolic pathways that yield precursors of pyochelin, tricarboxylic acid cycle intermediates, phenazines, and the *Pseudomonas* quinolone signal (PQS). Upon examination of these pathways, we identified structural similarity between the inhibitor and kynurenine, a precursor of anthranilate, which is necessary for PQS production and critical for *P. aeruginosa* virulence. Biochemical analysis revealed that *S*-phenyl-L-cysteine sulfoxide is a competitive inhibitor of kynureninase, the enzyme responsible for the conversion of kynurenine to anthranilate. By adding exogenous anthranilate, the QS-inhibitory effect of *S*-phenyl-L-cysteine sulfoxide could be reversed in reporter assays. Finally, it was shown that *S*-phenyl-L-cysteine sulfoxide significantly reduces PQS production in vivo. Several studies have previously shown that mutant strains deficient in the kynurenine pathway are associated with reduced QS and virulence factor expression; however, this is the first study to demonstrate a small molecule that inhibits *P. aeruginosa* kynureninase, and associated QS and virulence systems, suggesting a potential anti-virulence strategy.

Author Disclosure Block:

S.H. Kasper: None. **R.P. Bonocora:** None. **J.T. Wade:** None. **R.A. Musah:** None. **N.C. Cady:** None.

Poster Board Number:

FRIDAY-483

Publishing Title:

Assessing Target Engagement *In Vitro* and *In Vivo* of Mvfr Inhibitors in *Pseudomonas aeruginosa* (Pa)

Author Block:

A. RUBIO[1], M. Antolini[2], E. Benetta[2], V. Costantini[2], A. Felici[2], S. Fontana[2], L. Rahme[3], J. Teague[4], P. Thommes[4], P. Turnpenny[5], P. Warn[4], T. Parr[1], M. Pucci[1], R. Zahler[1]; [1]Spero Therapeutics, Cambridge, MA, [2]Aptuit, Verona, Italy, [3]Massachusetts Gen. Hosp., Boston, MA, [4]Evotec, Manchester, United Kingdom, [5]Evotec, Abingdon, United Kingdom

Abstract Body:

Objective: Pa is a common opportunistic human pathogen and has developed resistance to many classes of the available antibiotics. MvfR is a global transcriptional regulator responsible for expression of many virulence factors, including 4-hydroxy-2-heptylquinoline (HHQ), 3,4-dihydroxy-2-heptoquinoline (PQS) and pyocyanin (PYO). PQS, HHQ and PYO are produced both *in vitro* and *in vivo* and provide a surrogate measurement to the MvfR IC_{50}. Here we describe the *in vitro* properties of a novel MvfR inhibitor, SPR00305, and measure target engagement *in vivo* using both neutropenic and immunocompetent acute models of Pa infection. **Methods:** IC_{50} values against biomarkers on the MvfR pathway were determined in the Pa strain PA14. PYO IC_{50} was measured in a deep 96 well colorimetric assay format adapted from the method described in Essar *et al* J Bacteriol 172: 884. HHQ and PQS IC_{50} were measured using the isotope-dilution method followed by LC-MS/MS analysis. Target engagement was measured *in vivo* as HHQ and PQS levels quantified from murine thigh tissues (both immunocompetent and neutropenic) infected with PA14. **Results:** SPR00305 was found to be a potent *in vitro* inhibitor of the MvfR pathway with IC_{50} values of 93 nM against PYO, 115 nM against HHQ and 109 nM against PQS in PA14. *In vivo* the analytical methods quantified HHQ and PQS from the immunocompetent acute thigh infection model with a sensitivity of 5 ng/mL as the LLQ. After oral administration, 200 mg/kg SPR00305 was found to decrease HHQ by 40% and PQS by 50% compared to vehicle. The analytical methods quantified HHQ from the neutropenic acute thigh infection model with a sensitivity of 1 pg/mL as the LLQ. SPR00305 administered orally (up to 200 mg/kg tid) induced a slight decrease in HHQ levels compared to vehicle in the neutropenic acute thigh infection model. **Conclusion:** SPR00305 represents a novel, potent MvfR inhibitor *in vitro* that also displays target engagement *in vivo*.

Author Disclosure Block:

A. Rubio: D. Employee; Self; Spero Therapeutics. **M. Antolini:** D. Employee; Self; Aptuit. **E. Benetta:** D. Employee; Self; Aptuit. **V. Costantini:** D. Employee; Self; Aptuit. **A. Felici:** D. Employee; Self; Aptuit. **S. Fontana:** D. Employee; Self; Aptuit. **L. Rahme:** N. Other; Self;

Spero Therapeutics. **J. Teague:** D. Employee; Self; Evotec. **P. Thommes:** D. Employee; Self; Evotec. **P. Turnpenny:** D. Employee; Self; Evotec. **P. Warn:** D. Employee; Self; Evotec. **T. Parr:** D. Employee; Self; Spero Therapeutics. **M. Pucci:** D. Employee; Self; Spero Therapeutics. **R. Zahler:** C. Consultant; Self; Spero Therapeutics.

Poster Board Number:

FRIDAY-484

Publishing Title:

Inhibition Of *pseudomonas Aeruginosa* By Peptide-Conjugated Phosphordiamidate Morpholino Oligomers (Ppmos)

Author Block:

C. R. Sturge[1], J. Howard[1], M. M. Yabe-Gill[2], M. Labandeira-Rey[1], K. Marshall-Batty[3], M. Wong[4], B. L. Geller[5], D. E. Greenberg[1]; [1]UT Southwestern Med. Ctr., Dallas, TX, [2]Univ. of Missouri, Columbia, MO, [3]Frederick Natl. Lab. for Cancer Res., Frederick, MD, [4]Sarepta Therapeutics, Cambridge, MA, [5]Oregon State Univ., Corvallis, OR

Abstract Body:

P. aeruginosa is a highly virulent multidrug-resistant pathogen that causes significant morbidity and mortality in a variety of patients, particularly in those with Cystic Fibrosis (CF). We utilize antisense oligomers, specifically PPMOs, for sequence-specific, species-specific silencing of target genes. We have previously demonstrated *in vitro* and *in vivo* success in targeting essential genes in other Gram-negative pathogens, and here attempt to use PPMOs to inhibit *P. aeruginosa*.PPMOs were designed to complement known or putative essential genes in *P. aeruginosa* PAO1. When co-incubated with colistin (Polymyxin B) or Polymyxin B Nonapeptide (PMBN) at sub-inhibitory concentrations (2 μg/ml), a number of PPMOs were found to be inhibitory in a panel of *P. aeruginosa* strains with IC_{50} concentrations of 1-4 μM (in Mueller-Hinton media) for the essential genes *acpP* (acyl carrier protein), *rpsJ* (30S ribosomal protein S10) and *lpxC* (UDP-(3-*O*-acyl)-N-acetylglucosamine deacetylase). Growth inhibition was enhanced when PPMOs were screened in minimal media (MOPS). Lead PPMOs against these targets prevented *P. aeruginosa* biofilm formation. Importantly, PPMOs were capable of penetrating pre-formed biofilms, reducing biofilm thickness from 20-40 μM to <10 μM as assessed by confocal microscopy of GFP-expressing PAO1. Furthermore, examination of combinations of PPMOs and clinically relevant antibiotics (such as tobramycin) revealed that they could act synergistically to reduce the growth of *Pseudomonas*.These are the first studies demonstrating that PPMOs are effective against the *P. aeruginosa* essential gene targets *acpP*, *rpsJ* and *lpxC* and can be used synergistically with conventional antibiotics. Efficacy of PPMOs is enhanced by the presence of sub-inhibitory amounts of PMBN and may serve as a future co-delivery adjuvant.

Author Disclosure Block:

C.R. Sturge: None. **J. Howard:** None. **M.M. Yabe-Gill:** None. **M. Labandeira-Rey:** None. **K. Marshall-Batty:** None. **M. Wong:** D. Employee; Self; Sarepta Therapeutics. **B.L. Geller:** C. Consultant; Self; Sarepta Therapeutics. N. Other; Self; Inventor and Patents Pending,

Sarepta Therapeutics. **D.E. Greenberg:** E. Grant Investigator; Self; Research funding, Sarepta Therapeutics. N. Other; Self; Inventor and Patents Pending, Sarepta Therapeutics.

Poster Board Number:

FRIDAY-485

Publishing Title:

Inhibition Of Biofilm And Quorum-Sensing Pathways In *pseudomonas Aeruginosa* by Peptide-Conjugated Phosphorodiamidate Morpholino Oligomers (Ppmos)

Author Block:

C. R. Sturge[1], J. Howard[1], H. Justice[1], M. Wong[2], B. L. Geller[3], D. E. Greenberg[1]; [1]UT Southwestern Med. Ctr., Dallas, TX, [2]Sarepta Therapeutics, Cambridge, MA, [3]Oregon State Univ., Corvallis, OR

Abstract Body:

Given the global crisis of increasing antibiotic resistance, the development of novel antibiotics is of the utmost importance. We utilize antisense oligomers, specifically PPMOs, for sequence-specific, species-specific silencing of target genes. We have previously demonstrated *in vitro* and *in vivo* success in targeting essential genes in various Gram-negative pathogens. Here we focus on targeting virulence mechanisms, specifically biofilm production pathways in the major human pathogen *Pseudomonas aeruginosa*.*Pseudomonas* biofilms were grown utilizing a minimum biofilm eradication concentration (MBEC™) assay. Biofilm production was measured utilizing crystal violet staining with measurements at OD570. *P. aeruginosa* PAO1 was used to screen for the prevention of biofilm utilizing 25 PPMOs targeting various genes involved in biofilm or quorum-sensing. Polymyxin B nonapeptide (PMBN) at sub-inhibitory concentrations was used to enhance entry of PPMOs into *Pseudomonas*. Six potential lead PPMOs were identified and further screened for activity against multidrug-resistant clinical strains from patients with cystic fibrosis (CF). 3 PPMOs targeting the genes *algZ* (alginate biosynthesis), *algU* (sigma factor) and *lasR* (transcriptional regulator) displayed a greater than 50% reduction in biofilm formation at 18 hours (compared to no treatment controls) in 80% of the strains tested. In the strains that were tested, PPMO concentrations needed to reduce biofilm to this degree ranged from 8 μM to at or below 0.5 μM. Confocal microscopy of GFP-PAO1 displayed markedly reduced biofilm formation with these 3 PPMOs compared to no PPMO or a scrambled-sequence control PPMO. Lead PPMOs did not cause cytotoxicity in cell culture.The use of PPMOs to target non-essential genes has the theoretical advantages of circumventing classic pressures that can select for drug resistance. These are the first studies demonstrating that PPMOs are effective against virulence mechanisms (biofilm formation) in the major human pathogen *P. aeruginosa*.

Author Disclosure Block:

C.R. Sturge: None. **J. Howard:** None. **H. Justice:** None. **M. Wong:** D. Employee; Self; Sarepta Therapeutics. **B.L. Geller:** E. Grant Investigator; Self; Research funding, Sarepta Therapeutics. N. Other; Self; Inventor and Patents Pending, Sarepta Therapeutics. **D.E.**

Greenberg: E. Grant Investigator; Self; Research funding, Sarepta Therapeutics. N. Other; Self; Inventor and Patents Pending, Sarepta Therapeutics.

Poster Board Number:

FRIDAY-486

Publishing Title:

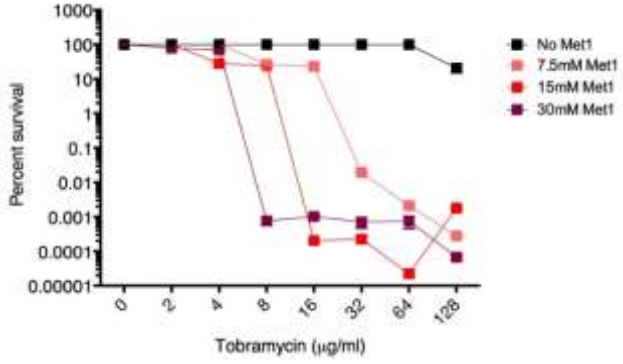
A Novel Combination of Tobramycin with a Potentiator for the Treatment of Chronic *Pseudomonas aeruginosa* Infections

Author Block:

M. Koeva[1], A. D. Gutu[2], F. M. Ausubel[2], **D. Joseph-McCarthy**[1]; [1]EnBiotix, Inc., Cambridge, MA, [2]Massachusetts Gen. Hosp., Boston, MA

Abstract Body:

Background: EBX-001, a combination of tobramycin with a bacterial metabolite as a potentiator, is being developed for the treatment of chronic *P. aeruginosa* infections in Cystic Fibrosis patients. The combination utilizes an anti-persisters strategy and is aimed at reducing recurrent infections.**Methods:** *P. aeruginosa* cultures in the planktonic stationary phase (PSP) were used to select for bacterial persisters, bacteria in a quasi-dormant state. In these PSP experiments, a range of tobramycin concentrations was tested with a range of metabolite concentrations to determine the potentiation effect of the metabolite under a variety of conditions. MICs were also determined for a variety of CF clinical isolates to select a diverse set of strains for inclusion in the study.**Results:** Enhanced killing of up to 6 orders of magnitude of *P. aeruginosa* persisters for a range of strains was observed (see example in the figure below).**Conclusions:** A combination of tobramycin with a potentiator remains an attractive therapeutic option for eliminating recurrent *P. aeruginosa* infections through the eradication of bacterial persisters.



Author Disclosure Block:

M. Koeva: D. Employee; Self; EnBiotix, Inc.. K. Shareholder (excluding diversitied mutual funds); Self; EnBiotix, Inc. **A.D. Gutu:** B. Collaborator; Self; EnBiotix. **F.M. Ausubel:** B. Collaborator; Self; EnBiotix. **D. Joseph-McCarthy:** D. Employee; Self; EnBiotix, Inc.. K. Shareholder (excluding diversitied mutual funds); Self; EnBiotix, Inc..

Poster Board Number:

FRIDAY-487

Publishing Title:

Alginate Oligomer Oligog Cf-5/20 Treatment of *Pseudomonas aeruginosa* Grown in Artificial Sputum Medium

Author Block:

L. C. Powell[1], M. F. Pritchard[1], A. A. Jack[1], E. Onsøyen[2], P. D. Rye[2], K. E. Hill[1], D. W. Thomas[1]; [1]Cardiff Univ., Cardiff, United Kingdom, [2]AlgiPharma AS, Sandvika, Norway

Abstract Body:

Background: Traditional *in vitro* antimicrobial testing of cystic fibrosis (CF) isolates has been shown to relate poorly to *in vivo* test conditions. An artificial sputum medium (AS) model has been developed to mimic the restricted nutritional environment of sputum, and more closely resemble growth conditions of mucoid *Pseudomonas aeruginosa* in the CF lung. The antimicrobial effects of the low molecular weight alginate oligomer, OligoG CF-5/20, (previously shown to disrupt pseudomonal biofilms and potentiate antibiotics) was tested in this low nutrient medium. **Methods:** Susceptibility of the CF clinical isolate *P. aeruginosa* NH57388**A** to OligoG (2-6%) ± the commonly-inhaled antibiotic colistin (0.05-0.2 µg/ml) was compared in Mueller-Hinton (MH) and AS media using growth curves (36 h; n=3), with antibiofilm effects visualized using LIVE/DEAD® confocal laser scanning microscopy (CLSM) and quantified using COMSTAT software analysis (n=6) and scanning electron microscopy. Acyl-homoserine lactones (AHLs) were extracted from planktonic cells grown in AS ±OligoG (24 h) and quantified using high performance mass spectrometry (n=3). **Results:** Growth curves demonstrated a synergistic decrease in bacterial growth when treated with OligoG and colistin in MH. OligoG maintained its effectiveness in AS, colistin alone however, was less capable of hindering bacterial growth. CLSM using MH, demonstrated cellular aggregation and decreased bacterial viability when OligoG was tested in conjunction with colistin, reflecting an enhanced combined antimicrobial effect ($P<0.05$). Growth was significantly lower in AS compared to MH, with a 4-fold decrease in the minimum inhibitory concentrations (MICs) for colistin (0.4 Vs 0.1 mg/ml in MH). Distinct bacterial micro-colonies formed in AS, which were considerably smaller when treated with OligoG ($P<0.05$). The effect of OligoG on pseudomonal quorum sensing was confirmed by dose-dependent decreases in C4-AHL production ($P<0.05$). **Conclusions:** The pronounced differences observed in the two media, reflect the role of environmental conditions on the effectiveness of antimicrobial therapies. The ability of OligoG to exhibit its anti-bacterial and anti-biofilm properties under these nutrient conditions was evident.

Author Disclosure Block:

L.C. Powell: I. Research Relationship; Self; AlgiPharma AS. **M.F. Pritchard:** I. Research Relationship; Self; AlgiPharma AS. **A.A. Jack:** I. Research Relationship; Self; AlgiPharma AS.

E. Onsøyen: D. Employee; Self; AlgiPharma AS. **P.D. Rye:** D. Employee; Self; AlgiPharma AS. **K.E. Hill:** I. Research Relationship; Self; AlgiPharma AS. **D.W. Thomas:** I. Research Relationship; Self; AlgiPharma AS.

Poster Board Number:

FRIDAY-488

Publishing Title:

Oligog Cf-5/20 Reduces *Pseudomonas aeruginosa* Biofilm Formation and Virulence via Quorum Sensing Inhibition

Author Block:

A. Jack[1], S. Khan[1], H. Sadh[1], E. Onsøyen[2], P. Rye[2], D. Thomas[1], **K. E. Hill**[1]; [1]Cardiff Univ., Cardiff, United Kingdom, [2]Algipharma AS, Sandvika, Norway

Abstract Body:

Background: *Pseudomonas aeruginosa* is a nosocomial, opportunistic pathogen, able to infect almost all areas of the human body. Virulence in *P. aeruginosa* is regulated by the quorum sensing (QS) Las and Rhl pathways. OligoG CF-5/20 is an alginate previously shown to reduce biofilm formation, as well as QS regulated motility and swarming in *P. aeruginosa.* OligoG could therefore directly affect QS signalling in *P. aeruginosa***Methods:** Cell-free supernatants of OligoG (0.2-10%)-treated *P. aeruginosa* PAO1 virulence factor (pyocyanin, rhamnolipids, elastase, protease) production was monitored using spectrophotometric assays (Mueller Hinton broth, MH; 24 h). A *Chromobacterium violaceum* CV026 bio-sensor assay determined expression of acyl homoserine lactones (AHLs), C4-AHL and C12-AHL (controlled by Las and Rhl respectively) in PAO1 ± OligoG (0.2-10%) with AHL extracts from PAO1 grown in MH (12, 18, 24, 30 h) and Artificial Sputum (AS) medium (30, 48 h) quantified using liquid chromatography mass spectrometry (LC/MS). SYBR-Green Real-time RT-PCR (qPCR) was used to characterise the effect of OligoG on expression of QS genes *lasI, lasR, rhlI, rhlR, pvdQ, pqsE* and *pilE*. Dry weight was used to normalise differences in culture biomass.**Results:** Virulence factor assays showed decreasing production of pyocyanin and rhamnolipid (at 0.2 and 10% OligoG; $P<0.05$) and of elastase and protease at 10% ($P<0.05$). Significant reductions at 2 and 10% were also seen in AHL production of both C12-AHL and C4-AHL. LC/MS showed decreasing production of C12-AHL and C4-AHL over time, (with a 3-fold greater overall yield of C4 than C12), and with the greatest concentrations seen at the earliest time point (12 h). In contrast, *P. aeruginosa* grown in nutrient limited AS medium showed extremely low concentrations of both AHLs which decreased over time for C12-AHL (being greatest at 10% OligoG 30 h) with surprisingly similar levels of C4-AHL seen for both time points. A significant decrease in gene expression was seen for *lasI/R* at 0.2, 2 and 10%, while a decrease was also seen for *rhlR* at 2 and 10% and *rhlI* at 10% ($p<0.05$).**Conclusions:** Expression of Las and Rhl QS pathways were altered in a time-dependent manner following OligoG treatment, offering insight into the molecular mechanism by which OligoG induces its antibiofilm effect.

Author Disclosure Block:

A. Jack: I. Research Relationship; Self; Algipharma AS. **S. Khan:** I. Research Relationship; Self; Algipharma AS. **H. Sadh:** I. Research Relationship; Self; Algipharma AS. **E. Onsøyen:** D. Employee; Self; Algipharma AS. **P. Rye:** D. Employee; Self; Algipharma AS. **D. Thomas:** D. Employee; Self; Algipharma AS. **K.E. Hill:** I. Research Relationship; Self; Algipharma AS.

Poster Board Number:

FRIDAY-489

Publishing Title:

Sasp: A Novel Antibacterial Targeting *Pseudomonas aeruginosa* Demonstrating a Rapid Mode of Action and Low Propensity for Resistance Development

Author Block:

A. L. Castillo, S. Cullen, A. S. Nepal, J. Cass, A. Wilkinson, **H. Fairhead**; Phico Therapeutics, Cambridge, United Kingdom

Abstract Body:

Background: For novel antibacterials to be successful it is critical that they demonstrate a low propensity for the development of resistance. SASPject is a unique antibacterial technology utilising engineered gene delivery vehicles to inject the gene encoding SASP, a protein found in bacterial spores, into target pathogens. The unique mode of action of SASP, which binds to DNA in a non-sequence specific manner to prevent DNA replication and gene transcription, coupled with rapid bactericidal activity, suggests SASP has a low propensity to induce resistance compared to other antibiotics. Here the potential for resistance of *Pseudomonas aeruginosa* (*Pa*) to develop in response to SASPject PT3.8 was measured under both a 24 h exposure regime and in a 52-day passaging study.**Methods:** Passaging studies: *Pa* 3503 and 2923 cultures at ~10^6 cfu/ml in Luria Bertani broth supplemented with magnesium, calcium, and glucose (LB+) were passaged daily after 24 h growth at 37 °C in the presence of *Pa* SASPject at 1 x 10^7, 5 x 10^6, 2.5 x 10^6 and 1.25 x 10^6 SASPject PT3.8 Units (U)/ml. A control without added PT3.8 was passaged in parallel. Susceptibility of the cultures to PT3.8 was assessed daily by 3 h kill assays. 24 h exposure regime: 4 *Pa* strains were assayed by time-kill curve over 24 h, and remaining bacterial survivors isolated and tested for sensitivity in a 3 h kill.**Results:** During long-term exposure studies, using 3 sub-inhibitory concentrations of PT3.8, no decrease in sensitivity was observed during 21 and 52 days of passaging *Pa* strains 2923 and 3503, respectively. During 24 hour exposure to bactericidal concentrations of PT3.8, bacterial kill was rapid with numbers declining to within the limit of detection within 1 hour for all strains tested. Survivors isolated after 24 hours of exposure to PT3.8 remained highly sensitive to PT3.8.**Conclusions:** The unique mode of action of SASP means that mutations in bacterial DNA cannot lead to resistance as SASP inactivates bacterial DNA in a sequence-independent manner. This is supported by the data presented here, where resistance did not develop in the *Pa* strains tested in either a 24 h kill assay or within 52 days of passaging with PT3.8. In conclusion the SASPject technology platform displays a low propensity for resistance development in the treatment of *Pa*.

Author Disclosure Block:

A.L. Castillo: None. **S. Cullen:** None. **A.S. Nepal:** None. **J. Cass:** None. **A. Wilkinson:** None. **H. Fairhead:** None.

Poster Board Number:

FRIDAY-490

Publishing Title:

Characterization of the Interaction with, and Disruption of Mucoid *Pseudomonas aeruginosa* Biofilms by the Alginate Oligosaccharide Oligog Cf-5/20

Author Block:

L. C. Powell[1], M. F. Pritchard[1], E. L. Ferguson[1], K. A. Powell[1], H. Sadh[1], E. Onsøyen[2], P. D. Rye[2], K. E. Hill[1], D. W. Thomas[1]; [1]Cardiff Univ., Cardiff, United Kingdom, [2]AlgiPharma AS, Sandvika, Norway

Abstract Body:

Background: Biofilm-associated infections are a major cause of morbidity in chronic respiratory diseases such as cystic fibrosis (CF); resisting antibiotic/antimicrobial therapy and host immune responses. A novel alginate oligosaccharide, OligoG CF-5/20, potentiates antibiotic activity against multi-drug resistant bacteria. In this study, the effects of OligoG on the establishment and disruption of pseudomonal mucoid biofilms was characterized and quantified.**Methods:** A mucoid *Pseudomonas aeruginosa* (NH57388A) CF isolate was used to determine the effect of OligoG (0.5, 2, 6%) on biofilm formation (24 h) and on disruption following OligoG treatment of pre-established biofilms (24 h), grown *in vitro* in Mueller Hinton broth (MHB) at 37ºC. OligoG was conjugated to the fluorophore Texas Red (TxRd®) cadaverine to determine the diffusion of OligoG through the biofilm extracellular polysaccharide (EPS) matrix. Confocal laser scanning microscopy (CLSM) with LIVE/DEAD®, Sypro Ruby Biofilm Matrix® and SYTO 9® stains was used for biofilm imaging and COMSTAT image analysis software used to quantify the physical properties of the biofilm. Supernatant removed from the biofilms was stained with crystal violet and the OD_{595} was measured to quantify displaced planktonic cells.**Results:** OligoG treatment impaired formation of *P. aeruginosa* biofilms in a dose-dependent manner, resulting in a significant reduction in bacterial biomass and mean biofilm thickness ($p<0.05$), with increased EPS matrix porosity. Planktonic cells from the biofilm supernatant were significantly increased at 0.2 and 0.5% OligoG (but not at 2 and 6%) compared to the control ($p<0.05$). Established biofilms were also disrupted following 24 h OligoG treatment, leading to biofilm height reduction ($p<0.05$) and disruption of the EPS matrix. TxRd®-labelled OligoG penetrated through the entire biofilm depth; TxRd® alone showed no evidence of biofilm disruption.**Conclusions:** The ability of OligoG to inhibit biofilm formation and diffuse into and disrupt established biofilm structures highlights the potential for this novel therapy to be used as a treatment of biofilm-associated infections in CF, and other biofilm-mediated diseases.

Author Disclosure Block:

L.C. Powell: I. Research Relationship; Self; AlgiPharma AS. **M.F. Pritchard:** I. Research Relationship; Self; AlgiPharma AS. **E.L. Ferguson:** I. Research Relationship; Self; AlgiPharma AS. **K.A. Powell:** I. Research Relationship; Self; AlgiPharma AS. **H. Sadh:** I. Research Relationship; Self; AlgiPharma AS. **E. Onsøyen:** D. Employee; Self; AlgiPharma AS. **P.D. Rye:** D. Employee; Self; AlgiPharma AS. **K.E. Hill:** I. Research Relationship; Self; AlgiPharma AS. **D.W. Thomas:** I. Research Relationship; Self; AlgiPharma AS.

Poster Board Number:

FRIDAY-491

Publishing Title:

Polymyxin (Pm) Resistance in *Pseudomonas aeruginosa* (Pa): Metabolomic Changes Underpin Lipid A Modifications

Author Block:

M-L. Han, Y. Zhu, S-E. Cheah, M. D. Johnson, H. H. Yu, H-H. Shen, D. J. Creek, T. Velkov, J. Li; Monash Univ., Melbourne, Australia

Abstract Body:

Background: Modification of lipid A with positively charged 4-amino-4-deoxy-L-arabinose (L-Ara4N) is a major mechanism of PM resistance in PA[1]. However, the metabolomic changes due to lipid A modifications have not been well characterized in PM-resistant PA.**Methods:** Structural analysis of lipid A was performed with ESI-MS in the negative ion mode with PM-susceptible PAK (PMB MIC 1 mg/L) and -resistant PAK*pmrB6* (MIC 16 mg/L)[2]. The influence of PM treatment on lipid A modifications was investigated with both strains (initial inoculum at ~10^6 CFU/mL) in the presence of 1×MIC PMB. The metabolomes of PAK and PAK*pmrB6* were studied using LC-MS with samples collected at mid-logarithmic phase (OD_{600nm} 0.5) without PMB treatment. Global metabolic changes in both strains were examined with multivariate and univariate analyses. The synthesis pathway of L-Ara4N involved in the lipid A modification was analyzed.**Results:** Structural analysis of lipid A from the wild-type PAK strain and the *pmrB* mutant (PAK*pmrB6*) showed penta- and hexa-acylated lipid A species, differing in the absence or presence of 3-hydroxydecanoate acyl chain. The structures of lipid A from PAK*pmrB6* without PMB treatment revealed modifications with one or two L-Ara4N, but not with the PAK strain. In the presence of 1×MIC PMB, L-Ara4N modified lipid A was detected in PAK at 24 h; however, in PAK*pmrB6*, the proportion of the modified lipid A did not changed over 24 h. Interestingly, the relative abundance of penta-acylated lipid A (i.e. removal of 3-hydroxydecanoate) in both strains substantially increased in response to PMB treatment at 24 h. The global metabolomics revealed 30% decrease of total glycerophospholipids in PAK*pmrB6*, compared to PAK. Remarkably, metabolic pathway analysis on the synthesis of L-Ara4N showed the intracellular level of UDP-glucuronate in PAK*pmrB6* increased ~400 folds compared to PAK, while UDP-L-Ara4N, UDP-L-Ara4FN and undecaprenyl phosphate-L-Ara4N were only detected in PAK*pmrB6*.**Conclusions:** For the first time, our study revealed significant metabolomic changes in the synthesis pathway of L-Ara4N in a PM-resistant PA strain. Importantly, besides addition of L-Ara4N, lipid A deacylation and reduction of membrane glycerophospholipids also contribute to PM resistance in PA.

Author Disclosure Block:

M. Han: None. **Y. Zhu:** None. **S. Cheah:** None. **M.D. Johnson:** None. **H.H. Yu:** None. **H. Shen:** None. **D.J. Creek:** None. **T. Velkov:** None. **J. Li:** None.

Poster Board Number:

FRIDAY-492

Publishing Title:

Phenotypic and Genomic Evaluation of Isogenic Mutants of *Pseudomonas aeruginosa* (Pa) Hypersusceptible to Multiple Classes of Antibiotics

Author Block:

L. B. Harrison[1], R. C. Fowler[2], N. D. Hanson[1]; [1]Creighton Univ., Omaha, NE, [2]Univ of NE Med Ctr, Omaha, NE

Abstract Body:

Background: : PA colonized in the lungs of cystic fibrosis patients are exposed to a myriad of antibiotics. Hypersusceptibility of PA to multiple antibiotic drug classes has been reported but the genetic mechanisms responsible for this phenotype remain unclear. The purpose of this study was to use phenotypic, molecular and genomic data to characterize chromosomal modifications leading to a hypersusceptible phenotype in isogenic mutants of PA. **Methods:** Mutants were generated from the clinical isolate PA34 by exposure to subinhibitory concentrations of doripenem or meropenem. MICs were determined by agar dilution. Two carbapenem-resistant mutants (PA34-822D and PA34-812M) hypersusceptible to non-carbapenem antibiotics were evaluated. Gene expression was evaluated by RT-qPCR and OprD production was evaluated by SDS-PAGE and Coomassie Blue. Strains were sequenced on the Illumina MiSeq and SNPs verified by IGV visualization. Lipid polysaccharide (LPS) profiles were evaluated by SDS-PAGE and visualized with ethidium bromide. **Results:** : MICs for imipenem, meropenem and doripenem in PA34 were 1, 0.12 and 0.25 µg/mL while both mutants had MICs of 8, 1 and 2 µg/mL, respectively. Both mutants were hypersusceptible to aminoglycosides, fluoroquinolones and non-carbapenem β-lactams. Compared to PA34 no change in expression was observed in ampC or the mex-opr efflux pump systems. Outer membrane protein analysis showed decreased OprD production. Genomic analysis revealed 4 SNPs in PA34-812M and 5 SNPs in PA34-822D compared to PA34. SNP induced amino acid substitutions in both mutants included E108D in AlgU and G77C in LptG. SNP induced truncations of OprD at loop 7 in PA-34812M and loop 8 in PA34-822D were observed. Preliminary LPS gel evaluation indicated modified rough LPS profiles in PA34-812M and PA34-822D compared to PA34.**Conclusions:** These data show that upon emergence of carbapenem resistance mutations can occur which lead to a hypersusceptible phenotype. Evaluation of these hypersusceptible isogenic mutants identified only two candidate genes for this phenotype, *algU* and *lptG*. Modificaitons in the LPS profiles observed in the mutants suggests that the amino acid substitutions found in LptG interfere with LPS transport and may contribute to the hypersusceptible phenotype observed.

Author Disclosure Block:

L.B. Harrison: F. Investigator; Self; Streck. **R.C. Fowler:** F. Investigator; Self; Johnson and Johnson. **N.D. Hanson:** E. Grant Investigator; Self; Streck.

Poster Board Number:

FRIDAY-493

Publishing Title:

Bacteriophage-Driven Inhibition of Biofilm in *Pseudomonas aeruginosa*

Author Block:

A. Negut[1], O. Sandulescu[1], **A. Streinu-Cercel**[1], C. Bleotu[2], D. Talapan[1], C. Grancea[3], O. Dorobat[1], C. Chifiriuc[4], M. Popa[1], A. Streinu-Cercel[1]; [1]Carol Davila Univ. of Med. and Pharm; Natl. Inst. Infect Dis Matei Balș, Bucharest, Romania, [2]Ș. Nicolau Inst Virol;Univ. Buc, Bucharest, Romania, [3]Ș Nicolau Inst Virol, Bucharest, Romania, [4]Univ. Buc, Bucharest, Romania

Abstract Body:

Background: *P. aeruginosa*, a Gram-negative opportunistic pathogen, has become a serious threat especially for immunocompromised patients, associating high biofilm burden. **Methods:** We have tested the impact of binary dilution of commercial phages on biofilm inhibition in 44 clinically relevant strains of *P. aeruginosa* collected from patients admitted in a Romanian national reference center following the protocol presented in Neguț et al., GERMS 2014.**Results:** All patients were Caucasian, 56.8% (25/44) were males, 15.9% (7/44) had diabetes mellitus and 59.1% (29/44) were evaluated as Carmeli 3. *P. aeruginosa* was isolated mostly from urinary infections (17/44) and skin infections (15/44). Antibiotic susceptibility was 51.4% for piperacillin < 52.5% piperacillin-tazobactam and ceftazidime < 56.8% imipenem < 100% colistin. Susceptibility to phages was 68.9% for PYO and 66.7% for INTESTI, with 35.6% for both PHAGYO and PHAGESTI.We performed the PYO and INTESTI assays for 31 and 32 strains, respectively. Most strains formed strong biofilm, 80.6% (25/31) in the first experiment and 78.1% (25/32) in the second one.In both experiments, all phage dilutions significantly decreased optical density (OD) of the culture (p=0.001) even if for the highest concentration of phages, complete biofilm inhibition occurred in 6/31 strains in the PYO assay, and 12/32 in the INTESTI assay, respectively. (Table 1)**Conclusion:** In our experiment a higher susceptibility occurred for phages compared with antibiotics, in clinically significant strains. Biofilm development significantly decreased when adding phages. All these data suggest that bacteriophages should be further investigated as options for hard to treat infections.Table 1. Effect of phages on biofilm

	OD culture	OD 1/64 phage dilution	OD 1/2 phage dilution
INTESTI	0.241	0.163	0.130
PYO	0.225	0.156	0.129
p-value INTESTI	NA	**0.000***	**0.000***

p-value PYO	NA	**0.001***	**0.000***

* p-value for comparing ODs for culture vs. phage dilution.

Author Disclosure Block:

A. Negut: None. **O. Sandulescu:** None. **A. Streinu-Cercel:** None. **C. Bleotu:** None. **D. Talapan:** None. **C. Grancea:** None. **O. Dorobat:** None. **C. Chifiriuc:** None. **M. Popa:** None. **A. Streinu-Cercel:** None.

Poster Board Number:

FRIDAY-494

Publishing Title:

Validation of a Porcine Wound Model for *Acinetobacter baumannii* Infection

Author Block:

C. C. Black, Y. Alamneh, R. K. Kim, C. L. Jones, M. G. Thompson, L. Biggeman, R. Patel, C. L. Honnold, X. Feng, C. Paranavitana, S. D. Tyner, **D. V. Zurawski**; Walter Reed Army Inst. of Res., Silver Spring, MD

Abstract Body:

Patients exposed to combat injury often require weeks of hospitalization increasing the risk of wound and surgical-site soft tissue infections (SSTI). Highlighting this, during the recent wars in Iraq and Afghanistan infection rates were 25% in casualties and approached 50% in patients admitted to an ICU. In particular, these infections are mediated in large part by multidrug-resistant (MDR) ESKAPE pathogens. Of these, *A. baumannii* is still relatively understudied, but novel antibacterials are needed because even colistin-resistant strains are now emerging. While many have developed murine models to evaluate antibacterials, a large animal model is lacking. To facilitate understanding *A. baumannii* pathogenesis and drug discovery, we present a porcine full thickness, excisional wound model of *A. baumannii* infection using a clinically-relevant MDR-*A. baumannii* isolate, AB5075, that proliferates to 10^8 CFU/g in tissue, forms biofilm, and can then be effectively treated with an antibiotic. The model requires cyclophosphamide-induced neutropenia to establish a SSTI that persists beyond seven days. Multiple 12 mm diameter wounds are created in the skin overlying the cervical and thoracic dorsum. Wound beds are inoculated with 5.0×10^4 AB5075 and covered with a dressing. The positive control, polymyxin B, is applied topically and endpoints are assessed using multiple quantitative and qualitative techniques to include gross pathology, weight gain, CFU burden, histopathology, peptide nucleic acid fluorescence in situ hybridization (PNA-FISH), and scanning electron microscopic (SEM) assessment of biofilm. Polymyxin B treatment resulted in a 3 $\log_{10}$ reduction of CFU/g tissue on Day 2 of treatment. By Day 6, there were on average 3.79×10^8 CFU/g AB5075 in wound tissue in untreated wounds, but no bacteria could be detected in treated samples. These measures provide a clear therapeutic window where novel antimicrobial therapies can be assessed. This model will be used to evaluate systemic and topical compounds for the ability to reduce pathogen load and clear biofilms. The combination of our porcine model with our murine wound model allows for a robust, multi-faceted *in vivo*, pre-clinical assessment of novel antibacterials for SSTI prior to testing in clinical trials.

Author Disclosure Block:

C.C. Black: None. **Y. Alamneh:** None. **R.K. Kim:** None. **C.L. Jones:** None. **M.G. Thompson:** None. **L. Biggeman:** None. **R. Patel:** None. **C.L. Honnold:** None. **X. Feng:** None. **C. Paranavitana:** None. **S.D. Tyner:** None. **D.V. Zurawski:** None.

Poster Board Number:

FRIDAY-495

Publishing Title:

Development and Validation of a Novel Ovine Model of Catheter Associated Urinary Tract Infections Caused by Uropathogenic *E. coli* (Upec)

Author Block:

P. J. Plummer, O. Sahin, K. Mullin, M. Beal, M. Ackermann, N. L. Barbieri, G. Li, Q. Zhang, C. Logue, and L. Nolan; Iowa State Univ., Ames, IA

Abstract Body:

Catheter associated urinary tract infections (CAUTI) are important and common cause of nosocomial infections in modern health care. While CAUTIs are typically polymicrobial in nature, uropathogenic *Escherichia coli* are commonly involved and can lead to significant clinical disease. Despite the clinical significance of CAUTIs, there are few clinically relevant animal models of the disease that can be used to study the host-pathogen interactions associated with this process. The goal of this study was to develop and validate an ovine (sheep) model of CAUTI that could be used to further our understanding of the host-pathogen interaction during infection with UPEC. For this study, eight mature female cross-bred sheep were divided into three groups, which included an uninoculated control group having two sheep , and two treatment groups each having three animals that were inoculated with UPEC. Prior to the start of the study all sheep had indwelling 14Fr x 9 inch radiopaque silicone Foley catheter with a 10 cc balloon placed through the external os of the urethra into the urinary bladder. The external end of the catheter was stabilized using a Bard Medical StatLock® Foley Device that was glued to the inner thigh of the ewe. On day zero of the study the two treatment groups were inoculated with one of two UPEC strains, UPEC CFT073 or *E. coli* ec958. CBC, serum chemistry, coagulation profiles and urinalysis were performed daily throughout the two-week study. Indwelling silicone Foley catheters were successfully maintained for the 16-day study in 7 of the eight sheep (one was removed at day 10 due to occlusion by a bacterial biofilm). Both strains of UPEC demonstrated immediate and long-term colonization of the bladder with urinalysis counts averaging between 10^5-10^8 CFU/ml from day 1 through day 14. At necropsy one treatment animal had severe pyelonephritis. All inoculated animals were culture positive in the ureters as were the majority in the renal pelvis, whereas neither of the control sheep developed any signs of infection nor yielded culture positive specimen.. This data indicates that the ovine indwelling urinary catheter model could replicate human CAUTI with high-level consistent colonization of the bladder with UPEC and subsequent development of clinical disease.

Author Disclosure Block:

P.J. Plummer: None. **O. Sahin:** None. **K. Mullin:** None. **M. Beal:** None. **M. Ackermann:** None. **N.L. Barbieri:** None. **G. Li:** None. **Q. Zhang:** None.

Poster Board Number:

FRIDAY-496

Publishing Title:

A Novel Foreign Body Osteomyelitis Model That Allows for Quantitative Cultures of the Infection Site Over Multiple Time-points

Author Block:

C. L. Brinkman, S. Schmidt-Malan, R. Patel; Mayo Clinic, Rochester, MN

Abstract Body:

In previous studies, we have utilized a rat model of foreign body osteomyelitis that involves implantation of a stainless steel wire into the intramedullary cavity of the rat tibia. One disadvantage to this model is that samples for culture are only collected at sacrifice, at which time bone and wire may be removed and quantitatively cultured. We sought to develop a model that would allow us to remove samples for culture at multiple time-points from the same animal.A novel rat foreign body osteomyelitis was established in six male Wistar rats using three stainless steel wires per animal. Three 1 mm holes were drilled into the left tibia using a bone drill. Stainless steel wires, 5 mm in length, were placed into the holes, perpendicular to the tibia, and dental gypsum was placed on the bone to secure the wires into place. Wires were seeded with either methicillin-resistant *Staphylococcus aureus* or *Staphylococcus epidermidis* by incubating wires in a 1.0 McFarland culture at 37°C, shaking at 140 rpm, for 2 hours prior to implantation. Infection was established for 28 days, after which wires were removed from the animals at one week intervals and quantitatively cultured to determine bacterial quantities.Wires from animals infected with *S. aureus* had a bacterial quantity of 4.81 $\log_{10}$ CFU/cm^2 at implantation and had an average bacterial quantity of 3.56, 3.60 and 5.51 $\log_{10}$ CFU/cm^2 at 5, 6 and 7 weeks post-infection, respectively. Wires removed from animals infected with *S. epidermidis* had an initial bacterial quantity of 4.61 $\log_{10}$ CFU/cm^2 and had an average bacterial quantity of 2.08, 2.17 and 2.62 $\log_{10}$ CFU/cm^2 at 5, 6 and 7 weeks post-infection, respectively. There was no contamination of either the wire or infection site over the course of the study.We have established a novel model using wires seeded with either *S. aureus* or *S. epidermidis*. This model will be used in the future to determine the kinetics of response to therapy and emergence of antimicrobial resistance during treatment of foreign body infection. Being able to sample bacteria at multiple time points from the same animal decreases the number of animals needed for an experiment and provides the ability to monitor foreign body infections in the same animal over time.

Author Disclosure Block:

C.L. Brinkman: None. **S. Schmidt-Malan:** None. **R. Patel:** None.

Poster Board Number:

FRIDAY-497

Publishing Title:

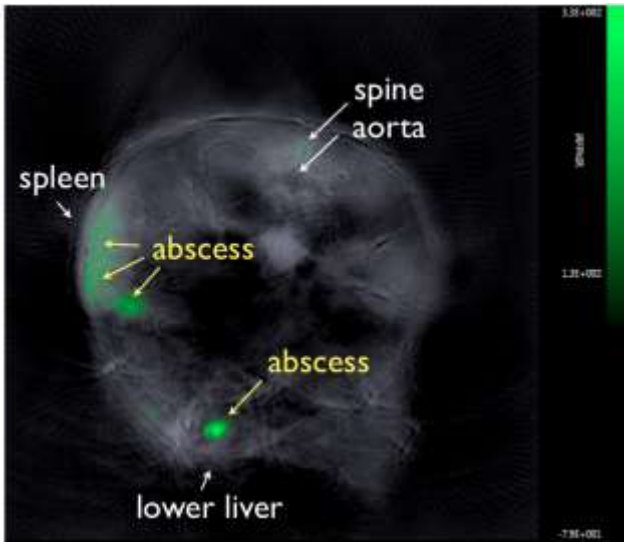
Multispectral Opto-Acoustic Tomography and Segmental 360-Degree Bioluminescent Imaging Both Show Potential for the Dynamic Study of Staphylococcal Infections

Author Block:

P. Panizzi; Auburn Univ., Auburn, AL

Abstract Body:

Background: Bio-luminescence imaging (BLI) of light-producing pathogens has made it possible to track infections non-invasively in mice. However, there exist inherent biases in tracking these infections as they spread. One of the primary issues is the consistent positioning of animal in the imager in relation to the detector. There are also cases where the signal from a deep tissue or bone infection has difficultly being seen, due to the light absorption and scattering properties of blood, fat, and muscle. Here, we use two techniques to overcome these issues and monitor *in vivo* propagation of a pathogen in mouse models over time. **Methods:** First, we used our newly developed semi-automated full 360-degree imager called the **M**ouse **I**maging **Spinner** (MiSpinner), which allows for the actuated rotation of the animal during the imaging process to reduce any positioning biases. Secondly, we used multispectral optoaccoustic tomography (MSOT) to image bacteria via their photo-acoustic signal generated after systemic administration of the near-infrared bacterial surface probe called XenoLight RediJect. This would allow for the detection of deep tissue signals. **Results:** In the images collected by the MiSpinner prototype housed in the IVIS Lumina XRMS system, we were able to appreciate liver signal in 1 of 5 mice on day 5 following bolus injection of *Staphylococcus aureus* Xen29 (1e5 CFUs). Using the MSOT imager and XenoLight RediJect agent, we could detect 2 of the 5 animals imaged had splenic and liver abscesses present on day 5 following Xen29 injection. A representative transverse plane image of a mouse is shown below with the green regions denoting the abscesses. **Conclusions**: Taken together, these results suggest new avenues available for the *in vivo* study Staphylococcal infections.



Author Disclosure Block:

P. Panizzi: None.

Poster Board Number:

FRIDAY-498

Publishing Title:

An *In Vitro* Urinary Catheterisation Model That Approximates Clinical Conditions for Evaluation of Innovations to Prevent Catheter Associated Urinary Tract Infections

Author Block:

R. Y. R. Chua[1], K. Lim[2], S. S. J. Leong[2], P. A. Tambyah[1], B. Ho[1]; [1]Natl. Univ. of Singapore, Singapore, Singapore, [2]Singapore Inst. of Technology, Singapore, Singapore

Abstract Body:

Catheter associated urinary tract infections (CAUTI), which account for about 25% of nosocomial infections globally, result in increased morbidity and healthcare cost. Biofilm colonized catheters also present opportunistic sources of increasingly antibiotics resistant bacteria. Since the practice of the closed drainage system, innovations to combat CAUTI have not produced significant improvement in clinical outcomes. The lack of a robust laboratory platform to test new CAUTI preventive strategies could be a key reason. We described an *in vitro* catheterization model (IVCM) in which a continuous supply of "urine medium" flows into a 250 mL polypropylene bottle (the "bladder") that is drained by a urinary catheter connected to a drainage container (the "drainage bag"). The set-up was carried out for 3 days to assess the anti-bacteria and anti-biofilm properties of catheters. On day 3, viable bacteria were detected from biofilm in a normal 100% silicone catheter (~10^4 CFU/cm^2, intraluminal), and "bacteriuria" reached a steady concentration of ~10^8 CFU/mL. In contrast, an anti-microbial peptide (AMP) CP116A coated catheter showed negligible biofilm colonization and no detectable "bacteriuria". The protection against biofilm and planktonic bacteria/"bacteriuria" offered by this novel AMP-based engineered catheter could be attributed to the rapid bactericidal effect of CP116A released from the catheter surface. Our IVCM has demonstrated to be a cost-effective preclinical approach to evaluate new strategies for the prevention of CAUTI.

Author Disclosure Block:

R.Y.R. Chua: None. **K. Lim:** None. **S.S.J. Leong:** None. **P.A. Tambyah:** C. Consultant; Self; AstraZeneca, GlaxoSmithKline and Johnson & Johnson. E. Grant Investigator; Self; Sanofi-Pasteur, Fabentech, Inviragen and Theravance. L. Speaker's Bureau; Self; MSD and Novartis. N. Other; Self; Teleflex, 3 M and Adamas.. **B. Ho:** None.

Poster Board Number:

FRIDAY-499

Publishing Title:

***Podoviridae* Bacteriophage (Phage) Infection Efficiency of *Staphylococcus aureus* Isolated from Prosthetic Joint Infection (Pji)**

Author Block:

D. J. Ferullo[1], R. Patel[2], J. Radding[1]; [1]EnBiotix, Inc., Cambridge, MA, [2]Mayo Clinic, Rochester, MN

Abstract Body:

Background: *Staphylococcus aureus* causes 20-40% of prosthetic hip and knee arthroplasty infections, often leading to multiple surgeries, replacement of the prosthesis, long term antibiotic therapy, and potentially, to arthrodesis or amputation of the limb. Debridement, antibiotics and implant retention (DAIR) is an effective approach in carefully selected patients with acute PJI. However, DAIR is not appropriate for chronic PJI. Novel approaches to target *S. aureus* associated with PJI are needed. EnBiotix is engineering phage to deliver biofilm-dispersing enzymes to *S. aureus* for use in combination with antibiotic therapy.**Methods:** The plaque-forming efficiency of 3 highly related virulent *Podoviridae* phages was determined using dilution agar overlay assays with *S. aureus* isolates, including 14 non-implant-associated isolates from multiple sources, and 27 isolates from PJIs (Mayo Clinic). Lytic efficiency was scored visually on a scale of 4 (total clearing) to 0 (no plaques) based on Kutter, Meth Mol Biol 2009:501:141-9. Phage genome sequences were obtained from NCBI and compared using Geneious v.6.1.4.**Results:** Two of the phages (GRCS and P68), but not 44AHJD, exhibited increased plaque-forming efficiency with *S. aureus* isolates from PJI compared to non-implant-associated isolates ($p<0.05$). Phage genome analysis revealed protein sequence divergence in a single ORF associated with a minor tail protein.

Phage	PJI isolates[1] % infected	Non-implant-associated isolates[1] % infected	p-value
GRCS	74%	14%	<0.001
P68	48%	7%	0.004
44AHJD	22%	21%	0.477

[1]Clearing at 10^4 dilution (from 10^9 pfu/ml)**Conclusions:** Two of three *Podoviridae* phages showed better plaque-forming efficiency with isolates from PJI compared to non-implant-associated isolates. Phage infectivity of *S. aureus* may depend upon expression, and cognate recognition by the minor tail phage protein, of determinants related to clinical source and/or the microenvironment of the isolates. The recognition of clinical isolates of *S. aureus* specifically

from PJI provides a starting point for engineering phage to deliver transgenes to *S. aureus* to disperse biofilms in implant-associated infections.

Author Disclosure Block:

D.J. Ferullo: D. Employee; Self; EnBiotix, Inc. **R. Patel:** B. Collaborator; Self; EnBiotix, Inc. **J. Radding:** D. Employee; Self; EnBiotix, Inc..

Poster Board Number:

FRIDAY-500

Publishing Title:

Isolation & Identification of Lytic Phages against Drug Resistant Pathogens: Finding Cure in Holy Rivers of Kathmandu, Nepal

Author Block:

R. NEPAL, R. Malla; Tribhuvan Univ., Kathmandu, Nepal

Abstract Body:

Antimicrobial resistance is a global problem & emergence of superbugs have threatened advances of modern medicine pushing us closer to post antibiotic era. Once easily treatable infections are now killing & alternatives to antibiotics are being explored. Among all alternatives, phage therapy - though used longest in clinics - is largely ignored by western world. Bacteriophages or phages are viruses that infect bacteria but leave animal & plant cells unscathed. As MDR becomes a threat, interest in phage therapy is revitalized & now even US-NIAID lists 'phage' as research priority to address antibiotic crisis. Here, we explore availability of phages against MDR bacteria in real world & assess their efficacy in-vivo followed by morphological identification.40 different drug resistant human pathogens representing 12 genera were collected from hospital after biochemical identification & antibiotic susceptibility test. Water sample from 5 holy rivers was screened for presence of phages by double layer agar assay. Phages were purified by successively sub-culturing single plaque thrice & standard spot assay was employed to determine multiple host range. Most potent phages were amplified & confirmed by TEM.34 lytic phages - 16 *E. coli*, 13 *Salmonella*, 2 *Shigella*, 2 *Klebsiella*, 1 *Citrobacter* - were isolated. Except 2 *Klebsiella* phages all 32 phages showed multiple host range (lysed >1 strains). All *Salmonella* phages showed interspecific (*S. typhi* & *S. paratyphi*) lytic ability. Bacteriophage induced mutants were only observed in *Klebsiella* phages. All except *Klebsiella* phage completely lysed bacterial host in their log & stationary phase of growth cycle & were Caudovirales (tailed virus) implying that multiple host range is a function of tail or tail fibers.Our findings show 'phages against MDR bacteria' that could be used therapeutically exists in real world & they are not extremely host specific as professed in scientific world. They've evolved to achieve broader host range & even possess interspecific lytic capability. Absolute absence of multiple host range in tailless phages but not in tailed led us to new hypothesis that phage evolution might have occurred from tailless to tailed. Conclusively - Phage Therapy is not a 'myth' but an alternative when antibiotics fail.

Author Disclosure Block:

R. Nepal: None. **R. Malla:** None.

Poster Board Number:

FRIDAY-502

Publishing Title:

Bacteriophages to Fight Respiratory Tract Infections - Pneumophage Project

Author Block:

H. Blois[1], N. Pallaoro[2], G. L'Hostis[1], E. Morello[2], A. Guillon[2], R. Respaud[2], L. Vecellio[3], N. Heuzé-Vourc'h[2], **J. Gabard**[1]; [1]Pherecydes-Pharma, Romainville, France, [2]Université François Rabelais, Tours, France, [3]Diffusion Technique Française, Saint-Etienne, France

Abstract Body:

Background: Hospital-acquired pneumonia is the second most frequent HAI infection and the first in terms of morbidity, mortality and cost (A. Torres, 2010). *P. aeruginosa* (*Pa*) is one of the predominant pathogens responsible for pneumonia acquired in hospital, and 30% of *Pa* strains are antibiotic multidrug resistant (MDR) (S.T. Micek, 2015). **Methods:** This project aims at developing a new strategy, based on bacteriophages (phages), to fight Pa pneumonia.**Results:** First, phages were isolated from sewage water and selected against a wide variety of *Pa* strains coming from several International collections and MDR patient isolates. They were characterized by transmission electronic microscopy and genome sequencing. Burst size ranges from 30 to 500 PFU/bacterium. The phage drug (PP1231) is a cocktail of 9 phages, including myoviridae, podoviridae and siphoviridae. When tested *in vitro* on 641 *Pa* strains, PP1231 provides 94 % efficacy. PP1231 was then tested in an animal model of *Pa* acute lung injury, using a bioluminescent strain (PAK-Lux). Animals were infected with 10^7 CFU/animal of PAK-Lux and treated, two hours post-infection, once intranasally with various concentrations of PP1231 (10^6 or 10^8 PFU/animal) or amikacin , used as a positive control. Lung infection was followed up over time, for up to 6 days, by bioluminescence imaging and *Pa* counted by microbiological assay in the lungs after animal sacrifice. All untreated infected animals died within 24h post-infection whereas, at a multiplicity of infection of 10, 100% of the animals treated with a single intranasal administration of PP1231 survived at day 2 and 60% at day 6. PP1231 cleared the lung from *Pa*, but secondary intestinal of ENT infections, probably due to ingestion, were detected in some animals and associated to death.**Conclusions:** Overall, local delivery of PP1231 is highly efficient at eradicating *Pa* in the lungs but may be unable to control secondary infections when locally administered only. The efficacy of PP1231 phage cocktail on *Pa* in this model suggests its potential use for treating pneumonia in humans.

Author Disclosure Block:

H. Blois: D. Employee; Self; Hélène Blois. **N. Pallaoro:** D. Employee; Self; Nikita Pallaoro. **G. L'Hostis:** D. Employee; Self; Guillaume L'Hostis. **E. Morello:** D. Employee; Self; Eric Morello. **A. Guillon:** D. Employee; Self; Antoine Guillon. **R. Respaud:** D. Employee; Self; Renaud Respaud. **L. Vecellio:** F. Investigator; Self; Laurent Vecellio. **N. Heuzé-Vourc'h:** F.

Investigator; Self; Nathalie Heuzé-Vourc'h. **J. Gabard:** A. Board Member; Self; Jérôme Gabard. K. Shareholder (excluding diversitied mutual funds); Self; Jérôme Gabard.

Poster Board Number:

FRIDAY-503

Publishing Title:

Genomic and Phenotypic Analysis of *Bacillus* Bacteriophage Endolysins

Author Block:

L. Harb[1], K. Eldridge[1], L. Rizkalla[2], R. Duong[2], **L. M. Temple**[1], A. Johnson[2], D. Nelson[3];
[1]James Madison Univ., Harrisonburg, VA, [2]Virginia Commonwealth Univ., Richmond, VA,
[3]Univ. of Maryland, College Park, MD

Abstract Body:

As antibiotic-resistant bacteria become more prevalent in clinical settings and the environment, the need for solutions to remedy this issue becomes of greater importance. Utilizing bacteriophages to control bacterial populations is a viable alternative to antibiotics and has been shown to be more effective at treating bacterial infections than antibiotics. Bacteriophage endolysins play a key role in host cell lysis and have their own potential therapeutic capabilities. In this study, we explored the efficacy and host range of different *Bacillus* phage endolysins. We annotated and identified putative endolysins from a collection of *Bacillus* phages and chose nine phages that represent different classes of endolysins by bioinformatics analysis. We synthesized these genes with His-tags, cloned them into inducible expression plasmids, and transformed them into *E. coli*. Cells were grown at 37°C to OD_{600} 0.40 and induced with 0.2% arabinose overnight. After induction, cells were mechanically lysed and the endolysins purified with nickel column chromatography using 500 mM imidazole. Whereas crude lysates of three phages (Waukesha92, Stitch, and Angel) only showed lytic activity by spot assay on one of eight different *Bacillus* strains tested, the purified endolysins fared much better, suggesting the crude lysates did not contain sufficient endolysin concentrations to observe lysis from without. Five of the nine purified endolysins demonstrated lytic activity on multiple strains of *Bacillus* including *B. simplex* RWR2, *B. thuringiensis* Kurstaki, and *B. cereus* 4342, a *B. anthracis* transition state strain. Our findings demonstrate that these bacteriophage endolysins are capable of lysing different strains of *Bacillus* independently. Future work will involve further characterization of the endolysins and exploring their full host range potential.

Author Disclosure Block:

L. Harb: None. **K. Eldridge:** None. **L. Rizkalla:** None. **R. Duong:** None. **L.M. Temple:** None. **A. Johnson:** None. **D. Nelson:** None.

Poster Board Number:

FRIDAY-504

Publishing Title:

Detection of Endotoxin Using LAL Test in Injectable Drugs Commonly Used for Parental Administration: A Blind Study from Karachi, Pakistan

Author Block:

S. G. NADEEM[1], M. Jan[1], S. T. HAKIM[2]; [1]MYCOLOGY RESEARCH & REFERENCE LABORATORY, JINNAH Univ. FOR WOMEN, KARACHI, Pakistan, [2]VIROLOGY & TISSUE CULTURE LABORATORY, JINNAH Univ. FOR WOMEN, KARACHI, Pakistan

Abstract Body:

Background: Toxins of the gram negative bacteria are considered a real threat, especially endotoxins (lipopolysacchrides) liberated on the death and cell lysis of gram negative bacteria particularly. An attractive hypothesis about endotoxic shock could be linked with administration of parenteral antibiotic drugs, lethal dose of endotoxin in the body, or massive bacterial killing that release endotoxin and patient deterioration. **Methodology: A** blind study was conducted for the detection and quantification of endotoxin in injectable drugs commonly used for parental administration and are easily available in the local markets in Karachi, Pakistan. A total of 100 pharmaceutical drugs were provided for the said research in injectable form. Each injectable drug samples was manufactured by a different pharmaceutical company and a total of 100 samples; 20 injectable drug samples each from each selected locality were obtained from the different localities, to quantify the amount of endotoxin by using Limulus Amoebocyte Lysate (LAL) endpoint chromogenic method. **Results:** Upon analysis, a total of 48% parental drugs were found endotoxin positive with above permissible limits; while 52 % were found negative that is below the permissible limits. The sensitivity and specificity of this kit ranges from 98.6% to 96.6%. On the basis of statistical analysis, this study showed that there is a significant linear correlation between absorbance and concentration of endotoxin found in intravenous drugs. The calculated Pearson coefficient of correlation value was 0.998 and found significant at < 0.100, that showed a positive correlation between absorbance and concentration of endotoxin. By testing several samples of intravenous drugs available in the local markets of metropolis, it was speculated that manufacturing of these products need to follow appropriate Good manufacturing practices (GMP), improve drugs quality delivery, reporting systems and establishment of quality-control testing of intravenous drugs. Large scale studies are recommended and needed for understanding the magnitude of this important problem and to reduce the impact of this infectivity of the parental drugs.

Author Disclosure Block:

S.G. Nadeem: None. **M. Jan:** None. **S.T. Hakim:** None.

Poster Board Number:

FRIDAY-505

Publishing Title:

The Impact of Rapid Identification of Influenza via Polymerase Chain Reaction (PCR) on Antimicrobial Management in Hospitalized Patients

Author Block:

S. Kuhn, K. Beadle, V. Creswell; Wesley Med. Ctr., Wichita, KS

Abstract Body:

Background: Early antiviral treatment of influenza has shown to decrease the duration of symptoms and may decrease the risk of complications. Antigen-based rapid diagnostic tests commonly result in false negatives. PCR has proven to be the most sensitive and accurate, as reflected in the current CDC recommendations. Recently, WMC implemented the use of the Biofire FilmArray®, which has the ability to test for 17 respiratory viruses (RV) via PCR. Rapid identification, along with pharmacist intervention, can lead to early appropriate antiviral use and may decrease the use of unnecessary antibiotics.**Methods:** This retrospective, quality improvement project aimed to describe the impact of PCR identification of RV on antimicrobial management in adult patients admitted for possible influenza and/or pneumonia during December 2014-March 2015. Data collection included antimicrobials initiated, discontinued or modified based on PCR results and changes in antimicrobial management based on a positive influenza result (PIR).**Results:** Of the 153 patients included, 73% had influenza A, 16% influenza B, and 11% were influenza negative. Median time between admission and PCR results was 13h11m. Twenty five percent of patients had an antiviral started empirically, and 71% had an antiviral started following PCR identification. Four percent never received an antiviral. Median time between influenza detection and start of antivirals was 1h30m. Following identification of influenza, 21% of patients had all antibiotics discontinued. Antibiotics were narrowed following PIR in 8% of patients. Median duration between PIR and antibiotic discontinuation was 4h7m. 71% of patients had antibiotics continued following a PIR. Of those, 22% of patients had antibiotics continued for an unrelated infection and 58% had antibiotics continued due to concern of a secondary pneumonia. Estimated cost savings due to discontinuation of inappropriate antimicrobials was $5200.**Conclusions:** Rapid identification of influenza via PCR combined with pharmacist intervention can reduce the inappropriate use of antimicrobials in patients with suspected respiratory infections. Further studies are needed to determine the clinical impact of these stewardship efforts on patient outcomes.

Author Disclosure Block:

S. Kuhn: None. **K. Beadle:** None. **V. Creswell:** None.

Poster Board Number:

FRIDAY-506

Publishing Title:

Survey of Antimicrobial Prescribing Practices and Interpretation of Rapid Blood Culture Identification Results

Author Block:

L. Donner, W. S. Campbell, E. Lyden, P. D. Fey, T. C. Van Schooneveld; Univ. of Nebraska Med. Ctr., Omaha, NE

Abstract Body:

Background: Microbiology reporting can be difficult to understand and new rapid pathogen identification technology has made it more challenging. Nebraska Medicine recently implemented the Biofire FilmArray Blood Culture Identification Panel (BCID) with stewardship-based education on test result interpretation. Provider BCID result interpretation and prescribing decisions were assessed via survey.**Methods:** An email survey was distributed to 382 physicians. Questions addressed physician characteristics, antibiotic prescribing practices, and interpretation of BCID results. Seven questions required respondents to interpret BCID results and make clinical decisions. Tallied correct responses resulted in a knowledge score. Descriptive statistics summarized survey responses. General linear models evaluated the effect of role and specialty on the mean knowledge score and correct responses to specific scenarios. Kappa statistics were used to evaluate the agreement between use of antimicrobial stewardship resources and correct responses to knowledge-based questions.**Results:** The response rate was 40.8% with a distribution of specialties of 57% internal medicine, 20% family medicine, and 25% other. Role included 41% residents, 5% fellows, and 53% faculty. Of those surveyed, 89.4% reported reviewing antimicrobial susceptibility results with 81.6% adjusting therapy based on this review while only 60% reported adjusting therapy based on BCID results. Correct response rates for interpretation questions ranged from 52 - 86%. Common errors included misinterpretation of *Enterobacteriaceae* and *Staphylococcus* genus results. After adjusting for role, the mean total knowledge score did not differ among specialties (p=0.13). After adjusting for specialty, the score did not differ among role (p=0.47). There was low agreement between correctly answering the knowledge questions and having reviewed antimicrobial resources on the BCID (all Kappa statistics <0.2).**Conclusions:** Misinterpretation of BCID results is relatively common and may result in ineffective treatment or missing the opportunity to narrow therapy. Improved reporting practices of BCID results with clinical decision support tools providing interpretation guidance available at the point of care may improve the clinical impact of this technology.

Author Disclosure Block:

L. Donner: None. **W.S. Campbell:** None. **E. Lyden:** None. **P.D. Fey:** None. **T.C. Van Schooneveld:** None.

Poster Board Number:

FRIDAY-507

Publishing Title:

Outcomes of Antimicrobial Stewardship Intervention (Asi) in Bloodstream Infections (Bsi) with & without a Rapid Pcr Based Blood Culture Identification Panel (Bcid)

Author Block:

S. H. MacVane, F. S. Nolte; Med. Univ. of South Carolina, Charleston, SC

Abstract Body:

Background: Studies have demonstrated the combination of ASI & rapid organism identification (ID) improves outcomes in BSI, but have not controlled for the contribution of the ASI element. We conducted a retrospective study comparing antimicrobial use & outcomes among patients with BSI that underwent either: conventional organism ID (controls, 8/1/10-10/31/10), conventional organism ID with ASI (ASA, 8/1/12-10/31/12), or BCID with ASI (ASB, 8/1/14-10/31/14). **Methods:** Hospitalized adult patients with positive blood cultures (BC) of organisms on BCID (19 bacteria, 5 Candida spp., & mecA, vanA/B, & KPC antimicrobial resistance genes) were studied. The primary outcome was time to effective therapy (TTET) & antimicrobial utilization in the first 96 h. Clinical endpoints included length of stay (LOS), mortality, readmission, & hospital cost. Patients with organisms not included on BCID, or those with blood culture contaminants were excluded. **Results:** There were 783 patients with positive BC, 364 met inclusion criteria (115 control, 104 ASA, 145 ASB) representing 404 BC pathogens (41.6% gram-positive, 50.5% gram-negative, 7.9% Candida spp.). BCs were polymicrobial in 9.3% of patients. Groups did not differ in Charlson comorbidity index or Pitt bacteremia score. Antimicrobial resistance was minimal & did not differ across the study periods (2 VRE, 5 ESBL, 0 KPC). Time from BC collection to organism ID was shorter in ASB (17.2 h; $P<0.001$) compared with control (57.4 h) or ASA (53.9 h). ASB had shorter TTET (4.9 h; $P<0.001$) than control (15.0 h) or ASA (13.0). ASA (56.7%) & ASB (52.4%) had significantly higher rates of antimicrobial de-escalation than control (33.9%), with time to 1st antimicrobial de-escalation occurring sooner in ASB (48.1 h; $P=0.034$) than ASA (60.5 h) or control (63.0 h). No difference occurred between control, ASA, or ASB in mortality (11.3%, 11.5%, 13.8%), 30-d readmission (25.5%, 26.1%, 18.4%), ICU LOS (7, 5, 5 d), post-culture LOS (8.4, 7.8, 8.2 d), or costs ($27,564, $21,222, $23,840). **Conclusions:** In patients with BSI, an ASI program was associated with improved antimicrobial utilization during the initial 96 h. Addition of BCID to ASI shortened time to effective therapy & further improved antimicrobial use compared to ASI alone, even in a setting of low rates of antimicrobial resistance.

Author Disclosure Block:

S.H. MacVane: None. **F.S. Nolte:** I. Research Relationship; Self; Biofire Diagnostics LLC., GenMark Diagnostics.

Poster Board Number:

FRIDAY-508

Publishing Title:

Strategizing Procalcitonin Assessment with Antibiotic Stewardship: Partering Microbiology

Author Block:

J. Kuzyck, K. Kasarda, T. Walsh, J. Thomas; Allegheny Gen. Hosp., Pittsburgh, PA

Abstract Body:

Background: Recently, rapid serum procalcitonin (PCT) assessment has emerged as a complimentary tool for focusing on the infectious etiology, virus versus bacteria, highlighting the importance of antibiotic stewardship. Here, we wanted to maximize the benefit of rapid TAT within the Microbiology Laboratory partnering with a new Antibiotic Stewardship Program charged with tailoring antibiotic use in LRI and sepsis. **Methods:** Although a consortium of six hospitals with approximately 2,000 beds, this pilot study focused on Allegheny General Hospital (AGH) and it's off-site consortium core Microbiology Laboratory. Lower Respiratory Infections ,LRI, and sepsis, were target hospital diseases compared with ER testing using the Biomereux (BMX) Mini Vidas, results provided to the ordering MD, the ER and Antibiotic Stewardship 24/7, starting in March 2015. Interpretation of PCT serum results was provided by BMX: LRI had three and sepsis two reporting categories, one each for "increased likelihood for bacterial etiology; antibiotics encouraged". A web based algorithm was provided by Antibiotic Ste**Results:**Average number of samples per week was 139 +/- 24, with Saturday/Sunday 19 +/- 6. ER provided essentially 15-40% of samples per week. Heaviest load was Tuesday-Thursday during daytime shift; evening shift assayed 1-2 times. ER specimens were treated "stat" with 2 hour TAT; all others were "batched" with 6 hour TAT window, with 91% compliance for all assays. For sepsis, an average of 34.7% PCT samples were above 0.5ng/ml and for LRI 42.3% above 0.25 ng/ml indicative of bacterial etiology and potential benefit of antibiotic intervention. The need for heightened education also focused on the approximate 20% unintended disease assessment, primarily UTI's. Repeat samples at 6-24 hours were minimal. **Conclusions:** We found incorporating PCT in Microbiology a logical partnering to the Antibiotic Stewardship Program, heightening the present armamentarium provided by Microbiology and antibiotic testing, featuring its central and controllable data management available 24/7.

Author Disclosure Block:

J. Kuzyck: None. **K. Kasarda:** None. **T. Walsh:** None. **J. Thomas:** None.

Poster Board Number:

FRIDAY-509

Publishing Title:

DailyAntimicrobial Stewardship Review of Positive Culture Results and Impact onAppropriateness of Antimicrobial Therapy - a Randomized Controlled Trial

Author Block:

B. Langford, A. Chan, M. Downing, J. Seah, J. Johnstone; St. Joseph's Hlth.Ctr., Toronto, ON, Canada

Abstract Body:

Antimicrobial stewardship (AS) interventions can minimize unnecessary exposure to antimicrobial therapy (AT) and limit the emergence of resistant pathogens. The optimal approach to identifying and communicating AS recommendations is unknown. Our site is a 400-bed community teaching hospital in Toronto, Canada. We examined whether a positive culture review system improved the appropriateness of AT for hospitalized adult patients.AS pharmacists reviewed all positive culture reports daily during weekdays from May 2015 to January 2016. For each patient, the electronic health record was reviewed to assess the appropriateness of AT and identify any specific recommendations for improvement. Each recommendation was written on a standardized form and then randomized (using a concealed random number table) to either the intervention group (send recommendation to clinical pharmacist caring for the patient) or control group (withhold recommendation). The primary endpoint was improvement in appropriateness of AT (as specified on the recommendation note) within 24 hours of the recommendation. Differences in proportions were analyzed using the chi-square test.From 170 recommendations randomized, 167 from 165 patients were evaluable. Seventy three (73) were randomized to intervention and 94 to control. Recommendations based on positive urine cultures were most common (51%), followed by positive blood cultures (25%). The most common recommendation was to streamline therapy (59%), followed by optimize duration (17%). The primary outcome of improvement in appropriateness of AT was achieved in 35 patients (48%) in the intervention group and 37 patients (39%) in the control group (p=0.27). A subgroup analysis found a marginally significant difference in improved therapy when only examining recommendations that suggested streamlining (65% vs. 45%, p=0.053).In conclusion, a daily positive culture review system did not significantly improve the appropriateness of AT in our patient population. However, recommendations focusing on streamlining therapy may be more useful. Further research is required to determine the optimal method of communicating AS recommendations.

Author Disclosure Block:

B. Langford: None. **A. Chan:** None. **M. Downing:** None. **J. Seah:** None. **J. Johnstone:** None.

Poster Board Number:

FRIDAY-510

Publishing Title:

Implementing Satellite Blood Culture in the Emergency Department Significantly Decreases Time to Incubation

Author Block:

G. Johnson; Lakeridge Hlth., Oshawa, ON, Canada

Abstract Body:

Background: Lakeridge Health is a 450 bed hospital servicing a community of over 600,000 people. The Emergency Department (ED) collects ~50% of blood cultures. When cultures were loaded 24/7 into the BD BACTEC™ FX Blood Culture System in the Core Laboratory, an average delay of 2.48 hours was noted from collection to loading. To reduce time to pathogen identification and improve outcomes for patients with sepsis; guidelines recommend cultures should be incubated within 2 hours from draw.* The implementation of a satellite BACTEC™ FX and EpiCenter™ Data Management System in the ED provided an opportunity to reduce time to detection and reporting results. **Methods:** Pre- and post-install time to incubate (TTI) data was collected from LIS and BACTEC plots (n=76, 29 positives) and LIS and EpiCenter - Specimen Registration (n=120, 51 positives), respectively. Data was extracted and analyzed for improvement in TTI. **Results:** Following implementation, an 82% decrease in TTI for all ED blood cultures over all shifts was observed: 2.48 hours from collection to incubation pre-install versus 0.45 hours post install. An 84% reduction for positive cultures was realized: 2.67 hours from collection to incubation pre-install versus 0.43 hours post install. The largest improvement was noted during the night shift: from 2.57 hours to 0.45 hours. EpiCenter monitored instrument status, capacity, contamination, positivity rates and blood volume. **Conclusions:** This study demonstrates that placement of a blood culture incubator close to the site of collection can dramatically reduce TTI allowing for alignment with best practices and improved patient care. In a recent survey**: 1. 1.Over 50% of Canadian physicians indicated they weekly encounter a patient with suspected sepsis. 2. Blood culture with subsequent identification/susceptibility was rated as the two most important lab tests for detection, antibiotic optimization and improved patient outcomes.3. Delays in TTI are inherent in many institutions.

Author Disclosure Block:

G. Johnson: None.

Poster Board Number:

FRIDAY-511

Publishing Title:

Daptomycin Dosing Optimization in Obese Patients: Experiences in a Large Community Teaching Hospital

Author Block:

S. Kuhn, V. Creswell; Wesley Med. Ctr., Wichita, KS

Abstract Body:

Background: Conventional daptomycin dosing has been based on actual body weight to achieve an effective area under the curve (AUC) to minimum inhibitory concentration ratio. Studies suggest obese patients will have an increased maximum concentration and AUC versus normal weight individuals. In recent years, alternative dosing regimens of daptomycin have been conducted including dosing based on ideal and adjusted body weight. These studies have shown no difference in clinical outcomes in patients dosed on ideal body weight or adjusted weight versus actual body weight. In 2014, Wesley Medical Center changed their standard dosing of daptomycin based upon this literature.**Methods:** This retrospective, observational quality improvement study described the safety and efficacy of dosing daptomycin based on ideal or adjusted body weight in obese patients. Patients were included if they received daptomycin between June-October 2014 and were obese (actual body weight ≥130% of ideal body weight.) Data collected included indication, patient characteristics, dosing, cultures, complications during therapy, and outcome measures.**Results:** Of the nine patients included, 67% were started on daptomycin to facilitate transition to outpatient care. Thirty-three percent were placed on daptomycin following an allergic reaction to vancomycin. *Staphylococcus aureus* was the most common pathogen isolated (50%). Most patients (89%) received a dose of 6 mg/kg. Seventy-eight percent of patients were dosed based on adjusted weight, with 22% dosed on ideal body weight. Sixty-six percent of patients had complete resolution of symptoms. Eleven percent discontinued daptomycin due to adverse reactions. Two patients (22%) completed their course, but were re-admitted for similar joint infections.**Conclusions:** Daptomycin dosed per adjusted body weight is safe and effective in most patients when implemented in a community hospital. Further studies should be conducted in patients with bone and joint infections prior to implementation of an adjusted body weight dosing strategy of daptomycin for this indication.

Author Disclosure Block:

S. Kuhn: None. **V. Creswell:** None.

Poster Board Number:

FRIDAY-512

Publishing Title:

Necessity of Carbapenem Use When Prescribed Per Infectious Diseases Specialists

Author Block:

K. J. Goodlet[1], S. M. Cardwell[2], M. D. Nailor[3]; [1]Hartford Hosp., Hartford, CT, [2]Flagstaff Med. Ctr., Flagstaff, AZ, [3]Univ. of Connecticut, Storrs, CT

Abstract Body:

Background: Combating antibiotic resistance requires stewardship programs aimed at reducing the overuse of broad-spectrum antibiotics. A common methodology employed to restrict the use of these agents is to require infectious diseases (ID) approval. The objective of this study is to assess the frequency at which ID appropriately identify patients requiring carbapenem therapy as opposed to other agents, specifically cefepime (FEP), and evaluate patient risk factors for multi-drug resistant organisms (MDRO) used by ID to justify carbapenems empirically. **Methods:** This study is a retrospective, descriptive chart review of patients ages 18 to 88 prescribed a carbapenem between January 1, 2014 and June 30, 2014, inclusive, by an ID physician. Patients with cystic fibrosis, definitive therapy, or protocol-concordant therapy were excluded. Necessary carbapenem use was defined as the presence of an organism susceptible to the prescribed carbapenem and resistant to FEP, the agent of choice at the study facility for MDRO.**Results:** 235 charts were reviewed, of which 84 were included. The most common infection source was intra-abdominal (38%) or respiratory (35%). In the past 90 days, 52% of patients had a prior hospitalization and 58% received prior antibiotic therapy. 19% of patients were immunosuppressed, 19% were admitted from a long-term care facility, and 20% had MDRO isolated in the past year. At the time of carbapenem prescribing, 49% of patients were in the intensive care unit, with 29% intubated and 24% on at least one vasopressor. Mean carbapenem therapy was 5.0 ± 4.0 days, with 44% prescribed as initial therapy and 56% as escalation of current therapy. Cultures were drawn for 94% of patients, of which 61% were positive. The most commonly isolated organism was *Escherichia coli* (44%). Carbapenem use was necessary for only 7% of patients. De-escalation occurred in 54% of culture-positive patients, with mean time to de-escalation of 0.73 ± 1.4 days.**Conclusions:** Carbapenem therapy by ID was generally in severely ill patients with risk factors for drug resistance. However, the factors selected by ID to utilize carbapenems were poor indicators for resistance to the hospital's first line agent, FEP. Hospital-specific risk factors may more appropriately identify patients requiring carbapenem therapy.

Author Disclosure Block:

K.J. Goodlet: None. **S.M. Cardwell:** None. **M.D. Nailor:** C. Consultant; Self; Astellas, Theravance. L. Speaker's Bureau; Self; Astellas.

Poster Board Number:

FRIDAY-513

Publishing Title:

Use of Intravenous versus Oral Antibiotics in the Emergency Department/Observation Unit (ED/OU) for the Treatment of Acute Bacterial Skin and Skin Structure Infections (ABSSSIS)

Author Block:

K. C. Claeys, E. J. Zasowski, A. M. Lagnf, S. L. Davis, M. J. Rybak; Wayne State Univ., Detroit, MI

Abstract Body:

Background: Perceived need for intravenous (IV) antibiotics (abx) to treat ABSSSIs has been shown to be a common reason for inpatient admission. In a recent prospective study this was the only cited reason for nearly half of patients. As with the decision to admit patients, administration of IV abx is largely subjective. This current study aimed to describe prescribing patterns in patients treated in the ED/OU setting without inpatient admission.**Methods:** Retrospective, single-centered, observational cohort of consecutive adult patients with primary diagnosis of ABSSSI treated in the ED/OU between April 2012 - 2015. Type of ABSSSI, baseline clinical characteristics and patient severity, and past medical conditions were collected and analyzed. Patient outcomes included abx received, 96-hour ED re-visit, and 30-day ABSSSI-related admission.**Results:** A total of 347 patients were included in the final analysis: 223 received IV abx, 124 received PO abx. Patients receiving IV abx were older [median age 42 (IQR 31 - 52) vs. 34 (IQR 25 - 49) years, $p = 0.019$], and had a higher median Charlson Comorbidity Score (CCS) [1 (IQR 0 - 2) versus 0 (IQR 0 - 1), $p = 0.011$]. Presence of at least one systemic inflammatory response syndrome (SIRS) criterion was significantly associated with the receipt of IV abx. Patients with primary ABSSSI sites of the head/neck or hand were more likely to receive IV abx (27.8% vs. 15.3%, $p = 0.004$). Among patients with CCS of zero and no SIRS criteria 41.7% received IV abx. With the exception of IV drug use (8.5% IV vs. 2.4% PO, $p = 0.025$), there were no significant differences based on individual comorbid conditions. MRSA coverage was more common in patients with PO abx (99.2% PO vs. 80.3% IV, $p < 0.001$), regardless of type of ABSSSI (i.e. cellulitis vs. abscess). A similar number of patients in both groups experienced 96-hour ED revisit (8.0% IV vs. 7.7% PO), and 30-day infection-related admission (1.6% vs. 2.3%).**Conclusions:** There is a large degree of heterogeneity in clinical presentation associated with the receipt of IV abx in the ED/OU, however at least one SIRS criterion, higher CCS, and infections of the head/neck or hand were more likely to receive IV abx. A significant portion of patients with no SIRS criteria and a CCS of zero received IV abx and were potential candidates for PO.

Author Disclosure Block:

K.C. Claeys: None. **E.J. Zasowski:** None. **A.M. Lagnf:** None. **S.L. Davis:** A. Board Member; Self; Allergen; Merck & Co.. C. Consultant; Self; Pfizer Pharmaceuticals. E. Grant Investigator; Self; Allergen; Merck & Co. **M.J. Rybak:** A. Board Member; Self; Actavis; Merck & Co; Bayer; Cempra inc; The Medicines Company; Sunovian; Theravance. C. Consultant; Self; Actavis; Merck & Co; Bayer; Cempra inc; The Medicines Company; Sunovian; Theravance. E. Grant Investigator; Self; Actavis; Merck & Co; Bayer; Cempra inc; The Medicines Company; Sunovian; Theravance; NIAID. L. Speaker's Bureau; Self; Actavis; Merck & Co; Bayer; Cempra inc; The Medicines Company; Sunovian; Theravanc.

Poster Board Number:

FRIDAY-514

Publishing Title:

Challenges in Antimicrobial Stewardship: Oritavancin for Acute Bacterial Skin and Skin Structure Infections (Absssis)

Author Block:

N. H. Chan-Tompkins[1], T. L. Trienski[1], D. N. Bremmer[2], T. L. Walsh[1]; [1]Allegheny Gen. Hosp., Allegheny Hlth.Network, Pittsburgh, PA, [2]Western Pennsylvania Hosp., Allegheny Hlth.Network, Pittsburgh, PA

Abstract Body:

Background: Oritavancin is FDA-approved for treatment of ABSSSIs as a one-time intravenous (IV) dose. Although the wholesale acquisition cost is $2,900.00, reimbursement of the average sale price plus 6% is possible for select patients (pts) in the outpatient setting. In August 2015, an Antimicrobial Stewardship Program (ASP) ABSSSI initiative was implemented on pts admitted to the hospital. If IV antibiotic therapy was warranted for the treatment duration, the ASP assessed the feasibility of discharging the pt and administering oritavancin in the outpatient setting one day post-discharge. The objective was to describe the insurance benefit coverage of oritavancin in pts with an ABSSSI. **Methods:** Pts identified for potential treatment with oritavancin were assessed based on submission of the physician request form to the oritavancin support program and review of the summary of benefits form. IRB exemption was obtained for this quality improvement project. **Results:** The ASP assessed 38 pts for potential oritavancin use between 8/1/15-12/31/15. Three (7.9%) pts without insurance and income were eligible for oritavancin via the pt assistance program. The remaining 35(92.1%) were assessed for insurance benefit coverage where all submissions were processed with the available generic C-code or J-code. Oritavancin was not covered in 4/35(11.4%) pts. Oritavancin was covered in 17/35(48.6%) pts. Prior authorization or additional processing was required in the remaining 14/35(40%) pts. Overall, 14 pts had deductibles listed for their insurance. Median annual deductible in 14 pts was $350 (range, $147-4000). Twelve pts had co-insurance requirements: 6/12(50%) had 20%; 6/12(50%) had 10%. **Conclusion:** Although oritavancin was found to be covered by most insurers, use often required prior authorization or additional processing. The presence of co-insurance presented a challenge with determination of a pt's true out-of-pocket cost. As of January 2016, oritavancin now has a permanent J-code, which warrants further assessment of insurance benefit coverage. Despite the challenges with insurance coverage of oritavancin, it continues to be a viable tool for our ASP ABSSSI initiative.

Author Disclosure Block:

N.H. Chan-Tompkins: None. **T.L. Trienski:** None. **D.N. Bremmer:** None. **T.L. Walsh:** None.

Poster Board Number:

FRIDAY-515

Publishing Title:

Incidence of and Risk Factors for Acyclovir-associated Nephrotoxicity

Author Block:

K. E. Barber, K. R. Stover, J. L. Wagner; Univ. of Mississippi Sch. of Pharmacy, Jackson, MS

Abstract Body:

Background: As obesity rates increase, it is unclear which acyclovir (ACY) dosing weight should be utilized for central nervous system and skin/soft tissue infections to optimize efficacy and limit toxicity. We evaluated the incidence of and risk factors for ACY-associated nephrotoxicity. **Methods:** This retrospective case-control included adult patients who received IV ACY for ≥ 72 hours in 2014. Excluded: receipt of renal replacement therapy (RRT) or vasoactive medications at start of ACY therapy. Incidence of nephrotoxicity defined by RIFLE criteria. Data collected: ACY medication use information, renal function, concomitant nephrotoxins, and clinical data. Risk factors were determined utilizing multivariate logistic regression analyses.**Results:** 62 patients included: 17 (27%) developed nephrotoxicity (cases). Groups were similar, but more cases were admitted to the ICU (53% vs 20%, p=0.025) and remained in ICU longer (12 days vs 3 days, p=0.008). Median daily ACY dose (1725 mg vs 2175 mg, p=0.559) and duration of therapy (10 days vs 5 days, p=0.129) were similar. No differences were found in mg/kg dose regardless of dosing weight used. Case patients classified as Risk (41.2%), Injury (23.5%), Failure (35.3%); 3 patients needed RRT. Nephrotoxicity led to more frequent ACY dose adjustments (47.1% vs 2.2%, p<0.001). 82% received at least 1 concomitant nephrotoxic agent; vancomycin was most frequently co-administered (50%). Case patients were more likely to receive vancomycin (71% vs. 42%, p=0.046), but total concomitant nephrotoxic agents was similar (p=0.712). No differences in fluid hydration (82% vs 76%, p=0.739). Patient characteristics related to ACY-associated nephrotoxicity are in Table 1.

Table 1. Patient characteristics associated with nephrotoxicity.	N (% Total Population)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
ICU admission	18 (29)	4.5 (1.36 – 14.94)	3.3 (0.90 – 12.27)
Concomitant vancomycin administration	31 (50)	3.3 (0.99 – 10.90)	2.1 (0.55 – 7.82)

Conclusion: Concomitant vancomcyin and ICU admission influenced ACY-associated nephrotoxicity in bivariate but not multivariate analyses. Further studies are warranted to determine optimal ACY dosing weight and additional concomitant risk factors.

Author Disclosure Block:

K.E. Barber: None. **K.R. Stover:** E. Grant Investigator; Self; Astellas Pharma. **J.L. Wagner:** None.

Poster Board Number:

FRIDAY-516

Publishing Title:

Impact of 2010 Cephalosporin CLSI Breakpoint Revisions for *Enterobacteriaceae* on Susceptibility and Antibiotic Utilization

Author Block:

S. S. Doyen, A. D. Junkins, A. M. Wilde; Norton Hlth.care, Louisville, KY

Abstract Body:

Background: In January 2015, Norton Healthcare adopted the 2010 Clinical and Laboratory Standards Institute (CLSI) breakpoints for 1st-3rd generation cephalosporins for Enterobacteriaceae but continued to detect and report ESBL and AmpC production. The purpose of this study is to assess the impact of the breakpoint implementation and describe the prevalence, treatments, and outcomes of third generation cephalosporin (TGC) susceptible, ESBL or AmpC positive Enterobacteriaceae. **Methods**: This was a retrospective, observational study performed at Norton Healthcare, a multihospital system in Louisville, Kentucky. Ceftriaxone susceptibilities were reviewed for inpatient and outpatient antibiograms and antibiotic utilization was collected as days of therapy (DOT) per 1,000 patient days pre- (2014) and post-implementation (2015) of breakpoints. Enzyme-producing, TGC-susceptible Enterobacteriaceae were reviewed for source, definitive therapy, and 30 day mortality. **Results**: Ceftriaxone susceptibility for Enterobacteriaceae minimally decreased post-implementation for inpatient [90% (6221/6936) to 89% (6912/7784), p=0.0856] and outpatient [96% (7652/7959) to 94% (9058/9643), p=0.0001] antibiograms. Carbapenem utilization decreased from 34.2 DOT/1000 patient days, 95% CI [33.5, 34.9] in 2014 to 32.6, 95% CI [31.9, 33.2] in 2015. There were 168 cases of enzyme-positive, TGC-susceptible isolates and 65% were from outpatients (109/168). The most common sources were urine (89%), wound (9%) and blood (2%). Definitive therapy most commonly used for enzyme-positive, TGC-susceptible isolates was nitrofurantoin (22%) and fluoroquinolones (22%). Patients with enzyme-positive, TGC-susceptible isolates that received a TGC as definitive therapy had a thirty day mortality rate of 0% (0/18) and 14% (1/7) for urine and non-urine sources, respectively. **Conclusion**: The implementation of the 2010 CLSI breakpoints had a small effect on ceftriaxone susceptibilities without increasing broad spectrum antibiotic use. TGC-susceptible, enzyme-positive organisms were isolated primarily in outpatient urine cultures. The detection and reporting of ESBL and AmpC enzymes may not be needed for patient care, especially from urine cultures. More data on treatment with TGC in non-urine sourced infections is needed.

Author Disclosure Block:

S.S. Doyen: None. **A.D. Junkins:** None. **A.M. Wilde:** None.

Poster Board Number:

FRIDAY-517

Publishing Title:

Comparative Rates of Acute Kidney Injury in Patients on Vancomycin Combined with Either Piperacillin/Tazobactam or Cefepime

Author Block:

B. Navalkele, J. Pogue, S. Karino, B. Nishan, M. Salim, J. Koons, W. Perry, T. Hussain, S. Solanki, A. Pervaiz, N. Tashtoush, H. Shaikh, S. Koppula, K. Kaye; Wayne State Univ., Detroit, MI

Abstract Body:

Background: Recent evidence suggests that receipt of concomitant piperacillin/tazobactam increases the risk of vancomycin-associated nephrotoxicity. Well-controlled, adequately powered studies comparing rates of acute kidney injury (AKI) in patients receiving vancomycin+piperacillin/tazobactam (VPT) compared to similar patients receiving vancomycin+cefepime (VC) are lacking. This study compared the incidence of AKI among patients receiving combination therapy with VPT to a matched cohort of patients receiving VC.**Methods:** A retrospective matched outcomes study was performed including patients cared for at Detroit Medical Center from 2011 to 2013. Patients were eligible if they a) received combination therapy with either VC or VPT; b) had antibiotics initiated within 24 hours of one another; and c) had combination therapy continued for ≥ 48 hours. Patients were excluded if their baseline serum creatinine was >1.2mg/dl or they were receiving renal replacement therapy at the initiation of combination therapy. Patients receiving VC were matched to patients receiving VPT in a 1:1 ratio based on severity of illness, ICU status at onset of combination therapy, duration of combination therapy, daily dose of vancomycin and number of concomitant nephrotoxins. The primary outcome was the incidence of RIFLE criteria-defined AKI. Multivariate modelling was performed using cox proportional hazards.**Results:** 558 patients were included (279 received VC, 279 VPT). The mean age was 55.9±16.6 years. There were more females in VC group (p=0.03) and more patients who were white in the VPT group (p=0.0005). AKI was significantly higher in the VPT than the VC group (81/279 (29%) vs. 31/279 (11%), HR=4.0, 95% CI=2.6-6.2). In multivariate analysis, therapy with VPT was an independent predictor for AKI (HR=3.7, 95% CI=2.3-6.0). Patients receiving VPT had greater median length of stay (LOS) compared to patients receiving VC (8 vs 6 days, p=0.01). Among patients who developed AKI, the median onset was more rapid in the VPT compared to the VC group (3 vs 5days, p=<0.0001).**Conclusions:** The VPT combination was associated with an increase in AKI risk, a more rapid onset of AKI, and a greater LOS compared to the VC combination.

Author Disclosure Block:

B. Navalkele: None. **J. Pogue:** None. **S. Karino:** None. **B. Nishan:** None. **M. Salim:** None. **J. Koons:** None. **W. Perry:** None. **T. Hussain:** None. **S. Solanki:** None. **A. Pervaiz:** None. **N. Tashtoush:** None. **H. Shaikh:** None. **S. Koppula:** None. **K. Kaye:** None.

Poster Board Number:

FRIDAY-518

Publishing Title:

Impact of Area Under the Curve-targeted Dosing (Auc-Td) on Vancomycin (Van)-Associated Nephrotoxicity (Vn)

Author Block:

N. Finch[1], K. Murray[1], E. Zasowski[2], R. Mynatt[1], J. Zhao[1], R. Yost[1], J. Pogue[1], M. Rybak[1]; [1]Detroit Med. Ctr., Detroit, MI, [2]Wayne State Univ., Detroit, MI

Abstract Body:

Background: Despite widespread VAN use, the optimal dosing strategy remains poorly defined. Guidelines recommend targeting trough (Tr) concentrations of 15-20 mg/L in complicated infections to maximize the likelihood of achieving an AUC_{24h}/ MIC ratio of ≥400 when the VAN MIC ≤1 mg/L. However, Tr of 15-20 mg/L have been associated with increased VN, and evidence suggests AUC_{24h} exposures can be met with lower Tr values. In 2015, the DMC converted from VAN Tr monitoring to AUC-TD for invasive infections. The primary objective of this study was to investigate VN rates with AUC-TD versus dosing for Tr concentrations of 15-20 mg/L. **Methods:** Retrospective quasi-experimental study from 9/2014 - 4/2015. Inclusion: ≥18 yr; ≥1 VAN Tr obtained in pre-intervention group or ≥2 VAN levels for AUC_{24h} determination in the post-intervention group; received VAN ≥72 h. Exclusion: baseline SCr ≥2 mg/dL or pre-existing need for renal replacement therapy, concomitant piperacillin-tazobactam, or the use of VAN for an indication excluded from AUC-TD. Primary outcome: nephrotoxicity as defined in VAN Guidelines, RIFLE, and AKIN criteria.**Results**: Outcomes were evaluated for 632 patients; 333 in the pre- and 299 in the post-intervention group. Baseline characteristics were similar. VAN Tr were higher in the pre-intervention group. VN rates were numerically higher with Tr-directed dosing compared to AUC-TD, though this did not reach statistical significance (Table).

	Tr Directed	AUC-TD	P
Age, yr	61 (49-74)	61 (50-72)	0.735
Baseline CrCl, ml/min	76 (53-112)	81 (53-109)	0.962
APACHE II	12 (8-18)	12 (9-19)	0.161
VN per Guideline Definition	30 (9.0%)	16 (5.4%)	0.077
RIFLE Class 2 or 3	39 (11.7%)	26 (8.7%)	0.213
AKIN Stage 2 or 3	33 (9.9%)	23 (7.7%)	0.327
Initial Tr, mg/L	14.6 (10.4-19.6)	12.6 (9.6-16.6)	0.001

Initial $AUC_{24h,}$ mg*h/L	n/a	492 (399-571)	

Data displayed as median (IQR) or *n* (%).**Conclusions**: This is the first comparison of VN with AUC-TD vs Tr-directed dosing. The AUC-TD approach was associated with a numerically lower rate of VN. Further study will determine whether this difference is statistically significant.

Author Disclosure Block:

N. Finch: None. **K. Murray:** None. **E. Zasowski:** None. **R. Mynatt:** None. **J. Zhao:** None. **R. Yost:** None. **J. Pogue:** None. **M. Rybak:** C. Consultant; Self; Allergan, Bayer, Cempra, Merck, Sunovian, The Medicine Company. I. Research Relationship; Self; Allergan, Cempra, Bayer, Merck, Sunovian, The Medicine Company. L. Speaker's Bureau; Self; Allergan, Bayer, Cempra, Merck, Sunovian, The Medicine Company.

Poster Board Number:

FRIDAY-519

Publishing Title:

Antibiotics for *Clostridium difficile* Infection: Impact of Drug Selection on Treatment Outcomes

Author Block:

S. L. Revolinski[1], J. N. Wainaina[2], M. Graham[2], L. S. Munoz-Price[1]; [1]Froedtert and the Med. Coll. of Wisconsin, Milwaukee, WI, [2]Med. Coll. of Wisconsin, Milwaukee, WI

Abstract Body:

Background: National guidelines from the IDSA stratify treatment (tx) for *Clostridium difficile* infection (CDI) by severity, with recommendations to treat severe CDI with vancomycin (VAN) and mild to moderate CDI with metronidazole (MTN). Recent literature has suggested VAN may be the drug of choice for CDI of any severity. The purpose of this study is to evaluate tx outcomes in patients (pts) with CDI treated with either VAN or MTN.**Methods:** A retrospective review conducted from 10/1/14 - 5/31/15 evaluated inpts initially treated with VAN or MTN for CDI, defined as diarrhea (≥3 liquid stools in 24 hours) and a positive *C. difficile* NAAT. Pts were excluded if tx began prior to admission, or received initial combination therapy or drug other than VAN or MTN. Outcomes (as binary variables) included: therapy escalation (TE) after 72 hours of initial treatment, lack of clinical cure (CC) defined as diarrhea at end of overall therapy (EOT), and CDI recurrence within 28 or 56 days. The effect of tx (modeled as a binary variable [VAN or MTN]), Charlson Comorbidity Index, age, sex, BMI, and CDI severity were using SAS.**Results:** In the final analysis, 77 patients received VAN and 166 MTN. TE evaluated using logistic regression. Regression analyses were performed occurred in 2.6% and 19.9% of pts receiving VAN and MTN, respectively (p=0.0001). The need for TE was associated with choice of drug (OR = 0.11 for pts treated with VAN compared to MTN; 95% CI: 0.03-0.47; p<0.0001). Age over 60 was also associated with TE, and drug choice remained significant after adjusting for age. Lack of CC at EOT occurred in 11.7% and 4.2% of pts treated with VAN and MTN, respectively (p=0.0478). Achieving CC was associated with choice of drug (OR=0.33 for treatment with VAN compared to MTN; 95% CI: 0.12-0.93; p<0.0001). No other factors were found to be significant. Recurrence at 28 or 56 days occurred in 9.1% of those treated with VAN and 13.9% with MTN (p=0.4021). CDI recurrence was not associated with choice of drug.**Conclusion:** Our data suggest that CDI tx with VAN may be associated with decreased need for therapy escalation. CC at EOT and recurrence may be skewed as these were evaluated at the end of overall therapy and TE could have impacted these results.

Author Disclosure Block:

S.L. Revolinski: None. **J.N. Wainaina:** None. **M. Graham:** None. **L.S. Munoz-Price:** None.

Poster Board Number:

FRIDAY-520

Publishing Title:

Systematic Review: Is Trimethoprim-Sulfamethoxazole (TMP-SMX) a Valid Alternative in the Management of Infections in Children in the Era of Community-Acquired Methicillin Resistant *Staphylococcus aureus*? : A Comprehensive Systematic Review

Author Block:

M. T. Rosanova, Cuellar Pompa Leticia MD. Fundación Canaria de Investigación Sanitaria (FUNCANIS), Tenerife, Spain., G. Perez, N. Sberna, P. Serrano Aguilar, G. Berberian, R. lede; Hosp. Garrahan, Buenos Aires, Argentina

Abstract Body:

Background: To evaluate the use of TMP-SMX compared with other options available for the treatment of children with CA-MRSA infections. **Methods:** Data sources: The following data base were searched: Medline and PreMedline (OivdSP interface); Excerpta Medica Database (EMBASE) (Elsevier interface); Cumulative Index to the Nursing and Allied Health Literature (CINAHL) (EbscoHost interface); Sciences Citation Index Expanded (SCI-EXPANDED) (Web of Science interface); Cochrane Library (Wiley interface); Scopus (Elsevier interface) and DARE, HTA (CRD interface). The search strategy was the one developed by SIGN to identify randomized clinical trials and systematic reviews. Also, we conducted a hand review of all reference lists of included studies. Nor language or data limits were added. The last search was done on October 1, 2015. Main key words were Trimethoprim or Trimethoprim-Sulfamethoxazole combination and *Staphylococcus aureus*. Study Selection: Only randomized controlled trials (RCT) comparing TMP-SMX vs any other antibiotic as the first-line treatment in CA-MRSA infections in children were included. Articles were reviewed by two reviewers and in case of discrepancy the final decision was made by the study coordinator. Data Extraction: Only 27 out of 364 papers identified were RCTs and only 4 fulfilled the eligibility criteria (Jadad score $\geq$3). Data synthesis: Evidence found only referred to use of TMP-SMX in soft-tissue infections **Conclusions:** Available evidence is not conclusive to promote or refuse TMP-SMX as first line treatment in CA-MRSA infections in children. Additional well-designed studies are required to further elucidate this issue.

Author Disclosure Block:

M.T. Rosanova: None. **G. Perez:** None. **N. Sberna:** None. **P. Serrano Aguilar:** None. **G. Berberian:** None. **R. lede:** None.

Poster Board Number:

FRIDAY-521

Publishing Title:

Implications of Reappraisal of Fluoroquinolone Minimum Inhibitory Concentration Susceptibility Breakpoints in Gram-negative Bloodstream Isolates

Author Block:

M. M. Brigmon[1], J. A. Justo[2], P. B. Bookstaver[2], J. Kohn[3], C. Troficanto[3], H. Albrecht[1], M. N. Al-Hasan[1]; [1]Univ. of South Carolina Sch. of Med./Palmetto Hlth., Columbia, SC, [2]South Carolina Coll. of Pharmacy/Palmetto Hlth., Columbia, SC, [3]Palmetto Hlth., Columbia, SC

Abstract Body:

Background: The National Antimicrobial Susceptibility Testing Committee for the United States (USCAST) has recently proposed changing ciprofloxacin minimum inhibitory concentration (MIC) susceptibility breakpoints for gram-negative bacilli from ≤ 1 μg/mL as per current Clinical and Laboratory Standards Institute (CLSI) and Food and Drug Administration (FDA) criteria to ≤0.25 for *Enterobacteriaceae* and ≤0.5 for non-fermenters. This study examines the impact of these proposed changes on ciprofloxacin susceptibility of gram-negative bloodstream isolates (GN-BSI). **Methods:** Matched pairs mean difference (MD) with 95% confidence intervals (CI) was calculated to examine change in ciprofloxacin susceptibility after reappraisal among GN-BSI at Palmetto Health Hospitals in Columbia, SC from 2010-2014. **Results:** Ciprofloxacin MIC distributions of 1055 GN-BSI are in Table as number (%). An overall downshift in *E. coli* ciprofloxacin susceptibility will occur after reappraisal (MD -5.5, 95% CI -7.5, -3.5) with more notable decline among residents of skilled nursing facilities (MD -9.3, 95% CI -15.6, -3.0) and patients with recent fluoroquinolone exposure (MD -14.3, 95% CI -26.5, -2.1). Reappraisal will have most impact on *P. aeruginosa* (MD -7.8, 95% CI -14.5, -1.1) and least on *Enterobacter* species (MD -3.0, 95% CI -7.2, 1.2). **Conclusion:** Reappraisal of current CLSI/FDA fluoroquinolone susceptibility breakpoints will significantly impact GN-BSI, particularly non-fermenters and in the presence of risk factors for fluoroquinolone resistance.

Table. Distribution of ciprofloxacin minimum inhibitory concentration in gram-negative bloodstream isolates

Bacteria	≤ 0.25	0.5	1	≥ 2
Enterobacteriaceae (n=967)	753 (78)	23 (2)	27 (3)	164 (17)
Escherichia coli (n=543)	386 (71)	16 (3)	14 (3)	127 (23)
Klebsiella species (n=205)	176 (86)	2 (1)	9 (4)	18 (9)
Proteus mirabilis (n=72)	60 (83)	2 (3)	1 (1)	9 (13)
Enterobacter species (n=67)	63 (94)	2 (3)	0 (0)	2 (3)
Non-fermenters (n=88)	62 (70)	9 (10)	7 (8)	10 (11)
Pseudomonas aeruginosa (n=64)	50 (78)	2 (3)	5 (8)	7 (11)

Author Disclosure Block:

M.M. Brigmon: None. **J.A. Justo:** J. Scientific Advisor (Review Panel or Advisory Committee); Self; Cempra Pharmaceutical. **P.B. Bookstaver:** J. Scientific Advisor (Review Panel or Advisory Committee); Self; Allergan. **J. Kohn:** None. **C. Troficanto:** None. **H. Albrecht:** None. **M.N. Al-Hasan:** None.

Poster Board Number:

FRIDAY-522

Publishing Title:

Colistin-Associated Renal Dysfunction: A Retrospective Analysis of 282 Patients

Author Block:

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Abstract Body:

Background: Colistin use has increased considerably in recent years. Studies examining colistin associated nephrotoxicity have yielded variable incidence rates and risk factors. The objective of this study was to assess the incidence and risk factors associated with the development of colistin associated nephrotoxicity among a large cohort of patients.**Methods:** A retrospective cohort study was performed among patients who received at least 24h of colistin intravenously during a ten-year period at a tertiary care academic medical center. Exclusion criteria included use of renal replacement therapy (RRT) at the start of therapy. The primary outcome was renal dysfunction, defined by RIFLE criteria, or the initiation of RRT. A multivariate analysis was performed to evaluate the influence of risk factors on the development of nephrotoxicity during colistin therapy.**Results:** 409 hospitalizations with colistin administration were identified, with 282 subjects eligible for study analysis. 78% of patients resided in the ICU. Results are in Table 1.

Table 1.				
	Renal Dysfunction (n=142)	No Renal Dysfunction (n=140)	Univariate OR (95% CI)	Multivariate OR (95% CI)
Age, mean, yrs	56	50	1.02 (1.01-1.03)	1.02 (1.01-1.03)
Dose, mg/kg/day	3.83	3.65	1.12 (0.93-1.36)	1.16 (0.95-1.41)
Concurrent Nephrotoxin	115	97	1.89 (1.09-3.29)	1.82 (1.04-3.21)
Duration of Therapy, days	10.2	8.9	1.03 (0.99-1.06)	
Charlson Comorbidity Index, mean	2.1	2.1	1.01 (0.90-1.11)	

Acute Physiology and Chronic Health Evaluation II (APACHE II)	19.7	18.5	1.02 (0.99-1.05)	

Conclusions: In the largest studied cohort to date using RIFLE criteria, half of patients who received colistin experienced renal dysfunction. There was no evidence of a relationship between dose and renal dysfunction. Providers should be especially cautious when using colistin in patients who are older or receiving concomitant nephrotoxins.

Author Disclosure Block:

J. Jacob: None. **K. Haynes:** None. **J.C. Gallagher:** None.

Poster Board Number:

FRIDAY-523

Publishing Title:

Tolerance of Ceftriaxone Administrated Subcutaneously - A French Prospective Multicenter Observational Study

Author Block:

C. Roubaud Baudron[1], E. Forestier[2], T. Fraisse[3], G. Gavazzi[4], L. Pagani[5], M. Paccalin[6]; [1]CHU Hopitaux de Bordeaux, Bordeaux, France, [2]Ctr. Hosp.ier Métropole Savoie, Chambéry, France, [3]Ctr. Hosp.ier Alès-Cévennes, Ales, France, [4]CHU Michallon, Grenoble, France, Grenoble, France, [5]Ctr. Hosp.ier Annecy, Annecy, France, [6]CHU Hopitaux de Bordeaux, Poitiers, France

Abstract Body:

BACKGROUND: Antibiotic administration through subcutaneous (SC) injection is common practice in France, especially with ceftriaxone (CRO). The aim of this study was to determine the tolerance of CRO administrated subcutaneously. **Methods:** This is an ancillary study focusing on CRO use from a large prospective non-interventional multicentre study including every adult patient treated at least one day with SC CRO from May to September 2014 in 50 French hospitals. Data on local adverse effects (AE) and clinical evolution were collected until the end of treatment.**RESULTS:** 163 patients, median age 86 (19-104), treated with CRO were included. Main indications were urinary [n=78 (48%)] and respiratory [n=45 (28%)] infections. Thirty-three patients (21.5%) experienced at least one AE: pain (n=21, 13%), induration (n=11, 7%), hematoma (n=13, 8%) and erythema (n=5, 3%). No cutaneous necrosis was observed. Local AE were mostly reversible, only one patient had a persistent pain associated with transient hematoma and induration contributing to his transfer from rehabilitation unit to acute yard. An injection > 5 min (p=0.005) and the use of non-rigid catheter (p=0.0021) were protective factors against AE. In 137 cases (84%), CRO was successfully injected until the scheduled end. CRO was switched to another more convenient antibiotic in 16 patients (10%). A clinical or bacteriological failure or death were observed in 4% (n=6). AE led to discontinuation of the SC infusion for 4 patients (2%).**CONCLUSIONS:** In a recent report, the European Medicines Agency noticed that data were lacking to recommend a subcutaneous administration of CRO. This original study supports the hypothesis that SC CRO administration may represent an interesting safe alternative to IV route, especially in some peculiar patient settings, such as elders. However this route needs to be further assessed especially for its efficacy and PK/PD data.

Author Disclosure Block:

C. Roubaud Baudron: None. **E. Forestier:** None. **T. Fraisse:** None. **G. Gavazzi:** None. **L. Pagani:** None. **M. Paccalin:** None.

Poster Board Number:

FRIDAY-524

Publishing Title:

Assessing Viral Populations and Predicted Proteins in Coprolites Via Ancient Dna Scaffolding

Author Block:

J. I. Rivera-Perez[1], R. J. Cano[2], Y. Narganes[1], L. Chanlatte[1], G. A. Toranzos[1]; [1]Univ. of Puerto Rico, Rio Piedras Campus, San Juan, PR, [2]California Polytechnic State Univ., San Luis Obispo, CA

Abstract Body:

We recently described for the first time DNA fragments (average length=220bp) obtained from coprolites belonging to two pre-Columbian Caribbean cultures. However, fragment lengths were too small for further analyses. In this study we used additional scaffolding and identified protein-coding viral genes in these paleofeces, a portion of the microbial community commonly ignored by paleomicrobiome studies. Briefly, DNA was extracted from the coprolite cores of each culture and subjected to shotgun sequencing. Quality-filtered DNA fragments underwent scaffolding via Omega (Minimum overlap = 100bp; Insertion and Deletion cost = 3; Mismatch cost = 2) and were submitted to METAVIR for protein analyses (e-value $\leq 10^{-7}$.)Approximately 8% and 25% of Saladoid and Huecoid contigs, respectively, were identified. The majority were dsDNA viruses, although a portion was from ssDNA viruses; retroviral DNA was also detected. Caudovirales, Phycodnaviridae and Mimiviridae, dominated both viromes. Caudovirales protein-coding genes detected included those associated to Siphoviridae, among others. In fact, Siphoviridae-like genes constituted 41% and 39% of the identified Huecoid and Saladoid contigs, the majority of which were from phages infecting *Bacillus sp*, followed by *Rhizobium*, *Staphylococcus*, *Streptococcus*, *Lactococcus* and *Mycobacterium sp*. Bacillus phage genome-coverage was the most complete, including genes encoding for potential replication proteins, a helix-turn-helix domain protein, tail proteins and several site-specific integrases. Interestingly, in Huecoid samples we obtained excellent coverage of possible Mimiviridae genomes, specifically from Acanthamoeba polyphaga moumouvirus and mimivirus. Genes detected from these giant viruses included those encoding for DNA topoisomerases, N-acetyltransferases, epimerases, DNA mismatch repair proteins, as well as several helicase and stomatin family proteins.In conclusion, this study emphasizes the importance of scaffolding in metagenome analyses pipelines, as alignments of ancient DNA sequences <400bp are likely to give refutable results. It also supplements currently limited data on the viromes of pre-Columbian ancient cultures in the Americas.

Author Disclosure Block:

J.I. Rivera-Perez: None. **R.J. Cano:** None. **Y. Narganes:** None. **L. Chanlatte:** None. **G.A. Toranzos:** None.

Poster Board Number:

FRIDAY-525

Publishing Title:

Presence of a Prophage Determines Temperature-Dependent Capsule Production in &Lt/&Gtstreptococcus Pyogenes&Lt/I&Gt Hsc5

Author Block:

K. CHO; Indiana State Univ., Terre Haute, IN

Abstract Body:

Hyaluronic acid capsule is a major virulence factor in the pathogenesis of *S. pyogenes*. It acts as an adhesin to keratinocytes and anti-phagocytic agent. The expression of the capsule is primarily regulated by the two-component regulatory system CovRS, which acts as a repressor of capsule transcription. The *covRS* genes are frequently mutated in many clinically isolated invasive strains, and this mutation confers visible capsule production on agar plates. We previously reported that capsule production of some invasive strains with *covRS* mutation was dependent on environmental temperature. These strains produce a large amount of capsule only at sub-body temperature, and this thermoregulation occurs at a post-transcriptional level. Here, we report that a prophage has a crucial role in capsule thermoregulation. The lab strain, HSC5, contains three prophages in the chromosome and the prophage ΦHSC5.3 is involved in capsule thermoregulation. In all the spontaneous capsule thermoregulation mutants generated by passaging cells in the lab, only ΦHSC5.3 was cured. Disruption of a gene in ΦHSC5.3 also abolished capsule thermoregulation and caused capsule overproduction in a temperature-independent manner, suggesting that a prophage gene product or products in ΦHSC5.3 participate in capsule thermoregulation. Taken together, these results indicate that the prophage ΦHSC5.3 plays a crucial role in capsule thermoregulation most likely by repressing capsule production at 37°C at a post-transcriptional level.

Author Disclosure Block:

K. Cho: None.

Poster Board Number:

FRIDAY-526

Publishing Title:

Phage-encoded Molecules Regulate Stec Virulence

Author Block:

D. Chakraborty, G. Koudelka; State Univ. of New York, Buffalo, NY

Abstract Body:

Background: Shiga toxin-encoding *Escherichia coli* (STEC) cause food poisoning in humans and have been the source of many outbreaks dating back to 1982, when *E. coli* O157:H7 was first isolated. More recent outbreaks of Shiga toxin (Stx) poisoning have been caused both by O157 and non O157 STEC strains. Although primary symptoms of STEC infections are caused by Stx, it is at yet unclear what factors modulate the severity of STEC-mediated disease. In STEC, Stx is encoded by lambdoid prophages. The population structure of *E. coli* O157:H7 lineage is highly homogenous, but the identities and gene content of Stx-encoding phages in these strains are highly variable. Since Stx production, and thus disease, depends on phage lytic growth, our central hypothesis is that induction is primarily regulated by phage-encoded molecules, which subsequently regulates the severity of STEC infections. Our work focuses on determining and comparing the role of Stx-encoding phage repressors affecting induction frequency in Stx-encoding (Φ272 and 933W) and -non encoding phages (434 and λ). We also examine the potential role of a phage-encoded methylase in modulating STEC virulence. **Methods:** UV induction was performed to determine susceptibility of induction in Φ272, 933W, 434 and λ. qRT-PCR was performed to quantify P_{RM} transcripts. In vitro transcription was performed to determine the effects of repressor binding on P_{RM} transcriptional activity. *Acanthamoeba* viability was assessed using a trypan blue exclusion test.**Results:** Stx-encoding phages, Φ272 and 933W, have a higher frequency of spontaneous induction and lower P_{RM} transcript levels than non Stx-encoding phages, 434 and λ. In Φ272 and 933W, the difference in repressor concentration needed to activate versus repress P_{RM} transcription is much smaller than in 434 and λ. Stx-encoding PA8 prophages kill *Acanthamoeba* more efficiently than PA2 prophages, despite both PA2 and PA8 producing similar amounts of Stx when exposed to an SOS inducer. Restoring methylase function increased the killing efficiency of PA2 prophages and affected induction frequency.**Conclusion:** Φ272 and 933W synthesize less P_{RM} transcripts owing to altered binding characteristics by the repressors. This implies lower levels of intracellular repressor, which leads to a higher susceptibility to induction. A phage-encoded specific methylase is responsible for Stx-mediated *Acanthamoeba* killing difference between PA2 and PA8.

Author Disclosure Block:

D. Chakraborty: None. **G. Koudelka:** None.

Poster Board Number:

FRIDAY-527

Publishing Title:

Myophage as an Effective Antimicrobial in a Gut-Derived Model of Bacteremia

Author Block:

S. Green, L. Ma, F. Ramig, B. Trautner, A. Maresso; Baylor Coll. of Med., Houston, TX

Abstract Body:

E.coli is a leading cause of gram-negative bacteremia and is an increasing threat due to the prevalence of multi-drug resistant strains in the population. Often arising from a patient's own gastrointestinal tract, strains referred to as ExPEC (extraintestinal pathogenic *E.coli*) are opportunistic organisms capable of translocating across the intestinal barrier into major organ systems and causing life-threatening infections. While immunocompromised patients are at high risk for these infections, chemoprophylaxis with antibiotics, a common treatment, invariably leads to resistant strains and is damaging to the GI microflora. Thus, new treatment avenues are needed. Bacteriophage therapy is an alternative treatment that has not been tested against multi-drug resistant strains in animal models that faithfully recapitulate these types of infections. Here, we report the discovery of a novel series of myoviruses that efficiently kill a panel of multi-drug resistant clinical isolates of ExPEC. These viruses were laboratory evolved using a host range expansion procedure to be further selective against a prominent globally circulating lineage of ExPEC known as ST131. Myophage are effective a reducing ExPEC translocation and levels in tissues and organs while improving health scores of the host in a murine model of neutropenia and gut-derived ExPEC bacteremia. In addition, we demonstrate myophage are safe and can penetrate major organ systems, highlighting their potential desirable qualities as therapeutics. Our results suggest therapeutic phages have potential as effective antimicrobials against MDR ExPEC during bacteremia in compromised hosts.

Author Disclosure Block:

S. Green: None. **L. Ma:** None. **F. Ramig:** None. **B. Trautner:** None. **A. Maresso:** None.

Poster Board Number:

FRIDAY-528

Publishing Title:

Investigating Impact of Mycobacterial Physiology on Mycobacteriophage Life Cycles by Mass Spectrometry

Author Block:

Y. Li, K. L. Clase; Purdue Univ., West Lafayette, IN

Abstract Body:

Phage lyse bacteria and can be used to design new antibacterial therapies and combat the growth of antibiotic resistant bacteria. Indeed, many current applications in food safety and antibacterial treatment exploit the lytic life cycle of the phage. Therefore, it is necessary to obtain better understanding of phage life cycles, especially the requirements for maintaining different life cycles in order to improve the efficiency of phage's lytic activity.A temperate phage has lytic and lysogenic life cycles. Lytic cycle is preferred when the phage infects exponentially growing bacteria, while the lysogenic cycle is preferred when the phage infects stationary bacteria [1]. Nutrient level of the growth medium impacts the transition of bacterial growth phase and subsequent alteration of bacterial protein expression pattern [2, 3]. Divalent cations may also assist in phage lytic cycle [4]. Thus, the phage life cycle is affected by the physiology of the host bacteria and the content of the growth medium. To explore how the bacterial physiology affects phage life cycles, the phage-host interaction epitomized in the protein expression patterns of both phage and bacteria needs to be investigated.In order to test this hypothesis, we selected three different phages to infect *Mycobacterium smegmatis* (*M. smegmatis*) in different growth phases for subsequent protein extraction. The protein expression patterns were analyzed by high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) with an in-house database. Our overall objective is to identify the phage proteins mediating phage lytic and lysogenic cycles and the corresponding bacterial proteins expressed during key transition points in the phage life cycles. The findings will provide a better understanding of phage-host interactions over time and contribute towards novel applications in biotechnology.

Author Disclosure Block:

Y. Li: E. Grant Investigator; Self; Merck, Roche, AstraZeneca. **K.L. Clase:** E. Grant Investigator; Self; Merck, Roche, AstraZeneca.

Poster Board Number:

FRIDAY-529

Publishing Title:

Characterizing Parb of Mycobacteriophage Redrock

Author Block:

W. L. Ng, J. Cervantes, R. M. Dedrick, V. Villanueva, T. N. Mavrich, D. Jacobs-Sera, G. F. Hatfull; Univ. of Pittsburgh, Pittsburgh, PA

Abstract Body:

Plasmids, bacteria, and some phages encode segregation systems for chromosomal partitioning at cell division. One of several such systems is *parABS*, in which ParB DNA-binding protein binds to conserved *parS* sites on the chromosome, and ParA-ATPase segregates ParB-bound DNA. We identified *parABS* systems in forty-six Cluster A bacteriophages belonging to *mycobacteria* and *gordonia* host species. All Cluster A bacteriophages are either temperate or recent derivatives of temperate parents, and most encode an integration system. *Par* phages are predicted to form circularly permuted extrachromosomal prophages and require the *parABS* system for prophage maintenance and stability. Characterization of ParB binding sites in phage *parABS* systems may provide valuable insights into phage diversity and contribute to the development of a potential research tool for the transformation and replication of large or potentially cytotoxic DNA into bacterial cells. To this end, we constructed consensus sequences for *parS* sites of the aforementioned phages and predict that there are at least four specificity groups. Representatives from each group, namely RedRock and Gladiator, which belong to the same group, and Alma, KatherineG, and Echild from each of the remaining three groups, were selected for further study. In support of our predictions, binding assays show RedRock ParB binds more tightly to Gladiator *parS* than to Alma, KatherineG, and Echild *parS*; these data are also consistent with phylogenetic analyses of ParB protein sequences. Contrary to predictions based on *parS* consensus sequences and phylogenies of ParA and ParB proteins, binding studies with Alma ParB demonstrate tight binding to RedRock *parS*, followed by Echild, Gladiator, and KatherineG *parS*. All three components of the *parABS* system can contribute to incompatibility, so further work is needed in order to directly assess *par* cassette compatibility.

Author Disclosure Block:

W.L. Ng: None. **J. Cervantes:** None. **R.M. Dedrick:** None. **V. Villanueva:** None. **T.N. Mavrich:** None. **D. Jacobs-Sera:** None. **G.F. Hatfull:** None.

Poster Board Number:

FRIDAY-530

Publishing Title:

Characterizing the Replication System of Extrachromosomal Mycobacterial Prophages

Author Block:

R. E. Rush, T. N. Mavrich, R. M. Dedrick, D. A. Russell, D. Jacobs-Sera, G. F. Hatfull; Univ. of Pittsburgh, Pittsburgh, PA

Abstract Body:

When studying mycobacteria, there is an ongoing need for the development of new biotechnological tools. Currently, there are only a few characterized mycobacterial replication systems that can be used for cloning. However, we have identified a group of over forty genetically related, temperate mycobacteriophages (phages) that may aid in the expansion of available genetic tools. Temperate phages have two life cycles, lytic and lysogenic. Typically, during lysogeny the phage's genome is integrated into the host chromosome where it can be passively replicated by host replication systems. However, our mycobacteriophages of interest do not have any apparent integration machinery and instead contain putative partitioning systems. Partitioning systems are used to ensure that DNA is stably inherited during replication. The observed substitution of partitioning machinery for integration machinery suggests that these phages are maintained as extrachromosomal prophages and may utilize novel replication systems as well. The identification and characterization of these replication systems would enable further development of mycobacterial genetic tools. In order to investigate the replication systems used by these prophages, we first employed whole genome sequencing and double lysogenization tests to determine if these prophages are extrachromosomal. Additionally, we conducted standard molecular cloning to determine if the partitioning locus is sufficient for replication. Our results confirm that these phages are indeed extrachromosomal, but that the partitioning locus is not sufficient to confer the ability to replicate. Further characterization of these mycobacteriophages is needed to facilitate the development of new tools and to provide valuable insight into phage biology.

Author Disclosure Block:

R.E. Rush: None. **T.N. Mavrich:** None. **R.M. Dedrick:** None. **D.A. Russell:** None. **D. Jacobs-Sera:** None. **G.F. Hatfull:** None.

Poster Board Number:

FRIDAY-531

Publishing Title:

This Chaperone Can Dance - A New Role for Phage Tail Fibre Assembly Proteins

Author Block:

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Abstract Body:

Background: Bacteriophages (phages) possess remarkable specificity for their hosts. In phages containing contractile tails this specificity is determined primarily by tail fibres - elongated trimeric proteins that mediate the initial physical interactions between a phage and a bacterium. In phage T4, the formation of the distal portion of these fibres requires a specific phage-encoded tail fibre assembly protein (TFA; product of gene 38), which is widely believed to act as a chaperone (Leiman, 2010). Genes encoding putative TFAs can be identified in at least 15% of all tailed phage genomes, however, their mechanism of action remains unknown. In this study, we investigated the role of the TFA in assembly of the tail fibres of *E. coli* phage Mu. **Methods & Results:** Through a series of protein expression, purification and *in vitro* binding assays we found that the TFA binds to the C-terminal portion of the monomeric fibre and triggers its oligomerization, preventing the tail fibre from forming intracellular aggregates. Intriguingly, analytical size exclusion chromatography revealed that the TFA does not dissociate from the complex with the fibre after oligomerization or after the assembly of the phage baseplate wedge. A stable complex between the fibre and the TFA is also formed in *E. coli* phage P2, and *Salmonella* phages ST64b and Fels-2, suggesting that the TFA may be a part of the mature tail fibre. Our receptor binding assays further support this idea. When Mu or P2 phages are premixed with their purified bacterial receptors they are subsequently blocked from infecting their bacterial hosts. This inhibition is relieved when the purified receptors are incubated with Mu or P2 TFAs prior to the phage addition. This data as well as our full cell-binding assays reveal that Mu and P2 TFAs are able to specifically bind receptors on bacterial cells. These TFAs share 93% amino acid sequence identity and yet demonstrate different receptor-binding preferences, consistent with those of full phage particles. **Conclusions:** Taken together, our data demonstrates that the TFA assists in tail fibre oligomerization, and that, either in combination with the tail fibre or alone, likely plays a role in determining host specificity in the phages studied. Utilizing TFAs as receptor-binding proteins may represent an evolutionarily conserved mechanism for determining the host range of tailed phages.

Author Disclosure Block:

O. Ivantsiv: None. **A.R. Davidson:** None.

Poster Board Number:

FRIDAY-532

Publishing Title:

Investigation Of The Accessory Region Of Bacteriophage Lambda's p_L Operon

Author Block:

K. Cascino, L. Thomason, N. Costantino, D. Court; NIH, Frederick, MD

Abstract Body:

Bacteriophage lambda is a temperate phage able to undergo both lytic and lysogenic cycles in its host *E. coli*. The p_L operon of lambda, expressed early after infection, contains an "accessory region" composed of eight open reading frames (ORFs) that are non-essential to lambda plaque formation, but are known to contribute to alterations in host cell physiology and cell cycle regulation. Previous work in this laboratory has shown that expression of the accessory region causes pleiotropic effects including a decrease in cAMP levels, an increase in ppGpp levels, and a block to initiation of *E. coli* DNA replication. The goal of this study is to precisely map the ORFs responsible for the changes observed in *E. coli* physiology and cell cycle regulation to gain insight into fundamental viral-host interactions. In order to identify the ORF(s) responsible for these changes, an isogenic set of strains containing nested deletions in the accessory region was constructed using recombineering technology. The effect on cAMP was monitored by assaying β-galactosidase (β-gal) activity. Strains containing both wild-type p_L and the proximal five ORFs of the accessory region inhibit β-gal levels sevenfold compared to strains deleted for the entire region, which do not inhibit β-gal levels. Therefore, the decrease in cAMP can be attributed to a function within the first five ORFs of lambda's accessory region: *orf62*, *orf63*, *orf61*, *orf29*, and *orf73*. In addition, we have previously shown through flow cytometry experiments that strains expressing the distal four ORFs of the accessory region, *orf73*, *ea22*, *ea8.5*, and *orf55*, produced distinct peaks of 4, 8, or 16 complete chromosomes, indicating a block to initiation of replication due to expression of these ORFs. Interestingly, of the eight ORFs composing the accessory region, *orf73* alone contains a recognizable motif. It has a C4-type zinc finger domain similar to TraR and DksA, which modulate gene expression by interacting with the secondary channel of RNA polymerase. Both the decrease in cAMP levels and block to initiation of DNA replication appear to require *orf73*, and we speculate that this gene product may be at least partially responsible for some of the effects observed from the accessory region.

Author Disclosure Block:

K. Cascino: None. **L. Thomason:** None. **N. Costantino:** None. **D. Court:** None.

Poster Board Number:

FRIDAY-533

Publishing Title:

Investigation of Two Putative Integrases Within *Gordonia terrae* Bacteriophages

Author Block:

B. R. Brown, M. Montgomery, University of Pittsburgh SEA-PHAGES Students, D. Jacobs-Sera, D. A. Russell, W. H. Pope, G. F. Hatfull; Univ. of Pittsburgh, Pittsburgh, PA

Abstract Body:

Comparative genomic analysis of a 1000+ actinobacteriophage collection at the University of Pittsburgh provides a rich source for understanding phage diversity and evolution. (Hatfull, 2010.) One of the hosts used in this endeavor is Gordonia terrae, a soil microbe known to degrade environmental pollutants. To date, 129 phages have been isolated using this host, with 44 genomes being sequenced. The robust diversity that has been found in the mycobacteriophage was also present amongst these Gordonia phages having 13 clusters and 16 singletons. Analysis of these genomes has identified two bacteriophages that contain two integrases each. The integrase gene enables the phage to integrate into the host genome, replicating along with the host. These phages, Bowser and Howe, contain two distinct integrases each, one of which they share in common. The other two integrases are found in 2 other phage genomes, Kita and Schwabeltier. While phages Bowser and Schwabeltier share 92% nucleotide similarity, they only share one of the 2 integrases. Comparing Howe, Kita, and Bowser, they share less than 50 % nucleotide identity placing them into distinct clusters. While functional studies have not been performed yet, the identity of each integrase, shared with Kita and Schwabeltier would suggest that they are functional. It is not yet known why a phage genome would contain two integrases, but this may suggest that Howe and Bowser were recently in an alternative host. In addition, two integration sites may provide additional integration vectors that can be propagated to use as genetic tools in the future.

Author Disclosure Block:

B.R. Brown: None. **M. Montgomery:** None. **D. Jacobs-Sera:** None. **D.A. Russell:** None. **W.H. Pope:** None. **G.F. Hatfull:** None.

Poster Board Number:

FRIDAY-534

Publishing Title:

***Tetrahymena* Phagosomes: Platforms for Bacterial Gene Transfer**

Author Block:

I. Aijaz, G. Koudelka; Univ. at Buffalo, Buffalo, NY

Abstract Body:

Background: The microbial community in natural environments such as soil, pond water or animal rumen is comprised of a diverse mixture of bacteria and protozoa such as ciliates or flagellates. In such microbiota, the main source of bacterial mortality is currently considered to be grazing by phagotrophic protist. Many protists are omnivorous heterotrophs, i.e., they can feed on a range of different bacterial species. Due to this indiscriminate feeding, different bacterial species are assembled together in the food vacuoles of the protists, where they can potentially exchange genetic material. Earlier investigations have used a voracious bacterivore ciliate, *Tetrahymena thermophila*, to demonstrate gene transfer via conjugation within protozoan phagosomes. In this study, we explore the transfer of a bacteriophage 933W, and an *E. coli-Corynebacterium* plasmid shuttle vector pECTAC-*gfp* from one bacterial strain to another within protozan phagosomes. **Methods:** To focus on ingested bacteria and bacteriophage we used differential centrifugation to separate bacteria encapsulated in *Tetrahymena* from planktonic bacteria. We monitored the bacterial population inside *Tetrahymena* using confocal microscopy and measured cfu to determine bacterial survival within *Tetrahymena* phagosomes. Bacteriophage induction within *Tetrahymena* phagosomes was measured using Selectable In-Vivo Expression Technology (SIVET). Bacteriophage activity within phagosomes was monitored *via* spot assay. Bacteriophage transfer was detected *via* PCR and quantified by qPCR. The transfer frequency of shuttle vector pECTAC-*gfp* was measured by counting cfu. **Results:** Bacterial strains, engulfed in the vesicles of protozoa, assemble together. A large fraction of the bacteria are not digested and are expelled to the outside environment. The bacteriophage contained within engulfed bacteria1) induces as early as the first 15 minutes post- ingestion 2) a large fraction of the release bacteriophage remains active, for up to 2 hours and 3) infects recipient strain at higher frequency within *Tetrahymena* phagosomes. Moreover, we found high frequency transfer of a bacteriophage, 933W, and a bacterial shuttle vector pECTAC-*gfp*, from one bacterial strain to another within *Tetrahymena* phagosomes.**Conclusion:** *Tetrahymena* phagosomes, serve as platforms for bacterial gene transfer, providing microbes with the means to evolve and adopt to changing environment.

Author Disclosure Block:

I. Aijaz: None. **G. Koudelka:** None.

Poster Board Number:

FRIDAY-535

Publishing Title:

Incidence and Characterization of Type II Crispr1-Cas and Crispr2 Systems in *Enterococcus*

Author Block:

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Abstract Body:

CRISPR-Cas systems, a specific and adaptive immune response in prokaryotes, obstruct both viral infection and incorporation of mobile genetic elements by horizontal transfer. As horizontally-acquired genes may provide rapid adaptation to habitat change, and thus increase survival, antiviral CRISPR protection may represent a fitness cost to its host. To date, investigations into the prevalence of CRISPR have primarily focused on pathogenic and clinical bacteria, while less is known about CRISPR dynamics in commensal and environmental species.New PCR primers and DNA sequencing were used to detect the presence of *cas1*, a universal CRISPR-associated gene, in environmental and non-clinical *Enterococcus* isolates. Type II CRISPR1-*cas1* was detected in approximately 33% of the 275 strains examined, and significant differences in CRISPR1 carriage between species was observed. Incidence of *cas1* in *E. hirae* was 73%, nearly three times that of *E. faecalis* (23.6%) and 10 times more frequent than in *E. durans* (7.1%). This is the first report of CRISPR1 presence in *E. durans*, as well as in the plant-associated species *E. sulfureus* and *E. casseliflavus*. Significant differences in CRISPR1-*cas1* incidence among *Enterococcus* species support the hypothesis that there is a tradeoff between protection and adaptability. Differences in the habitats of these enterococcal species may exert varying selective pressure, resulting in a species-dependent distribution of CRISPR-Cas systems.Additionally, previously designed primers were used to detect and characterize the CRISPR2 array in isolated *E. faecalis* strains. CRISPR2 is presumed to be an inactive system, due to its lack of associated *cas* genes, and is thus referred to as an "orphan" locus. CRISPR2 loci were detected in 95% of the 99 *E. faecalis* strains analyzed, and 39 of these arrays were fully sequenced for spacer content. Of these, 14 strains contained at least one spacer identifiable as either phage or plasmid, suggesting a past immune role for the presumptively inactive CRISPR2 array. However, further work is needed to ascertain a function for these highly conserved orphan CRISPRs in *E. faecalis*.

Author Disclosure Block:

C. Lyons: None. **N. Raustad:** None. **M.A. Bustos:** None. **M. Shiaris:** None.

Poster Board Number:

FRIDAY-536

Publishing Title:

Phenotypic Observations Upon Deleting a *Toxoplasma gondii*-Specific Expanded Locus

Author Block:

G. W. Alfonse, A. L. Borges, A. N. Klinghoffer & J. P. Boyle; Univ. of Pittsburgh, Pittsburgh, PA

Abstract Body:

Disseminating throughout host cell types and species with intact capacity to cause disease in warm-blooded animal and human hosts, the interaction of the eukaryotic protozoan parasite *Toxoplasma gondii* with its host is in dynamic flux mediated by stage-specific protein effectors. Unlike microbes which have one mode of host cell entry: being endocytosed by the host cell, *T. gondii* also actively facilitates its entry into the host cell through a subset of protein effectors, for instance. On the genome level *T. gondii* matches its closest protozoan relatives by over 80%, yet its transmission across intermediate host organism species and virulence within each of several species studied surpasses theirs. Aside from varied transcriptional expression, an array of differentially diversified protein effectors could account for the transmission-and-virulence advantage of *T. gondii*. We report the genetic deletion of a tandem duplicated-and-diversified gene cassette present in *T. gondii* and not in its closest protozoan relatives which we have termed a *Toxoplasma gondii*-specific expanded locus (TSEL), one known gene of which encodes for a KRUF (Lysine-Arginine rich unidentified function) family protein with a putative role in translation. Through the generation of independent *T. gondii* knockout clones, the removal of the reported TSEL did not prevent parasite growth in tissue culture (human foreskin fibroblasts). Further tissue culture growth assays and *in vivo* studies are impending.

Author Disclosure Block:

G.W. Alfonse: None.

Poster Board Number:

FRIDAY-537

Publishing Title:

***Toxoplasma gondii* Infections Alter Host Neuronal Gabaergic Signaling**

Author Block:

I. Blader[1], J. Brooks[1], M. Fox[2]; [1]Univ. at Buffalo, Buffalo, NY, [2]Virginia Tech, Roanoke, VA

Abstract Body:

BACKGROUND: Patients suffering from microbial infections of the central nervous system often develop seizures. Breakdown of the blood brain barrier, inflammatory cell influx, altered ionic homeostasis, and glial cell activation are amongst the changes that occur during infections that have been proposed to contribute to seizure development. But how these changes impact neuronal structure and function to cause seizures is not understood. Gamma-aminobutyric acid (GABA) is the primary inhibitory neurotransmitter that regulates the flow and timing of excitatory neurotransmission and when this pathway is altered, seizures develop. We therefore hypothesized that neuronal infections alter GABAergic signaling and tested this in mice infected with the protozoan parasite *Toxoplasma gondii*. **METHODS:** We used immunohistochemistry and electroencephalography/electromyography to assess GABAergic synapses and seizure activity in *Toxoplasma*-infected mice. **RESULTS:** Here, we show that infection with type II *Toxoplasma* tissue cysts led to global changes in the distribution of GAD67, a key enzyme that catalyzes GABA synthesis in the brain. The effect of *Toxoplasma* on GAD67 was specific since excitatory presynaptic markers were unaltered by infection. Alterations in GAD67 staining were not due to decreased expression but rather a change from GAD67 clustering at presynaptic termini to a more diffuse localization throughout the neuron. Consistent with a loss of GAD67 from the synaptic terminals, *Toxoplasma*-infected mice develop spontaneous seizures and are more susceptible to drugs that induce seizures by antagonizing GABA receptors. Interestingly, GABAergic protein mislocalization and the response to seizure inducing drugs were observed in mice infected with type II but not type III strain parasites indicating a role for polymorphic parasite factor(s) in regulating GABAergic synapses. We are currently examining the role that *Toxoplasma*-induced neuroinflammation plays in GAD67 mislocalization and seizure development. **CONCLUSIONS:** Taken together, these data support a model in which seizures and other neurological complications seen in *Toxoplasma*-infected individuals are due, at least in part, to changes in GABAergic signaling.

Author Disclosure Block:

I. Blader: None. **J. Brooks:** None. **M. Fox:** None.

Poster Board Number:

FRIDAY-538

Publishing Title:

Exploring the Host Targets of MPR1, a Fungal Metalloprotease That Facilitates the Transmigration of *Cryptococcus neoformans* Across the Blood-Brain Barrier

Author Block:

S. Na Pombejra[1], C. Zhang[2], M. Salemi[1], B. S. Phinney[1], A. Gelli[1]; [1]Univ. of California, Davis, CA, [2]Second Military Med. Univ., Shanghai, China

Abstract Body:

Cryptococcus neoformans can cause a life-threatening fungal infection in immunocompromised patients. The high mortality of cryptococcosis is associated with cryptococcal invasion of the central nervous system (CNS) via the blood brain barrier (BBB). By examining the extracellular proteome of *C. neoformans*, we identified a novel secreted metalloprotease, Mpr1 that facilitates the migration of *C. neoformans* across the BBB. We sought to identify the host targets of Mpr1 and resolve the molecular mechanism of Mpr1 during BBB crossing.Remarkably, we found that *Saccharomyces cerevisiae* (Sc - normally cannot cross the BBB) expressing Mpr1 (Sc<CnMPR1>) gained the ability to attach to brain endothelial cells and migrate across the in vitro BBB model. The transmigration of Sc<CnMPR1> occurred without tight junction disruption confirmed by transendothelial electrical resistance (TER) and FITC-dextran permeability. This suggested that Sc<CnMPR1> penetrated through the BBB transcellularly similar to *C. neoformans*. Moreover, SEM imaging of both wild type Sc (ScWT) and Sc<CnMPR1> strains showed no morphological difference on the yeast cell surfaces. Based on these observations, we hypothesized that instead of altering the fungal cell surface Mpr1 might interact with surface proteins of the BBB and thereby facilitate the transmigration of fungal cells. We exploited the gain function of Sc<CnMPR1> to identify specific host targets of Mpr1. Cell surface proteins of hCMEC/D3 cells (human brain endothelial cell line) were labeled with sulfo-NHS-SS-biotin and then isolated via magnetic beads coupled to a biotin antibody. The labeled proteins were incubated with Sc<CnMPR1> or ScWT (control). Following incubation, yeast were washed to remove non-specific binding & unbound proteins. Host proteins that remained attached to the yeasts were eluted and identified by MALDI-TOF MS/MS. Proteomics analysis revealed hits corresponding to surface proteins of brain endothelial cells with roles in cytoskeleton-plasma membrane rearrangement and endosomal trafficking – process that mediate transcellular migration of *C. neoformans*.

Author Disclosure Block:

S. Na Pombejra: None. **C. Zhang:** None. **M. Salemi:** None. **B.S. Phinney:** None. **A. Gelli:** None.

Poster Board Number:

FRIDAY-539

Publishing Title:

An Eph-Tyrosine Kinase Receptor-Activated Pathway Mediates Transmigration of *C. neoformans* across the Blood-Brain Barrier

Author Block:

M. Jamklang, **P. A. Robins**, A. Gelli; Univ. of California, Davis, Davis, CA

Abstract Body:

Cryptococcus neoformans is the leading cause of fungal meningoencephalitis, a devastating disease that occurs primarily in immunocompromised individuals and claims more than 600,000 deaths per year. Colonization of the brain occurs because *C. neoformans* manages to breach the blood-brain barrier (BBB) and although mounting evidence supports a transcellular mechanism of crossing, the genes and gene products that mediate this process remain largely unknown. Given that the initial association and attachment of cryptococcal cells to the BBB is a key step in the development of meningoencephalitis, we sought to resolve the transcriptome of the BBB during this early stage of transcytosis. To do this we exposed human brain microvascular endothelial cells (hCMEC/D3) to *C. neoformans* and performed high-throughput sequencing on the Illumina platform (RNA-seq). Among the many canonical signaling pathways identified as mediating molecular interactions during the association of *C. neoformans* with brain endothelial cells, we found that the EPH tyrosine kinase receptor-activated pathway is a key player. The EPH receptors and their membrane-tethered ligands (ephrins) mediate several different cellular processes including modulation of the cytoskeleton, cell-substrate adhesion and activation of signaling. Using siRNA to silence the gene encoding an EPH tyrosine kinase receptor in hCMEC/D3 cells, we found that the transmigration of *C. neoformans* in the *in vitro* model of the BBB was significantly reduced. Accordingly, the addition of a chemical agonist (doxazosin) known to activate the EPH pathway promoted greater transmigration of *C. neoformans* across the BBB. Similar transmigration results were observed upon the addition of an EPH ligand (ephrinA1). Our results also suggest that the enhanced transmigration of cryptococci observed upon activation of the EPH receptor did not alter the integrity of the tight junctions consistent with a transcellular mechanism. Taken together our results suggest that the EPH receptor may serve as a link that couples *C. neoformans*' penetration to cytoskeleton–remodeling by serving as an entry and intracellular signaling receptor for *C. neoformans*.

Author Disclosure Block:

M. Jamklang: None. **P.A. Robins:** None. **A. Gelli:** None.

Poster Board Number:

FRIDAY-540

Publishing Title:

Understanding The Murine Immunological Susceptibility To Candida Albicans

Author Block:

D. P. Agustinho[1], F. Sedlazeck[2], M. Oliveira[3], A. L. Bocca[3], A. A. H. Tavares[3], I. Silva-Pereira[3]; [1]Albert Einstein Coll. of Med., New York, NY, [2]Simons Ctr. for Quantitative Biology, Cold Spring Harbor Lab., New York, NY, [3]Univ. of Brasilia, Brasilia, Brazil

Abstract Body:

The commensal fungal pathogen *Candida albicans* can cause lethal systemic infections in immunocompromised patients. Macrophages and monocytes are the inate immune cells that represent the first line of defense against *C. albicans* infection, and they recognise pathogens via Pattern Recognition Receptors (PRRs). Activation of these receptors induces a signaling pathway that culminates in specific cytokine production, that orchestrates a specific immune response. Understanding the patterns of gene expression that results in resistance to this pathogen could be key in unraveling the infection process and in elaborating novel and more efficient treatments for this disease.In this study we focused on analyzing the molecular patterns of susceptibility to *C. albicans* by investigating the differences in gene expression response of macrophages from two mouse strains, one susceptible and the other resistant to this fungus. This is the first study analyzing the global gene expression in different models of suscetibility to *C. albicans*, and the first to investigate both host macrophage and pathogen cell at the same time. Bone marrow-derived macrophages from the susceptible and resistant strains (DBA/2J and BALB/c, respectively) were co-cultured with *C.albicans* yeasts for different time points and RNA from both pathogen and host cells were extracted and sequenced by Dual RNA-seq. Differential expression analysis showed that many PRR pathways are altered between the resistant and the suceptible mouse strain. The resistant strain (BALB/c) shows a more defined inflamatory response, while the suceptible strain (DBA/2J) shows a down-regulation of TLR and MAPK signalling pathways elements, as well as pro-inflamatory cytokines. The pathogen, on the other hand, shows distinct metabolic differences when interacting with both mouse strains. Theses results led thus to formulate a model of the differences of interaction between macrophages from these two mouse strains and *C. albicans*, possibly highlighting possible new targets for *C. albicans* infection therapy.

Author Disclosure Block:

D.P. Agustinho: None. **F. Sedlazeck:** None. **M. Oliveira:** None. **A.L. Bocca:** None. **A.A.H. Tavares:** None. **I. Silva-Pereira:** None.

Poster Board Number:

FRIDAY-541

Publishing Title:

***Cryptococcus neoformans* Releases Proteins into Macrophages That Are Associated with Host Cell Metabolic Changes**

Author Block:

E. H. Jung, L. Huang, L. Santambrogio, A. Casadevall; Albert Einstein Coll. of Med., Bronx, NY

Abstract Body:

Cryptococcus neoformans is a facultative intracellular pathogen that can persist and replicate in macrophages. The outcome of the *C. neoformans*-macrophage interaction is a key determinant contributing to the outcome of infection. Previous studies have shown that vesicles released by *C. neoformans* under normal laboratory growth conditions contain polysaccharide and protein components associated with virulence[1]. In this study we analyzed fungal and host proteins in macrophages infected with *C. neoformans*. We used two cell types to study mammalian interaction with *C. neoformans*; an immortalized murine macrophage-like cell line (J774.16) and primary bone marrow macrophages derived from C57BL/6 mice to identify proteins released from cryptococcal cells into macrophages. Mass spectrometry and proteomic analysis allowed us to identify one hundred and twenty six proteins of fungal origin in the lysate of *C. neoformans* infected macrophages. The functions of these proteins ranged from transcription factors to virulence factors like urease. In addition, we analyzed macrophage proteome in macrophages infected with *C. neoformans* and found significant differences in the protein expression of multiple pathways including cell death. These results indicate that quantifiable amounts of proteins are secreted by *C. neoformans* during infection and that host macrophages alter their expression patterns based on the viability of the pathogen.

Author Disclosure Block:

E.H. Jung: None. **L. Huang:** None. **L. Santambrogio:** None. **A. Casadevall:** None.

Poster Board Number:

FRIDAY-542

Publishing Title:

***Candida parapsilosis* Cell-wall Adhesins Similar to Agglutinin-like Sequence (Als) Proteins are Involved in Binding of Fibronectin and Adhesion to Human Epithelial Cells**

Author Block:

A. Kozik, D. Zajac, G. Bras, J. Karkowska-Kuleta, O. Bochenska, M. Rapala-Kozik; Jagiellonian Univ., Faculty of Biochemistry, Biophysics and Biotechnology, Krakow, Poland

Abstract Body:

Background: Adhesion to host tissues, mediated by pathogen surface-exposed molecules is a critical step in the pathogenesis of infections caused by fungal pathogens of humans. At the cell surface of *Candida parapsilosis*—a widespread commensal commonly isolated from human skin, but also an emerging infectious agent that causes systemic candidiasis in immunocompromised individuals—several proteins similar to *C. albicans* agglutinin-like sequence proteins (Als) have recently been identified and considered as potentially important virulence factors. However, their actual interactions with the host surfaces such as the epithelium or extracellular matrix (ECM) has not been satisfactorily characterized. **Methods:** In this work two Als-like *C. parapsilosis* proteins—Cpar2_404780p and Cpar2_404800p—were isolated from a fraction of beta-1,6-glucanase-extractable cell-wall proteins and purified by ion-exchange and gel filtration chromatography. Adhesive properties of these proteins towards an ECM protein—fibronectin—were characterized with the use of surface plasmon resonance measurements, and their ability to bind to human epithelial cells of the A-431 line was tested by microtiter assays with the use of pre-labeled fungal proteins.**Results:** The dissociation constants determined for the complexes of Cpar2_404780p and Cpar2_404800p with human fibronectin were of 7.76×10^{-10} M and 5.54×10^{-9} M, respectively, suggesting a very high affinity of these fungal proteins to the host ECM. Moreover, both fungal proteins strongly adhere to the surface of epithelial cells, to a capacity of 0.1 and 0.05 pmoles of Cpar2_404780p and Cpar2_404800p bound per 1×10^{6} of human cells.**Conclusion:** Characterization of the interactions of two Als-like fungal adhesins with fibronectin, as well as evidence for their adherence to the human epithelium, deepen our understanding of the mechanisms of host-pathogen interactions during candidiasis caused by *C. parapsilosis*.

Author Disclosure Block:

A. Kozik: None. **D. Zajac:** None. **G. Bras:** None. **J. Karkowska-Kuleta:** None. **O. Bochenska:** None. **M. Rapala-Kozik:** None.

Poster Board Number:

FRIDAY-543

Publishing Title:

Biological Control of *Eichhornia crassipes* Using Fungal Plant Pathogens

Author Block:

J. P. B. Abengaña, G. R. Dedeles; Res. Ctr. for Natural and Applied Sci., Univ. of Santo Tomas, Manila, Philippines

Abstract Body:

Eichhornia crassipes (Mart.) [Solms] or commonly known as water hyacinth is an aquatic weed, found in lakes, rivers, and ponds of countries with temperate climates. Apart from environmental problems arising from water hyacinth infestation, it also impose a negative impact on the socioeconomic status of infested areas.Thus, In this study, fungal plant pathogens were tested for their potential as biological control agents to prevent the rapid proliferation of water hyacinth.Healthy water hyacinth plants with no trace of any disease were collected from aquatic weed-infested areas and were cultivated in greenhouse conditions. Fungal pathogens used in the experiment were isolated from water hyacinth plant parts with observable disease manifestation or necrosis using tissue plating and moist chamber technique. Purified cultures of all emergent fungi were subjected to pathogenicity testing on healthy water hyacinth plants using two inoculation methods, namely, agar block and spore suspension method. Ratings for the infection were measured using a grid method to calculate for total percentage of infected surface area. After 2 weeks of incubation period, all fungal isolates which exhibited disease manifestation were further tested for host-specificity test.Out of 92 isolated fungi, 17 of which exhibited a potential for use as biological control agents. And of the 17, 6 isolates exhibited the most promising results. The six of which with tentative identification are coded as isolates BL1 with a 39% infected leaf surface, BL2 with a 68% infected leaf surface, BL3 with a 45% infected leaf surface, BL6 with a 19% infected leaf surface, WH1 with a 55% infected leaf surface, WH4 with a 38% infected leaf surface, and WH5 with a 64% infected leaf surface. On the other hand, host-specificity test conducted on aquatic plants *Pistia stratiotes* and *Ipomoea aquatica* revealed that isolates WH1 and WH5 are not host-specific for water hyacinth which is an undesirable characteristic for fungal pathogens to be considered as an effective biological control agent. The results indicated that isolates BL1, BL2, BL3, BL6, and WH4 may have a potential as effective biological control agents for water hyacinth. Further experiment is recommended to see if there will be an increased in the effectivity of these fungal pathogens when applied in combinations.

Author Disclosure Block:

J.P.B. Abengaña: None. **G.R. Dedeles:** None.

Poster Board Number:

FRIDAY-544

Publishing Title:

A New Murine Model of Cutaneous Aspergillosis in a Large Skin Defect

Author Block:

A. M. Tatara[1], N. D. Albert[2], A. G. Mikos[1], D. P. Kontoyiannis[2]; [1]Rice Univ., Houston, TX, [2]The Univ. of Texas MD Anderson Cancer, Houston, TX

Abstract Body:

Background: Cutaneous aspergillosis is a clinically challenging condition to resolve. In part, this is due to the ability of *Aspergillus* to invade local blood vessels and suppress host angiogenesis, resulting in large necrotic ulcers with poor innate regenerative capacity[1]. In order to better understand this disease process, as well as build a platform for developing improved therapeutic strategies, we have created a new murine model of cutaneous aspergillosis featuring a large skin defect.**Methods:** Chemically immunosuppressed BALB/c mice[1] were inoculated with subcutaneous injections of either saline or $1.75x10^6$ conidia/mL *A. fumigatus* Af293. After 72 hours, a full-thickness surgical wound of 5mm was created with a sterile biopsy punch and the wound was re-inoculated at the same dosage. Every 3 days, the size of the wound bed was measured. Mice were euthanized at Day 9 (n=5 per group, performed in duplicate) and Day 18 (n=5 per group). After euthanasia, the wound bed was harvested subjected to histologic analysis for host inflammation and presence of fungi.**Results:** As early as day 9, the rate of cutaneous wound healing was significantly inhibited by the presence of *Aspergillus* infection (wound area reduction of 13.5%, p=0.55), whereas saline groups demonstrated significant reduction of wound area (39.0%, p=0.017). Viable *A. fumigatus* could still be recovered from the wound bed at least two weeks after the initial infection. Histology revealed the presence of hyphae and inflammation in infected wound beds at time of euthanasia.**Conclusions:** We have successfully created a murine model of chronic cutaneous aspergillosis infection of a large tissue defect with impaired wound healing. This model reproducibly recapitulates the impaired wound healing seen in immunocompromised patients. Unlike previous work, this model allows for 1) real-time macroscopic assessment of healing of a large cutaneous *Aspergillus*-infected wound, 2) chronicity of infection, and 3) the ability to test novel local therapies for fungal-infected wound beds in the future.

Author Disclosure Block:

A.M. Tatara: None. **N.D. Albert:** None. **A.G. Mikos:** None. **D.P. Kontoyiannis:** None.

Poster Board Number:

FRIDAY-545

Publishing Title:

Immune Recognition of *Candida albicans* in Zebrafish

Author Block:

M. E. Theriault, R. T. Wheeler; Univ. of Maine, Orono, ME

Abstract Body:

Candida albicans is an opportunistic fungal pathogen that causes diseases ranging from oral thrush to candidemia in immunocompromised individuals. Recognition of pathogens, like *C. albicans*, during infection is poorly characterized primarily due to the difficulties in visualizing the host/pathogen interaction without killing the host. Transparent animal hosts, such as *Danio rerio* (zebrafish), are necessary to accomplish the task of imaging pathogen recognition while maintaining host viability. For pathogen recognition, zebrafish likely use immune receptors similar to mammalian receptors including C-type lectin receptors. Human C-type lectin receptors have already been shown to be crucial in recognition of fungal pathogens like *C. albicans*, and our goal was to identify and characterize cognate receptors crucial for fungal recognition in zebrafish. Fusion proteins of recently identified receptors were expressed via cell culture and purified with HiTrap Protein A columns. Characterization of receptor binding was conducted using fluorescence microscopy as well as with a high-throughput luminescence binding assay. Recognition by the purified zebrafish receptors of *C. albicans* was observed using fluorescence microscopy. Optimization of the luminescence assay has proved a highly efficient way to detect receptor-ligand binding *in vitro*. Due to its efficiency, this assay will be used to determine the binding affinities of the purified receptors to various other microbes to allow for increased characterization. These studies have contributed to a better understanding of fungal pathogen recognition in zebrafish, an animal model widely used to study disseminated *C. albicans* infection.

Author Disclosure Block:

M.E. Theriault: None. **R.T. Wheeler:** E. Grant Investigator; Self; Regeneron.

Poster Board Number:

FRIDAY-546

Publishing Title:

Human Gingival Fibroblasts from Patients Infected with Periodontal Pathogens are Less Sensitive to *Candida albicans* Infection

Author Block:

M. Rapala-Kozik[1], D. Ostrowska[1], J. Sykut[1], O. Bocheńska[1], J. Karkowska-Kuleta[1], K. Seweryn[1], W. Aoki[2], M. Ueda[2], A. Kozik[1]; [1]Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian Univ., Krakow, Poland, [2]Div. of Applied Life Sci., Graduate Sch. of Agriculture, Kyoto Univ., Kyoto, Japan

Abstract Body:

Background: Within the gingival tissue, fibroblasts not only constitute the most common cell type that regulate the homeostasis of the connective tissue but are also involved in contacts with periodontal pathogens that form a network of mutualistic or antagonistic interactions at the site of infection. *Candida albicans*—an opportunistic fungal pathogen—is present in the oral cavities of significant part of the population. In particular, it has been identified in gingival tissues and periodontal pockets where it may interact with bacteria to colonize the host. The analysis of the responses of bacteria-infected fibroblasts to the virulence factors of *C. albicans* can provide novel insights into the regulation of host responsiveness to fungal infections.**Methods:** Primary gingival human fibroblasts, obtained from healthy donors and chronic periodontitis patients, were treated with whole *C. albicans* cells or selected aspartic proteases (Saps), glucans, mannans and the whole mixture of isolated cell wall proteins. Lipopolysaccharide served as a positive control. After incubation for different time interval, RNA and protein were extracted and the transcript or protein levels were determined for interleukins and myofibroblast markers**Results:** Two aspartic proteases—Sap3 and Sap6—were identified as the main *C. albicans* factors that activated gingival fibroblasts to produce cytokines and induced the myofibroblast formation. The cell wall proteins and glucans or mannans were less effectively recognized by these cells. Fibroblasts isolated from patients with chronic periodontitis were less sensitive to contact with *C. albicans* cells and Sap treatment.**Conclusions:** Determination of the nature of cytokine influence on the interactions between *C. albicans* and bacteria-stimulated gingival fibroblasts as well as possible changes of the proteome of these host cells may lead to the development of novel strategies to prevent or treat oral candidiasis.

Author Disclosure Block:

M. Rapala-Kozik: None. **D. Ostrowska:** None. **J. Sykut:** None. **O. Bocheńska:** None. **J. Karkowska-Kuleta:** None. **K. Seweryn:** None. **W. Aoki:** None. **M. Ueda:** None. **A. Kozik:** None.

Poster Board Number:

FRIDAY-548

Publishing Title:

***in Vitro* Dopamine Activity On Gram Negative Bacilli (Gnb) And *candida* spp Biofilm (Bf) From Urogenital Infections (Ugti)**

Author Block:

A. E. FARINATI, Sr., R. Villanueva, M. Marques, C. Layño; SALVADOR Univ., MARTINEZ, BUENOS AIRES, Argentina

Abstract Body:

Background: The majority of microbial endocrinology investigations have focused on the bacteria interaction of stress-associated molecules. Some authors affirm and others denied that dopamine (DA) can modulate growth and virulence factors production in planktonic *E. coli* but they have not assayed with microorganisms (MO) in BF. Aim: to study the DA influence on GNB and *Candida* biofilms from UGTI.**Methods:** We used 8 clinical isolates; 2 GNB: *Escherichia coli* (EC) and *Proteus mirabilis* (PM); yeast: 3 *Candida albicans* (CA) and 3 Non *albicans Candida* (NAC). The BF was investigated using a glass coupon (GC). GNB isolates were inoculated in Trypticase Soy Broth (TSB) and Peptone Broth (PB) with GC. CA and NAC isolates were inoculated in Sabouraud Broth with GC. We used DA (6.0 ± 1.1 ng/ml) that simulate plasma concentration and 12.0 ± 1.1 ng/ml (elevate DA levels) as stress agents. The controls without DA were used with similar techniques. Assays were performed in triplicate; everything was read by optical microscopy previous crystal violet tinction after 6, 12 and 24 hours. To evaluate and compare the results we used a scoring system (SS) based on BF growth (BFSS) from 0 to 5. A 0 is given for the absence of BF, 1 to 5 are given to different growing BF phases.**Results:**DA at plasma level increased the EC BF at early formation (BFSS 2 *vs* control 1) and the increasing is more evident at 24 hours (BFSS 4 vs control 2). There is not variation with TSB and PB. Under influence of elevate DA the BFSS are 3 and 5 respectively vs 1 and 3 of controls. The influence over CA BF is not evident in early stages and at 24 hours. In NAC BF appears a variation of 3 to 5 in the score vs 1 to 3 in 24 hours controls under the two DA levels. **Conclusions:** The present data suggests that DA increased the GN BF growth at least in EC and PM but the influence on *Candida* BF is different. The interaction between DA and *Candida* spp BF is not evidenced in some CA isolates, even though the highest concentration was used. The BF increased in NAC under influence of DA. The association between stress hormones and urogenital infections are well known. Most of them have been related to the suppressing immune function. It is important to take into consideration the influence of DA on microorganisms BF, mainly under stress condition.

Author Disclosure Block:

A.E. Farinati: None. **R. Villanueva:** None. **M. Marques:** None. **C. Layño:** None.

Poster Board Number:

FRIDAY-549

Publishing Title:

Induction of Th2 Responses to Ingested Antigen Following *Candida albicans* Colonization/Infection of the Gastrointestinal Tract

Author Block:

H. E. Lust, N. R. Falkowski, E. M. Hunter, R. A. McDonald, G. B. Huffnagle; Univ. of Michigan, Ann Arbor, MI

Abstract Body:

Background: The interplay between the microbiome and the gastrointestinal (GI) immune system protects against pathogenic organisms, while concurrently providing an environment tolerant to the indigenous microbiota and dietary antigens. Thus, major alterations in the microbiome ("dysbiosis") have the potential to dysregulate GI mucosal immunity and lead to the development of immune responses to ingested antigens. The goal of this study was to investigate the development of Th2 immunity to oral antigen exposure following broad spectrum antibiotic treatment and subsequent *Candida albicans* colonization.**Methods:** Two groups of C57BL/6 mice were given ovalbumin (OVA) in their drinking water and via oral gavage. One group (OCC) was treated with ceftriaxone in the drinking water and subsequently inoculated orally with *C. albicans* CHN1; the other group (OUU) was untreated. The mice were followed over a 3-week period.**Results:** In the OCC mice, *Candida* was cultured from the stomach, throughout the intestinal tract and in the feces. Histologically, there was *Candida* colonization in the limiting ridge of the stomach with the presence of yeast, hyphae and neutrophils. In contrast, there was no detectable tissue inflammation or fungal invasion of the intestinal tract, suggesting that *Candida* was colonizing the lumenal/mucus layer. Following exposure to OVA, there was a 5 log increase in microaerobic bacteria in the feces of OCC mice. Additionally, there was a 1 log increase in lactic acid bacteria and a 7 log increase in *C.albicans* in the feces. By the end of the three week study, there was a >100 fold increase in serum IgE levels and evidence of T cell activation in the spleens of OCC mice.**Conclusions:** These results indicate that *C.albicans* infection of the stomach mucosa and colonization of the intestinal tract can modulate mucosal immunity to an oral antigen.

Author Disclosure Block:

H.E. Lust: None. **N.R. Falkowski:** None. **E.M. Hunter:** None. **R.A. McDonald:** None. **G.B. Huffnagle:** None.

Poster Board Number:

FRIDAY-550

Publishing Title:

A Role of *Candida albicans* Als3 in Gut-Derived Fungal Sepsis

Author Block:

Y. Nanjo, A. Yamamoto, Y. Ishii, K. Tateda; Toho Univ., Tokyo, Japan

Abstract Body:

Background: *Candida albicans* is one of the most common causes of nosocomial bloodstream infections. Especially in cancer patients undergoing immune suppression, translocation of *C. albicans* from gut to bloodstream is reportedly a major cause of fungemia. However, there is limited information available to explain the mechanisms of *C. albicans* entry and virulence factors involved. In the present study, we compared occurrence of *C. albicans* translocation and lethality in several clinical isolates in mice model. The presence and expression of *C. albicans* virulence factors were determined by quantitative RT-PCR (qRT-PCR). **Methods:** A total of 68 clinical isolates of *C. albicans* (Toho University Hospital) and 2 standard strains were used. *C. albicans* was administered via the drinking water (approximately $1.0x10^7$ CFU/mL) to mice for 5 days, and mice were treated with cyclophosphamide to induce translocation through the gut to systemic infection. Total 16 virulence genes in *C. albicans* were examined in clinical isolates by qRT-PCR. An association between virulence gene expression and fungal translocation was analyzed to identify the virulence factor responsible for the fungemia. **Results:** Immne suppression in addition to mucosal damage induced systemic *C. albicans* translocation in the gut in varying degrees of challenged organisms. The number of fungi in the livers undergoing immume suppression and endogenous fungemia was varied in clinical isolates examined. Among 16 virulent genes examined in *C. albicans*, the most striking association was observed in Als-3 (the agglutinin-like sequence-3) between translocation from gut and virulence factors expressed. **Conclusions:** The present data demonstrated that colonization of virulent *C. albicans* may be a prerequisite of endogenous gut-derived *C. albicans* fungemia, although wide range's of mortalitiy differences were observed in strains examined. Among *C. albicans* virulence factors investigated, Als-3 was strongly associated with translocation from gut in mice model of C. *albicans* fungemia. Our data refocused endogenous *C. albicans* translocation in the gut of immunocompromised individuals. Also, Als-3 gene expression was closed up as a responsible virulence factor for gut-derived *C. albicans* sepsis.

Author Disclosure Block:

Y. Nanjo: None. **A. Yamamoto:** None. **Y. Ishii:** None. **K. Tateda:** None.

Poster Board Number:

FRIDAY-551

Publishing Title:

Immune Responses in *"Candida"* Vaginitis and Vaccine Development

Author Block:

F. De Bernardis, S. Graziani, S. Norelli; ISTITUTO SUPERIORE DI SANITA', Rome, Italy

Abstract Body:

Vulvovaginal candidiasis affects at least 75% of women. the recurrent forms of the disease (RVVC) has a prevalence of 7-15% and devastates the quality of life, causes high cost of medical visits and large use of drugs with possible subsequent upsurge of drug resistance. The aim of our work was to investigate the host defence mechanisms in vaginal candidiasis and to identify "*Candida"* molecules potentially useful for vaccination or immunotherapy of this disease. **"***Candida"* vaginitis has been reproduced in mice and rats. Animals were injected subcutaneously with of estradiol and were inoculated intravaginally with *C.albicans* strains To characterize the interaction between innate and adaptative immunity, the role of the Toll like receptors (TLRs) and C-type lectin like receptors (CLRs) in vaginal candidiasis was evaluated in mice with different TLR knocked out genes. The results suggest that TLRs and CLRs have a role in protection to "*Candida"* at vaginal level. In particular, TLR4 and Dectin 1 are both required for the induction of acquired protective immunity to the fungus.The evidence of an immune response in the vaginal compartment was very encouraging to identify the proper targets for new strategies for vaccination or immunotherapy of vaginal candidiasis. We were working with a recombinant protein: an aspartyl-proteinase (Sap2) which is an important immunodominant antigens and virulence factors of "*C.albicans"* acting in mucosal infections. The recombinant protein Sap2 was assembled with virosomes and a vaccine PEV7 was obtained. The results evidenced that the vaccine PEV7, intravaginally administered, confers protection against "*C. albicans"* vaginal infection as exemplified by the accelerated clearance of the fungus from the vagina and resolution of the infection at least one week before infection in controls (administration of empty virosomes).The results evidenced the contribution of innate and adaptive immune responses in vaginal candidiasis. The vaccine PEV7 has an encouraging therapeutic potential for the treatment of recurrent vulvovaginal candidiasis. This opens the way to a modality for anti-"*Candida"* protection at mucosal level.

Author Disclosure Block:

F. De Bernardis: None. **S. Graziani:** None. **S. Norelli:** None.

Poster Board Number:

FRIDAY-552

Publishing Title:

The Entomopathogenic Fungus *Aspergillus sclerotiorum* as a Potential Biocontrol Agent of Western Subterranean Termites (R*eticulitermes hesperus*)

Author Block:

T. S. Laird, S. M. Richart; Azusa Pacific Univ., Azusa, CA

Abstract Body:

Termites have an important ecological role in regards to nutrient cycling and soil quality. However, termites, especially subterranean termites, are pests that cause billions of dollars in structural damage worldwide. Methods of termite control typically involve the use of hazardous pesticides. However, alternative methods exist, including the use of entomopathogenic fungi. Our previous work showed that *Aspergillus sclerotiorum* was pathogenic to western drywood and western subterranean termites. This study establishes that in Petri dish bioassays, spores of *A. sclerotiorum* were able to be transmitted between exposed and unexposed subterranean termites (*Reticulitermes hesperus*) which affected termite survivability. With 50%, 27%, and 10% of termites initially exposed to spores, survivability was reduced by 80%, 71%, and 35% respectively compared to the control. Further experimentation was conducted in conical tubes filled with wet sand, whereby only 10% of termites were exposed to fungal spores. In this model, however, which better mimics the trap and treat field application of fungus, there was only 16% reduction in survivability compared to the control.. This suggests that *A. sclerotiorum* may not be an ideal biocontrol agent for use against western subterranean termites in pest control scenarios, but still has potential as a model for understanding insect host-pathogen interactions.

Author Disclosure Block:

T.S. Laird: None. **S.M. Richart:** None.

Poster Board Number:

FRIDAY-553

Publishing Title:

Fumarate and Nitrate Reduction Regulatory Protein-Dependent Proliferation of *V. vulnificus* in Intestine Is Required for Effective Dissemination to Further Organs

Author Block:

T. Kado, T. Kashimoto, K. Yamazaki, S. Ueno; Kitasato Univ., Towada, Aomori, Japan

Abstract Body:

Vibrio vulnificus (*V. vulnificus*) causes a severe sepsis for human after ingestion of sea foods or direct exposure of a wound to seawater. Fatal cases in *V. vulnificus* infection mostly associate with underlying diseases such as chronic liver diseases, immunological dysfunction, and diabetes. The average of incubation period in primary septicemia is only about 36 h. Besides being a short time incubation period, the mortality rate is quite high (exceed 50%). Such fast growing and disseminating abilities of *V. vulnificus* in vivo are bring into a poor clinical outcome in patients. Therefore, elucidation of the proliferation mechanisms of this organism in vivo may lead to development of an effective therapeutic strategy. In our previous study, the significantly higher numbers of *V. vulnificus* were detected from the intestine in the dead mice than that of the surviving mice after orogastric challenge of *V. vulnificus*, suggesting that proliferation of *V. vulnificus* in intestine is the initial critical event for lethal sepsis in oral infection. In this study, we focused on the low oxygen concentration in intestinal milieu because of a drastic change from that of in the air. FNR has been known as the global transcriptional regulator for adaptation to anaerobic condition in various bacteria. When the *fnr* deletion mutant of *V. vulnificus* (*fnr*::*erm*) was tested in competition assay against its isogenic parent (WT) in vivo, the competitive index of *fnr*::*erm* to WT were 0.378±0.096 (Mean±S.E.M) and 0.243±0.0615 in the intestinal loop and liver respectively. Thus, we found that the FNR-dependent proliferation of *V. vulnificus* in intestine is required for effective dissemination to further organs.

Author Disclosure Block:

T. Kado: None. **T. Kashimoto:** None. **K. Yamazaki:** None. **S. Ueno:** None.

Poster Board Number:

FRIDAY-554

Publishing Title:

The Effect of Oxygen On Acid Tolerance of Listeria Monocytogenes

Author Block:

H. N. Jenkins, D. McClung, B. Roberts, J. R. Donaldson; Mississippi State Univ., Mississippi State, MS

Abstract Body:

Background: *Listeria monocytogenes* is a gram-positive facultative anaerobe that is responsible for the foodborne illness listeriosis. This bacterium is resistant to the broad pH range and oxygen availability encountered within the gastrointestinal (GI) tract. Several virulence factors are known to facilitate this pathogen's survival in these conditions. Recent evidence has indicated that anaerobic conditions can influence the expression of virulence genes, suggesting that oxygen availability impacts virulence potential. Therefore, the purpose of this study was to determine if anaerobiosis impacted the acid tolerance of *L. monocytogenes* using conditions that mimic those encountered within the GI tract. The hypothesis was that tolerance to acidic pH would be enhanced under anaerobic conditions, particularly in regards to strains isolated from listeriosis cases. **Methods:** In order to test this hypothesis, 20 strains of *L. monocytogenes* representing various serotypes and isolation sources were cultured in tryptic soy broth (TSB) to mid-logarithmic phase in aerobic or anaerobic conditions and then cultured in media at a either a pH of 7 or 3 for 2 hours in the same aerobic or anaerobic conditions. Viable plate counts were determined. Fold changes in the expression of putative oxygen sensors were determined by RT-qPCR.**Results:** The results showed an increase in viability at a pH of 3 when strains were cultured under anaerobic conditions in comparison to aerobic conditions. Several virulent strains showed remarkable increases in survival (<1% aerobically to >30% anaerobically). The expression of *resD* was also found to increase anaerobically among these strains.**Conclusions:** These data suggest that oxygen availability influences acid tolerance, but the impact varies among strains of *L. monocytogenes.* Further research is needed to determine the mechanism by which anaerobiosis impacts the stress response of *L. monocytogenes.*

Author Disclosure Block:

H.N. Jenkins: None. **D. McClung:** None. **B. Roberts:** None. **J.R. Donaldson:** None.

Poster Board Number:

FRIDAY-555

Publishing Title:

Defining the Genetic Basis of Growth Promotion of *Staphylococcus lugdunensis* by Hemoglobin, Stress Hormones, and Siderophores

Author Block:

J. R. Brozyna, D. E. Heinrichs; Univ. of Western Ontario, London, ON, Canada

Abstract Body:

Staphylococcus lugdunensis is capable of causing an array of serious infections reminiscent of the highly virulent *S. aureus*. Iron is an essential nutrient and the host restricts its access as a form of nutritional immunity. Success of *S. lugdunensis* as a pathogen must be, in part, attributable to its success at stealing iron from the host. Uniquely for staphylococci, *S. lugdunensis* does not produce siderophores - small iron-chelating molecules that strip iron from host glycoproteins, including the major iron source in serum (transferrin). Rather, *S. lugdunensis* can pirate siderophores from other bacterial species and can use heme/human hemoglobin (Hb) as an iron source. It may be metabolically favourable to simply use exogenous siderophores or obtain heme-iron to support *S. lugdunensis* growth in the host. We have demonstrated that serum is growth inhibitory to *S. lugdunensis*, unless it is supplemented with heme, Hb, siderophores or catecholamine stress hormones (e.g. epinephrine, dopamine). We have identified the genetic basis of these transport processes through construction and characterization of genetic deletion mutants, which are impaired for growth with the various metabolites. These mutants allow us to assess whether *S. lugdunensis* preferentially steals iron from transferrin via exogenous siderophores and circulating catecholamines, or whether it prefers to scavenge heme-iron in various host niches. We have coupled mutations into a single strain that is incapable of using heme, hemoglobin, siderophores and stress hormones as sources of iron. This mutant is impaired in its ability to cause subcutaneous lesions, and significantly impaired for its ability to colonize the kidneys of mice. This research provides insight into the utility of various iron acquisition strategies employed by *S. lugdunensis*, and ongoing work will elucidate the role of the various mechanisms in organ-specific infection niches.

Author Disclosure Block:

J.R. Brozyna: None. **D.E. Heinrichs:** None.

Poster Board Number:

FRIDAY-556

Publishing Title:

Between-Patient Evolution of Sepsis *Escherichia coli*

Author Block:

D. Kisiela[1], S. Paul[1], M. Aziz[2], S. Shevchenko[1], K. Polukhina[1], T. Johnson[3], J. R. Johnson[4], L. B. Price[2], E. Sokurenko[1]; [1]Univ. of Washington, Seattle, WA, [2]George Washington Univ., Washington, DC, [3]Univ. of Minnesota, Saint Paul, MN, [4]Univ. of Minnesota, Minneapolis, MN

Abstract Body:

Escherichia coli is one of the major causes of bloodstream infections and death due to sepsis. However, the virulence traits promoting systemic dissemination of *E. coli* are not known. To investigate the evolution of virulence of sepsis strains we performed whole-genome/transcriptome comparative analysis of two within-household transmitted isolates of *E. coli* associated with vividly different clinical illnesses, i.e. fatal sepsis and chronic UTI. We showed that genomes of these two isolates differed only by 8 SNPs, of which three occurred in the cystitis strain and five occurred in the blood strain, with the latter additionally having duplicated 47-kbp region comprising the fec operon. Interestingly, the majority of detected SNPs targeted transcriptional regulators involved in control of stress responses and virulence loci, which resulted in abrogation of the transcriptional regulation. While the cystitis isolate acquired a mutation in cold shock protein CspE, the blood isolate carried mutations in RpoS, LacI, DeoR-family type regulators and LrhA. LrhA is known to be a major repressor of type 1 fimbriae and flagella, with the latter being critical for invasion of renal ducts leading to bacterial systemic dissemination. Interestingly, the RNAseq studies revealed that inactivation of LrhA was not only associated with increased transcription of flagellar genes in urine but also the activation of the temperate phage integrated in the bacterial genome. Thus, LrhA inactivation might be associated with increased lysis of bacterial cells during infection and higher likelihood of sepsis. Indeed, as detected for other *E. coli* strains, the inactivation of LrhA appeared to be a frequent signature of blood isolates. Taken together, the results indicate that *E. coli* undergoes evolutionary changes in the host that might be niche-specific and the inactivation of transcriptional regulator, LrhA, might be of an adaptive nature for blood isolates.

Author Disclosure Block:

D. Kisiela: None. **S. Paul:** None. **M. Aziz:** None. **S. Shevchenko:** None. **K. Polukhina:** None. **T. Johnson:** None. **J.R. Johnson:** None. **L.B. Price:** None. **E. Sokurenko:** None.

Poster Board Number:

FRIDAY-557

Publishing Title:

The Adaptation of *Pseudomonas aeruginosa* in Non-Cystic Fibrosis Bronchiectasis (Ncfb) Patients

Author Block:

T. Woo, J. Duong, H. R. Rabin, M. D. Parkins, D. G. Storey; Univ. of Calgary, Calgary, AB, Canada

Abstract Body:

Background: *Pseudomonas aeruginosa* is a major pathogen in suppurative lung diseases like cystic fibrosis (CF) and non-cystic fibrosis bronchiectasis (nCFB) patients. Colonization of these individuals with *P. aeruginosa* is linked with a decline in respiratory function and an increased risk in morbidity and mortality. While the natural history of *P. aeruginosa* in CF has been well studied, much less research has been done on *P. aeruginosa* from nCFB. As such, we set out to directly investigate the adaptation of *P. aeruginosa* in nCFB. **Methods:** We characterized 84 nCFB and 83 CF isolates for virulence potential which included protease, lipase, and elastase production, 'mucoidy', swarm and swim motility, biofilm production, and for the presence of *lasR* mutants. Of the 84 nCFB isolates we then examined 40 early (defined as the earliest isolate in the biobank) isolates to 41 late (defined as the latest available isolate in the biobank) isolates to observe the natural history of adaptation in nCFB patients.**Results:** Interestingly, we found that the natural history of *P. aeruginosa* remained similar in both chronic lung diseases, with a decline in virulence factor production between early and late time points. Within our cohort, we observed heterogeneity in production of virulence factors both within and between isolates from each disease. We also observed similar average virulence factor production with the exception of lipase and elastase expression, which were significantly lower in *P. aeruginosa* found in nCFB patients relative to CF patients.**Conclusion:** Overall, our data suggests that *P. aeruginosa* adapts similarly to the nCFB lung environment as that in CF with a few notable exceptions. In particular, *P. aeruginosa* from nCFB patients seems to down regulate elastase and lipase production more than that in CF patients. Differential expression of these enzymes may allude to the unique selective pressures within the lung environment of each disease.

Author Disclosure Block:

T. Woo: None. **J. Duong:** None. **H.R. Rabin:** E. Grant Investigator; Self; Gilead Sciences. **M.D. Parkins:** E. Grant Investigator; Self; Gilead Sciences. **D.G. Storey:** None.

Poster Board Number:

FRIDAY-558

Publishing Title:

***Pseudomonas aeruginosa* Co-Regulates a Bifunctional, Bactericidal Chitinase/Lysozyme with Virulence Factors**

Author Block:

J. Flynn[1], K. Kelly[2], R. Hunter[2]; [1]Univ. of Minnesota, Saint Paul, MN, [2]Univ. of Minnesota, Minneapolis, MN

Abstract Body:

The opportunistic pathogen *Pseudomonas aeruginosa* is a causative agent of wound and chronic airway infections. Within these environments, *P. aeruginosa* secretes a number of proteins that have been implicated in their pathogenicity. For example, ChiC, an endochitinase, has been shown to be expressed and secreted during exposure to mucus from cystic fibrosis patients. However, the specific role(s) that a chitinase may play in lung disease is unclear given the lack of chitin in the airways. In this study, we sought to determine the means by which *chiC* expression is regulated and characterize its activity in an effort to determine its role in infection. Using a transposon mutagenesis screen, we found a number of genetic determinants, including the master virulence regulator, MvfR, to be responsible for the regulation of *chiC* expression. Additionally, with enzymatic techniques we confirmed the endochitinase activity of ChiC and found it that it also has lysozyme activity against *Micrococcus lysodeikticus*. Based on these results, we hypothesized these activities were consistent with the bactericidal activity of other 'virulence factors' (*i.e.* pyocyanin, cyanide, proteases) expressed by *P. aeruginosa*. This hypothesis was supported by the ability of purified ChiC to inhibit a number of other bacteria found within the lower airways during chronic CF lung infections. The work presented here suggests the reason for the existence of ChiC in the 'virulence' repertoire of *P. aeruginosa* is to prevent competition for resources in the lower airway environment.

Author Disclosure Block:

J. Flynn: None. **K. Kelly:** None. **R. Hunter:** None.

Poster Board Number:

FRIDAY-559

Publishing Title:

Potential Diversity of Pneumococcal Capsule Based on O-Acetyltransferase Functionality

Author Block:

B. L. Spencer, K. A. Geno, J. S. Saad, M. H. Nahm; Univ. of Alabama-Birmingham, Birmingham, AL

Abstract Body:

Background: Since anti-capsular antibodies confer serotype-specific protection against *Streptococcus pneumoniae* (pneumococcus), understanding capsular diversity is important for anticipating emerging serotypes following vaccine implementation. One mechanism of capsular diversity is O-acetylation, which alters epitopes and may allow evasion of vaccine-elicited antibodies. Serotype 33A has two O-acetyltransferase genes: *wciG* and *wcjE*. A serotype 33A variant, 33F, is *wcjE*-deficient and occurs naturally, but no *wciG*-deficient variants have been reported. Serotype 33F will be included in the next pneumococcal conjugate vaccine, and we hypothesize that vaccine pressure will select for *wciG*-deficient 33F variants in nature. **Methods:** We created three 33A variants by inactivating *wciG* (serotype 33X), *wcjE* (serotype 33F), or both *wciG* and *wcjE* (serotype 33Y) in the serotype 33A strain TIGR33A. Sequencing confirmed O-acetyltransferase gene inactivation. Bacterial cells were stained with serological reagents and analyzed by flow cytometry. Purified capsules were studied with ^{1}H-NMR.**Results:** All variants have serologically-distinct capsules based on rabbit antisera and Hyp33FG1 (monoclonal antibody) binding. ^{1}H-NMR confirmed that TIGR33A capsule has one WciG- and two WcjE-mediated O-acetyl additions (2.14 ppm and 2.10, 2.18 ppm, respectively) and that TIGR33AΔ*wcjE* (functionally 33F) capsule lacks both WcjE-mediated O-acetyl additions. Finally, ^{1}H-NMR showed that TIGR33AΔ*wciG* lacks WciG-mediated O-acetylation (2.14 ppm).**Conclusions:** We conclude that TIGR33AΔ*wciG* and TIGR33AΔ*wciG*Δ*wcjE* express novel serotypes (termed 33X and 33Y, respectively) which can be distinguished by our in-house monoclonal antibody Hyp33FG1. These serotypes are not differentiated by currently used serotyping techniques like Quellung or PCR, and therefore may be present, but undetected in nature. This study describes a possible mechanism of capsular diversity via O-acetylation and highlights the importance of sensitive serological tools in uncovering novel variants.

Strain (serotype)	**O-acetyltransferase genes**	**fs33b**	**fs20b**	**Hyp33FG1**
TIGR33A (33A)	*wciG*, *wcjE*	+	+	+/-
TIGR33AΔ*wcjE* (33F)	*wciG*	+	+/-	+
TIGR33AΔ*wciG* (33X)	*wcjE*	+/-	+	-

TIGR33AΔ*wciG*Δ*wcjE* (33Y)	none	+	-	+

Author Disclosure Block:

B.L. Spencer: None. **K.A. Geno:** None. **J.S. Saad:** None. **M.H. Nahm:** None.

Poster Board Number:

FRIDAY-560

Publishing Title:

Ftsh Function Is Essential to Survival of the Lyme Disease Spirochete B*orrelia burgdorferi* *In Vivo* and *In Vitro*

Author Block:

P. E. STEWART, C. Chu, A. Bestor, B. Hansen, P. Rosa; ROCKY MOUNTAIN LABORATORIES, NIAID, NIH, HAMILTON, MT

Abstract Body:

In many bacteria, the protease FtsH and its modulators, HflK and HflC, form a complex that contributes to membrane quality control and regulation of the cellular response to environmental stress. All three proteins have chromosomally-encoded homologs in *Borrelia burgdorferi*, the Lyme disease spirochete. An obligate pathogen, *B. burgdorferi* depends on crucial membrane functions to infect and persist in both the mammalian host and the *Ixodes* tick. Using an inducible promoter system to control expression, we demonstrate that FtsH is essential for *B. burgdorferi* survival in vitro and in vivo. Depletion of FtsH protein in vitro resulted in arrested cell growth and morphological defects. Overexpression of FtsH did not have detectable adverse effects on spirochete growth in vitro, suggesting that FtsH does not proteolytically overwhelm its substrates. In vivo infection studies demonstrated that FtsH was also required for survival of the spirochete in both the mammalian host and the tick vector. Surprisingly, analysis of the *B. burgdorferi* HflK/C double-mutant in vitro did not identify any defect in morphology, growth rate or growth under stress conditions. The phenotype of the HflK/C double mutant in vivo, including the ability to complete the mouse-tick infectious cycle and ID_{50}, were indistinguishable from wild type. These results demonstrate that FtsH provides an essential function for *B. burgdorferi* survival both in vitro and in vivo, but that the modulators HflK and HflC do not detectably affect FtsH function.

Author Disclosure Block:

P.E. Stewart: None. **C. Chu:** None. **A. Bestor:** None. **B. Hansen:** None. **P. Rosa:** None.

Poster Board Number:

FRIDAY-561

Publishing Title:

Characterization of Bile Induced Membrane Damage in Listeria Monocytogenes

Author Block:

O. Paul[1], D. Clark[2], A. Thopmson[1], J. Wilson[1], J. R. Donaldson[1]; [1]Mississippi State Univ., Mississippi State, MS, [2]Tougaloo Coll., Tougaloo, MS

Abstract Body:

Background: *Listeria monocytogenes* is a Gram positive, facultative intracellular organism responsible for the foodborne disease listeriosis. *Listeria monocytogenes* must survive a variety of stressors encountered within the gastrointestinal (GI) tract, including variations in pH, oxygen availability, and bile. Though it is known that bile induces oxidative damage under aerobic conditions, it is not known whether this differs under anaerobic conditions. **Methods:** Previous studies from our laboratory indicated extensive membrane damage occurs in the presence of bile salts and that the damage is significantly different under anaerobic conditions. To characterize these differences, membrane induced damage was analyzed by transmission electron microscopy (TEM) and fatty acid analysis in strains representing three different serovars of *L. monocytogenes*: F2365, 10403S, and HCC23.**Results:** TEM observations indicated that the size of all strains under both aerobic and anaerobic conditions decreased following bile exposure. However, no differences in cell envelope width were observed with HCC23 under aerobic or anaerobic conditions; 10403S increased under both conditions and F2365 width increased only under anaerobic conditions. Analysis of fatty acid profiles indicated that saturated fatty acids palmitic acid and stearic acid, as well as the unsaturated fatty acids linoleic acid and oleic acids, increased under aerobic and anaerobic culture conditions following bile exposure. Pre-treatment of strains to a commercial lipid mixture containing palmitic acid, oleic acid, stearic acid, and linoleic acid increased the survival of HCC23 and 10403S to bile, but not F2365 under aerobic conditions. Interestingly, an impact on survival was not observed under anaerobic conditions.**Conclusions:** These data suggest that incorporation of exogenous lipids induces alterations to the cell membrane and may improve bile survival under aerobic conditions in a strain dependent manner. Additional research is needed to determine the mechanism by which *L. monocytogenes* is resistant to bile induced membrane damage under anaerobic conditions.

Author Disclosure Block:

O. Paul: None. **D. Clark:** None. **A. Thopmson:** None. **J. Wilson:** None. **J.R. Donaldson:** None.

Poster Board Number:

FRIDAY-562

Publishing Title:

***Vibrio cholerae* toxr Increases Cyclic Di-Gmp Levels in Response to Bile**

Author Block:

J. C. Shook[1], B. J. Koestler[2], C. M. Waters[1]; [1]Michigan State Univ., East Lansing, MI, [2]The Univ. of Texas at Austin, Austin, TX

Abstract Body:

Vibrio cholerae, the causative agent for the disease cholera, survives in a wide range of environments from aquatic reservoirs to animal digestive tracts. In order to survive in these different environments, *V. cholerae* responds to various cues to appropriately regulate downstream phenotypes. One mechanism by which *V. cholerae* adapts to an ever-changing environment is through the second messenger signal cyclic dimeric guanosine monophosphate (c-di-GMP). *V. cholerae* has over 60 enzymes that are predicted to synthesize and degrade c-di-GMP; however, the environmental signals controlling the activity of these enzymes are poorly understood. Bile is a major component of the human intestinal tract encountered by *V. cholerae* during infection. We recently showed that bile increases c-di-GMP levels and promotes biofilm formation. This is partially due to the repression of the HD-GYP VC1295, which degrades c-di-GMP, in the presence of synthetic human bile (SHB). Bioinformatic analysis of the VC1295 promoter predicted four putative binding sites for ToxR, the master virulence regulator of *V. cholerae.* We therefore hypothesized that ToxR represses the transcription of VC1295 in the presence of SHB. Deletions from the upstream region of the VC1295 promoter were constructed to define the active promoter. Repression of VC1295 by SHB is abolished in a **Δ***toxR* mutant, while complementation with ToxR restores SHB-mediated repression. Site-directed mutagenesis of the four putative ToxR binding sites allows for the expression of luciferase regardless of the presence of SHB. Our results show that ToxR further enhances *V. cholerae* adaptation to the intestinal environment by modulating the levels of c-di-GMP in response to bile.

Author Disclosure Block:

J.C. Shook: None. **B.J. Koestler:** None. **C.M. Waters:** None.

Poster Board Number:

FRIDAY-563

Publishing Title:

Regulation of *Coxiella burnetii* Type IV Secretion by the Pmrab Two-Component System

Author Block:

S. C. O. Reed, C. R. Roy; Yale Sch. of Med., New Haven, CT

Abstract Body:

Coxiella burnetii encode a Type IV secretion system (T4SS) that translocates bacterial effector proteins into the host cell as the phagosome matures into a lysosome-like compartment, remodeling the lysosome to creating a fusogenic, replicative *Coxiella*-containing vacuole. Expression of the T4SS and subsequent intracellular growth requires the PmrAB two-component system. We hypothesized that the PmrB histidine kinase senses and responds to changes in pH and ion concentration in the maturing lysosome. We assayed light production by axenically grown *Coxiella burnetii* Nine Mile Phase II strains expressing bacterial luciferase under the control of PmrAB-dependent (icmW, cig2) and independent (311, TetRA) promoters. PmrAB-dependent promoters were strongly expressed during early exponential phase growth (2-4 d post-inoculation or post-infection of HeLa cells). Surprisingly, there was no relationship between relative PmrAB-dependent promoter activity and changes in pH or composition of axenic growth media, suggesting that the Coxiella PmrB-activating signal is distinct from other iron or pH-responsive bacterial *pmrB* genes. Further screening may reveal the host-derived signal necessary for activation of this critically important regulator of intracellular growth.

Author Disclosure Block:

S.C.O. Reed: None. **C.R. Roy:** None.

Poster Board Number:

FRIDAY-564

Publishing Title:

Investigation of the High Rates of Extra Pulmonary Tuberculosis in Ethiopia Reveals No Single Driving Factor and Minimal Evidence for Zoonotic Transmission of *Mycobacterium bovis* Infection

Author Block:

G. E. Degu, **G. E. Degu**; St. Paul's Hosp. Millennium Med. Coll., Addis Ababa, Ethiopia

Abstract Body:

Background: Ethiopia has one of the highest burdens of human tuberculosis (TB) in the world, and reports one of the highest incidence rates of extra-pulmonary TB, dominated by cervical TB lymphadenitis (TBLN). Infection with Mycobacterium bovis, usually associated with extrapulmonary disease, has previously been excluded as the main reason for the high rate of extrapulmonary TB in Ethiopia.**Methods:** Here we examined demographic and clinical characteristics of 953 pulmonary and 1198 TBLN patients visiting 11 health facilities in distinct geographic areas of Ethiopia. Clinical characteristics were also correlated with genotypes of the causative agent, Mycobacterium tuberculosis.**Results:** No major patient or bacterial strain factor could be identified as being responsible for the high rate of TBLN, and there was no association with HIV infection. Regular direct contact and sharing a household with live animals and drinking raw milk were more associated with TBLN than with pulmonary TB, although no M. bovis was isolated from patients with TBLN.**Conclusions:** The study suggests a complex role for multiple interacting factors in the epidemiology of extrapulmonary TB in Ethiopia, including possible zoonotic transmission of M. tuberculosis and genetic features of the pathogen and/or the host population.

Author Disclosure Block:

G.E. Degu: None. **G.E. Degu:** None.

Poster Board Number:

FRIDAY-565

Publishing Title:

Genomic Effects of Host-Specific Specialization in *Mycobacterium shottsii*

Author Block:

J. H. Doss[1], A. Biswas[1], R. Karls[2], F. Quinn[2], M. Ryan[1], D. Ranjan[1], M. Zubair[1], D. T. Gauthier[1]; [1]Old Dominion Univ., Norfolk, VA, [2]The Univ. of Georgia Coll. of Vet. Med., Athens, GA

Abstract Body:

Striped bass (*Morone saxatilis*) is a finfish species of importance to both commercial and recreational fishing on the US east coast. Mycobacterial infections in this species have been observed in striped bass since 1997, and the impacts of mycobacteriosis in this species may result in profound economic and ecological impacts. Two novel mycobacteria, *Mycobacterium shottsii* and *Mycobacterium pseudoshottsii*, have been described in infected striped bass. Whereas most fish-infecting mycobacteria including *M. pseudoshottsii* are environmental residents and facultative pathogens, *Mycobacterium* appears to be specialized to infect only moronids, and may be an obligate pathogen. A comparison of the *M. shottsii* genome to those of its closest relatives (*M. marinum* complex bacteria) has revealed that approximately 500 genes of their common ancestor have deteriorated into pseudogenes, presumably in order to promote this host-specific specialization. Many of these pseudogenes are the result of insertion sequences that have functionally disrupted the genes; these insertion sequences are comprised of eight different varieties, most unique to *M. shottsii*. Other genomic adaptations in *M. shottsii* are similar to those of the human adapted specialists *M. ulcerans* and *M. tuberculosis*, indicating common patterns of vertebrate specialization in this bacterial group.

Author Disclosure Block:

J.H. Doss: None. **A. Biswas:** None. **R. Karls:** None. **F. Quinn:** None. **M. Ryan:** None. **D. Ranjan:** None. **M. Zubair:** None. **D.T. Gauthier:** None.

Poster Board Number:

FRIDAY-566

Publishing Title:

Identification and Genomic Characterization of a Novel *Pseudomonas* Species (*Pseudomonas galenii* sp. Novus) Associated with Human Lung Disease

Author Block:

B. S. Scales, J. R. Erb-Downward, R. P. Dickson, N. R. Falkowski, J. J. LiPuma, G. B. Huffnagle; Univ. of Michigan Med. Sch., Ann Arbor, MI

Abstract Body:

There are over 52 *Pseudomonas* species that taxonomically group within the *Pseudomonas fluorescens* species-complex. Members of this complex are gram-negative bacteria (gammaproteobacteria) that have the ability to flourish in many environmental niches due their metabolic adaptability. Using culture-independent analysis, our laboratory has identified abundant levels of *P. fluorescens*-related species in lungs of individuals with chronic lung disease. To further investigate the genomic characteristics of *P. fluorescens*-species complex bacteria from clinical samples, we sequenced a collection of *P. fluorescens*-related strains isolated from individuals with chronic lung disease. This included 28 strains that taxonomically grouped within the *P. fluorescens*-species complex by 16S rRNA gene homology and multi-locus sequence analysis with housekeeping genes. Full genome sequencing, assembly and annotation was performed on all 28 strains. Twelve of the 28 strains grouped within previously defined subclades I, II and III of the *P. fluorescens* species- complex. However, the other 16 strains were distinct and clustered on a phylogeneticlly unique branch that lacked any representative genomes (clinical or environmental) in Genbank. The 16 strains also displayed phenotypic differences *in vitro* from the other subclades. Additional comparative genomic analysis found that its members displayed significant reductions in genome size, gene diversity and GC content compared to other *P. fluorescens* strains. Average nucleotide identity (ANI) analysis between the 16 strains and previously sequenced *Pseudomonas* strains revealed that their shared ANI was below the cutoff required to be grouped within any previously defined species. Despite being most phylogenetically similar to members of the *P. fluorescens* species-complex, the 16 unique strains contained all the elements of a Ysc family type III secretion system, most homologous to that found in *P. aeruginosa.* Analysis of the flagellin gene also revealed structural differences that support the concept that the 16 strains represent a distinct species within the *P. fluorescens* species-complex. We have designated these 16 unique strains as the new *Pseudomonas* species- *Pseudomonas galenii* sp. novus.

Author Disclosure Block:

B.S. Scales: None. **J.R. Erb-Downward:** None. **R.P. Dickson:** None. **N.R. Falkowski:** None. **J.J. LiPuma:** None. **G.B. Huffnagle:** None.

Poster Board Number:

FRIDAY-567

Publishing Title:

Profile-Based Comparison of *Salmonella* Genomes Reveals Signatures of Host Adaptation

Author Block:

N. E. Wheeler[1], L. Barquist[2], P. P. Gardner[1]; [1]Univ. of Canterbury, Christchurch, New Zealand, [2]Univ. of Würzberg, Würzberg, Germany

Abstract Body:

Comparative genomics investigations have yielded an abundance of information on the genetic variation between organisms, however understanding the consequences of this variation for protein function has proved challenging. A small non-synonymous change to or a short indel within a coding sequence can have varying effects on protein function, depending on both the nature of the change and the context of the change within the protein. These effects can range from maintaining native protein function to a complete abrogation of function, or even in rare cases altering protein function. The increasing availability of complete genome sequences for collections of closely related bacteria presents an opportunity to study the fine-grain functional evolution of protein sequences in nature. We present a profile HMM-based method for assessing the functional significance of mutations in protein coding sequences. We demonstrate an application of the method to comparative analysis of bacterial genomes to scan for functionally significant genetic variation. As a model system, we have chosen the well-studied species *Salmonella enterica*, of which several lineages are known to have undergone host-restriction events characterized by a narrowing of host tropism with a concurrent accumulation of pseudogenes. We show that our method is able to detect functional degradation of genes associated with host-adapation that are not detected by conventional pseudogene analysis, and suggest that this approach offers a sensitive measure of the loss-of-function mutations that may occur as a result of adaptation to a new niche.

Author Disclosure Block:

N.E. Wheeler: None. **L. Barquist:** None. **P.P. Gardner:** None.

Poster Board Number:

FRIDAY-568

Publishing Title:

Measuring Differential Growth Rates of Uropathogenic *Escherichia coli* Strains *In Vitro* and During Experimental Murine Urinary Tract Infection

Author Block:

S. N. Smith, C. Armbruster, A. Springman, S. Himpsl, H. L. T. Mobley; Univ. of Michigan, Ann Arbor, MI

Abstract Body:

Uropathogenic *Esherichia coli* (UPEC), the causative agent of most uncomplicated urinary tract infections (UTIs), is a member of a subset of bacteria broadly known as *extraintestinal pathogenic E. coli* (EXPEC). EXPEC infections include urinary tract infections, meningitis, sepsis, pneumonia and surgical site infections. The versatility of EXPEC isolates may be due, in part, to differences in their ability to grow and survive in different host environments. While measurement of growth *in vitro* has been routinely determined for as long as scientists have studied bacteria, the growth rate of bacteria during experimental infections has only been cursorily examined. In this study, we used a previously constructed marker plasmid (pGTR902) (a gift of Paul Gulig) that replicates only in the presence of arabinose, to investigate growth rates of various EXPEC isolates *in vitro* and in a murine model of ascending urinary tract infectionUTI. The average copy number of pGTR902 was determined to be ~26 by differential plating to verify segregation of the plasmid under non-permissive (arabinose-negative) conditions. Female mice were transurethrally inoculated at a dose of 10^8 CFU/ mouse and sacrificed after 48 hours to compare infecting doses (ID_{50}) between EXPEC isolates. Subsequent infection studies were conducted to compute doubling time of each isolate by determining the proportion of bacteria that maintained the marker plasmid over time. Our data indicate that UPEC isolates, such as CFT073, have much higher colonization levels and faster growth rates during experimental UTI than asymptomatic bacteriuria (ABU) and commensal isolates, indicating that UPEC strains have an enhanced ability to colonize and grow at a faster rate within this particular host niche. Closer examination of the dynamics of growth and colonization rates between EXPEC strains will give us deeper insight into the differences of these closely-related strains and their abilities to cause infection.

Author Disclosure Block:

S.N. Smith: None. **C. Armbruster:** None. **A. Springman:** None. **S. Himpsl:** None. **H.L.T. Mobley:** None.

Poster Board Number:

FRIDAY-569

Publishing Title:

Transposon Insertion Sequencing to Identify *Proteus mirabilis* Fitness Factors for Murine Catheter-Associated Urinary Tract Infection

Author Block:

V. S. DeOrnellas, C. E. Armbruster, S. N. Smith, H. L. T. Mobley; Univ. of Michigan, Ann Arbor, MI

Abstract Body:

The most common healthcare-associated infections are urinary tract infections (UTI), ~80% of which are associated with indwelling catheters. Long term catheter use inevitably results in bacteriuria, which can lead to the development of fever, cystitis, pyelonephritis, bacteremia. Historically, *Proteus mirabilis* commonly causes catheter-associated UTI (CAUTI), present in up to 44% of cases. During CAUTI, bacteria colonize the bladder to cause infection of the lower urinary tract, but may ascend to the kidney causing infection of the upper urinary tract. The ability to cause infection in two different environments likely requires expression of organ-specific fitness factors. Identification of the full complement of *P. mirabilis* virulence and fitness factors is required to elucidate novel therapeutic targets for treatment of CAUTI. The classical virulence factors of *P. mirabilis* include fimbrial adhesins, flagellar-mediated motility, hemolysin and urease. Previously a library of 2,088 signature-tagged mutants was used to identify additional fitness factors during non-catheterized infection, including genes for nutrient and iron acquisition. These findings are not likely to represent a comprehensive list of fitness factors since ~34,000 mutants are required for full genome coverage, and it does not account for potential differences in infection with an indwelling catheter. In this study, we utilized transposon directed insertion site sequencing (TnSeq), which makes use of a modified mariner transposon, to generate a saturating transposon mutant library and capture flanking chromosomal DNA for identification. A random library of 50,000 transposon mutants was generated and validated. CBA/J mice were infected by transurethral inoculation through an indwelling catheter with pools of 10,000 transposon mutants at $1x10^5$ CFU/mouse, resulting in a median bacterial burden of $2.7x10^7$ CFU/g tissue in the bladder and $9.3x10^5$ CFU/g tissue in the kidneys at 4 d.p.i. Application of next-generation sequencing of mutants harvested from bladders and kidneys after CAUTI infection allowed determination of the relative abundance of individual mutants. The subpopulation of mutants underrepresented in each site following infection represent genes required to colonize bladder and kidneys of catheterized mice.

Author Disclosure Block:

V.S. DeOrnellas: None. **C.E. Armbruster:** None. **S.N. Smith:** None. **H.L.T. Mobley:** None.

Poster Board Number:

FRIDAY-570

Publishing Title:

Ethanolamine Utilization Genes are Novel Virulence Determinants of Uropathogenic *E. coli* During Human Urinary Tract Infection

Author Block:

A. Sintsova[1], S. Subashchandrabose[2], S. Smith[1], H. Mobley[1]; [1]Univ. of Michigan, Ann Arbor, MI, [2]Wake Forest Univ., Winston-Salem, NC

Abstract Body:

Uropathogenic *Escherichia coli* (UPEC) is the major causative agent of uncomplicated urinary tract infections (UTIs). The high incidence of UTIs makes them a global heath concern that is further exacerbated by the rise of antibiotic-resistant strains. Accordingly, identification of novel virulence factors and research aimed at understanding UPEC pathogenesis is of the utmost importance in our drive to discover new therapeutic strategies. To this end, we recently compared the gene transcription profiles of UPEC isolated directly from UTI patients to those of the same strains cultured in sterile urine, and identified genes specifically up-regulated during human UTI. Intriguingly, this led to identification ethanolamine utilization (*eut*) genes as important host-specific virulence factors. Using a well-established mouse model of UTI, we show that a UPEC construct in which *eut* genes were deleted has a severe defect in bladder colonization, and is outcompeted by wild type UPEC strain 20-fold in the murine model of ascending UTI. Ethanolamine utilization has been linked to the pathogenesis of a number of bacterial species, including *Salmonella typhimurium* and *Listeria monocytogenes*. In these bacteria, all *eut* genes are up-regulated during intestinal infections, and various *eut* mutants have attenuated virulence *in vivo*, however, the exact role of *eut* operon in facilitating colonization remains enigmatic. While in some instances ethanolamine seems to serve as a useful source of nutrition for *E. coli*, our preliminary data show no growth defect in a *eut* mutant strain cultured under conditions that mimic the *in vivo* environment. This suggests that the ethanolamine utilization pathway might function to impede proper functioning of the innate immune response and help UPEC evade detection and clearance.

Author Disclosure Block:

A. Sintsova: None. **S. Subashchandrabose:** None. **S. Smith:** None. **H. Mobley:** None.

Poster Board Number:

FRIDAY-571

Publishing Title:

Genome Wide Identification of *Escherichia coli* K1 Genes Essential for Growth *In Vitro* and Pathogenesis by Transposon Insertion Site Sequencing

Author Block:

A. J. McCarthy[1], R. A. Stabler[2], B. W. Wren[2], P. W. Taylor[1]; [1]Univ. Coll. London, London, United Kingdom, [2]London Sch. of Hygiene & Tropical Med., London, United Kingdom

Abstract Body:

Background: *Escherichia coli* K1 is a major causative agent of neonatal bacterial meningitis and associated sepsis. The genes important for initial colonisation of the gastrointestinal tract, entry and survival in the blood and invasion are poorly understood. **Objective:** to construct an *E. coli* K1 A192PP-*Tn5* mutant library for high-throughput screening, to identify genes essential for growth in nutrient broth, for gastrointestinal (GI) colonization in the neonatal rat and for survival in serum using transposon insertion site sequencing. **Methods:** A library of >280,000 unique mutants was constructed using the EZ-Tn5 transpome kit. Transposon insertion sites were sequenced using the Illumina MiSeq platform, and bioinformatics analyses used to assess the distribution of mutants across the genome. Single gene mutants were created using lamda-red recombineering, and their fitness was assessed using *in vitro* and/or *in vivo* rat models of colinsation/infection. **Results:** Analysis identified 370 genes in *E. coli* K1 A192PP essential for growth *in vitro*; 113 of these genes were not considered essential for the growth of *E. coli* K12. A library of 50,000 distinct mutants was also screened for genes that decreased the capacity of *E. coli* A192PP to colonize the GI tract of neonatal rats and to survive in human serum. A short-list of >200 genes was identified as important for colonisation and serum survival. A subset of newly identified genes was selected for further characterisation and deletion mutants were constructed in A192PP. Each mutant was tested *in vitro* and/or *in vivo* to confirm their attenuated phenotypes. **Conclusions:** We have developed and validated a large robust *E. coli* K1-A192PP Tn*5* library for the study of physiology and pathogenesis of this neonatal pathogen. Our data has revealed genes not previously considered essential for *in vitro* growth and identified genes important for colonization and serum survival. Further characterisation of the genes will improve understanding of physiology and pathogenesis, and may aid the identification of novel targets for therapeutic intervention.

Author Disclosure Block:

A.J. McCarthy: None. **R.A. Stabler:** None. **B.W. Wren:** None. **P.W. Taylor:** None.

Poster Board Number:

FRIDAY-572

Publishing Title:

Demonstration of the Effect of pH on Competition within a Vaginal Co-culture

Author Block:

D. W. Koenig[1], P. Anunson[1], R. Bartell[1], L. Peed[2], J. Li[2], R. Vongsa[1]; [1]Kimberly Clark Corp., Neenah, WI, [2]Kimberly Clark Corp., Roswell, GA

Abstract Body:

Background: Human microbiome research has revealed that interspecies interactions play an important role in influencing host health. We have developed a simplified *in vitro* co-culture model to assess the ecological impact of growth factors on the key members of the vaginal microbiome. Vaginal microflora of healthy women are dominated by *Lactobacillus* spp. Changes in the vaginal environment will reduce the dominance of *Lactobacillus* to favor harmful bacteria. The aim of this study was to use a vaginal co-culture model to demonstrate pH regulation of bacterial growth. **Methods:** Cultures of two bacteria, *Lactobacillus crispatus* ATCC 33820 and *Escherichia coli* ATCC 10798 were grown in batch bio-reactors to evaluate the change in cell yields as a function of medium type and pH. Growth was recorded by plate count and OD 800nm. The pH was controlled for multiple pH sets between 4.5 and 6.0. Finally, two lactobacilli media were used, LAPT-G (yeast extract, peptone, tryptone, and Tween without glucose) which enhances *E. coli* growth and LAPT-G plus sucrose which enhances *Lactobacilli* growth. **Results:** In LAPT-G single and co-cultures, *E. coli* did not grow when held at pH 4.5, and both *E. coli* and *L. crispatu*s grew when pH was held at 6.0. The pH of unregulated co-cultures grown in LAPT-G did not lower with growth, staying at 6.5. In unregulated pH LAPT-G single cultures, L. *crispatus* dropped the pH from 6.6 to 5.6, while the pH remained at 6.4 with *E. coli*. In LAPT-G plus sucrose single and co-culture, both organisms grew when the pH was held at 6.0. *E. coli* did not grow when pH was held at 4.5 in either single or co-culture and did not grow when pH was unregulated in co-culture with a starting pH of 6.4. *L. crispatus* grew slowly at pH 4.5 and required 50 hrs to reach max cell yield in co-culture. In unregulated pH single cultures of LAPT-G plus sucrose, *L. crispatus* dropped the pH from 6.7 to 4.2 and remained at 6.4 for *E. coli*. **Conclusions:** The binary system described here confirms the reported *in vivo* observations of the effect of pH on vaginal community structure. pH drives the selective growth of *L. crispatus* over *E. coli* in a co-culture system. Use of this model will allow for the pre-clinical assessment of prebiotics and other biologicals that have the potential to alter the vaginal consortium.

Author Disclosure Block:

D.W. Koenig: D. Employee; Self; Kimberly Clark. **P. Anunson:** D. Employee; Self; Kimberly Clark. **R. Bartell:** D. Employee; Self; kimberly clark. **L. Peed:** D. Employee; Self; Kimberly

Clark. **J. Li:** D. Employee; Self; Kimberly Clark. **R. Vongsa:** D. Employee; Self; Kimberly Clark.

Poster Board Number:

FRIDAY-573

Publishing Title:

Plant Cell Wall Xyloglucans are Utilized by Human Gut Colonizing Bifidobacteria as a Sole Carbon Source

Author Block:

E. Özcan, D. A. Sela; Univ. of Massachusetts, Amherst, MA

Abstract Body:

Human-associated bifidobacteria are beneficial members of gut microbial community and capable of using dietary and host-derived glycans (e.g. milk oligosaccharides and mucins) that are impervious to host digestion. The genomes of *Bifidobacterium infantis* and *Bifidobacterium longum* encode an array of glycosyl hydrolases and oligosaccharide transporters to utilize dietary oligosaccharides that are encountered in their respective ecological niches. These phylogenetically near-neighbors use divergent mechanisms to utilize oligosaccharides, while both secrete acetate and lactate as end-products of fermentative metabolism. In this study, we tested the hypothesis that *B. longum* and *B. infantis* utilize plant-derived xyloglucans extracted from the cranberry cell wall using the characteristic phosphoketolase pathway common to the genus *Bifidobacterium*. Accordingly, we observed strain-level phenotypic variation with respect to growth phenotype while utilizing xyloglucans as a sole carbon source. Interestingly, bifidobacterial strains subsisting on xyloglucans varied in the theoretical yield of 3:2 acetate to lactate sectered in contrast to glucose catabolism. Xyloglucan utilization results in carbon shunted towards 1C metabolism as higher concentrations of formate is secreted at the expense of lactate. In order to determine if overlapping mechanisms are deployed to capture xyloglucans, we characterized the expression of several target genes previously implicated in oligosaccharide transport. Previous comparative genomics and functional experiments have indicated that *B. longum* has likely adapted to utilize plant-derived products. However, our results indicate that *B. infantis* strains that are typically associated with infants are highly competent for plant xyloglucan metabolism.

Author Disclosure Block:

E. Özcan: None. **D.A. Sela:** None.

Poster Board Number:

FRIDAY-574

Publishing Title:

Interactions between Two Human Gut Symbionts in Coculture

Author Block:

M. Smith, N. Buan; Univ. of Nebraska Lincoln, Lincoln, NE

Abstract Body:

The human gut is home to 10^{14} microorganisms. They digest polysaccharides we are unable to and exist symbiotically with our cells. Though not an abundant phyla, methane producing archaea are ubiquitous in the human gut. One species, *Methanobrevibacter smithii*, can be detected in 95.7% of individuals. The *M. smithii* genome encodes adhesion like proteins predicted to bind to chondroitin sulfate (CS), a component of the luminal intestinal extracellular matrix. Because CS is not a known carbon source for methanogens, I hypothesize that *M. smithii* uses the proteins in order to co-localize with fermenting bacteria such as *Bacteroides thetaiotaomicron*. *B. theta* has been demonstrated to use CS as a sole carbon and energy source, and is also known to associate with gut epithelia. To test my hypothesis, I developed a coculture of *B. theta* and *M. smithii*.In the syntrophic coculture, the end products excreted by *B. theta* (H_2, CO_2, acetate, and formate), are carbon and energy sources for *M. smithii*. This benefits *B. theta* by removing waste, allowing cells to continue to proliferate. Samples of the coculture at stationary phase were observed using an EVOS confocal microscope. Large aggregates of *M. smithii* and *B. theta* cells could be seen. Whole granules appeared to have coenzyme F_{420} fluorescence diffusing from *M. smithii* cells. Putative F_{420} dehydrogenase genes were found in *B. theta* suggesting a possibility of how the two organisms coordinate their metabolisms.To determine if *M. smithii* binds CS directly, or uses the adhesion like proteins to bind dietary polysaccharides or to other microbial cells, a carbohydrate binding assay was developed using *B. theta*. Carbo-bind plates were coated with glucose or CS. Stationary-phase *B. theta* cells were added and binding was measured using a Coommassie stain on bound protein. When grown on glucose, *B. theta* cells have decreased binding from 57% on a glucose coated plate to 19% on a CS coated plate ($p<0.01$). However, CS grown cells experienced no change in binding (18.5% to 16.3%, $p=0.2$). The assay will be used to measure carbohydrate and *B. theta* binding by *M. smithii*. Should *M. smithii* and/or *B. theta* influence polysaccharide binding of each other, it would support my hypothesis that *M. smithii* attaches itself to polysaccharides to co-localize with *B. theta*, and the idea that *M. smithii* actively seeks syntrophic partners for growth.

Author Disclosure Block:

M. Smith: None. **N. Buan:** None.

Poster Board Number:

FRIDAY-575

Publishing Title:

Horizontal Gene Transfer of Accelerated Protein Evolution in the Human Gut Microbiome

Author Block:

Y. Wang[1], K. Sasaninia[1], X. Yu[2], B. Paul[3], C. Wei[2], S. Mazmanian[4], J. F. Miller[1]; [1]Univ. of California-Los Angeles, Los Angeles, CA, [2]Shanghai Jiaotong Univ., Shanghai, China, [3]Univ. of California-Santa Barbara, Santa Barbara, CA, [4]California Inst. of Technology, Pasadena, CA

Abstract Body:

The human gastrointestinal (GI) tract houses approximately 10^{14} microbes of more than 1500 species, and represents one of the most diverse and complex microbial ecosystems in the biosphere. The mechanisms by which these microbes interact, exchange genetic information, and evolve new traits remain largely unknown. Diversity-generating retroelements (DGRs) are a novel family of genetic elements that diversify protein-encoding genes and accelerate protein evolution. Bioinformatic analysis discovered numerous DGRs in the human gut microbiome, including within the genome of *Bacteroides fragilis* 638R. In this study, we have characterized the functionality and dissemination of a model retroelement *B. fragilis* 638R DGR, which encodes a diversified target protein, BdtA, with a lipoprotein signal sequence. Proteinase K sensitivity and immunostaining studies showed that BdtA is localized in the outer leaflet of the outer membrane and surface-exposed, supporting the hypothesis that *B. fragilis* has exploited DGRs to generate new ligand-binding specificities. Further examination of the 638R DGR locus and flanking regions suggested that it is carried on an integrative and conjugative element (ICE) subject to tight and complex regulation. *In vitro*, upon induction by BdiA (BF638R_2082), an AraC-like transcriptional regulator, the DGR-containing ICE was readily excised as detected by PCR assays. During colonization of gnotobiotic mice, ICE excision was also detectable in 638R cells even in the absence of BdiA. During *in vitro* mating, the ICE was horizontally transferred to *B. fragilis* 638R or *B. thetaiotaomicron* VPI5482 at a frequency of 10^{-6} to 10^{-7}, and next-generation sequencing (NGS) successfully detected DGR activity in 638R cells grown *in vitro* or during colonization of the mouse GI tract. Colon tissue-associated 638R cells demonstrated the highest DGR activity, followed by mucus-associated cells. Thus far, we have elucidated a molecular mechanism underlying the wide distribution of DGR-mediated protein evolution cassettes, which has the potential to increase bacterial fitness within the dynamic environment of the host gut.

Author Disclosure Block:

Y. Wang: None. **K. Sasaninia:** None. **X. Yu:** None. **B. Paul:** None. **C. Wei:** None. **S. Mazmanian:** None. **J.F. Miller:** None.

Poster Board Number:

FRIDAY-576

Publishing Title:

A Reduced Response to Bacterial Muramyl Dipeptide: Comparison of Nod2 Mutations Associated with Crohn's Disease and Blau Syndrome

Author Block:

E. R. Stiles, R. Ewer, C. P. Bocian, L. Nguyen, **A. M. Domina**; Husson Univ. Sch. of Pharmacy, Bangor, ME

Abstract Body:

NOD2 is a human pattern recognition receptor for muramyl dipeptide (MDP), a component of the bacterial peptidoglycan layer of Gram positive and Gram negative bacteria. MDP binding to NOD2 results in the activation of several intracellular signaling pathways, including the NF-κB and MAP kinase pathways. Some mutations in the NOD2 gene predispose individuals to Crohn's Disease, while other NOD2 mutations cause Blau Syndrome, which is associated with childhood onset of granulomatous dermatitis, arthritis, and uveitis. It has been proposed that the Crohn's-associated mutations result in decreased NOD2 activation, perhaps due to its instability, while the Blau-associated mutations result in constitutive NOD2 activation; however, some studies have called into question the clinical relevance of constitutive NOD2 activation in Blau syndrome. Therefore, the goal of this work is to compare the effects of Crohn's- versus Blau-associated NOD2 mutations on its activation. To achieve this goal, Crohn's-associated NOD2 mutations (R702W, G908R), Blau-associated mutations (R334W, E600A, T605N, T605P), and appropriate controls were constructed and transfected into HEK-Blue cells, which are HEK 293 cells containing a gene for secreted embryonic alkaline phosphatase (SEAP) under the control of multiple NF-κB binding sites and AP-1 sites. Transfected cells were treated with or without MDP, followed by quantification of SEAP activity, which is reflective of NOD2 activation. The results indicated that all Blau-associated and Crohn's-associated NOD2 mutations exhibited a reduced ability to respond to MDP compared to wild type NOD2. These results indicate that Crohn's- and Blau-associated NOD2 mutations induce a common cellular effect in that they have a reduced ability to respond to MDP. Future work will explore the possibility that the Blau-associated mutations result in an activation-induced NOD2 inactivation.

Author Disclosure Block:

E.R. Stiles: None. **R. Ewer:** None. **C.P. Bocian:** None. **L. Nguyen:** None. **A.M. Domina:** None.

Poster Board Number:

FRIDAY-577

Publishing Title:

Enteroviruses in the Pancreas of Live Adult Patients Newly Diagnosed with Type 1 Diabetes - The DiViD Study

Author Block:

A. Q. Toniolo[1], A. P. Genoni[1], D. Campani[2], S. Sansonno[1], L. H. Krogvold[3], K. Dahl-Jorgensen[4]; [1]Univ. of Insubria, Varese, Italy, [2]Univ. of Pisa, Pisa, Italy, [3]Oslo Univ. Hosp., Oslo, Norway, [4]Univ. of Oslo, Oslo, Norway

Abstract Body:

Background: The DiViD study aims at assessing whether viral agents are present in the pancreas at the onset of type 1 diabetes (T1D). Pancreas samples of 6 DiViD cases and 11 nondiabetic controls were analyzed for 20 groups of viruses, including enteroviruses (EVs). **Methods:** Detection methods combining cell culture and gene amplification were used. Frozen pancreas was obtained from 6 adult T1D cases (University of Oslo, Norway) and 11 cases of pancreatic carcinoma without diabetes (University of Pisa, Italy). Tissue homogenates were produced in DMEM supplemented with antimicrobials. Cell lines (AV3, RD, HEp-2, LLC-MK2) were exposed to pancreas homogenates. DNA and RNA were extracted from cell cultures. DNA viruses (6 herpesviruses, parvovirus B19, HBV) and RNA viruses (HCV, GBV-C, rubella, influenza A/B, parainfluenza 1-4, RSV, astrovirus, norovirus, rotavirus, HAV, EVs, poliovirus) were searched for by real time PCR. End-point EV PCR was performed with four primer pairs targeting the 5'UTR region of the A, B, C, D species. Amplicons were directly sequenced. Expression of EV VP1 capsid protein was evaluated by immunofluorescence (mAbs 5D-8.1 and 9D5) in cultured cells exposed to pancreas homogenates. **Results:** EV genomes were detected in the pancreas of 6/6 T1D cases vs. 2/11 control carcinoma cases ($p<0.05$). In the T1D group, sequences of 5'UTR amplicons were compatible with EVs of the A and B species. One EV of the B and one of the C species were found in controls. Clear cytopathic effects were not perceived in EV-carrying cell cultures. Signals of viruses other than EVs were rare (occasional positivity for EBV, HHV6, parvovirus B19). Cytoplasmic expression of VP1 in cells exposed to pancreatic extracts was concordant with results of PCR assays. **Conclusion:** Results demonstrate that detection of EVs in the pancreas is a constant finding in T1D cases at the clinical onset and a possible finding in cases of pancreatic carcinoma. This study could not establish the location of EVs within the pancreas. However, previous investigations of DiViD cases pointed to infection of islets of Langerhans as the virus source (Krogvold et al., 2015). The findings reinforce the idea of a non-chance association of EV infections with the origin of T1D.

Author Disclosure Block:

A.Q. Toniolo: None. **A.P. Genoni:** None. **D. Campani:** None. **S. Sansonno:** None. **L.H. Krogvold:** None. **K. Dahl-Jorgensen:** None.

Poster Board Number:

FRIDAY-578

Publishing Title:

Infectobesity and Cancer Risk: Could Adenovirus 36 Infection Increase Your Risk for Breast Cancer?

Author Block:

T. Gray, W. C. Webley; Univ. of Massachusetts Amherst, Amherst, MA

Abstract Body:

Obesity is linked to a higher risk of recurrence of breast cancer despite optimal treatment. Recent studies confirm that obesity increases the risk of recurrence in women with hormone receptor-positive breast cancer. More recently, studies confirmed a significant increase in adiposity, and subsequent obesity, in chickens, mice, rats, and non-human primates infected with human adenovirus 36 (Adv36). These viruses are thought to increase the replication, differentiation, lipid accumulation, and insulin sensitivity in fat cells while reducing leptin secretion. Adv36 is the only human adenovirus to date that has been directly linked with human obesity; however, Adv37 and Adv5 have also been suspected. The current study sought to determine the prevalence of obesogenic adenovirus serotypes in breast tissue and blood from patients undergoing breast biopsy or mammoreduction surgery.**Methods:** We utilized serotype-specific PCR amplification to determine the presence of adenovirus species in patient samples obtained from human mammary epithelial cells (HMEC), buffy coat, and formalin-fixed paraffin-embedded (FFPE) breast tissue.**Results**: The average BMI of the patient cohort was 31.45, average age 37.2 years old and there was no significant association between the presence of obesogenic adenovirus and parity. Adv36 DNA was isolated from the breast tissue and blood of 55% (25/45) of all HMEC and buffy coat samples. Adv37 was found in 13% (6/45), Adv5 in 71% (32/45) and Adv2, 9 and 37 in one patient each. Since archived FFPE breast tissue biopsy samples are more readily available, we also evaluated these for the presence of obesogenic adenoviruses. Twenty nine percent (11/44) FFPE samples were positive for Adv36 and 76% (32/44) for Adv5. Data from the FFPE samples suggest an association between the prevalence of Adv36 and BMI in this patient cohort. The average BMI of patient samples positive for Adv36 was 33.83 compared to 30.8 for Adv36 negative samples.**Conclusion:** Our data shows, for the first time, that Adv36 DNA was present more frequently in breast tissue of women with higher BMI. We also confirmed previously published serological data that Adv5 is prevalent in the population and now demonstrate that its DNA is readily detected in breast tissue and white blood cells. Our initial findings support a role of infectobesity in the risk for breast cancer development.

Author Disclosure Block:

T. Gray: None. **W.C. Webley:** None.

Poster Board Number:

FRIDAY-579

Publishing Title:

A Cluster of Cases with Melioidosis Following Chennai Floods

Author Block:

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Abstract Body:

Background: Devastating floods were recorded in the city of Chennai and surrounding areas, South India, on November 8-9 and December 1, 2015; We describe a case series of melioidosis following these floods.**Methods:** Retrospective chart review of patients with melioidosis to analyze the clinical profile and association with flood water exposure. All patients with blood or any other sterile site specimen growing *Burkholderia pseudomallei* between 9th November 2015 to 8th January 2016 were studied. Blood cultures were performed using the BacT-Alert automated blood culture system (BioMerieux).**Results:** 11 patients with blood culture growing *B. pseudomallei* were identified during this 2 month period. All were from the flood affected area. 9 cases (81 %) had flood water exposure. Only 3 had occupational risk in the form of farming. Majority were diabetics (81%), 2 had skin lesions (eczema and diabetic foot infection). All presented with fever, 2 with pneumonia, 4 had septic foci elsewhere. All had bacteremia, 3 had one other site culture positivity as well. 36% mortality was noted. **Conclusions:** In a previous study done from this institution, between 2005-2010, out of 32 cases, only 11 were from South India. Thus there is definite increase in number of cases of meliodosis post flood. Understanding the epidemiology, rainfall and flood exposure as risk factors for meliodosis will help early recognition and starting appropriate therapy.

Epidemiology of the cases				
	Place of flood exposure	Date of flood exposure	Date of onset of symptoms	Outcome
Case 1	Neyveli	Nov 10	Nov 15	expired
Case 2	Karaikal	Nov 9	Nov 14	expired
Case 3	Nellore	Nov 10	Nov 16	improved
Case 4	Neyveli	Nov 11	Nov 18	improved
Case 5	Kadappa	Nov 11	Nov 25	improved
Case 6	Chennae	no	Dec 12	improved
Case 7	Chennai	Dec 1	Dec 6	expired
Case 8	Neyveli	Dec 2	Dec 16	improved
Case 9	Nellore	Dec 3	Dec 27	improved
Case10	Neyveli	Nov 10	Dec 24	expired
Case 11	Tirupathi	no	Dec 12	improved

Author Disclosure Block:

A. Rajalakshmi: None. **A. Ghafur:** None. **D. Vidya:** None. **P. Senthur Nambi:** None. **V. Ramasubramanian:** None. **D. Suresh Kumar:** None. **R. Gopalakrishnan:** None.

Poster Board Number:

FRIDAY-580

Publishing Title:

Neuroendocrine Factors Dictate *Streptococcus pneumoniae* Serotypes-Specific Responses

Author Block:

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Abstract Body:

Background: Among the 92 known serotypes of *Streptococcus pneumoniae (S. pneumoniae)*, few are considered virulent. Moreover, invasive serotypes are not contagious (e.g. pneumonia, otitis media and sepsis). Rather, transmission of disease stems from the reservoir of resident asymptomatic pneumococci along the nasal passages. Previous studies have shown that hormonal factors may be influential in regulating *S. pneumoniae's* transition from a non-pathogen to a pathogenic state. The current study investigated the effects of corticotropin-releasing hormone (CRH) and Norepinephrine (NE), on the virulence properties across *S. pneumoniae* serotypes. **Methods:** *S. pneumoniae* serotypes 3 and 19 were treated with either Corticotropin-releasing hormone (CRH) or Norepinephrine (NE) and allowed to grow in the incubator at 37º C, 5% CO_2. *S. pneumoniae's* response to CRH and NE was quantified by colony forming units, fluorescence analysis of metabolic activity and capsule staining using Congo red.**Results:** We demonstrated serotype-specific responses to both CRH and NE in terms of metabolic activity, bacterial growth and capsular formation. Specifically, CRH significantly increased the metabolic activity of serotypes 3 and 19. In contrast, NE significantly decreased their activity. Bacterial growth of serotype 3 was significantly increased by CRH. However, CRH did not influence the growth of serotype 19. In contrast, NE significantly decreased bacterial growth of serotype 19. NE did not influence the bacterial growth of serotype 3. Both CRH and NE promoted capsule formation of serotype 19 but had no significant effect on serotype 3.**Conclusion:** These results demonstrate the potential diversity of pneumococcal responsiveness to neuroendocrine factors that in turn may influence serotype phenotype with potential implications of pathogenicity.

Author Disclosure Block:

C.G. Ngo Ndjom: None. **H.P. Jones:** None.

Poster Board Number:

FRIDAY-581

Publishing Title:

Methicillin-Resistant *Staphylococcus aureus* in People Living with HIV/Aids Visiting a Tropical and Infectious Disease Hospital in Nepal

Author Block:

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Abstract Body:

The emergence of Methicillin-resistant *Staphylococcus aureus* (MRSA) infections in people living with HIV/AIDS (PLWHA) has become a major challenge in clinical settings worldwide. MRSA infections in PLWHA is more frequently acquired in the community, may lead to increase risk of multi-drug resistance to opportunistic infections. Early identification of pathogen and proper antibiotic treatment can play a significant role in reducing the disease burden among PLWHA. Studies on MRSA infections in PLWHA in Nepal are, however, scarce. The objective of this study, hence, was to understand antimicrobial resistance of MRSA infections in patients living with HIV/AIDS in Nepal.A total of 324 nasal swabs were extracted from PLWHA through September 2014 to February 2015 in Sukraraj Tropical and Infectious Disease Hospital according to the protocol of Clinical Laboratory Standard Institute. Antibiotic susceptibility test was performed by using modified Kirby-Bauer disk diffusion technique and agar dilution method was performed for oxacillin. CD4 cell counting was done using computerized GeneQuant spectrophotometer. Of these, 91 were found positive for *S. aureus,* of which 38 were MRSA (41.76%) isolates. 76.3% (29/38) of them, had CD4 counts less than 500 cells/mm^3. D-test shown that inducible clindamycin resistant MRSA were 28.94 %. All of the isolated *S. aureus* were sensitive to vancomycin while 100% resistance to penicillin. *S. aureus* isolates were susceptible to cephalosporin (94%), tetracycline (89%), clindamycin (87%), gentamicin (53%), and erythromycin (45%) while ciprofloxacin (21%) and cotrimoxazole (10%) were less effective. Multidrug resistance (MDR) was significantly distributed with methicillin resistance where 22 MSSA (41.5%) and 35 MRSA (92.1%) were MDR. Clindamycin is a reserve option and decreed in severe MRSA infection. D-test is a simple laboratory method to portray inducible and constitutive clindamycin resistance *S.* aureus. Low CD4 cell count may increase incidence of MRSA in PLWHA and cause difficulties in treating opportunistic infections. The present study would be useful in bridging knowledge gap on MRSA infections in PLWHA in Nepal.

Author Disclosure Block:

B. Rayamajhee: None. **J. Aacharya:** None. **L. Ghimire:** None. **S.B. Pun:** None.

Poster Board Number:

FRIDAY-582

Publishing Title:

Obesity and Microbiota among Healthy Saudi Females with Various Degrees of Obesity

Author Block:

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Abstract Body:

Background: Obesity is a modern global epidemic and is a risk factor for diabetes and cardiovascular diseases (CVD). The prevalence of overweight and obesity in Saudi Arabia is on the rise, placing a huge burden on health and economic resources. Recently, gut microbiota has been reported to be involved in the pathogenesis of many metabolic disorders and diseases, including obesity, diabetes, and CVD.**Objective:** The objective of this study was to identify obesity-associated gut microbiota dysbiosis and their relationship to body mass index (BMI) among healthy Saudi females with different degrees of obesity.**Methodology:** A total of 120, below the age of 30 years, healthy females with different degrees of obesity were recruited. All those filled out a questionnaire related to their nutritional habits, health conditions and demographics. Their height, body weight, hip and waist circumference were measured (BMI and age). Stool samples were collected and genomic DNA was extracted from those samples. The 16S rRNA were amplified and sequenced via next generation sequencing (MiSeq), sequencing reads were trimmed, analyzed and filtered and assigned to taxonomic units.**Results:** The results indicated the presence of various bacteriological groups including Firmicutes, *Actinomyces odontolyticus*, *Escherichia coli* and *Ruminococcus obeum* and others. Work is in progress to correlate the prevalence of those bacterial groups with BMI.**Conclusion/Recommendations:** The results indicated that a variety of bacterial strains and microbiota populations are present among our study subjects. Bioinformatical data analysis will help to identify certain microbiota marker populations to be associated with different stages of obesity among the female Saudi population. Final goal is an early prediction of obesity and to target those patient groups to treat obesity.

Author Disclosure Block:

S. Harakeh: None. **S. Bahijri:** None. **G. Ajabnoor:** None. **A. Al-Hejin:** None. **M. Pfaffl:** None. **S. Farraj:** None. **G. Hegazy:** None. **S. Masaudi:** None. **E. Azhar:** None.

Poster Board Number:

FRIDAY-583

Publishing Title:

Immune Response to Bk Virus in Renal Transplant Recipients

Author Block:

D. DeWolfe, 02215[1], J. Gandhi[1], M. Mackenzie[1], T. A. Broge, Jr[1], E. Bord[1], D. A. Mandelbrot[1], R. Viscidi[2], F. Cardarelli[1], M. Pavlakis[1], **C. S. Tan**[1]; [1]Beth Israel Deaconess Med. Ctr., Boston, MA, [2]Johns Hopkins Univ., Baltimore, MD

Abstract Body:

Background: The adaptive immune characteristics associated with BK Virus (BKV) infection and control remain incompletely described. In this study we further elucidate the immune response to BKV.**Methods:**29 patients undergoing renal transplantation from two institutions were followed prospectively for one year to evaluate the recipients' BKV specific humoral and cellular immune responses. ELISA assay detected IgG antibody titers against BKV 1 and 4 and intracellular staining for interferon gamma (IFN-γ) determined BKV-specific $CD4^+$ and $CD8^+$ T lymphocytes. We quantified T lymphocyte phenotypes and expression of activation and exhaustion markers to demonstrate lymphocyte functions.**Results:**7 patients developed BK viruria and 3 progressed to BK viremia. There were no clinical differences between BKV positive and negative patients. Viremic patients had an increase in IFN-γ producing $CD8^+$ T Cells with time (P= 0.034) and a trend toward increased IFN-γ positive $CD4^+$ T Cells. Prednisone was associated with fewer BKV specific $CD8^+$ T Cells (P=0.027). Viremic patients had an increase in IgG titers against both BKV 1 and 4(P= 0.044). Viremic patients also had higher titers against BKV 4 at 6 (P=0.035) and 12 months (P=0.004) and a similar trend toward higher BKV 1 titers. Viremic patients had fewer $CD4^+$ central memory cells expressing activation markers CD38 (P=0.042) and HLA-DR (P= 0.027) at 1 month. Viremic patients had more $CD8^+$ effector cells at baseline (P = 0.002) and 12 months (P = 0.009).**Conclusions:**The response to BK virus includes both a BKV specific humoral and cellular component, dominated by $CD8^+$ T lymphocytes, and may be impaired by prednisone. Viremia was associated with fewer activated $CD4^+$ central memory cells at 1 month, perhaps indicating an early memory deficit; and significantly more $CD8^+$ effector cells at 12 months, potentially reflecting the cytotoxic response to viremia. Monitoring and modulating specific immune factors against BKV post transplant could help identify risk of BKV infection and factors to harness as immunotherapy against BKV.

Author Disclosure Block:

D. DeWolfe: None. **J. Gandhi:** None. **M. Mackenzie:** None. **T.A. Broge:** None. **E. Bord:** None. **D.A. Mandelbrot:** None. **R. Viscidi:** None. **F. Cardarelli:** None. **M. Pavlakis:** None. **C.S. Tan:** None.

Poster Board Number:

FRIDAY-584

Publishing Title:

Antibiotic Disruption of a Fish Model Microbiome with Negative Host Effects

Author Block:

J. M. Carlson, T. P. Primm; Sam Houston State Univ., Huntsville, TX

Abstract Body:

Human and veterinary medicine practices the application of broad spectrum antibiotics because of their robust ability in fighting an assortment of bacterial infections. Yet in clinical settings, patients can exhibit serious side effects, including antibiotic-associated enterocolitis, which presumably results from dysbiosis of the microbiome. Study utilizing a well-controlled system is needed. Therefore, we observed the compositional changes within the skin and gut bacterial communities from an antibiotic and how this treatment affects the host. Samples of the two mucosal microbiomes, skin and gut, were collected from *Gambusia affinis* before, during, and after exposure to rifampicin, a broad spectrum antibiotic. In both the skin and gut microbiomes, 16S rRNA profiling revealed a decrease in community evenness and diversity, and dominance of the genus *Myroides*. This was followed by a recovery period resulting in a drop in *Myroides* and stabilization to a taxonomic distribution different than pre-exposure. Several host effects were measured on fish with altered microbiomes. Treated fish given a standardized diet gained less weight over the duration of a month than control fish. Susceptibility to the fish pathogen *Edwardsiella ictaluri* increased in treated fish rather than in the control. In contrast, polymicrobial water challenges from feces and soil, or with addition of the toxin nitrate, observed no survival differences between fish groups. A novel observation was that treated fish became more sensitive to high salinity concentration. Findings from this study reveal a tractable system that can be used to determined mechanisms of confirmed antibiotic-derived negative host effects.

Author Disclosure Block:

J.M. Carlson: None. **T.P. Primm:** None.

Poster Board Number:

FRIDAY-585

Publishing Title:

Acute *Montipora W*hite Syndrome and Its Potential Causative Agents, Environmental Drivers, and the Importance of Coral Health

Author Block:

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Abstract Body:

Disease is a threat to coral reefs and exacerbates the widespread impacts of climate change. Prevalence of a number of coral diseases are increased by environmental stressors including elevated water temperatures, pollution, and runoff, which can lead to destructive outbreaks. Numerous outbreaks of the tissue loss disease acute *Montipora* white syndrome (aMWS) have occurred in *Montipora capitata*, a major reef-building coral in Kaneohe Bay, Hawaii, which may have been triggered by sewage runoff associated with winter rainfall and pre-existing stress from a chronic tissue loss disease (cMWS). One of the potential etiological agents of aMWS is *Pseudoalteromonas* sp. OCN003, a strain isolated from diseased *M. capitata*. Under controlled conditions, 20% of the healthy *M. capitata* fragments exposed to OCN003 developed acute tissue loss within three weeks post-inoculation. When fragments already compromised by cMWS were exposed to OCN003, 60% of the fragments developed acute tissue loss within four days post-exposure. Thus, OCN003 is more successful as a secondary pathogen in laboratory experiments. Chronic MWS infections are observed year-round in Kaneohe Bay, while aMWS outbreaks have only occurred during the rainy winter months. The bacterial communities of samples from healthy and diseased *M. capitata* during an aMWS outbreak were analyzed with high-throughput sequencing. Bacteria from the family Enterobacteriaceae dominated all samples, healthy and diseased, and the most abundant OTU was similar to species that inhabit the human gastrointestinal tracts, suggesting sewage and/or freshwater input. This study describes the first *Pseudoalteromonas* sp. pathogenic to coral, evidence for OCN003 as a secondary pathogen, and possible bacterial communities consistent with anthropogenic stressors associated with a fatal coral disease.

Author Disclosure Block:

S. Beurmann: None. **A.M. Smith:** None. **B. Ushijima:** None. **C.M. Runyon:** None. **P. Videau:** None. **S.M. Callahan:** None. **G.S. Aeby:** None.

Poster Board Number:

FRIDAY-586

Publishing Title:

Identification of Urine Metabolites as Novel Biomarkers of Early Lyme Disease

Author Block:

A. Pegalajar-Jurado[1], L. v. Ashton[2], J. T. Belisle[2], K. Webb[2], G. P. Wormser[3], C. R. Molins[1]; [1]Ctr.s For Disease Control and Prevention, Fort Collins, CO, [2]Colorado State Univ., Fort Collins, CO, [3]New York Med. Coll., Valhalla, NY

Abstract Body:

Over 300,000 cases of Lyme disease (LD) are reported annually in the United States. Current diagnostics for LD are antibody-based and test patient serum samples. Limitations of this approach include a sensitivity that is dependent upon the stage of infection (low in early LD and high for late LD), the utilization of immunoblotting that requires advanced training for proper execution and interpretation, and the inability to distinguish between current and past infections. As an innovative diagnostic approach, we applied metabolomics to determine the presence of dysregulated metabolites in urine from patients with early LD. Urine samples (n=42) were obtained from well-characterized patients diagnosed with early LD (EL), 1 month treated early LD (1mthEL) and healthy controls (HC). Urine samples were analyzed using liquid chromatography-mass spectrometry (LC-MS) in positive- and negative-ion mode, resulting in the resolution of several thousand metabolites. Comparisons between EL, 1mthEL and HC were performed using the Agilent Mass Profiler Professional software. Metabolites that significantly differed ($p<0.05$) between the comparative groups, and were consistently observed in multiple LC-MS analyses, were selected to establish group-specific metabolic profiles. Specifically, biosignatures that differentiated EL vs HC, and EL vs 1mthEL were produced. These biosignatures consisted of 327 to 548 metabolites. Currently, *in silico* analyses are being performed with the METLIN database and the web-based Pathos software to provide a structural identity for the metabolites and predict metabolic pathways altered during EL and 1mthEL. Further refinement of the biosignatures and structural conformation of the selected metabolites is currently undergoing. This evaluation offers an opportunity for the identification of early LD and 1mthEL biomarkers that can be applied towards the development of novel diagnostic tools that utilize a non-invasive patient specimen. These studies will also provide insight into the host response of early LD.

Author Disclosure Block:

A. Pegalajar-Jurado: None. **L.V. Ashton:** None. **J.T. Belisle:** None. **K. Webb:** None. **G.P. Wormser:** None. **C.R. Molins:** None.

Poster Board Number:

FRIDAY-588

Publishing Title:

Association of *Staphylococcus aureus* Exotoxin Antibody Concentrations to Clinical Outcomes in Patients with Bacteremia

Author Block:

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Abstract Body:

Patients with *Staphylococcus aureus* bacteremia (SaB) have variable clinical responses attributed to multi-drug resistance, virulence, and immune evasion organism characteristics. These factors contribute to a dysregulated host immune response during the initial infection course. This study correlates the staphylococcal exotoxin (SE) antibody response to primary clinical outcomes in patients with confirmed SaB. Adult patients with SaB at a single academic medical center were included prospectively. Serum samples were collected on the initial day of patient presentation. Serum antibody to 10 SEs were measured using an electrochemiluminescence based multiplex immunoassay. Patients were compared according to outcomes of rapid bacteremia clearance ($\leq$ 4 d), persistence (> 4 d), and in-hospital mortality. Patient demographics, severity of illness, source of infection, and initial antibiotic treatment were collected. SE concentrations were compared by patient outcome (Mann Whitney U) and adjusted for collected covariates (logistic regression). Consecutive patients (n=98) were included in the study for analysis. 39 patients (39.8%) had persistent bacteremia > 4 days and 14 (14.3%) died during the hospital course. Patients with prolonged SaB often had lower antibody responses. Of interest, antibodies to staphylococcal enterotoxin A (SEA) were significantly lower with prolonged SaB compared to the rapid clearance (median 1.95 vs 2.37 log Ab U/ml; p=0.046). Duration of SaB was inversely related to SEA (Pearson coefficient 0.249; p=0.013). A similar trend was observed with SED in prolonged versus rapid SaB (0.88 vs 1.09; p=0.069). Patients who died also had lower SE antibody responses, most strikingly with alpha hemolysin (death vs survival 3.14 vs 3.33, respectively), but none of these comparisons were statistically significant. The SE antibody responses in SaB are lower in patients with poor outcomes of prolonged SaB and mortality. This is consistent with earlier studies demonstrating lower SE antibodies in patients with sepsis. These results provide further data on the dysfunctional host immune response in SaB and may be a potential biomarker for a precision approach to treatment.

Author Disclosure Block:

W. Rose: C. Consultant; Self; Visante, Inc. E. Grant Investigator; Self; Merck. J. Scientific Advisor (Review Panel or Advisory Committee); Self; Theravance. L. Speaker's Bureau; Self;

The Medicines Company, Merck. **R.P. Adhikari:** D. Employee; Self; Integrated Biotherapeutics, Inc.. **R. Koralkar:** None. **S. Krishnan:** D. Employee; Self; Integrated Biotherapeutics, Inc.. **R. Proctor:** None. **M.J. Aman:** D. Employee; Self; Integrated Biotherapeutics, Inc.. **S. Shukla:** None.

Poster Board Number:

FRIDAY-589

Publishing Title:

Prognostic Role of Toll-Like Receptor 2 and Cytokines in Patients with *Staphylococcus aureus* Bacteremia

Author Block:

J. Sung[1], J. Cho[2], Y. Choi[2], S. Choi[3], M. Kim[2], N-H. Kim[2], C-J. Kim[4], K-H. Song[2], P. Choe[3], W. Park[3], E. Kim[2], K. Park[2], N. Kim[3], M-d. Oh[3], H. Kim[2]; [1]Yonsei Univ. Coll. of Med., Seoul, Korea, Republic of, [2]Seoul Natl. Univ. Coll. of Med., Seongnam, Korea, Republic of, [3]Seoul Natl. Univ. Coll. of Med., Seoul, Korea, Republic of, [4]Ewha Womans Univ. Mokdong Hosp., Seoul, Korea, Republic of

Abstract Body:

Background: Toll-like receptor 2 (TLR2) is the main pattern recognition receptor recognizing pathogen-associated molecular patterns in *Staphylococcus aureus* infection, playing an important role in innate immune response. The cytokines released by TLR2 signaling are either beneficial or detrimental. We aimed to examine the relationship of TLR2 expression and cytokines with prognosis in patients with *S. aureus* bacteremia (SAB).**Methods:** Whole blood samples were collected at several time points (≤ 5 days, 6-9 days, 10-13 days, 14-19 days and ≥ 20 days) after the onset of bacteremia, from patients with SAB in 2 teaching hospitals in Korea. The relative mRNA expression of TLR2 and the concentration of IL-6 and IL-10 were measured to identify associations with 30-day mortality and severity.**Results:** The level of TLR2 expression during the clinical course after the onset of bacteremia was variable among the 62 patients with SAB. In 10 patients with 30-day mortality, TLR2 expression was down-regulated and showed less dynamic changes throughout the whole period than in 52 survivors. Within 7 days of bacteremia, the mortal group showed significantly elevated IL-6 level (755.8 vs. 97.8 pg/mL, P=0.017) and more patients (90%, 9/10) in the mortal group showed measurable IL-10 (>2.49 pg/mL) compared with the survived group (40%, 20/50). IL-6/IL-10 ratio tended to be either low or high in patients with 30-day mortality, which implies excessive immune response of either pro-inflammation or anti-inflammation. The level of TLR2 expression within 5 days of bacteremia was higher in patients with complicated bacteremia or septic shock.**Conclusion:** Down-regulated TLR2 expression and elevated level of either IL-6 or IL-10 at early stage of bacteremia were associated with 30-day mortality in SAB patients. TLR2 expression within 5 days of bacteremia was higher in patients with severe presentations. Host immune response including TLR2 expression and secretion of cytokines may be a potential prognostic factor in SAB.

Author Disclosure Block:

J. Sung: None. **J. Cho:** None. **Y. Choi:** None. **S. Choi:** None. **M. Kim:** None. **N. Kim:** None. **C. Kim:** None. **K. Song:** None. **P. Choe:** None. **W. Park:** None. **E. Kim:** None. **K. Park:** None. **N. Kim:** None. **M. Oh:** None. **H. Kim:** None.

Poster Board Number:

FRIDAY-590

Publishing Title:

Dysregulation of Bile Acids Occurs at Different Stages of Lyme Disease

Author Block:

L. V. Ashton[1], C. R. Molins[2], K. Webb[1], G. P. Wormser[3], J. T. Belisle[1]; [1]Colorado State Univ., Fort Collins, CO, [2]Ctr.s for Disease Control, Fort Collins, CO, [3]New York Med. Coll., Valhalla, NY

Abstract Body:

Lyme disease (LD) is the most common tick-borne disease in the United States. Early disease is characterized by the presence of an erythema migrans (EM) lesion and general symptoms of malaise. However, multiple EMs, cardiac involvement or neurological symptoms may develop as the infection disseminates, and Lyme arthritis presents in the late stage of LD. Liquid chromatography-mass spectrometry-based metabolomics was applied to sera obtained from well-characterized patients diagnosed with various stages of LD (early localized and disseminated LD, late LD, and one month treated early LD) as well as from healthy controls (HC), to elucidate metabolic pathways perturbed for LD. This effort resulted in the resolution of 2,027 unique molecular features (MFs) that significantly differed in abundance with at least a two-fold change between early LD and HC, or between the various disease states. Although individual metabolic biosignatures were discovered for the various LD stage comparisons, an overlap (44 to 66%) in the molecular features that comprised these biosignatures was observed. *In silico* analyses using the METLIN database and the web-based Pathos software were applied to predict metabolic pathways altered in LD. Within the predicted pathways, bile acid metabolism and secretion, among others, was found to be perturbed in LD. A total of 38 MFs with putative structural identifications aligned to this pathway, and seven of these metabolites have been confirmed. These data are consistent with previous clinical findings demonstrating abnormal liver function in LD patients, and the frequency of these abnormalities being greater in early disseminated LD versus early localized LD. The structural conformation of additional bile acid metabolites is currently being performed, as well as investigation of LD stage-specific differences in the bile acid pathway.

Author Disclosure Block:

L.V. Ashton: None. **C.R. Molins:** None. **K. Webb:** None. **G.P. Wormser:** None. **J.T. Belisle:** None.

Poster Board Number:

FRIDAY-591

Publishing Title:

Comparison and Characterization of Usa300 Clones by Whole Genome Sequencing and Mice Pneumonia Model

Author Block:

S. Sonoda[1], T. Yamaguchi[2], K. Aoki[2], C. Kajiwara[2], S. Kimura[2], Y. Akasaka[2], Y. Ishii[2], K. Tateda[2]; [1]Tokyo Med. and Dental Univ., Tokyo, Japan, [2]Toho Univ. Faculty of Med., Tokyo, Japan

Abstract Body:

Background: Highly virulent community-acquired MRSA, especially USA300 clone, has become epidemic in the world. Types and severities of diseases by USA300 clones varied from mild skin infection to fetal necrotizing pneumonia, although the exact mechanisms still remained unknown. In the present study, by applying whole genome sequence (WGS) analysis, we compared genetic backgrounds of virulence factors in several USA300 clones, such as isolates from severe pneumonia (IP) and mild skin diseases (IS). In vivo comparative virulence was characterized in bacterial burdens, cytokine responses, pathological changes in the lung and the survival in mice model of pneumonia.**Methods:** IP and IS isolates, in addition to two reference strains (BAA-1556, USA300; N315, USA100), were used. In pneumonia model, mice (BALB/c, 6-8 weeks old) were intra-tracheally challenged with $1\text{-}3\times10^8$ CFU/mouse of bacteria, and sacrificed at 12 hours after infection for bacterial burdens, cytokine responses and pathological analysis in the lungs. WGS analysis of IP and IS was performed, and transcriptional levels of several virulence factors (ex. α-toxin) were examined in several conditions.**Results:** In pneumonia model, IP strain demonstrated significantly stronger virulence than that of IS strain (mortality rate of IP 100% vs IS 0%). Well correlated with the mortality, significantly stronger cytokine productions and bacterial burdens (IP 1.6×10^9 vs IS 2.7×10^7 CFU/lungs) were observed in the lungs of IP-challenged group. Interestingly, strong bacterial aggregation and tissue destructions were exhibited in the lungs of IP-group. WGS analysis showed that the IP isolate possessed 16 additional ORFs, comparing to IS isolate. Although not all genes were examined yet, we observed higher expressions of α-toxin in IP isolate than that of IS isolate.**Conclusions:** The present data demonstrated that USA300 IP isolate was more virulent than IS isolate in pneumonia model. WGS analysis showed presence of 16 additional ORFs in IP isolate, although remaining genes detected were similar to those of IS isolate. Our data suggested that gene expression experiments directing several virulence factors, such as α-toxin, are warranted for better understanding a virulence difference observed among USA300 clones.

Author Disclosure Block:

S. Sonoda: None. **T. Yamaguchi:** None. **K. Aoki:** None. **C. Kajiwara:** None. **S. Kimura:** None. **Y. Akasaka:** None. **Y. Ishii:** None. **K. Tateda:** None.

Poster Board Number:

FRIDAY-592

Publishing Title:

Transcription Factor Efg1 Regulates Biofilm Formation and Contributes to Palatal Tissue Damage in *Candida albicans* Associated Denture Stomatitis

Author Block:

J. Yano, A. Yu, D. Stoute, P. L. Fidel, Jr, M. C. Noverr; LSU Hlth. Sci. Ctr. Sch. of Dent., New Orleans, LA

Abstract Body:

Background: Denture stomatitis (DS), a condition characterized by inflammation of the oral mucosa in direct contact with dentures, affects a significant number of denture wearers. The disease is a fungal infection predominantly caused by *Candida albicans,* which readily colonizes and form biofilms on denture materials. While evidence for biofilms on abiotic and biotic surfaces initiating *Candida* infections is accumulating, a role for biofilms in DS remains unclear. Using an established animal model incorporating a novel intraoral denture system, the purpose of this study was to determine the role of biofilm formation in mucosal damage during pathogenesis of DS *in vivo*. **Methods:** Denture materials or rat palatal tissues were inoculated with wild-type *C. albicans* or mutants defective in morphogenesis (*efg1*$^{-/-}$) or biofilm formation (*bcr1*$^{-/-}$) and evaluated for the presence of biofilms by scanning electron and confocal microscopy. For *in vivo* analyses, rats fitted with custom dentures, consisting of fixed and removable parts, were inoculated with wild-type *C. albicans,* mutants or reconstituted strains and monitored weekly for fungal burden (denture and palate), body weight and tissue damage (LDH) for up to 8 weeks. **Results:** *C.albicans* wild-type and reconstituted mutants formed biofilms on dentures and palatal tissues under *in vitro, ex vivo* and *in vivo* conditions as indicated by microscopy demonstrating robust biofilm architecture and colocalization of extracellular matrix (ECM) and *C. albicans*. In contrast, both *efg1*$^{-/-}$ and *bcr1*$^{-/-}$ mutants exhibited poor biofilm growth with little to no ECM . In addition, quantification of fungal burden showed reduced colonization on dentures and palates of rats inoculated with *efg1*$^{-/-}$ mutant throughout the infection period compared to rats inoculated with wild-type, reconstituted mutants or *bcr1*$^{-/-}$ mutant. Finally, rats inoculated with *efg1*$^{-/-}$ had minimal palatal tissue damage/weight loss while those inoculated with wild-type, reconstituted mutants or *bcr1*$^{-/-}$ mutant showed elevated levels of tissue damage and substantial weight loss. **Conclusions:** These data suggest that biofilm formation is not required to induce damage during DS; however, regulation by *C. albicans* Efg1, a central regulator of virulence, has a pivotal role in pathogenesis of DS.

Author Disclosure Block:

J. Yano: None. **A. Yu:** None. **D. Stoute:** None. **P.L. Fidel:** None. **M.C. Noverr:** None.

Poster Board Number:

FRIDAY-593

Publishing Title:

A Quorum Sensing Signal Promotes Host Tolerance Training Through Chromatin Modifications

Author Block:

A. Bandyopadhaya, A. Tsurumi, D. Maura, K. L. Jeffrey, L. G. Rahme; Massachusetts Gen. Hosp. & Harvard Med. Sch., Boston, MA

Abstract Body:

The quorum sensing excreted volatile small molecule 2-aminoacetophenone (2-AA), amply produced by the human opportunistic pathogen *Pseudomonas aeruginosa* (PA), dampens host innate immune responses, modulates host metabolism, while promoting host tolerance against high bacterial burden, ultimately rescuing mice from PA induced mortality. The biological mechanisms by which pathogens act upon hosts to maintain their burden without affecting host fitness, and the causes and consequences of variation in tolerance are still underdeveloped. Similarly, the precise underlying mechanisms by which innate immune training can be triggered and the outcome of this "training" for the pathogen remains elusive. Here, we have used 2-AA molecule paradigmatically to uncover the mechanism by which bacterial molecules may "train" the host to become tolerant to high bacterial burden, while having a positive effect on host fitness. The training was induced by 2-AA pretreatment and the training capacity was investigated following exposure to the specific or non-specific molecule and/or PA itself *in vitro* and *in vivo*. Chromatin modifications were analyzed by biochemical, molecular assays and ChIP-PCR. We show that 2-AA regulates histone deacetylase (HDAC)1, resulting in H3K18 hypoacetylation at proinflammatory cytokine loci. 2-AA was capable of reprogramming the innate immune cells via the induction of specific alterations in histone acetylation at critical immune cytokines *in vivo* and *in vitro*. The host epigenetic reprograming generated by 2-AA, maintained for up to seven days, resulted in a significantly dampened response to subsequent exposure to 2-AA or other pathogen-associated molecules, lipopolysaccharide or peptiodoglycan. This process is a distinct molecular mechanism of host chromatin regulation by a QS excreted small molecule. Genetic and pharmacological inhibition of HDAC1 prevented the immunomodulatory effect of 2-AA. These observations provide the first mechanistic example of a QS excreted small molecule that trains the host tolerance against infection via epigenetic reprogramming. A solid understanding of patho-epigenetic changes following host-pathogen interactions will benefit the development of therapeutic interventions for acute and chronic infection.

Author Disclosure Block:

A. Bandyopadhaya: None. **A. Tsurumi:** None. **D. Maura:** None. **K.L. Jeffrey:** None. **L.G. Rahme:** None.

Poster Board Number:

FRIDAY-594

Publishing Title:

***Streptococcus pneumoniae* Forms Biofilms Within the Heart**

Author Block:

A. T. Shenoy[1], R. P. Gilley[2], N. Kumar[3], S. Ott[3], S. C. Daugherty[3], L. J. Tallon[3], H. Tettelin[3], C. J. Orihuela[1]; [1]Univ. Of Alabama at Birmingham (UAB), Birmingham, AL, [2]Univ. Of Texas Hlth.Sci. Ctr. at San Antonio (UTHSCSA), San Antonio, TX, [3]Inst. for Genome Sci., Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract Body:

Streptococcus pneumoniae, (the pneumococcus) is a leading cause of opportunistic diseases such as community-acquired pneumonia, bacteremia, and meningitis. During severe infections the pneumococcus also contributes towards cardiac complications such as arrhythmia and heart failure. Along such lines, we previously reported that pneumococci invade the myocardium during invasive pneumococcal disease (IPD) forming bacteria-filled microlesions that disrupt cardiac functionality. Herein we expand upon our previous microscopic observations and detail changes in the number and size of microlesions within the ventricular tissue. Fluorescent and electron microscopic analysis revealed equidistantly spaced non-encapsulated pneumococci with a striking absence of infiltrating immune cells; a finding that is suggestive of biofilms as described in the nasopharynx. A biofilm-like phenotype was confirmed by the presence of extracellular matrix components poly-N-acetylglucosamine and both eukaryotic and prokaryotic extracellular DNA within the microlesions. Furthermore, pneumococci isolated from the myocardium were more resistant to antibiotics, a hallmark of the biofilm phenotype. Pneumococci isolated from the heart were found to retain unique properties following their isolation. These included an increased capacity to adhere and invade cardiomyocytes *in vitro*, and increased tropism for the heart on subsequent infections. Comparative gene expression profiling of the pneumococci within the myocardium using RNA-Seq identified numerous differentially expressed genes including established virulence determinants. Ongoing studies are focused on examining the requirement for these determinants in experimentally challenged mice and are intended to provide a deeper insight into the host-pathogen interactions *in vivo*, identify new strategies to prevent pneumococcal invasion of the myocardium, and allow development of treatment strategies for IPD- associated cardiac complications.

Author Disclosure Block:

A.T. Shenoy: None. **R.P. Gilley:** None. **N. Kumar:** None. **S. Ott:** None. **S.C. Daugherty:** None. **L.J. Tallon:** None. **H. Tettelin:** None. **C.J. Orihuela:** None.

Poster Board Number:

FRIDAY-595

Publishing Title:

Cystic Fibrosis and Immunodeficiency Disease

Author Block:

R. Harbeck, K. Lucey; Natl. Jewish Hlth., Denver, CO

Abstract Body:

Chronic, pulmonary infections are the most prominent cause of the increased morbidity and mortality in cystic fibrosis (CF). In immunodeficiency diseases, an individual is often unable to mount an immune response to microorganisms. Common variable immune deficiency (CVID) is a frequently diagnosed immunodeficiency, especially in adults, characterized by low levels of serum immunoglobulins and antibodies, which causes an increased susceptibility to infection. In this study we examined the immune profile of patients diagnosed with immunodeficiency and CF to determine if an immunodeficiency influenced the profile of microorganisms recovered from the respiratory secretions of CF patients.Between the years of 2008 and 2015 we identified ten patients with a diagnosis of CF and an immunodeficiency. Two of these patients were diagnosed with CVID. All patients had immunoglobulin levels performed and some also were tested for pneumococcal polysaccharide and tetanus antibody titers. In addition CD4, CD8, CD19 and memory B cells were determined. We compared the different organisms that were identified in this population to the organisms recovered from our population of patients diagnosed with only cystic fibrosis.Four of the ten patients had multiple organisms identified in their respiratory cultures including *Pseudomonas aeruginosa,* methicillin resistant *Staphylococcus aureus, Staphylococcus aureus, Haemophilus influnzae, Candida albicans* and mold. One patient was only positive for *Pseudomonas aeruginosa* in multiple cultures that were collected. The other five patients had cultures with normal flora present or had no cultures performed. The profile of organisms compares with those recovered from patients with only cystic fibrosis where predominately *Pseudomonas aeruginosa* and *Staphylococcus aureus* were identified. In most cases the immunological profile of these patients support the diagnosis of immunodeficiency or CVID.Patients diagnosed with both cystic fibrosis and immunodeficiencies have many of the same pathogens recovered as do patients diagnosed only with cystic fibrosis. It does not appear that an immunodeficiency alters the microbial populations in the airways of CF patients.

Author Disclosure Block:

R. Harbeck: None. **K. Lucey:** None.

Poster Board Number:

FRIDAY-596

Publishing Title:

Human Genetic Variation in *vac14* Regulates *S. typhi* Invasion and Typhoid Fever Susceptability

Author Block:

M. Alvarez[1], S. Dunstan[2], P. Luo[1], S. Oehlers[1], E. Walton[1], L. Glover[1], L. Wang[1], D. Tobin[1], D. Ko[1]; [1]Duke Univ., Durham, NC, [2]Univ. of Melbourne, Melbourne, Australia

Abstract Body:

Background: Human genetic variation influences many aspects of our lives including susceptibility to infectious diseases. Our lab has developed a novel cell-based screen of human genetic variation (Hi-HOST: High-throughput human in vitro susceptibility testing) for identifying genetic differences that impact host-pathogen interactions. Specifically, we examined genetic variation in *S.* Typhi invasion of cells and the impact this has on risk of typhoid fever in people.**Methods and Results:** We carried out a Hi-HOST screen of genotyped cell lines from 351 individuals for invasion by *S.* Typhi. A human single nucleotide polymorphism (SNP) in the gene encoding the phosphoinositide scaffolding protein VAC14 was associated with invasion (p=0.0006). While this association suggested that VAC14 regulates invasion, we experimentally tested this with RNAi, CRISPR knockouts, and plasmid complementation. RNAi against VAC14 in HeLa cells caused an increase of *Salmonella* invasion (p=0.02). CRISPR knockout of VAC14 completely abolished protein expression and nearly doubled invasion (p=0.005). To determine the mechanism by which VAC14 alters invasion, assays of membrane docking, actin-based ruffling, and early intracellular survival were carried out on *vac14*$^{-/-}$ cells. Of these steps in early *S.* Typhi infection, only docking was increased in *vac14*$^{-/-}$ mutant cells (p=0.0002). Cellular lipid measurements suggest the *vac14*$^{-/-}$ cells have an altered plasma membrane composition that then impacts *Salmonella* docking and invasion. To extend these studies *in vivo*, mutant *vac14* zebrafish are being evaluated for *Salmonella* dissemination and outcome. Finally, the *vac14* SNP was tested for association with typhoid fever in a case-control study (n=500 cases, 496 controls). Remarkably, the allele that decreases invasion is associated with protection against typhoid fever (p=0.0098, OR=1.4).**Conclusion:** Through this multi-disciplinary project, we discovered VAC14 to be an inhibitor of *Salmonella* invasion. We propose that this effect on invasion is mediated by VAC14 regulation of phosphoinositides that impact plasma membrane lipid composition. Ultimately, we hope to use these findings to develop new therapeutic options.

Author Disclosure Block:

M. Alvarez: None. **S. Dunstan:** None. **P. Luo:** None. **S. Oehlers:** None. **E. Walton:** None. **L. Glover:** None. **L. Wang:** None. **D. Tobin:** None. **D. Ko:** None.

Poster Board Number:

FRIDAY-597

Publishing Title:

Integrative Analysis of Gwas, Transcriptomices, Metabolites, and Cytokines Reveals Methylthioadenosine Is a Prognostic Biomarker for Death in Sepsis

Author Block:

L. Wang[1], E. Ko[1], J. J. Gilchrist[2], A. Rautanen[2], S. Jaslow[1], A. V. S. Hill[2], E. T. Tsalik[1], D. C. Ko[1]; [1]Duke Univ., Durham, NC, [2]Univ. of Oxford, Oxford, United Kingdom

Abstract Body:

Background: Sepsis is a systemic, deleterious inflammatory response to infection. Sepsis is a leading cause of death, and identifying sensitive and specific biomarkers could improve diagnosis, prognosis, and treatment. This study reports a novel and robust biomarker for sepsis death based on analysis combining multiple "omics" datasets.**Methods:** Three patient datasets were used to examine the association between genetic, transcriptional, metabolite, and cytokine markers and sepsis: A GWAS analysis was performed from 218 cases of non-typhoidal Salmonellae (NTS) bacteremia patients and 3000 controls. Metabolites, RNA-seq, and cytokine levels were examined in SIRS patients from the previously published CAPSOD study and an independent cohort with temporal data, the VAP study (157 samples).**Results:** A pathway-based GWAS of NTS bacteremia showed a strong statistical enrichment for SNPs near genes of the methionine salvage pathway. Measurement of the pathway's substrate, 5'-methylthioadenosine (MTA), in two independent cohorts of sepsis patients demonstrated that plasma levels are increased in nonsurvivors compared to survivors and controls. High plasma MTA was correlated with high levels of inflammatory cytokines (IL-6 and IL-8), suggesting elevated MTA could mark a subset of patients with excessive inflammation. Finally, we evaluated a machine-learning model by combining MTA and other clinical variables and measured approximately 80% accuracy (based on AUC) in cross-validation and testing of independent samples. Notably, MTA alone was nearly as successful in predicting sepsis outcome.**Conclusions:** Based on integrative analysis of panomics approaches, we identified and validated that MTA is a robust prognostic biomarker of sepsis death. Our approach combining genetic association data with biomolecule measurements can shape our understanding of sepsis and lead to the development of accurate biomarkers.

Author Disclosure Block:

L. Wang: None. **E. Ko:** None. **J.J. Gilchrist:** None. **A. Rautanen:** None. **S. Jaslow:** None. **A.V.S. Hill:** None. **E.T. Tsalik:** None. **D.C. Ko:** None.

Poster Board Number:

FRIDAY-598

Publishing Title:

Semen-Derived SEM1 Amyloid Fibrils Modify Immune Responses to Vaginal Anaerobes

Author Block:

S. N. Ryan[1], H. S. Yamamoto[1], N. R. Roan[2], R. N. Fichorova[1]; [1]Brigham and Women's Hosp., Boston, MA, [2]Univ. of California, San Francisco, San Francisco, CA

Abstract Body:

Semen harbors amyloids fibrils, including those made up of peptide fragments from semenogelin 1 (SEM1).The physiologic function of SEM1 is unknown, but it has been shown to increase HIV infectivity *in vitro*. Because the majority of HIV infections in women occur through vaginal sexual contact, it is of significant interest to study how factors in semen, such as SEM1, interact with the genital immune environment and the vaginal microbiota, which plays an important role in the formation of the mucosal barrier against sexually-transmitted diseases. The disturbance of the vaginal microbiota leads to replacement of beneficial *Lactobacillus* species with anaerobic bacteria, and inflammation, which in turn facilitates HIV. Whether SEM1 fibrils affect the vaginal microbiota and influence vaginal immunity has not been studied. We treated female genital tract epithelial cells treated with SEM1 fibrils at doses previously demonstrated to enhance HIV infection followed by incubation with *Lactobacilli* or with anaerobes representing the disturbed vaginal microbiota (*P. bivia* and *A. vaginae).* Vaginal colonization was determined by colony forming units attached to the epithelial cells. Inflammatory responses were measured by NF-κB activation and chemokine secretion. Both lactobacilli and pathogenic bacteria colonized the vaginal epithelium in the presence of SEM1 fibrils, which did not affect immune responses to *Lactobacilli* but induced NF-κB activation and enhanced the proinflammatory activities of *A. vaginae*. The fibrils increased levels of the neutrophil chemoattractant GRO-α, decreased levels of the dendritic cell chemoattractant MIP-3α, and did not affect levels of RANTES, which is known to block HIV entry. Our experiments support our hypothesis that SEM1 fibrils may increase the risk of HIV acquisition in part by inducing an inflammatory response and enhancing the proinflammatory activities of vaginal bacteria that thrive in a disturbed vaginal environment. The proinflammatory activities of the SEM1 fibrils are in line with the beneficial effects of semen-induced inflammation on reproductive success; however, these activities present a problem to women at high risk of HIV who seek pregnancy.

Author Disclosure Block:

S.N. Ryan: None. **H.S. Yamamoto:** None. **N.R. Roan:** None. **R.N. Fichorova:** None.

Poster Board Number:

FRIDAY-599

Publishing Title:

Adjuvant Hyperbaric Oxygen Therapy Augments Tobramycin Treatment in Left-Sided *Staphylococcus aureus* Endocarditis in an Experimental Rat Model

Author Block:

C. J. Lerche, L. Christophersen, M. Kolpen, P. O. Jensen, N. Høiby, C. Moser; Clin. Microbiol., RigsHosp.et, Copenhagen, Denmark

Abstract Body:

Background: *Staphylococcus aureus* endocarditis (SA IE) still presents a clinical challenge and remains a disease with high mortality. SA IE is a rapid evolving and complex infectious disease. Tobramycin single agent antibiotic therapy in SA IE has been shown to be suboptimal. We hypothesized, that hyperbaric oxygen therapy (HBOT) could enhance the efficacy of tobramycin treatment in experimental SA IE. **Methods:** SA IE was established in male Wistar rats (n=47). Animals were randomized in two groups: 1. group received a combination of HBOT and tobramycin (n=23) and 2. group received tobramycin as monotherapy (non-HBOT, n=24). Animals exposed to HBOT were treated for 90 minutes at > 99% O_2 at a pressure of 280 kPa (2.8bar) in a hyperbaric chamber (OXYCOM 250 ARC, Hypcom). Tobramycin was given s.c. 20 mg/kg once a day. Treatments were initiated 1 day post infection (DPI) and cardiac valves were evaluated at 3DPI or 4DPI. The animals of the two treatment groups were divided into two subgroups a low-grade IE and a high-grade IE, defined by bacterial valve burden at the time of evaluation with a cut-off at 10^7 and 10^6 CFU/g valve at 3DPI and 4DPI, respectively. Low-grade IE < and high-grade IE > cut-off. **Results:** HBOT was well-tolerated and no adverse effects were observed. In the group of low-grade IE there was a significant reduction of bacterial load in valve vegetations in the HBOT group (3.4 ± 0.2, n=5) as compared to the non-HBOT group (4.8 ± 0.2, n=5)(p=0.03) already at 3DPI, but only a trend at 4DPI (p=0.06). In the high-grade IE group a significant reduction in bacterial load in the HBOT group (7.2 ± 0.3, n=8) was not observed until 4DPI as compared to the non-HBOT group (8.2 ± 0.4, n=6)(p=0.03). Animals, in the high-grade IE group exposed to HBOT, showed significantly reduced bacterial load in valve vegetations from 3DPI (8.4 ± 0.28, n=5) to 4DPI (7.2 ± 0.3, n=8)(p=0.014). The group treated with tobramycin alone was stationary in bacterial load in valve vegetations, from 3DPI (n=8) to 4DPI (n=6). **Conclusion:** Consecutive HBOT reduced the bacterial load in valve vegetation in experimental SA IE in tobramycin treated rats. The effect was postponed in animals with high-grade IE. The present results suggests HBOT may prove beneficial as adjunctive treatment of IE.

Author Disclosure Block:

C.J. Lerche: None. **L. Christophersen:** None. **M. Kolpen:** None. **P.O. Jensen:** None. **N. Høiby:** None. **C. Moser:** None.

Poster Board Number:

FRIDAY-600

Publishing Title:

Evidence of Bacterial Genomic Variation Contributing to Invasive Pneumococcal Disease Manifestation

Author Block:

Y. Li, B. Metcalf, S. Chochua, P. A. Hawkins, R. William, T. Pilishvili, L. Mcgee, B. W. Beall, Active Bacterial Core Surveillance Team; CDC, Atlanta, GA

Abstract Body:

Background: Bacteremia without focus (BwoF) and pneumonia with bacteremia (PB) are the two most common invasive pneumococcal disease (IPD) manifestations in children. It remains unclear whether specific bacterial factors contribute to the development PB over BwoF. Here we aim to identify pneumococcal variants associated with PB among pediatric IPD patients. **Methods:** Blood isolates from patients aged 5 or younger with either BwoF (n=665) or PB (n=346) were obtained through the Active Bacterial Core surveillance in years 1998, 1999, 2009, 2012, and 2013. Short reads from Illumina whole genome sequencing were mapped onto 3225 non-redundant pneumococcal coding DNA sequences (CDSs) to identify single nucleotide polymorphisms (SNPs), insertion/deletions, and gene absence. Association between PB and each variant was assessed by a Mantel-Haenszel (MH) test conditioned on patient age and sample year. Frequency of candidate variant was also examined in 17 pleural fluid (PF) isolates from the same patient population.**Results:** After Bonferroni correction, 5 non-synonymous SNPs in 3 CDCs showed p-value<0.05 in the MH test, hence identified as candidate variations. Two candidate SNPs were found in the polar amino acid ABC uptake transporter substrate binding protein gene (*paat*) and they exhibited strong linkage disequilibrium (r^2=0.98). In a multivariate analysis, patient age, surveillance year, and serotype were significant predictors of IPD syndrome. After controlling for these covariates, snp2401844A allele in *paat*, and no variant in other genes, showed association with PB (OR=2.25, 95% CI 1.08-4.67). The frequency of snp2401844A allele in the 17 PF isolates (0.29, 95% CI 0.10-0.56) was also significantly higher than that of the BwoF isolates (0.03, 95% CI 0.02-0.05).**Conclusion:** Genomic variation in pneumococcus appears to contribute to IPD manifestations among patients aged 5 years or younger. Serotype was a major but not the only pneumococcal determinant of IPD syndrome. How the snp2401844A allele in *paat* may facilitate lung infection deserves further investigation, including confirmation of this association.*The findings and conclusions in this report are those of the author(s) and do not necessarily represent the official position of the Centers for Disease Control and Prevention.*

Author Disclosure Block:

Y. Li: None. **B. Metcalf:** None. **S. Chochua:** None. **P.A. Hawkins:** None. **R. William:** None. **T. Pilishvili:** None. **L. Mcgee:** None. **B.W. Beall:** None.

Poster Board Number:

FRIDAY-601

Publishing Title:

Inhibition of Epithelial-Mesenchyme Transition Prevents Genital Chlamydia-Induced Complications

Author Block:

J. U. Igietseme[1], Y. Omosun[2], O. Stuchlik[1], M. Reed[3], C. M. Black[3], J. Pohl[3], J. Pohl[3], F. O. Eko[1], Q. He[2]; [1]CDC/NCID, Atlanta, GA, [2]Morehouse Sch. of Med., Atlanta, GA, [3]CDC/NCEZID, Atlanta, GA

Abstract Body:

Chlamydia trachomatis genital infection in women causes severe reproductive complications and is a strong co-factor for human papilloma virus (HPV)-associated cervical carcinoma. We have recently showed that chlamydia infection induces epithelial-mesenchyme transition (EMT) through caspase-mediated miRNA dysregulation that drives the development of complications. We tested the hypothesis that EMT inhibitors will prevent the key complications of chlamydial infection, especially reproductive tract inflammation, fibrosis, infertility, and promotion of HPV-related cervical carcinoma. Here we report that caspase inhibitors prevented chlamydia-induced EMT and the associated pathologies, including inflammation and infertility. Additionally, we found that, similar to estrogen, chlamydial infection promoted cervical epithelial hyperplasia in the HPV 16 E6/E7 transgenic mouse model of cervical carcinoma, which requires estrogen as co-factor for accelerated development of invasive cervical carcinoma. Therefore, the use of EMT antagonists provides a therapeutic strategy against the complications of genital chlamydial infections, as well as against HPV-related cervical carcinoma. Furthermore, EMT components are potential biomarkers for monitoring the development of complications of chlamydia genital infection and for assessment of the therapeutic efficacy of candidate treatment regimens.

Author Disclosure Block:

J.U. Igietseme: None. **Y. Omosun:** None. **O. Stuchlik:** None. **M. Reed:** None. **C.M. Black:** None. **J. Pohl:** None. **J. Pohl:** None. **F.O. Eko:** None. **Q. He:** None.

Poster Board Number:

FRIDAY-602

Publishing Title:

High Dietary Salt Intake Exacerbates *helicobacter pylori*-induced Iron Deficiency Anemia And Gastric Ulceration

Author Block:

A. C. Beckett, M. B. Piazuelo, J. M. Noto, R. M. Peek, Jr., H. M. S. Algood, T. L. Cover; Vanderbilt Univ. Sch. of Med., Nashville, TN

Abstract Body:

Background: Iron deficiency is the most prevalent nutritional disorder in the world, and often results in iron deficiency anemia (IDA). Human epidemiologic and animal studies suggest that *H. pylori* is linked to iron deficiency, but data regarding IDA are conflicting. High salt and low iron diets augment *H. pylori* virulence and are known to be risk factors for gastric cancer. Therefore, we hypothesized that *H. pylori*-associated IDA is promoted by increased dietary salt concentrations. **Methods:** Mongolian gerbils (either *H. pylori*-infected or uninfected) received one of four diets: normal, high salt, low iron, or combination high salt/low iron. Hematologic parameters and gastric histology were analyzed at 14 weeks post-infection. **Results:** *H. pylori*-infected animals had significantly lower hemoglobin values than their uninfected counterparts ($P<0.0001$). The greatest reduction in hemoglobin occurred in infected animals consuming a combination diet, indicating a role for both dietary iron and salt concentrations ($P<0.05$). Mean corpuscular volume (MCV) and serum ferritin values were also significantly reduced among *H. pylori*-infected animals when compared to uninfected cohorts. ($P<0.0001$). IDA (defined based on analyses of hemoglobin and serum ferritin) occurred only in infected animals, and increased in a diet-dependent fashion among the infected cohorts. Infected animals maintained on a combination diet experienced the highest rate of IDA (90%). A subset (30%) of the *H. pylori*-infected animals maintained on a normal diet developed IDA, indicating that *H. pylori* infection alone can cause IDA. Interestingly, gastric ulceration was detected in 67% of infected animals on a high salt diet compared to 30% of infected animals on a normal or low iron diet. Uninfected animals did not develop gastric ulcers, demonstrating an essential role for *H. pylori* in this process. Gastric pH was elevated in all infected cohorts. **Conclusions:** *H. pylori* infection promotes the development of IDA, and the risk of IDA is influenced by composition of the diet. We propose a model in which *H. pylori* promotes development of IDA through multiple mechanisms, including iron loss through bleeding ulcers and reduced absorption of dietary iron due to increased gastric pH.

Author Disclosure Block:

A.C. Beckett: None. **M.B. Piazuelo:** None. **J.M. Noto:** None. **R.M. Peek:** None. **H.M.S. Algood:** None. **T.L. Cover:** None.

Poster Board Number:

FRIDAY-603

Publishing Title:

Does Repeated Administration and Accumulation of Nanoparticles Increase Susceptibility of Mice to *Listeria monocytogenes*?

Author Block:

S. A. KHAN[1], K. Sung[1], B. Marasa[2], M. Nawaz[1], C. Cerniglia[1], A. Paredes[1], T. Ingle[1], P. Howard[1], A. Patri[1], Y. Jones[1], K. Tyner[2]; [1]Natl. Ctr. for Toxicological Res., JEFFERSON, AR, [2]Ctr. for Drug Evaluation and Res., Silver Spring, MD

Abstract Body:

It has been previously demonstrated *in vitro* that the accumulation of gold, silver, and silica nanoparticles (NPs) within macrophages reduces their ability to phagocytize bacteria. Impaired macrophage function may lead to an increased risk of infection. Whether this reduction in macrophage activity was preserved *in vivo* in non-clinical models and/or has a potential clinical impact has not been established to date. The focus of this study was to determine whether animals exposed up to 8-weeks to pharmacologically-relevant levels of gold (10 nm), silver (50 nm), and silica (10 nm) nanoparticles are more susceptible to *Listeria monocytogenes* (LM) infection. Eighty female BALB/c mice were divided in to 5 groups of 16 mice each. Animals in the vehicle control, silver and gold NP groups were subjected to weekly injections with 5% dextrose water, or 5 or 10 mg/kg body weight (BW) of NPs via tail vein for up to 8 weeks; the animals in silica NP group received weekly injections at 5 mg/kg BW for two weeks. This was followed by intraperitoneal injections of cyclophosphamide (CY) (80 mg/kg) in the positive control group of animals. All the animals were injected with 2,500 colony forming units (CFU) of LM via tail vein at 24 h after CY injections. On day 3 and 10 after LM injections, the animals were sacrificed and their livers, lungs, and spleens were collected for elemental analysis, electron microscopy, and viable LM count determination. The infection with LM resulted in an enlargement of spleen, liver, and lung. On day 3, the highest LM counts ($1\text{-}10^6$ CFU/gm tissues) were observed in spleen followed by liver and lungs. On day 10, most animals cleared *Listeria* from all the tissues suggesting that repeated administration and bioaccumulation of NPs in macrophages did not increase animal's susceptibility to bacterial infections. The data from this *in vivo* study is important to determine the impact of nanoparticle-based therapeutics and the immunological impact of accumulation of the nanoparticle-carrier. This will assist regulatory agencies in determining (i) the impact of nanomaterials and (ii) methodological approaches to evaluate the biological impact and risk.

Author Disclosure Block:

S.A. Khan: None. **K. Sung:** None. **B. Marasa:** None. **M. Nawaz:** None. **C. Cerniglia:** None. **A. Paredes:** None. **T. Ingle:** None. **P. Howard:** None. **A. Patri:** None. **Y. Jones:** None. **K. Tyner:** None.

Poster Board Number:

FRIDAY-604

Publishing Title:

Differential Characterization of Gene Expression Patterns in Pbmc of Children Who Have Recurrent Middle Ear Infections Compared to Non-Otitis Prone

Author Block:

R. Kaur[1], R. Kennedy[2], J. Casey[3], M. E. Pichichero[1]; [1]Rochester Gen. Hosp., Rochester, NY, [2]Mayo Clinic, Rochester, MN, [3]Legacy Pediatrics, Rochester, NY

Abstract Body:

Background: We used transcriptomic high-dimensional Next-Generation Sequencing to characterize the profile of peripheral blood mononuclear cells (PBMCs) from children who got recurrent middle ear infections called otitis-prone (OP) and non-OP during their healthy stage and during acute otitis media (AOM) episodes.**Methods:** RNA was extracted from PBMC of 12 children age between 9-15 months and sequenced using the Illumina HiSeq 2000 platform. Differential expression analysis comparing the OP subjects relative to the non-OP subjects was done using the edgeR package in R.**Results:** Results are reported within the healthy and AOM visits, as $\log_2$ of the fold change for the OP relative to the NOP, with corresponding p-values and false discovery rates. Following normalization 13,528 genes in healthy and 13248 genes during AOM visits were analyzed for differential expression between OP and NOP children respectively. Despite the relatively small sample size we identified significant expression differences in 39 and 31 genes ($p < 0.0003$ and $q \leq 0.1$) in OP children during healthy and AOM state respectively. A KEGG pathway enrichment analysis identified the complement and coagulation cascades pathway as being differentially downregulated (p=0.0006) in OP children during healthy state including genes C1, C2, C1qA and C3AR1. This pathway is a mediator of innate immunity leading to opsonization of pathogens, the recruitment of inflammatory and immunocompetent cells, and the direct killing of pathogens. Chemokine signaling pathway also showed differential trend (p=0.08) with the analysis. During the AOM state only one pathway, "Pathogenic Escherichia coli infection" emerged as different in OP (p=.08). Long Intergenic Non-Protein Coding RNA gene and some ubiquitin (USP32) encoding genes showed downregulation. Gene DSP encode for Desmoplakin was 10 fold upregulated that has shown to regulate the functions of chemical signaling pathways, cell differentiation and apoptosis.**Conclusions:** OP children are characterized by differential expression of selected genes in PBMC in both their healthy as well as during AOM state. The differential genes are mostly related to innate immune system.

Author Disclosure Block:

R. Kaur: None. **R. Kennedy:** None. **J. Casey:** None. **M.E. Pichichero:** None.

Poster Board Number:

FRIDAY-605

Publishing Title:

Nutritional Cues And Genetic Determinants For Biofilm Formation By An *acetobacter* Isolate From The Fruit Fly *drosophila Suzukii*

Author Block:

P. D. Newell, S. Magalhães Moreira; Oswego State Univ. of New York, Oswego, NY

Abstract Body:

The fruit fly *Drosophila melanogaster* has proven to be a successful model animal for studying host-microbiota interactions, thanks in part to a gut microbiota that is easily cultured and manipulated *in vitro.* While substantial progress has been made in characterizing the composition and function of the gut microbiota in this model animal, less is known about that of its cousin *Drosophila suzukii* (a.k.a Spotted-wing *Drosophila*), an emerging agricultural pest that infests fresh and ripening soft fruit. To explore the biology of bacteria associated with *D. suzukii*, *Acetobacter* sp. DsW_54 was isolated from the gut of a fly caught on raspberries in the field and its genome sequenced. In this study we characterized its growth and capacity for biofilm formation in dilute media amended with various carbon sources. Next, the bacteria were subjected to transposon mutagenesis followed by screening and enrichment strategies to isolate mutants with reduced or increased biofilm formation *in vitro*. Of 3,000 mutants screened, 19 showed a reduced ability to form a biofilm while 7 showed increased levels compared to wildtype. Transposon insertion sites were mapped by arbitrarily primed PCR and found within genes predicted to affect numerous cellular processes, including some typically associated with biofilm formation, e.g. flagellar motility. Finally, we utilized the newly isolated mutants to examine whether the capacity for biofilm formation *in vitro* correlates with the ability of *Acetobacter* to colonize and persist within *Drosophila.* The results show that some but not all biofilm-deficient mutants are affected for these phenotypes, and suggest that colonization is influenced by the diet of the host. In summary, *Acetobacter* sp. DsW_54 is useful strain for investigating the genetic requirements for colonization of abiotic surfaces as well as interactions between fruit flies and their gut microbiota. Future studies will investigate the mechanisms of colonization as well as the role that switching between sessile and motile lifestyles might play during the various life stages of the host.

Author Disclosure Block:

P.D. Newell: None. **S. Magalhães Moreira:** None.

Poster Board Number:

FRIDAY-606

Publishing Title:

Manipulating the Host-Microbe Phenotype in *Nasonia* Through Environmental Stressors

Author Block:

R. Brucker; Harvard Univ., Cambridge, MA

Abstract Body:

Background: The innate immune system functions to protect the animal from complications arising from injury, combating disease, and regulating the microbial community. A dynamic environment can influence the regulation of the host immune system by stimulating or suppressing regulation during stressful conditions; however, when the environment stresses the microbial community, how does the host respond? Do phenotypic traits arise when host-microbe relationships are stressed by the environment? Using the model system *Nasonia*, we use xenobiotics to stimulate changes to‘ the microbial community and determine host immune response and adaptation across host generations due to the changes in the microbial community.**Methods/Results:**Successive generations of xenobiotic-exposed animals were used to observe changes in microbial communities and innate immune regulation. Using NGS approaches, we determine that microbial community changes as well as variations in host immune expression are responding to acute and long-term exposure to environmental stresses. Likewise, metabolite production, mating behavior, and fitness were compared between populations. Furthermore, we compare immune regulation in germfree experiments from derived populations of xenobiotic and control *Nasonia* to conventional and artificial microbiota inoculated wasps and observe clear adaptation of the microbiome to prolonged exposure to environmental toxins.**Conclusion:**We observe heritable changes in the microbial community in response to xenobiotic exposure. The link between host microbiota and innate immunity during successive generations of xenobiotic exposure can result in positive adaptation by the host to environmental stressors.

Author Disclosure Block:

R. Brucker: None.

Poster Board Number:

FRIDAY-607

Publishing Title:

Antimicrobial Activity in Actinobacteria Associated with Monarch Butterflies (*Danaus plexippus*)

Author Block:

D. J. Desautels, A. J. Book, C. R. Currie; Univ. of Wisconsin-Madison, Madison, WI

Abstract Body:

There is a current crisis involving the evolution of antibiotic resistance in human bacterial and fungal pathogens. Multi-drug resistant pathogens are emerging at an alarming rate and the discovery of new antimicrobial compounds is slowing. Thus, discovery of novel antibiotic compounds is essential for maintaining health standards across the world. One major problem with the classic discovery pipeline is that it relies heavily on free-living soil microbes and the discovery of novel compounds from this source is increasingly rare. Previous research from our lab has shown that insects such as leaf-cutter ants and bark beetles harbor microbial symbionts that protect them from pathogens by producing antimicrobial compounds, some of which may are effective against human pathogens. The migratory lifestyle of the Monarch butterfly (*Danaus plexippus*) makes it an ideal candidate for symbiotic association with a protective symbiont. Each year the Monarch embarks on a long migration to Mexico, where millions of butterflies overwinter in trees in extremely dense populations. We propose this is an evolutionary pressure to select for microbial symbionts that protect the dense population from infection. Currently, little is known about the microorganisms associated with Monarch butterflies. We collected Monarch butterflies in Eastern Wisconsin after their migration north and used culture-dependent methods to obtain over 60 Actinobacteria isolates based on 16S sequencing. We will utilize bioassays with these Actinobacteria against ecologically relevant pathogens of monarch butterflies along with human pathogens to test for antimicrobial activity. Priority strains will be analyzed by LC-MS metabolomics and whole genome sequencing to identify novel secondary metabolites and gene clusters. This work will provide insight into the role of symbiotic Actinobacteria and Monarch hosts, as well as identifying antimicrobial compounds that may be useful treatments for human pathogens.

Author Disclosure Block:

D.J. Desautels: None. **A.J. Book:** None. **C.R. Currie:** None.

Poster Board Number:

FRIDAY-608

Publishing Title:

AI-2 Mediated Quorum Sensing by Microbial Symbionts within the European Honey Bee (*Apis mellifera*)

Author Block:

K. I. Miller, I. L. G. Newton; Indiana Univ., Bloomington, IN

Abstract Body:

Background: The European honey bee (*Apis mellifera*) is a charismatic species that plays a critical role in the pollination of agriculturally important crops and native flora. The recent and dramatic decline in colony numbers maintained in the United States has spurred researchers to both examine potential causes of the decline and identify factors that contribute to the health and well-being of the honey bee. One emerging field of research is that of the host-associated honey bee microbiome: a group of bacterial phylotypes consistently found within the honey bee and are thought to play critical roles such as protection from pathogens and nutrient acquisition. In other model systems, host-associated microbial communities are known to participate in a form of bacterial communication known as quorum sensing. This type of communication allows a bacterial community to sense their environment and respond with changes in gene expression, controlling a number of factors including virulence, biofilm formation, and cell motility. **Methods:** For the first time, we have investigated the production of quorum sensing molecules from honeybee specific microbiota *in vivo* and *in vitro*. We specifically looked for the presence of an important type of communication molecule: the inter-species autoinducer-2 (AI-2). To determine this, we used genetic data from the honey bee meta- genome and transcriptome and biosensors to look for the production of AI-2 both *in vivo* and *in vitro*. **Results:** The metagenome and the metatranscriptome showed the presence and transcription of the LuxS gene, respectively. Using biosensors, we confirmed the production of AI-2 both *in vivo*, using whole gut sections, and *in vitro*, using honey bee specific bacterial isolates. Additionally, using honey bee specific bacterial isolates, we have shown an increase in biofilm production when isolates are exposed to increased levels of AI-2. **Conclusions:** Together, these data provide multiple lines of evidence for the presence of quorum sensing inside the honey bee host. The effect of AI-2 on biofilm formation from honey bee specific bacteria shows one potential avenue for quorum sensing to affect host health. Future research will further investigate these interactions and their impacts on honey bee health.

Author Disclosure Block:

K.I. Miller: None. **I.L.G. Newton:** None.

Poster Board Number:

FRIDAY-609

Publishing Title:

Characterization of the *Trachymyrmex septentrionalis* Fungus Garden Microbial Community

Author Block:

K. M. Lee, J. L. Klassen; Univ. of Connecticut, Storrs, CT

Abstract Body:

The multi-partite fungus-growing ant symbiosis of *Trachymyrmex septentrionalis* is an established model for coevolution and secondary metabolite ecology. Like all fungus-growing ants, *Trachymyrmex* cultivate and maintain a "fungus garden" consisting primarily of a specific *Leucoagaricus sp.* (cultivar) fungus. The ants provide food for this fungus in the form of fresh leaf cuttings, oak seed pods (catkins), and frass (feces) from caterpillars. In return, the fungus produces specialized hyphal swellings that the ants consume as their primary food source. Such a rich nutrient source is not without predators, mainly other fungal species. To combat predation, the ants host a bacterial symbiont from the genus *Pseudonocardia* within specialized crypts that chemically defends the cultivar fungus. The cultivar fungus itself also hosts its own microbial community. What is less well understood is the composition and potential function of this microbial community. Using the *Trachymyrmex septentrionalis* fungus-growing ant system we are characterizing the composition, diversity, and potential interaction networks of the fungus garden microbial community. Because most of the food items incorporated into the fungus garden are either plant based (leaves and catkins), or derived from plant material (frass), the garden material contains many potential inhibitors of enzymes used in PCR. To overcome this difficulty, we have adapted previous methods to separate fungus garden bacteria from the fungus garden matrix to reduce and overcome PCR inhibition. Our community amplicon sequencing data generated from these techniques suggest that the microbial community associated with the fungus garden may be partitioned into two main fractions, one being highly transient and the other persistent. The sampled "microbiome" may therefore be a composite of"symbionts" that are important for fungus garden growth and immigrants that have neutral or detrimental effect on community function.

Author Disclosure Block:

K.M. Lee: None. **J.L. Klassen:** None.

Poster Board Number:

FRIDAY-610

Publishing Title:

Microbe Vs. Microbe: Bacterial and Fungal Cultivar Interactions in a Fungus-Farming Ant System

Author Block:

S. M. Kopac, J. Klassen; Univ. of Connecticut, Storrs, CT

Abstract Body:

Microbes live in close association with one another in the *Trachymyrmex septentrionalis* fungus-farming ant system, providing a framework for potentially undiscovered and understudied microbial interactions. Leafcutter ants, which belong to the same tribe as *Trachymyrmex*, are characterized by evolutionarily conserved interactions between pathogens and protective bacteria carried by the ants; similar dynamics might exist in the *T. septentrionalis* system. Interestingly, the role of bacteria living in the fungus garden (the cultivar) are not well characterized in either system. From a preliminary survey of 33 strains of fungus garden bacteria we identified over a dozen species, including Actinomycetales, Enterobacteriaceae, Lactobacillales and Bacillales. We found that many of these microbes have negative or positive effects on each other's growth. In the present study we focus on the interactions between these bacteria and with that of the cultivar.We characterized bacterial isolates to the species level and determined if they are persistent or transient members of the cultivar community by identifying conserved clades in a phylogeny of isolates from multiple fungus gardens. Conserved clades could be the result of bacterial-bacteria or cultivar-bacteria interactions on the assembly and maintenance of the fungus garden bacterial community or the result of abiotic conditions.Interactions between microbes from our system were assessed by growth assays; cross-streak growth assays for inhibition and spot plate co-cultures for beneficial and mutually beneficial interactions. The effect of bacteria on cultivar growth was tested using pour plate assays with an overlay. We tested for coevolution at the colony level by comparing the number of interactions between bacteria and bacteria/cultivar from the same colony vs. from different colonies. We also tested close relatives of bacterial isolates to determine if specialization to fungus gardens occurs at the clade level.In collaboration with JGI, the genomes of several isolates were sequenced using SMRT technology developed by PacBio. Genome content comparisons were made, with specific focus on genes associated with secondary metabolites and other forms of microbial interaction and communication. Secondary metabolites possess a variety of functions including antibacterial, antifungal, anti-malarial and anti-cancer properties.

Author Disclosure Block:

S.M. Kopac: None. **J. Klassen:** None.

Poster Board Number:

FRIDAY-611

Publishing Title:

Diversity, Ecology, and Evolution of *Trachymyrmex septentrionalis* Associated *Pseudonocardia*

Author Block:

S. L. Goldstein, J. L. Klassen; Univ. of Connecticut, Storrs, CT

Abstract Body:

The discovery of antibiotics was one of the chief scientific breakthroughs of the 20th century. However, the indiscriminate use of antibiotics has lead to antibiotic resistance becoming one of the greatest scientific crises of the 21st century. Although the natural diversity and function of these compounds remains largely unknown, an improved understanding of the natural functions of antibiotics will allow us to target discovery efforts and understand how antibiotic resistance is overcome in the natural environment. Here, we use the *Trachymyrmex septentrionalis* fungus-growing ant as a model system to study the diversity, ecology and evolution of antibiotics in their natural environment. *T. septentrionalis* participates in a multipartite mutualism consisting of the ants, a specific "cultivar" fungus used as the ants' main food source, and the antibiotic producing bacterium *Pseudonocardia*. *T. septentrionalis* nurtures its fungal cultivar in underground "fungus gardens. *Pseudonocardia* symbionts reside on the propleural plates of the ants and act as "crop sprayers" that use antibiotics to protect the fungus garden from pathogen infection. The antagonistic relationship that exists between *Pseudonocardia* and its target parasites that is mediated by secondary metabolites suggests that *Pseudonocardia* might be a valuable untapped source of natural products. We sampled ~100 *T. septentrionalis* colonies along a transect following the Atlantic coast of the USA. We isolated strains of *Pseudonocardia* from each of these colonies and identified these bacteria using 16S rRNA gene sequencing and phylogenetic analysis. These results indicate that all *T. septentrionalis*-associated *Pseduonocardia* fall within one clade, and were confirmed by genomic data having higher phylogenetic resolution. Together, these data suggest that *Pseudonocardia* symbionts are propagated between generations of their ant hosts with high fidelity. Our results also suggest that secondary metabolite biosynthetic genes differ between geographic locations. These differences in secondary metabolite biosynthetic gene clusters indicate a geographically localized response to pathogen pressure. Tapping into this evolutionary dynamic will grant a better understanding of how antibiotics evolve in the presence of their natural pathogens, and reveal novel chemical diversity that can be exploited for human use.

Author Disclosure Block:

S.L. Goldstein: None. **J.L. Klassen:** None.

Poster Board Number:

FRIDAY-612

Publishing Title:

Indications for Sulfur-Oxidizing Bacterial Ectosymbionts Inhabiting the Water Scorpion *Ranatra fusca*

Author Block:

K. Garcia[1], S. Kearney[2], S. Bhatnagar[3], S. Dawson[4], J. Leadbetter[5], D. Newman[6], E. Ruff[7]; [1]Howard Hughes Med. Inst., Chevy Chase, MD, [2]Dept. of Biological Engineering, Massachusetts Inst. of Technology, Cambridge, MA, [3]Microbiol. Graduate Group, Univ. of California Davis, Davis, CA, [4]Dept. of Microbiol., Univ. of California Davis, Davis, CA, [5]Linde Ctr. for Global Environmental Sci., California Inst. of Technology, Pasadena, CA, [6]Div.s of Biology and Biological Engineering, Geological, and Planetary Sci., California Inst. of Technology, Pasadena, CA, [7]Dept. of Molecular Ecology, Max Planck Inst. for Marine Microbiol., Bremen, Germany

Abstract Body:

Symbioses between bacteria and eukaryotic hosts are widespread in nature and are found in all realms from the deep-sea to the human gut. In most cases the bacterial partners have essential roles being involved in e.g nutrition, niche adaption, and detoxification. Yet, only few symbioses and the involved host-microbe interactions are well understood. Here we investigated a so far unknown association between bacteria and the water scorpion *Ranatra fusca*, an insect that is very common in freshwater and brackish ecosystems in North America. The R. fusca specimens originated from Trunk River, a brackish, sulfide and methane-rich pond close to Woods Hole, MA. We observed that the insects had a thick biofilm on their carapace, which is uncommon for this species, and hypothesized that it could be a layer of sulfur-oxidizing bacteria. To analyze bacteria and host in detail we used cultivation approaches, 16S rRNA gene libraries, fluorescence *in situ* hybridization as well as high-resolution fluorescence and scanning electron microscopy. Our results show that the biofilm indeed consists of aggregates of sulfur-oxidizing *Gammaproteobacteria*, most likely of the genus *Thiothrix*, that are embedded in a matrix of algae and other bacterial assemblages. Considering the very high sulfide concentrations in the ecosystem and the predatory life-style of the insect we propose the following mutually beneficial association: The bacteria are involved in sulfide detoxification for the host, while being attached to the insect enables them to hitch rides between oxic and sulfidic layers, having the ideal niche for sulfur oxidation. Although further investigation is needed our results indicate a novel association between bacteria and insects that may be widespread in brackish ecosystems along the American East Coast.

Author Disclosure Block:

K. Garcia: None. **S. Kearney:** None. **S. Bhatnagar:** None. **S. Dawson:** None. **J. Leadbetter:** None. **D. Newman:** None. **E. Ruff:** None.

Poster Board Number:

FRIDAY-613

Publishing Title:

Acetogenesis and Fermentation in the Gut of the Methane-Emitting Earthworm Eudrilus Eugeniae

Author Block:

S. Hunger[1], C. Bruss[1], R. Conrad[2], H. L. Drake[1]; [1]Univ. Bayreuth, Bayreuth, Germany, [2]MPI für terrestrische Mikrobiologie, Marburg, Germany

Abstract Body:

The earthworm gut represents a saccharide rich and anoxic habitat. The earthworm *Eudrilus eugeniae* emits H_2 and methane, indicating that anaerobic processes such as fermentation and methanogenesis are ongoing in the earthworm gut. Based on the occurrence of strictly anaerobic processes, we hypothesized that acetogenesis might also be an ongoing process in the gut of *E. eugeniae*. This hypothesis was addressed by (a) RNA-based stable isotope probing with [^{13}C]-glucose as a model saccharide in anoxic slurries with gut contents of *E. eugeniae*, (b) molecular analysis of bacterial 16S rRNA and *fhs* (encoding formyl-H_4F synthetase), and (c) cultivation-dependent methods. The degradation of [^{13}C]-glucose yielded various fermentation products that were enriched in ^{13}C (such as acetate, CO_2). Methane was produced in similar amounts in unsupplemented and [^{13}C]-glucose-supplemented slurries, and was enriched in ^{13}C during the degradation of [^{13}C]-glucose. H_2 and CO_2 that were added as co-substrates to [^{13}C]-glucose-supplemented slurries drove the production of acetate and decreased the enrichment of ^{13}C in acetate (in comparison to [^{13}C]-glucose-supplemented slurries without H_2), indicating that acetate was derived in part from H_2-dependent acetogenesis. An acetogenic enrichment that was obtained from gut contents converted H_2 to acetate in a stoichiometric ratio that was indicative for acetogenesis. Taxa most closely related to fermenters (*Robinsoniella peoriense*, *Citrobacter murliniae, Erwinia persicina*) were labeled by glucose-derived carbon, indicating that those taxa were involved in the anaerobic metabolism of glucose. *Fhs* sequences closely related to acetogens (*Clostridium glycolicum*, *Blautia producta*) were detected in [^{13}C]-glucose-supplemented slurries, suggesting that these taxa were participants in the methanogenic wood web in the gut of *E. eugeniae*.

Author Disclosure Block:

S. Hunger: None. **C. Bruss:** None. **R. Conrad:** None. **H.L. Drake:** None.

Poster Board Number:

FRIDAY-614

Publishing Title:

Inactivation of the Major Hemolysin Gene Influences Expression of Nonribosomal Peptide Synthetase *swra* in the Insect Pathogen *Serratia* sp. Strain Scbi

Author Block:

T. J. Koloski[1], L. M. Petersen[1], K. LaCourse[1], R. Audette[1], T. A. Schöner[2], H. Bode[2], L. S. Tisa[1]; [1]Univ. of New Hampshire, Durham, NH, [2]Goethe Univ. Frankfurt, Frankfurt, Germany

Abstract Body:

A novel *Serratia* sp. (termed SCBI) forms a mutualistic association with South African *Caenorhabditis briggsae* nematodes isolated through bait trapping within the greater wax moth, *Galleria mellonella.* This association represents a newly identified microbe-nematode relationship and shows potential as a nascent entomopathogenic system. Genetic and genomic approaches were used to understand the physiology of the insect pathogen *Serratia* sp. strain SCBI. Screening a strain SCBI fosmid library in *Escherichia coli* EPI300 cells revealed that several fosmids were virulent to our insect model system, *Manduca sexta*, the tobacco hookworm. One fosmid, A1-A8, was lethal to 73% of injected *M. sexta* and conferred hemolytic activity. Deletion of the major hemolysin gene in this fosmid removed the phenotype. A previously generated SCBI transposon library was screened and five hemolytic transposon mutants were identified with inserts within the major hemolysin gene. Inactivation of this hemolysin gene resulted in the loss of hemolysin activity, but did not attenuate insecticidal activity. Unexpectedly, these hemolysin mutants showed increased motility and antimicrobial activity. qPCR analysis of the hemolytic mutants showed an increased in mRNA levels of the nonribosomal peptide synthetase *swrA*, whose product produces serrawettin W2. Mutation of the *swrA* in strain SCBI resulted in highly variable antibiotic activity, motility, and hemolysis phenotypes dependent on the site of the disruption within the 17.75 Kb *swrA* gene. RT-PCR and qPCR analysis revealed differential expression patterns of the *swrA* gene for the three *swrA* transposon mutants. Two mutants with insertions in later portions of the gene showed expression of the gene up to the disruption site. However, one mutant with an insertion in the initial portion of the gene allowed expression of the gene downstream of insertion site, suggesting an internal regulatory element enhancing gene expression. While it is unclear how inactivation of the major hemolysin gene influences *swrA* expression, these results suggest that *swrA* plays an important role in motility and antimicrobial activity in *Serratia* sp. strain SCBI.

Author Disclosure Block:

T.J. Koloski: None. **L.M. Petersen:** None. **K. LaCourse:** None. **R. Audette:** None. **T.A. Schöner:** None. **H. Bode:** None. **L.S. Tisa:** None.

Poster Board Number:

FRIDAY-615

Publishing Title:

ahpc* Expression in *Vibrio fischeri

Author Block:

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Abstract Body:

Vibrio fischeri is a bioluminescent marine bacterium that forms a beneficial symbiosis with the Hawaiian bobtail squid, *Euprymna scolopes*. During the interaction, *V. fischeri* experiences oxidative stress when exposed to host derived reactive oxygen species (ROS). This bacterium contains a high abundance of Alkyl hydroperoxide reductase (AhpC), which is predicted to degrade peroxides, suggesting that it may be important during host interaction. In this study we investigated *ahpC* expression in different *V. fischeri* mutant backgrounds, different growth phases and oxidative stress conditions caused by methyl viologen. The mutant backgrounds used were *ahpC* mutant, catalase mutant and an *ahpC*/catalase double mutant. *ahpC* expression increased as the growth phases progressed, was significantly higher in the mutant backgrounds than in the wild-type parental strain, and was further induced when grown in the presence of methyl viologen. Among the mutant backgrounds, on average, *ahpC* expression was highest in the *ahpC* mutant strain. Together, these results suggest that the mutant strains are experiencing oxidative stress and that this stress induces the expression of *ahpC*. Future work will include expression studies of catalase gene.

Author Disclosure Block:

A. Sánchez Peña: None. **W.X. Torres Pérez:** None. **Z. Flores-Cruz:** None.

Poster Board Number:

FRIDAY-616

Publishing Title:

The Symbiotic Microbiome of Staghorn Coral *A. cervicornis* is Affected by Solar Radiation

Author Block:

A. Rivera[1], B. Cabera[1], F. Godoy-Vitorino[1], C. Toledo[2]; [1]Inter American Univ. of Puerto Rico, San Juan, PR, [2]Univ. of Puerto Rico, San Juan, PR

Abstract Body:

Background: Coral reefs are the most diverse ecosystems in the marine realm and among them, *A. cervicornis,* plays crucial services to an array of different species. In recent years however, this coral has suffered unprecedented mortality with no significant recovery across its entire range. The causes are variable and included diseases and climate change related stresses such as bleaching. Based on this, *A. cervicornis* is considered a threatened species under the US Endangered Species Act. In this project we aim to address the effect of depth-associated factors such as solar radiation, on the microbiome of *Acropora cervicornis* at two water depths. **Methods:** Six *A. cervicornis* fragments from different individuals at two depths (three at 1.5 and three at 11m) were collected from Palomino an island 3.2km off the northeast coast of Puerto Rico. Each fragment was used for genomic DNA extractions and the 16S rRNA gene region V4 was sequenced with the Illumina platform. Data analyses was done using the QIIME pipeline with the Greengenes database. **Results:** A total of 1,314,293 good-quality sequences were binned into 2,427 OTUs. Overall we found a total of 16 Phyla with a dominance of *Proteobacteria (Rhicketsiales)* (90%), followed by *Firmicutes* (4%), *Planctomycetes* (2.5%) among other taxa at lower abundance. Considering taxa with <10% relative abundance, we found that corals receiving at shallower depths had 11 phyla with a higher amount of Proteobacteria (Pseudomonas, Acinetobacter and Rhodobacteraceae)(~50%), Planctomycetes (Pirellulaceae) (19%) and Cyanobacteria (Oscillatoria and Rivularia)(~8%); while those at higher depths had 15 phyla with a dominance of Proteobacteria (*Psychrobacter, Acinetobacter* and unclassified Campylobacterales) (~43%), Firmicutes (*Streptococcus* and *Lactobacillus* mainly) (~33%), and Actinobacteria (*Corynebacterium* and *Micrococcus*) (10%). **Conclusion:** The microbiome of *A. cervicornis* is dominated by a Rickettsiales-like bacterium with changes in low dominance taxa according to water depth. Corals at shallow depths have a higher abundance of ammonia oxidizers and nitrogen-fixing taxa revealing bacterial-mediated nitrogen cycling in the coral-microbiome holobiont which may confer an advantage over the depth zone individuals in restoration procedures.

Author Disclosure Block:

A. Rivera: None. **B. Cabera:** None. **F. Godoy-Vitorino:** None. **C. Toledo:** None.

Poster Board Number:

FRIDAY-617

Publishing Title:

Genomic and Metabolite Divergence in Cyanobacterial Symbionts of Dysideidae Sponges

Author Block:

J. M. Blanton, S. Podell, V. Agarwal, B. S. Moore, E. E. Allen; Univ. of California San Diego, La Jolla, CA

Abstract Body:

Certain members of the tropical marine sponge family Dysideidae have co-evolved with strains of cyanobacteria identified as *Oscillatoria spongeliae* living within their tissues. These sponges are of interest as natural sources of potentially toxic polybrominated diphenyl ethers (PBDEs). Here we investigate the relationship between host genotype, symbiont genotype and PBDE biosynthesis using targeted gene sequencing and shotgun metagenomics. Samples from 17 sponges initially identified as *Dysidea granulosa* and *Lamellodysidea herbacea* were collected from Guam coastal reefs and evaluated for the presence and composition of PBDEs by LC-MS/MS. Ribosomal gene markers were amplified and sequenced to reconstruct host and symbiont phylogenies. As *Oscillatoria spongeliea* has yet to be cultivated, whole sponge samples were sequenced using shotgun metagenomic methods. Symbiont genomes and PBDE biosynthetic gene clusters were reconstructed through de novo metagenome assembly and phylogenetic binning analyses. Sponge-symbiont chemotypes, including the absence of PBDE chemistry, were identified and correlate strongly with both host and symbiont clades. Analysis of binned metagenomic assemblies indicate that the PBDE biosynthetic cluster is of cyanobacterial origin, not derived from the host sponge. Comparison of the symbiont genomic data analyzed to date shows varying degrees of divergence between strains (95-99% average nucleotide identity). The chemical variability of Dysideidae sponges is linked to diversification of the sponge-bacteria holobiont. While the *Oscillatoria* symbiont is responsible for production of polybrominated diphenyl ethers in sponges, host sponge phylogeny is a stronger predictor of the primary chemotype. This work demonstrates the utility of metagenomic methods in performing comparative genomic analysis on uncultured microbial symbionts of metazoan hosts.

Author Disclosure Block:

J.M. Blanton: None.

Poster Board Number:

FRIDAY-618

Publishing Title:

Unprecedented Diversity of Myxotroph Communities in Three Species of Crabs in Puerto Rican Red and Black Mangroves

Author Block:

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Abstract Body:

Background: The karst region of Puerto Rico contains mature forests of red (*Rhizophora mangle*) and black (*Avicenia germinans*) mangroves with a diversity of crabs that are distributed according to salinity and tree diversity. Red mangroves harbor *Goniopsis cruentata in their flooded roots,* and *Aratus pisonii* in the canopy, while black mangroves sustain *Uca rapax fiddler* crabs. Mangrove leaves and derived detritus are an important food source for these macroinvertebrates and their foraging and feeding activities directly influence the availability of organic carbon and nutrient cycling. The present study was carried out to understand the microbiome community composition and functional diversity among three different species of crabs in response to their feeding behaviors and trophic niches. **Materials:** A total of 10 samples were obtained from *Aratus pisonii*, *Goniopsis_cruentata* and *Uca_rapa*x, which underwent genomic DNA extractions and the 16S rRNA gene region V4 was sequenced with the Illumina platform. Data analyses was done using the QIIME pipeline with the Greengenes database. **Results:** A total of 3,953,258 good-quality sequences were binned into 411,484 OTUs. Overall we found a dominance of Proteobacteria and Tenericutes in *A. pisonii* and *G. cruentata with other 20 and 29 phyla respectively. U. rapax* crabs had a dominance of Tenericutes (~80%) (unclassified Mollicutes family), Gammaproteobacteria and Planctomycetes (Pyrellulaceae) with 58 other rarer phyla. A total of 3,800 archaeal OTUs were found with 90% belonging to *Uca* rapax with a dominance of *Nitrosopumilus* and *Cenarchaeum,* while *Aratus* and *Goniopsis* had abundant *Nitrosphaera* OTUs. **Conclusion:** Preliminary results indicate that the zonation recognized for the mangrove crabs is mimicked by the crab-associated microbiome that is species-specific, very diverse with dominant and novel Tenericute-associated taxa. Overall red mangrove crabs had dominant halotolerant bacteria with carbohydrate degrading taxa as well as ammonia oxidizers. Black mangrove associated *U. rapax* had a very high microbial diversity with a dominance of sulfate reducers, nitrate reducers and ammonia-oxidizers suggesting and adaptation of the crab microbiomes to high sulfate and low ammonia concentrations and a possible mixotrophic lifestyle.

Author Disclosure Block:

Y. Diaz: None. **G. Ortiz:** None. **M. Cafaro:** None. **F. Godoy-Vitorino:** None.

Poster Board Number:

FRIDAY-619

Publishing Title:

Multilocus Sequence Typing Analysis of the *Francisella*-like Endosymbiont of Ixodid Ticks

Author Block:

Z. C. Holmes, M. J. Pung, M. L. Zambrano, J. R. Hensley, G. A. Dasch; Rickettsial Zoonoses Branch, CDC, Atlanta, GA

Abstract Body:

The *Francisella*-like endosymbionts (FLE) of Ixodid ticks have been detected and distinguished from free-living *Francisella* (FLA), primarily by sequencing of the 16S rRNA gene. In some populations of ticks, a number of single nucleotide polymorphisms (SNP) have been detected; however, only 4 of 11 FLA multilocus sequencing typing assays (MLST) gave products with *Dermacentor variabilis* FLE, and their sequences differed greatly from FLA. We developed primers for nine FLE MLST targets and sequenced those amplicons in order to define the population diversity of FLE in *Amblyomma maculatum* from Georgia and Oklahoma, *D. occidentalis* from California, *D. andersoni* from Montana, and three populations of *D. variabilis* from Georgia, Ohio, and California. In order to select MLST sites, we performed a sliding window analysis of the nine MLST targets and determined the most informative 400 bp window from each gene. The sliding window analysis was performed with the R package "spider" on sequence alignments of FLA for which whole genome sequences were available and our FLE data. This approach informed us of the sites which exhibited the greatest rates of polymorphisms in FLA and therefore had a higher probability of exhibiting polymorphisms in the FLE. Analysis of 2048 bp of sequence from five of these MLST targets detected 59 SNPs, 13 of which occurred in more than one tick FLE sample and were therefore phylogenetically informative. Each species of surveyed tick harbors a specific dominant genotype of FLE, and the FLE from East Coast populations of *D. variabilis* (GA and OH) are distinct from FLE from West Coast populations (CA). A 408 bp region of the *fop*A1 outer membrane protein and a 257 bp region of the *atp*G ATP synthase gamma chain genes contained 14 and 7 SNPs, respectively. All sequenced FLE populations were distinguishable from the others by a minimum of two SNPs in both gene regions. Across all five gene regions, each FLE population is distinguishable from all others by a minimum of 7 SNPs and up to 41 SNPs. FLE in California *D. occidentalis* differed from those of *D. variabilis* collected from the same site. The inferred phylogeny of the FLE suggests that different species of ticks harbor distinct genotypes of FLE which are not shared, even when two species occur sympatrically, and that some ticks contain more than one genotype of FLE.

Author Disclosure Block:

Z.C. Holmes: None. **M.J. Pung:** None. **M.L. Zambrano:** None. **J.R. Hensley:** None. **G.A. Dasch:** None.

Poster Board Number:

FRIDAY-620

Publishing Title:

Assessing Exchange of *Rickettsia, Coxiella,* and *Francisella* Agents Between *Amblyomma Americanum* and *Amblyomma maculatum* Ticks in Georgia

Author Block:

J. R. Hensley, M. L. Zambrano, A. J. Williams-Newkirk, G. A. Dasch; CDC, Atlanta, GA

Abstract Body:

Two abundant species of aggressive ticks commonly feed on humans in Georgia: the Gulf Coast tick (*Amblyomma maculatum*) and the lone star tick (*A. americanum*). *A. maculatum* is the primary vector of *Rickettsia parkeri*, *Candidatus* Rickettsia andeanae, and a *Francisella*-like endosymbiont (FLE), whereas *A. americanum* is the primary vector for *R. amblyommii*, *Ehrlichia chaffeensis*, *E. ewingii*, and a *Coxiella*-like endosymbiont (AmCox). Horizontal transmission of *R. parkeri* from *A. maculatum* to *A. americanum* via co-feeding has been described, and *R. amblyommii* has been found infrequently in *A. maculatum* ticks. We assessed whether exchange of tick-associated bacteria is common between *A. maculatum* and *A. americanum* from the same site.Unengorged ticks were collected May-August 2014 in central Georgia from a 4.14 acre site by flagging and from humans and canines. Ticks were identified, washed, and their DNA extracted with the Wizard SV 96 Genomic DNA Purification System (Promega). All DNA samples were screened with qPCR assays for these bacteria found in both ticks, and the species of *Rickettsia* was identified by species-specific TaqMan assays or by sequencing of the *ompA* gene using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) on an ABI 3130xl Genetic Analyzer.Only *R. amblyommii* (14) and AamCox (39) were detected in 39 *A. americanum*, while the 74 *A. maculatum* only contained *R. parkeri* (30), *Candidatus* Rickettsia andeanae (3), and FLE (74). Neither tick species had either *Ehrlichia*.Consequently, we obtained no evidence for the frequent exchange of these tick-borne agents in a natural setting despite high levels of carriage of each agent and the common observance of infestation with both tick species of dogs and humans at this site. Based on these data, exchange of these *Rickettsia*, *Coxiella*, and *Francisella* agents between *A. maculatum* and *A. americanum* is not common, and if it occurs, it does so at low frequencies, undetectable except with much larger sample sizes.

Author Disclosure Block:

J.R. Hensley: None. **M.L. Zambrano:** None. **A.J. Williams-Newkirk:** None. **G.A. Dasch:** None.

Poster Board Number:

FRIDAY-621

Publishing Title:

***Aedes aegypti* Midgut Microbiota May Affect Mosquitoes' Dengue Virus Refractoriness**

Author Block:

E. Molina Henao, Y. Graffe, E. De La Cadena, I. Serrato, A. Correa, C. Ocampo; CIDEIM, Cali, Colombia

Abstract Body:

Dengue is a tropical disease with major distribution in the world, principally transmitted for the mosquito *Aedes aegypti*. We do not have a vaccine for dengue yet, so efforts for decreasing its incidence have been focused in mosquito populations' control. A new promising field has begun to explore vector competence manipulation for endosymbiont microorganisms, which influences mosquito's biology, immune response and the capacity to infect with the virus. So, we looked for identifying culturable A. aegypti midgut microorganisms in three selected strains (susceptible to dengue virus, Cali-S; refractory, Cali-BIM; and wild, Paso del Comercio). We evaluated whether there are differences between their microbial communities, and whether those differences affect mosquitoes' vector competence. In order to do that, we dissected adult mosquitoes midgut from the three strains, plated pools of 5 guts into LB broth and, after 72 hours, isolated bacterial colonies in blood, chocolate and McConkey agars. Bacteria identification was carried out using MALDI-TOF, Vitek, BD Phoenix and 16S rRNA gene sequences. Also, we fed 30 individuals from each strain with a mix of water, sugar and antibiotics and other 30 individuals without antibiotics during 10 days, and then we fed them with blood and dengue virus, and finally we tested vector competence by indirect immunofluorescence in the midgut and salivary glands. The highest bacterial diversity was presented in CALI-BIM with the genera *Pseudomonas*, *Serratia*, *Stenotrophomonas* and *Escherichia*. Meanwhile, Cali-S presented only *Bacillus* and a predominance of *Candida* yeasts. Paso del Comercio strain had also poor diversity and presented only *Bacillus megaterium*. Additionally, the refractory strain (Cali-BIM) was the only one that presented significant differences between the control and antibiotics treated population regarding dengue susceptibility. There were also differences between microbial communities in Cali-S and Cali-BIM, possibly due to a higher antimicrobial immune response in Cali-S; then, elimination of the midgut microbiota probably increases dengue susceptibility in CALI-BIM. Thus, this work suggests that the presence of bacteria in the midgut is influencing the vector competence of *A. aegypti*.

Author Disclosure Block:

E. Molina Henao: None. **Y. Graffe:** None. **E. De La Cadena:** None. **I. Serrato:** None. **A. Correa:** None. **C. Ocampo:** None.

Poster Board Number:

FRIDAY-622

Publishing Title:

Contribution of SopB, SptP and PphB in the Intracellular Survival of *Salmonella* Typhimurium in *Dictyostelium discoideum* and RAW264.7 Macrophages

Author Block:

I. M. Urrutia, C. A. Santiviago; Univ. de Chile, Santiago, Chile

Abstract Body:

Recently, we demonstrated that *Salmonella* Typhimurium is able to survive in the amoeba *Dictyostelium discoideum*. However, the molecular mechanisms involved in this process have not been characterized. In this work, we evaluated the contribution of SopB, SptP and PphB in the intracellular survival of *S.* Typhimurium in *D. discoideum* and RAW264.7 macrophages. SopB and SptP are well known *Salmonella* virulence factors. SopB is a phosphatidyl phosphatase required for invasion of epithelial cells. SptP is a bifunctional protein harboring a GAP domain involved in Rho GTPases inactivation, and a tyrosine phosphatase domain that contributes to intracellular replication of *Salmonella*. On the other hand, PphB is a putative serine-threonine phosphatase not characterized in pathogenicity. We constructed Δ*sopB*, Δ*sptP* and Δ*pphB* derivatives of the *S.* Typhimurium virulent strain 14028s using the Red Swap method. Then, we evaluated the intracellular survival of the wild-type and mutant strains by means of an infection assay *in vitro* using *D. discoideum* AX4 and RAW264.7 macrophages. The infections were carried out for 1 h at 22°C (*D. discoideum*) or 37°C (RAW264.7) using a MOI of 100 bacteria/cell. Infected cells were lysed with 0,2% Triton X-100 and the number of intracellular bacterial at 0, 1, 3 and 5 hours post-infection was determined by serial dilution and plating on LB agar. Our results showed that the three mutants are defective for intracellular survival in *D. discoideum* at 1, 3 and 5 h post-infection, as compared to the wild-type strain. The phenotype of the mutants was complemented *in trans*. On the other hand, we observed that the mutant strains present wild-type levels of intracellular survival in RAW264.7 macrophages. Thus, virulence factors SopB, SptP and PphB contribute to intracellular survival of *S.* Typhimurium in *D. discoideum*, but not in RAW264.7 macrophages.

Author Disclosure Block:

I.M. Urrutia: None. **C.A. Santiviago:** None.

Poster Board Number:

FRIDAY-623

Publishing Title:

Salmonella* Typhimurium Δ*sopB* and Δ*sifA* Mutants are Impaired in Intracellular Survival in the Amoeba *Dictyostelium discoideum

Author Block:

C. Valenzuela, A. Sabag, S. Riquelme, C. A. Santiviago; Univ. de Chile, Santiago, Chile

Abstract Body:

The ability of *Salmonella* to survive intracellularly in eukaryotic cells is explained, in part, by the modification of the intracellular niche within the cell in a compartment called the *Salmonella* containing vacuole (SCV). For this purpose, *Salmonella* employs a number of effector proteins secreted by the type-three secretion systems encoded in *Salmonella* pathogenicity island 1 (T3SS $_{SPI-1}$) and 2 (T3SS $_{SPI-2}$). Previously, we reported a role for these T3SS in the survival of *Salmonella* Typhimurium in the social amoeba *Dictyostelium discoideum*. In this work, we evaluated the role played by two effector proteins in this process. SopB (secreted by T3SS $_{SPI-1}$) is a phosphoinositide phosphatase that in other models acts increasing the PI(3)P species in newly formed SCV. SifA (secreted by the T3SS $_{SPI-2}$) is involved in the formation of *Salmonella*-induced filaments (Sifs), a complex network of membrane filaments that extends from the SCV. We performed phagocytosis assays using axenic cultures of *D. discoideum* strain AX2 co-cultured with *S.* Typhimurium wild-type strain 14028s or derived mutants Δ*sopB* and Δ*sifA*. Viable bacterial cells were recovered from *D. discoideum* at 0, 3 and 6 hours post-infection and titrated by serial dilution and plating in selective media. In parallel, viable cell counts of infected *D. discoideum* were obtained by Trypan blue exclusion. Our results show that Δ*sopB* and Δ*sifA* mutants are recovered ~10-fold less than the wild-type strain at 6 hours post-infection, indicating that these mutants are defective in intracellular survival in the amoeba. These results are in accordance with their reduced intracellular survival in murine macrophages *in vitro*. Altogether, our results suggest that the modification of a SCV-like compartment by SopB and SifA is required for *S.* Typhimurium to survive within *D. discoideum.*

Author Disclosure Block:

C. Valenzuela: None. **A. Sabag:** None. **S. Riquelme:** None. **C.A. Santiviago:** None.

Poster Board Number:

FRIDAY-624

Publishing Title:

Identification of Regulatory Mechanisms That Contribute to Novel Cell Cycle Outcomes During Host-Microbe Symbiosis

Author Block:

C. Puerner[1], C. Sadowski[2], K. Schallies[1], K. Gibson[1]; [1]Univ. of Massachusetts Boston, Boston, MA, [2]Symbiota Inc., Cambridge, MA

Abstract Body:

Sinorhizobium meliloti has emerged as a key model organism for understanding the link between cell cycle regulation and chronic host colonization because it undertakes novel cell cycle outcomes during symbiosis within the root nodules of legumes. The DivJ-like histidine kinase CbrA is a necessary regulator for both symbiosis and cell cycle progression. CbrA indirectly regulates the activity and degradation of CtrA, a master cell cycle transcription factor. We isolated a spontaneous Δ*cbrA* symbiosis suppressor strain from a rare phenotypically wild type root nodule, which contains a mutation that suppresses both symbiotic and free-living cell cycle defects of the Δ*cbrA* mutant. Whole genome sequencing identified the mutation responsible for Δ*cbrA* suppression to be located within a PAS domain of *divL* (*divL*^{Q362P}), which encodes a modified kinase that likely mediates CbrA regulation of CtrA by serving a bridge between the CbrA cognate response regulator DivK and the CckA/ChpT phosphorelay. The *divL*^{Q362P} allele shows temperature-sensitivity, a slow-growth defect, and importantly has a drastic reduction of endogenously expressed CtrA in an otherwise wild-type background. Genetic experiments are underway to further characterize the nature of *divL*^{Q362P} function. Recently, ten additional Δ*cbrA* symbiosis suppressor strains were isolated. These ten strains fall into three linkage groups and have been characterized further. Preliminary observations suggest that one group also suppresses Δ*cbrA* cell cycle defects, while the other two do not and appear to be symbiosis-specific. Specifically, Western blot analysis has revealed these symbiosis-specific suppressors do not restore CtrA levels to that of wild type and retain cell morphology defects. We plan to use whole-genome sequencing of these strains in order to identify the genes responsible for suppression. Through this study, I aim to better characterize the role DivL plays in symbiosis and regulation of the cell cycle and identify additional symbiosis-specific mechanisms of cell cycle regulation in *S. meliloti*.

Author Disclosure Block:

C. Puerner: None. **C. Sadowski:** None. **K. Schallies:** None. **K. Gibson:** None.

Poster Board Number:

FRIDAY-625

Publishing Title:

Packaging of *Campylobacter jejuni* into Multilamellar Bodies by the Ciliate *Tetrahymena pyriformis*

Author Block:

H. Trigui[1], V. E. Paquet[2], S. J. Charrette[2], S. P. Faucher[1]; [1]McGill Univ., Montréal, QC, Canada, [2]Laval Univ., Quebec, QC, Canada

Abstract Body:

Background: *Campylobacter jejuni* is a foodborne pathogen recognized as the major cause of human bacterial enteritis. The survival and persistence *of C. jejuni* in food processing environments are poorly understood. Some amoebae and ciliates ingest pathogenic bacteria, package them into multilamellar bodies (MLBs) and release them in the milieu. Packaged bacteria are protected from various stresses and are more able to survive than free bacteria. **Hypothesis:** We propose that *C. jejuni* could be packaged after internalization and then expelled into MLBs by protozoa known to be able to package other pathogens. **Results:** Co-cultures of *C. jejuni* strain 81116 with *Acanthamoeba castellanii* or the ciliate *Tetrahymena pyriformis* were processed for epifluoresence and transmission electron microsopy. *A. castellanii* did not expel packaged bacteria even when we tested multiplicity of infections higher than those reported in previous studies. In contrast, *T. pyriformis* is able to package *C. jejuni* into expelled spherical vesicles in less than one hour after internalization. According to their size and structures these MLBs were reminiscent to those excreted by ciliates as already described for *Salmonella enterica*, *E. coli* and *L. pneumophila*. Live/dead staining and CFU counts indicate that *C. jejuni* were present as viable cells in MLBs for up to 60 hours, and that this packaging increased the survival of *C. jejuni*. **Conclusions:** Our study suggests that *T. pyriformis* may increase the risk of persistence of *C. jejuni* in the environment and that the packaging could be involved in the transmission from farm water distribution systems or slaughterhouse chillers to the final product.

Author Disclosure Block:

H. Trigui: None. **V.E. Paquet:** None. **S.J. Charrette:** None. **S.P. Faucher:** None.

Poster Board Number:

FRIDAY-626

Publishing Title:

***biomphalaria Havanensis* (Syn. *obstructa*) Identified As New Snail Host For *drepanocephalus Auritus* (Digenea: Echinostomatidae) In Catfish Aquaculture**

Author Block:

N. R. Alberson[1], T. G. Rosser[1], L. H. Khoo[2], S. K. Buddenborg[3], E. S. Loker[3], T. D. Richardson[4], E. T. Woodyard[1], L. M. Pote[1], M. J. Griffin[5]; [1]Mississippi State Univ. Coll. of Vet. Med., Starkville, MS, [2]Thad Cochran Natl. Warmwater Aquaculture Ctr., Mississippi State Univ., Stoneville, MS, [3]Univ. of New Mexico, Albuquerque, NM, [4]Univ. of North Alabama, Florence, AL, [5]Thad Cochran Natl. Warmwater Aquaculture Ctr., Mississippi State Univ., Starkville, MS

Abstract Body:

Digenetic Trematodes are considered a major impediment to catfish aquaculture in the southeastern United States. Catfish production ponds make an ideal feeding ground for piscivorous birds due to the constant availability of a confined food source and are the perfect habitat for a variety of aquatic snails. These characteristics provide an optimal environment for the propagation of digenetic life cycles. In July 2014, two endemic snail species (*Planorbella trivolvis* and *Biomphalaria* sp.) were collected from two separate catfish production ponds in East Mississippi. Both snail species were observed shedding cercariae morphologically consistent with *Drepanocephalus auritus*, a parasite of the double crested cormorant, *Phalacrocorax auritus,* and channel catfish *Ictalurus punctatus*. Sequencing of five different gene targets (SSU, ITS, LSU, ND1, and CO1) confirmed these cercariae to be *D. auritus*. Sequencing of the mitochondrial cytochrome c oxidase subunit 1 (CO1) and nicotinamide adenine dinucleotide dehydrogenase subunit 1 (ND1) genes showed the snails in this study released cercariae belonging to both North American and South American genotypes. Juvenile channel catfish were challenged with ~275 *D. auritus* cercariae shed from *Biomphalaria havanensis* (syn. *obstructa*). Few mortalities (2 out of 10 fish) were observed, and surviving fish were euthanized 7 days post-challenge. Histology confirmed the presence of metacercariae, and pathology was consistent with previous studies. This work identifies a second snail species endemic to catfish aquaculture ponds that release cercariae pathogenic to juvenile channel catfish and is the first report of a *Biomphalaria* sp. being an intermediate host for *Drepanocephalus* sp. in North America.

Author Disclosure Block:

N.R. Alberson: None. **T.G. Rosser:** None. **L.H. Khoo:** None. **S.K. Buddenborg:** None. **E.S. Loker:** None. **T.D. Richardson:** None. **E.T. Woodyard:** None. **L.M. Pote:** None. **M.J. Griffin:** None.

Poster Board Number:

FRIDAY-627

Publishing Title:

Symbiont Communities in Submerged Cave Ecosystems: Molecular and Microscopical Investigations of Shrimp-Associated Bacteria

Author Block:

J. P. Nelson[1], L. M. Ortiz[2], G. Rouse[3], C. C. Cavanaugh[1]; [1]Harvard Univ., Cambridge, MA, [2]Univ. of Quintana Roo, Cozumel, Cozumel, Mexico, [3]Scripps Inst. of Oceanography, UCSD, La Jolla, CA

Abstract Body:

Dark, submerged caves contain complex habitats and endemic fauna. Several studies have suggested that in anchialine caves, in which landlocked marine water is often overlain by groundwater, chemosynthetic microbial communities support invertebrate dominated food webs. Yet, while chemosynthetic symbiosis in an anchialine cave shrimp has been reported based on electron microscopy and stable isotope enrichment experiments, symbiont identity and diversity have not yet been investigated. This study aims to identify the bacteria associated with members of the blind cave shrimp genus *Typhlatya* from 12 caves in the Yucatan Peninsula, Mexico. Sequencing of bacterial 16S rRNA genes and fluorescence in situ hybridization were employed to determine symbiont phylotypes and the diversity of the microbial community in the gills of several host species (N=5). These data were compared with geochemical analyses (salinity, dissolved oxygen, temperature, pH) of sampled shrimp habitats in order to assess whether environmental factors might correlate with symbiont association. Analyses of 16S rRNA sequences revealed that the dominant phylotypes were *alphaproteobacteria*, gammaproteobacteria, or spiroplasma and varied between host species. Proportions of proteobacterial clades and firmicutes that were associated with shrimp differed by cave site based on Illumina Miseq data. These findings give us insight into how host-associated microbes vary within and between anchialine shrimp species. The integration of geochemistry, microbial community structure, and symbiont studies in these shrimp will help to elucidate the roles of environment vs. host phylotype in determining symbiont composition. In particular, these investigations will add to recent reports of microbial niche partitioning in a variety of chemosynthetic symbioses.

Author Disclosure Block:

J.P. Nelson: None. **L.M. Ortiz:** None. **G. Rouse:** None. **C.C. Cavanaugh:** None.

Poster Board Number:

FRIDAY-628

Publishing Title:

Presence of Virulence Factor-Associated Genes in *Aeromonas* Pathovars, Cell Cytotoxicity and Nematode Lethality

Author Block:

W. Almonte, P. Kim, D. J. McGarey; Kennesaw State Univ., Kennesaw, GA

Abstract Body:

Background: Aeromonads are Gram-negative, chemoheterotrophic bacteria globally distributed in aquatic environments with some species, particularly *Aeromonas hydrophila*, able to cause various diseases including gastroenteritis, septicemia, soft tissue infections and acute necrotizing fasciitis. The severity of disease may be dependent on expression of specific virulence factors, which vary among species and strains within species (pathovars).**Methods:** Polymerase chain reaction was used to detect the presence of 16 genes encoding specific enzymes, toxins, surface components and secretion system components reported to contribute to virulence in clinical and environmental isolates of *A. hydrophila* (n = 22), *A. bestiarum* (n = 4), and *A. salmonicida* subsp *salmonicida* (n = 3). Based on gene amplification profiles the isolates were assigned into pathovar groups with representative members tested for cell cytotoxicity (rainbow trout gonad cells; RTG-2) and lethality using *C. elegans* challenge.**Results:** Eighty-three percent of strains inducing significant ($p < .05$) cell cytotoxicity were positive for genes encoding major surface adhesion (*aha1*), type VI secretion system (T6SS) effectors valine-glycine repeat G protein (*vgrG1*) and hemolysin co-regulated protein (*hcp*), and repeat in toxin exotoxin (*rtxA*). Genes for Type III secretion system (T3SS) toxins AexT and AexU (*aexT*, *aexU*) were present in 40% of highly cytotoxic strains, and 50% of strains with no significant cytotoxicity. Nematode lethality was greatest (43-53% lethal) in strains positive for *ashA* (S-layer) and toxin genes *vgrG1*, *hcp* and/or *rtxA*, and moderate (25-39%) when the same toxin genes were present without the *ashA*. It is likely the S-layer (gene expression confirmed) provided protection against clearance and lysis by the host's innate immune factors. Virulence factor genes common to all strains tested included elastase (*ahyB*), serine protease (*ser*), lipase (*lip*), and hemolysin (*ahh1*). Although these factors may contribute to disease, they did not appear to be correlated to cytotoxicity or nematode lethality.**Conclusions:** Based on these results, a multiplex PCR was developed for the simultaneous amplification of genes encoding toxins *rtxA*, *hcp*, *vgrG1*, *aexT*, and *aexU* and surface components *aha1* and *ashA* to differentiate potentially high virulence pathovars from those less virulent.

Author Disclosure Block:

W. Almonte: None. **P. Kim:** None. **D.J. McGarey:** None.

Poster Board Number:

FRIDAY-629

Publishing Title:

Recovery of a Diverse Gut Microbiota Occurs Gradually Following Fecal Microbiota Transplantation

Author Block:

J. J. Farrell[1], D. K. Martin[2], S. V. Thompson[3], A. Bogner[2], J. Bonello[2], H. Holscher[3]; [1]Univ. of Illinois Coll. of Med., Peoria, IL, [2]OSF Med. Ctr., Peoria, IL, [3]Univ. of Illinois, Urbana, IL

Abstract Body:

Background: Fecal microbiota transplantation (FMT) for treatment of *Clostridium difficile* infection (CDI) is associated with success rates >90%. The presumed mechanism of action is restoration of intestinal microbial diversity, but the time course and process of evolution to restoration of gut microbiota following FMT is not well understood. **Methods:** IRB approved FMT for patients with CDI who failed conventional treatment. FMT began November 2013, and in October 2014, patients submitted a stool sample or rectal swab prior to FMT. Designated donors (DD) also provided stool samples. Fecal DNA was extracted followed by generation of archaeal, bacterial, and fungal amplicon pools using a Fluidigm Access Array, sequencing on an Illumina MiSeq, and QIIME 1.8 analysis. Successful treatment was defined as resolution of symptoms of CDI following FMT. Failure was defined as diarrhea with > 3 stools/day or retreatment (either oral vancomycin or a 2nd FMT) within 30 days.**Results:** Between November 2013 and May 2015 48 FMT patients were enrolled: 38 women, 10 men; mean age=68 (median=69). CDI resolved for 44/48 (91.7%) patients following FMT (2 patients required a second FMT to achieve resolution of symptoms). There was no significant change in patient weight following FMT. Microbiome analysis was performed on stool from both of the DD, as well as 8 patients at day 0, 4/8 patients at day 30, and the same 4/8 patients at day 90 (only 2/4 patients that failed FMT provided follow-up stool specimens). Fecal bacterial operational taxonomic units (OTUs) and phylogenetic diversity (PD) were greater in DD than patients at day 0 ($p<0.05$). Patient OTUs and PD increased from day 0 to day 90 ($p<0.05$). OTUs and PD were not different in DD vs. patients at day 90 ($p>0.05$). Among the 4 patients that submitted stool at baseline, 30, and 90 days, PD was significantly greater at 90 days compared to baseline (27 vs. 13; $p=0.006$). Principal coordinates analysis (PCoA) of UniFrac distances between samples based on their 97% OTU composition and abundances indicated the enteric bacterial communities changed over time ($p=0.001$).**Conclusions:** Gut microbiota recovery following FMT was not instantaneous, but appeared to be a process of evolution following FMT that occurs over ≥ 3 months.

Author Disclosure Block:

J.J. Farrell: None. **D.K. Martin:** None. **S.V. Thompson:** None. **A. Bogner:** None. **J. Bonello:** None. **H. Holscher:** None.

Poster Board Number:

FRIDAY-631

Publishing Title:

The Gut Microbiome Drives Individual Differences in Metabolism of Anti-Cancer Drugs

Author Block:

L. Guthrie, S. Gupta, J. Daily, L. Kelly; Albert Einstein Coll. of Med., Bronx, NY

Abstract Body:

The ways in which the human gut microbiome shapes drug metabolism, efficacy and adverse drug responses (ADRs) is understudied and merits urgent investigation. As an example, severe diarrhea is a dose-limiting side effect of the colorectal cancer (CRC) chemotherapeutic and prodrug Irinotecan (CPT-11). Damage to intestinal epithelial cells and diarrhea can occur when a dominant, inactive metabolite of the drug, SN-38G, is converted to the active form by microbial β-glucuronidases (BGs) in the gut. ADRs to CPT-11 are variable in patient populations, potentially reflecting inter-individual variation in gut microbiota, which contribute to metabolism of the excreted drug. We find that diverse and mobile BGs populate the healthy human gut. We hypothesize that patterns of BG gene abundance, expression, and variability in the gut microbiome are linked to distinct metabolic phenotypes and may therefore be used to predict patient responses to drugs that are glucuronidated as a part of their metabolism. To assess the variation in functional activity of BGs among healthy individuals, we used LC-MS/MS to quantify microbiota-generated metabolites during ex-vivo drug administration of SN-38G to fecal samples. We found that microbial metabolism drives variable turnover phenotypes. We are further addressing the variation in functional activity of BGs between individuals using metatranscriptomic analysis to determine 1) whether BGs are expressed in response to drug administration and 2) to identify novel genes involved in drug metabolism. Integrating metabolomic, biochemical, evolutionary, and meta'omic data may identify new links between microbial metabolism and variable drug response. Finally, we compared the phylogenetic diversity and abundance of BGs in individuals with CRC and healthy controls and found distinct patterns of BG abundance and phylogeny in CRC patients in comparison to healthy individuals. BGs, and potentially other carbohydrate active enzymes may therefore be indicative of disease states. An overlapping set of microbial carbohydrate active enzymes mediate the metabolism of dietary substrates and xenobiotics, and, as a secondary consequence of metabolism, exert both protective and detrimental effects on carcinogenesis. Metagenomic assessment of CAZymes represents a novel and non-invasive approach to developing biomarkers for CRC diagnosis and progression.

Author Disclosure Block:

L. Guthrie: None. **S. Gupta:** None. **J. Daily:** None. **L. Kelly:** None.

Poster Board Number:

FRIDAY-632

Publishing Title:

Drug Metabolism Potential of the Human Microbiome Varies by Age

Author Block:

T. D. D. LeBlanc, M. G. I. Langille; Dalhousie Univ., Halifax, NS, Canada

Abstract Body:

Background: The human microbiome is the collection of bacteria living in and on the human body. Several studies have demonstrated that the gut microbiome can alter the metabolism of commonly used medications. However, there has yet to be a broad-scope analysis to determine the presence and variance of microbial genes with drug metabolizing potential. The aim of this study is to survey the drug metabolising potential of the human microbiome. We examine what drug metabolising genes are present in these microbes, and how the abundance of these genes changes based on body site, gender, and age. **Methods:** A subset of 50 microbial genes with the potential to metabolize drugs was manually curated from the Kyoto Encyclopedia of Genes and Genomes. These genes were annotated for two publicly available metagenomic data sets, including the Human Microbiome Project dataset with 118 gut microbiome samples[1], and a study examining type 2 diabetes and age in Chinese individuals with 370 gut microbiome samples[2] . Relative gene abundance were compared across body site, gender, and age of the individuals with ANOVA using Benjamini-Hochberg FDR multiple test correction.**Results:** All 50 genes were found to be present in the human microbiome, and 46 were different in proportion within the gut compared to other body sites. Although there were no significant differences based on gender, we identified 22 genes that were significantly different based on the individual's age, including alcohol dehydrogenase, azoreductase, and several methyltransferase genes. Varying levels of these genes in the microbiome could have clinical implications for a wide array of therapeutic treatments.**Conclusions:** The ability of the human microbiome to alter drug metabolism is diverse and variable across the human population and changes with age. Future studies will test other factors that could alter the drug metabolising potential of the gut microbiome, and this knowledge will be incorporated into personalized medicine applications.

Author Disclosure Block:

T.D.D. LeBlanc: None. **M.G.I. Langille:** None.

Poster Board Number:

FRIDAY-633

Publishing Title:

Metatranscriptome Analysis to Study the Response of the Human Gut Microbiota to Live Oral-Attenuated Vaccines and Subsequent Challenge with Wild-Type *Salmonella typhi*

Author Block:

Y. Zhang[1], A. Brady[1], C. Jones[1], Y. Song[1], A. J. Pollard[2], M. B. Sztein[1], C. M. Fraser[1]; [1]Univ. of Maryland Sch. of Med., Baltimore, MD, [2]Univ. of Oxford, Oxford, United Kingdom

Abstract Body:

Successful development of oral attenuated vaccines against *Salmonella* Typhi (*S.* Typhi) requires a better understanding of host immunity and its interaction with the resident microbiota in the human gastrointestinal tract; the combined responses of the microbiota and host immunity are likely to determine host susceptibility to or protection from typhoid disease (TD). This study was directed to characterize the structure and function of the gut microbiota in a human challenge model of TD in order to identify associations between the gut microbiota and host immune responses.Healthy adult volunteers received orally Ty21a (a licensed live attenuated typhoid vaccine, strain Ty21a), M01ZH09 (an attenuated live typhoid vaccine candidate strain) or placebo, followed by a subsequent challenge with wild-type (wt) *S.* Typhi and monitoring of clinical symptoms and immune responses. A total of 331 longitudinal stool specimens from 30 volunteers were collected. Messenger RNA were extracted from stool and sequenced on the Illumina HiSeq 2000 platform. Analysis of taxa abundances revealed that the gut microbiota was clustered into three enterocommunities dominated by *Ruminococcus*, *Methanobrevibacter* or *Firmicutes*. Enterocommunities were stable within an individual over the collection period, regardless of treatments or clinical outcomes. In contrast, differential gene expression analysis revealed that flagella genes, mainly from *Eubacterium* and *Roseburia*, were up-regulated in the volunteers who developed TD after 24 hr and 6 days following challenge with wt *S.* Typhi. In volunteers who did not develop TD, genes from COG group O (post-translational modification, protein turnover, chaperones) were up-regulated 24 hr after challenge with wt *S.* Typhi, consistent with activation of general defense mechanisms. This is the first study that investigates the functional responses of the human gut microbiota to challenge with wt *S.* Typhi. Future work will correlate changes in the gene expression profile of the gut microbiota with immune responses measured in the same volunteers over time.

Author Disclosure Block:

Y. Zhang: None. **A. Brady:** None. **C. Jones:** None. **Y. Song:** None. **A.J. Pollard:** None. **M.B. Sztein:** None. **C.M. Fraser:** None.

Poster Board Number:

FRIDAY-634

Publishing Title:

Effects of Two Polyphenols on the Gut Microbiome and Associated Weight Gain in Mice

Author Block:

J. E. Wilkinson[1], J. D. Hanson[2], C. D. Phillips[1], E. J. Rees[2], G. D. Mayer[1]; [1]Texas Tech Univ., Lubbock, TX, [2]RTL Genomics, Lubbock, TX

Abstract Body:

Recently, changes in gut microbial assemblages are being investigated as underlying causes of many health issues. Even minuscule changes in gut bacterial population structures have been correlated to conditions such as glucose intolerance, diabetes, and autism. We studied two polyphenols that are regularly consumed by humans and examined their effects on the gut microbiome and weight gain, using mice as a model. Natural sweeteners (Stevioside) are derived from plants (*Stevia rebaudiana*), and have sweetening effects ~300-fold greater than sucrose. Sugar substitutes are known to play a pivotal role in both obesity and diabetes and are consumed regularly as an alternative to sugar. Epigallocatechin-3-gallate (EGCG) is a polyphenol found in green tea that has been reported to impact health, including involvement in gut microbial metabolism and weight loss. However, no DNA sequencing studies to date have documented the gut microbial composition after the regular consumption of Stevioside or EGCG. C57BL/6J mice were divided into 5 groups: control, EGCG treatment (12.5 mg/kg bw/day, and 3 Stevioside treatments (10, 25, and 50 mg/kg bw/day). Mice were weighed and feces was collected daily. Four mice were sacrificed upon arrival to the lab and 6 mice at the start of the study (after a 3-day acclimation). All groups were divided 50/50 male and female. 16S amplicon sequencing was performed on gDNA of collected fecal and colon samples. We observed shifts in Firmicutes and Bacteroidetes ratios. Differences were noted between fecal and colon samples, which is to be expected. Trends in weight gain were present between groups, and between sexes within groups. We also observed a shift in OTU numbers from arrival until day 30, even in controls. Our results indicate that both Stevioside and EGCG affect the gut microbiome and weight gain. This was dose dependent for Stevioside. Notably, change of habitat from arrival until the start of the study (no treatment) altered the gut bacterial composition dramatically, which should be noted for other studies that only report changes before and after treatment without pre- and/or post-acclimation sampling. The findings of this study will inform people of potential health effects of these two polyphenols, and provide researchers knowledge of how acclimation and change in habitat affect gut microbial communities of mice.

Author Disclosure Block:

J.E. Wilkinson: None. **J.D. Hanson:** None. **C.D. Phillips:** None. **E.J. Rees:** None. **G.D. Mayer:** None.

Poster Board Number:

FRIDAY-635

Publishing Title:

Antibiotics Indirectly Disrupt Gut Microbiota Through Environmental Shifts

Author Block:

A. T. Reese[1], H. Durand[1], E. H. Cho[1], M. M. Villa[1], S. Nimmagadda[1], M. A. Deshusses[1], S. P. Nichols[2], N. A. Wisniewski[2], B. Klitzman[1], L. A. David[1]; [1]Duke Univ., Durham, NC, [2]Profusa, South San Francisco, CA

Abstract Body:

Antibiotic use can lead to major shifts in composition of the gut microbiota that persist after treatment has ceased. It is unknown, however, whether abiotic conditions also change, and, if so, whether such changes contribute to community dynamics. The microbiota are known to alter the environment of the gut, including maintaining the anoxic state of the distal lumen, so feedbacks between the community and environment after disturbance are predicted. Specifically, we hypothesized that changes in composition due to differential mortality would result in abiotic changes that induce further changes in composition. We sought to test this hypothesis with mouse and *in vitro* models. **Methods:** We measured colonic oxygen tension *in vivo* and redox potential in fecal pellets daily during treatment of C57BL-6 mice treated with a broad-spectrum antibiotic cocktail for five days. Oxygen was measured with a novel fluorescent biosensor inserted rectally. Redox potential was measured using a microelectrode. An *in vitro* bioreactor model of the human colon was subjected to increased oxygen and/or antibiotic treatment. Composition was evaluated by high-throughput sequencing of bacterial 16S ribosomal RNA using the Illumina MiSeq platform. Total bacterial load was estimated with qPCR of the 16S rRNA gene. **Results:** Antibiotics resulted in a two-fold decrease in bacterial load that persisted throughout treatment, with a significant reduction apparent within 12 hours. We observed an increase in reduction potential within 16 hours of the first dose of antibiotics *in vivo*, and reduction potential remained elevated (258±72mV) throughout treatment. In contrast, oxygen increased only transiently roughly one day after the initial dose. These changes coincide with a shift to a community dominated by Proteobacteria. A similar transient increase in oxygen is observed *in vitro*, coinciding with an increase in facultative anaerobes, including Proteobacteria, even in the absence of antibiotics. Treatment with antibiotics alone was not sufficient to recapitulate the changes in composition observed *in vitro*. **Conclusions:** Treatment with antibiotics results in a major environmental change that causes compositional shifts beyond those resulting from antibiotic-induced mortality.

Author Disclosure Block:

A.T. Reese: None. **H. Durand:** None. **E.H. Cho:** None. **M.M. Villa:** None. **S. Nimmagadda:** None. **M.A. Deshusses:** None. **S.P. Nichols:** D. Employee; Self; Profusa, Inc. **N.A. Wisniewski:** D. Employee; Self; Profusa, Inc. **B. Klitzman:** None. **L.A. David:** None.

Poster Board Number:

FRIDAY-636

Publishing Title:

Beta-Glucans As Novel Prebioitcs And Their Effects In Mouse Microbiome Modulation

Author Block:

K. LAM, H. Kwan, P. C. K. Cheung; The Chinese Univ. of Hong Kong, Shatin, Hong Kong

Abstract Body:

Background: Bifidobacteria are gram-positive, hetero-fermentative, non-motile, non-spore forming rods. Nowadays, many research projects have demonstrated that bifidobacteria constitute a healthy and balanced microbiome in human guts. In consideration of having evolved as a successful competitor in human gastrointestinal tract (GIT), which harbors at least 160 common bacterial species, bifidobacteria are believed to have specific mechanism in the utilization of carbohydrate, especially prebiotics for survival. Beta-glucans are complex non-digestible carbohydrates of D-glucose monomers linked by beta-glycosidic linkages. Structurally, beta-glucans are non-starch polysaccharides with repeating glucose residues with a linear chain having none or multiple side branches. Beta-glucans have long been shown to have health promoting effects including anti-tumor, immuno-stimulatory, and anti-obesity. More recently, beta-glucans are considered to have the potential to be used as novel prebiotics. **Methods:** Balb/c mouse were used as the animal model for the investigation of the effects of microbiome modulation by beta-glucans. Beta-glucans were used as prebiotic, *Bifidobacterium longum* subsp. *infantis* was used as probiotics and a combination of two used as synbiotics. The animals were randomly assigned to four groups, control, prebiotic, probiotic, and synbiotic. Prebiotics, probiotics and synbiotics were administered to the respective animal groups by intra-gastric gavage. After two weeks of gavage, fecal contents were collected. DNA from the fecal samples was extracted and the 16S rDNA region was sequenced. Sequences were analyzed with the QIIME pipeline to find out the alpha and beta diversity.**Results:** Our finding suggests that beta-glucans can shift the mouse gut microbiome with a slight increase of Bifidobacterium population. Beta-glucans could potentially be one of the novel prebiotics that not only confer beneficial effects to host via direct action such as immune-stimulatory effects, but also via an indirect route as the modulation of gut microbiome, bringing health benefits to host.**Conclusions:** Beta-glucans could potentially be one of the novel prebiotics.

Author Disclosure Block:

K. Lam: None. **H. Kwan:** A. Board Member; Self; Director, HSK GeneTech Limited. **P.C.K. Cheung:** None.

Poster Board Number:

FRIDAY-637

Publishing Title:

Increased *Akkermansia muciniphila* and *Bacteroides fragilis* Group Abundance After Islamic Fasting

Author Block:

C. Erdogdu[1], M. Y. Cirak[2], T. Karakan[2]; [1]Hacettepe Univ., Ankara, Turkey, [2]Gazi Univ., Ankara, Turkey

Abstract Body:

Metabolic disorders such as obesity is often associated with the changes in microbiota composition and quantity. It has been well known that dietary habits also effects intestinal microbiota. Here we hypothesized that long-term fasting may have a distinct effect on intestinal microbiota. Since the experimental design in human subjects is hard to perform for this purpose, we thought that Islamic fasting before-after specimens may give clues about the effect of long term fasting on intestinal microbiota. Total of 8 subjects were included in this study during Ramadan between the dates June 18 and July 16, which is approximately 17 hours of fasting per day during a 29 day period.The stool samples from volunteers were collected the day before the beginning of Islamic fasting and the day end of the Ramadan. 16S rRNA qPCR assay has been performed for quantification of *Akkermansia muciniphila, Faecalibacterium prausnitzii, Bifidobacterium spp, Lactobacillus spp, Bacteroides fragilis group, Enterobacteriaceae*. Blood samples were also collected to test for metabolic and nutritional parameters. The before and after Islamic fasting results were statistically analyzed using Wilcoxon Signed-Rank test.All of the subjects were normal weight according to the calculated BMI. The overall bacterial count did not significantly change over time in none of the subjects. However significantly increased abundance of *A. muciniphila* and *B. fragilis* group was observed in all subjects after Islamic fasting when compared to the baseline levels ($p=0.0078$ and 0.0156; respectively). Serum fasting glucose and total cholesterol levels in all subjects is also significantly reduced in all of the subjects ($p<0.001$ and $p=0.009$; respectively).Islamic fasting which has been approximately 17 hours of fasting for each day lead to a change in microbiota members.Figure 1. Increased levels of A. muciniphila and B. fragilis group before and after Islamic fasting

Author Disclosure Block:

C. Erdogdu: None. **M.Y. Cirak:** None. **T. Karakan:** None.

Poster Board Number:

FRIDAY-638

Publishing Title:

***Faecalibacterium prausnitzii* Prevents from the Non-Alcoholic Fatty Liver- Underlying Mechanisms**

Author Block:

E. Munukka[1], A. Rintala[1], R. Toivonen[1], A. Hänninen[1], S. Jalkanen[2], P. Huovinen[1], S. Pekkala[3]; [1]Univ. of Turku, Turku, Finland, [2]Medicity, Univ. of Turku, Turku, Finland, [3]Univ. of Jyväskylä, Jyväskylä, Finland

Abstract Body:

Current worldwide epidemic of obesity associates with an increase in the non-alcoholic fatty liver disease (NAFLD). The causal mechanisms of NAFLD are not entirely clear. We have previously shown in humans that the high liver fat content associates with a low fecal abundance of *Faecalibacterium prausnitzii* and a higher abundance of enterobacteria. To investigate *in vivo* the effect of *F. prausnitzii* (ATCC 27766) and *Enterobacter cloacae* subspecies *cloacae* (ATCC 13047) that served as a candidate enterobacteria, on NAFLD, experimental liver fat accumulation was induced by a high-fat diet (HFD) over 13 weeks in C57BL/6N female mice (n=6/group). Control group received normal chow. During the intervention mice received either live bacteria or PBS twice a week every two weeks via intragastric administration. After the intervention, subcutaneous and visceral adipose tissue, liver and *gastrocnemius* muscle were collected. All the tissues were pulverized in liquid nitrogen for the subsequent analyses of protein and gene expression and fat content measurements. Serum glucose and glycerol were analysed and liver sections were imaged by neutral lipid dying and confocal microcopy (Carl Zeiss). Gut microbiota composition was profiled with Illumina MiSeq 16S sequencing. The overall fat mass was heavier in *F. prausnitzii*-treated mice compared to HFD control. However tissues were metabolically healthier namely more insulin sensitive and less inflamed than in HFD control. *F. prausnitzii*-treated HFD mice were protected from fatty liver through the mechanisms involving increased fatty acid oxidation and adiponectin signaling. *E. cloacae* did not increase the liver fat content. Interestingly, *F. prausnitzii* also increased *gastrocnemius* muscle mass, which refers to an enhanced mitochondrial respiration. In addition, *F. prausnitzii* improved the intestinal integrity by increasing the expression of *Tjp1* mRNA, which may be to some extent due to the increased intestinal *TLR5* mRNA expression in response to *F. prausnitzii* and adjusted the gut microbiota composition, which may be to some extent due to the increased intestinal *TLR5* mRNA expression in response to *F. prausnitzii*. These findings show that *F. prausnitzii* prevents NAFLD induced by HFD.

Author Disclosure Block:

E. Munukka: None. **A. Rintala:** None. **R. Toivonen:** None. **A. Hänninen:** None. **S. Jalkanen:** None. **P. Huovinen:** None. **S. Pekkala:** None.

Poster Board Number:

FRIDAY-639

Publishing Title:

Probiotic Supplementation Alters Gut and Systemic Immune Activity and Energy Consumption of Growing Chickens

Author Block:

A. Ballou, R. Ali, M. Koci; North Carolina State Univ., Raleigh, NC

Abstract Body:

Probiotics have been the focus of considerable scientific and public interest as we learn how manipulation of the gut microbiome impacts host health and physiology. Previous research in our lab has demonstrated supplementation with a lactic acid bacteria probiotic product results in shifts in microbial colonization of the gut as measured by 16s rDNA sequencing. Reduced abundance of pro-inflammatory species like *Candidatus Savagella*, increased expression of IL-10 and decreased expression of IL-12RB2 in the ileum suggests this probiotic affects the host immune system. Further study of this relationship reveals probiotics stimulate circulating immune cells as well. Probiotic-treated animals generate antigen specific antibodies faster than control animals, and their circulating lymphocytes generate and consume more ATP. To better understand how oral administration of a probiotic leads to changes in systemic immune cells serum was collected at days 7, 14, 21 and 28 from animals fed control or probiotic diets (approximately $3x10^5$ CFU probiotic containing *Lactobacillus acidophilus, Lactobacillus casei, Enterococcus faecium,* and *Bifidobacterium thermophilus*/g of feed). Serum from treated and control animals was used to supplement RPMI media (10% v/v) in which chicken T-cell line CU205s were cultured for 2 or 4 days. Cells were assessed for changes in viability, proliferation and ATP content. Cells exposed to serum from probiotic-treated animals show consistently higher levels of ATP ($P < 0.05$). Partial size fractionation of the serum suggests the lymphocyte ATP-inducing factor is below 30 kDa in weight, and may be lipid associated. A study of cellular metabolism in these cells indicated the increased ATP may be a result of increased glycolytic activity. To begin to understand the specific effect of probiotics on lymphocytes cells, serum from control and treated animals was used to stimulate CU205 cells in triplicate and total RNA was isolated for transcriptional analysis. Transcript sequencing was performed on the Illumina Hi-Seq and data were processed using tools available from Galaxy. Analysis of differentially expressed genes networks will help identify the source of the metabolic stimulation, as well as other cellular processes affected by probiotics. These genes and gene pathways could serve as novel therapeutic targets for future microbial products.

Author Disclosure Block:

A. Ballou: None. **R. Ali:** None. **M. Koci:** None.

Poster Board Number:

FRIDAY-640

Publishing Title:

Correlation Between Commensal Intestinal Microorganisms Including Probiotics and Carbapenem-resistant *Klebsella pneumoniae* and Extended Spectrum Beta-lactamase Producing *Escherichia coli*

Author Block:

H. Kunishima[1], N. Ishibashi[2], K. Wada[3], M. Hirose[1], K. Oshima[2], T. Aoyagi[2], S. Endo[2], K. Oka[3], M. Takahashi[3], T. Matsuda[1], M. Kaku[2]; [1]St. marianna Univ., Kawasaki, Japan, [2]Tohoku Univ. Graduate Sch. of Med., Sendai, Japan, [3]Miyarisan Pharmaceutical Co., Ltd, Tokyo, Japan

Abstract Body:

Background: Increase of antibiotic resistant bacteria and its risks has been reported worldwide. Previous reports has shown that the commensal intestinal microbiota may have a role of reservoir of the antibiotic resistant properties. However, it is unclear about correlation between intestinal microbiota including probiotics and antibiotic resistant organisms. Therefore, we examined the effects of intestinal microbiota and/or probiotics on the growth, beta-lactamase activity and resistance gene transfer of antibiotics resistant bacteria.**Methods:** Six strains of *K. pneumoniae* (*bla*$_{\text{IMP-1}}$) and *E. coli* (*bla*$_{\text{CTX-M-15}}$) were used as a resistant bacteria. Type strains of *C. butyricum*, *C. difficile*, *C. perfirngens*, *Enterococcus faecium*, *Bifidobacterium longum*, *Bacteroides fragilis*, *Lactobacillus plantarum* and *C. butyricum* MIYAIRI588 (CBM) were also used. Resistant bacteria was incubated in a liquid medium supplemented with or without culture supernatant of probiotics or intestinal microbiota. Viable counts, beta-lactamase activity, *bla* gene expression and resistance transfer frequency of antibiotic resistant bacteria were evaluated.**Results:** Some of culture supernatant of intestinal microbiota including CBM suppressed the growth and beta-lactamase activity of resistant bacteria and decrease of resistance transfer frequency. In terms of mode of action of the reduction of beta-lactamase activity, CBM supernatant significantly suppressed expression of *bla* gene. However, some of the organism such as *E. faecium* did not affect to those of the properties.**Conclusion:** These results indicate that some certain intestinal microbiota may affect an expression of resistant mechanism and resistant gene transfer of antibiotic resistant bacteria. In addition, probiotics possibly attractive to reduce the risk of antibiotic resistant bacterial infection.

Author Disclosure Block:

H. Kunishima: None. **N. Ishibashi:** None. **K. Wada:** None. **M. Hirose:** None. **K. Oshima:** None. **T. Aoyagi:** None. **S. Endo:** None. **K. Oka:** None. **M. Takahashi:** None. **T. Matsuda:** None. **M. Kaku:** None.

Poster Board Number:

FRIDAY-641

Publishing Title:

Individual and Combined Administration of Galactooligosaccharide and *Bifidobacteria adolescentis* Ivs-1 During High Fat Feeding Causes Differential Adaptation of the Microbiome and Metabolic Profile

Author Block:

R. R. Segura Munoz; Univ. of Nebraska-Lincoln, Lincoln, NE

Abstract Body:

Human and animal studies have linked disturbances in the gastrointestinal microbiota to the worldwide epidemics of obesity and metabolic syndrome. Consequently, development of dietary strategies that reconstruct a beneficial microbiota has gained much momentum. One such strategy is the parallel administration of a prebiotic fiber with a probiotic bacterium, known as a synbiotic. Synbiotics may synergistically provide benefits to the host. The goal of this study was to systematically compare a novel synbiotic composed of *Bifidobacterium aldolescentis* IVS-1 and galactooligosaccharide (GOS) with its individual components to determine how their interactions impact host metabolism and influence gut microbiota composition. To achieve this goal, we compared the effects of feeding mice a high fat diet alone or supplemented with GOS, *B. adolescentis* IVS-1, or the combination of both. Although feeding IVS-1 had no effect on host metabolic phenotype, administering GOS alone significantly reduced both plasma glucose and insulin levels. Interestingly, feeding IVS-1 together with GOS abrogated the benefits of the prebiotic and adversely affected fat deposition and plasma glucose levels. In addition, feeding IVS-1 together with GOS prevented GOS-induced microbiome shifts that were positively correlated with an improved insulin resistance index. Together, these findings indicate that synbiotic approaches may lead to antagonistic interactions, possibly by the administered strain competing against the health-promoting organisms within the resident microbiota that are supported by the prebiotic.

Author Disclosure Block:

R.R. Segura Munoz: None.

Poster Board Number:

FRIDAY-642

Publishing Title:

Effect of Palm Kernel Cake Supplementation on Cecal Bacterial Communities of Pigs

Author Block:

Y. Lee; Natl. Inst. of Animal Sci., Wanju-gun, Korea, Republic of

Abstract Body:

Unpleasant odors in pig slurries have become an issue to resolve in the Republic of Korea because they can affect the health and well-being of people. Production of unpleasant odors in pig slurries is associated with bacterial species producing volatile compounds. Our previous study showed that odor was mitigated in pigpen pits of pigs fed diet supplemented with palm kernel cake. Therefore, intestinal bacterial communities associated with malodor production in pigs remain to be identified. The objective of this study was to examine effect of palm kernel cake supplementation on cecal bacterial communities of pigs. Cecal samples were collected from 24 newly weaned crossbred pigs (Landrace X Large White X Duroc) fed 1 of 3 diets: 1) 8 pigs fed control diet containing no palm kernel cake, 2) 8 pigs fed control diet supplemented with 2% palm kernel cake, and 3) 8 pigs fed control diet supplemented with 4% palm kernel cake. Bacterial communities were investigated from individual cecal samples using next-generation pyrosequencing technology. A total of 13,113 16S rRNA gene sequences were assigned to 22 phyla, where *Firmicutes* was the first dominant phylum and accounted for more than 80% of the total sequences in all 3 diet groups. *Bacteroidetes* was the second dominant phylum and accounted for about 10% of the total sequences in all 3 diet groups. *Peptostreptococcaceae* was the most dominant family in all 3 diet groups and its abundance. Tended to decrease in response to palm kernel cake supplementation, indicating that *Peptostreptococcaceae* may produce odor-causing compounds. Additional efforts to elucidate roles of *Peptostreptococcaceae* in pigs are necessary.

Author Disclosure Block:

Y. Lee: None.

Poster Board Number:

FRIDAY-643

Publishing Title:

Chenodeoxycholicacid Induces the Synthesis of Intestinal Antimicrobial Peptides and Attenuates Infection by Enteric Pathogens

Author Block:

S. Tremblay[1], G. Romain[1], M. Roux[1], X-L. Chen[1], K. Brown[2], S. Ramanathan[1], D. L. Gibson[2], A. Menendez[1]; [1]Univ. of Sherbrooke, Sherbrooke, QC, Canada, [2]Univ. of British Columbia Okanagan, Kelowna, BC, Canada

Abstract Body:

Background. Intestinal epithelial cells play a critical role in gut homeostasis. One of their major mechanisms is the synthesis and secretion of antimicrobial peptides and proteins (AMPP), which control the number and composition of the intestinal microbiota and protect from infections. Recent data have implicated the nuclear bile acid receptor FXR in the immunity of the small intestine. We hypothesized that bile acids (BA) contribute to the immunological homeostasis of the gut through the global regulation of intestinal AMPP expression.**Methods**. *Ex vivo* intestinal explants were treated with several BA to evaluate the BA impact on the synthesis of AMPP. C57BL/6 mice fed with a chenodeoxycholic acid (CDCA)-supplemented diet for 16 hours were used to determine the effect of CDCA on AMPP synthesis *in vivo*. Alterations in the immune cell populations of the intestinal mucosa were also studied using FACS. Susceptibility of CDCA-fed animals to enteric infections was tested with the BA-resistant pathogens *C. rodentium* and *S. typhimurium*.**Results.** Intestinal explants treated with CDCA demonstrated the highest and most consistent up-regulation of AMPP expression. CDCA feeding increased the base-line synthesis of intestinal AMPP *in vivo*; it also had an effect on the mucosal immune cell populations, inducing a decrease in the abundance of macrophages ($CD68^+$) and neutrophils ($Ly6G^+$) and an increase in the percentage of B cells ($IgG\kappa^+$). Gene expression analyses using DNA microarrays revealed an anti-inflammatory gene expression pattern in the ilea of CDCA-fed mice. Feeding with CDCA promoted a significantly faster clearance of *C. rodentium* and limited the establishment of systemic *S. typhimurium* infection.**Conclusion.** Our results demonstrate the existence of a novel mechanism for the control of intestinal AMPP expression and reveal a new aspect of the antimicrobial nature of bile acids *in vivo*. The regulation of intestinal AMPP synthesis by bile acids is physiologically relevant and seems to facilitate resistance to enteric pathogens, which has the potential to be exploited in therapeutic applications.

Author Disclosure Block:

S. Tremblay: None. **G. Romain:** None. **M. Roux:** None. **X. Chen:** None. **K. Brown:** None. **S. Ramanathan:** None. **D.L. Gibson:** None. **A. Menendez:** None.

Poster Board Number:

FRIDAY-644

Publishing Title:

Fatty Acid Control of *Salmonella* Virulence

Author Block:

Z. J. Zhang, H. C. Hang; The Rockefeller Univ., New York, NY

Abstract Body:

The challenge to global health imposed by microbial pathogens demands a better understanding of host-microbe interactions, and understanding how small molecules (metabolites) from environmental factors (diet, microbiota) control host immunity and microbial pathogenesis is a major frontier of infectious disease. While metabolites from the diet and microbiota have been reported to directly inhibit pathogen virulence, the direct mechanism(s) are often unclear. Notably, short-chain fatty acids (SCFAs) produced by commensal bacteria and long-chain fatty acids (LCFAs) from the diet have been shown to inhibit key bacterial virulence pathways, such as type III secretion system (T3SS) in enteric microbial pathogens,(1) but the molecular mechanism(s) are not well studied. Chemical reporters of SCFAs and LCFAs developed in our laboratory have enabled the characterization of covalent protein targets of fatty acids in bacteria,(2) yeast,(3) and mammalian cells.(4) With these tools, we identified the protein targets of SCFAs and LCFAs in *Salmonella enterica* serovar Typhimurium (*S. typhimurium*) and discovered that fatty-acylation of a specific transcription factor may control the expression of virulence genes in *S. typhimurium* and attenuate bacterial pathogenesis. The site-specific effects of protein fatty-acylation on this key transcriptional regulator was further evaluated by installing a stable fatty-acylation mimic with genome editing(5) and amber suppression technology(6). Using these chemical approaches, we found that exogenous fatty acids derived from the host microbiota or diet may directly modify specific proteins involved in virulence regulation and control bacterial pathogenesis. These studies are crucial for understanding how key metabolites that are generated in the gut affect specific virulence pathways of enteric Gram-negative bacteria pathogens and may facilitate the design of novel anti-infectives.

Author Disclosure Block:

Z.J. Zhang: None. **H.C. Hang:** None.

Poster Board Number:

FRIDAY-645

Publishing Title:

Sucralose Produces a Species Specific Dose Dependent Bacteriostatic Growth Attenuation on Members of the Human Microbiota

Author Block:

R. T. Kalyanam, L. S. Manly, D. T. Ha, M. Osmani, V. S. Volante, C. B. Coughlin; Univ. of North FLorida, Jacksonville, FL

Abstract Body:

Sucralose (Splenda®) is widely used as a means to help curb sugar intake. Previous studies in our lab observed concentration dependent bacteriostatic effects of sucralose on select environmental bacteria. It was hypothesized that similar sucralose-dependant inhibition of microbe growth could be measured in key species affecting human health. This experiment used clinically significant human gastrointestinal species: Bacteroides fragilis, Salmonella enterica, Klebsiella pneumoniae, and Akkermansia muciniphila. The species were challenged with incremental concentrations of sucralose within gently agitated Hungate cultures at 37°C; simulating conditions of the human gastrointestinal tract. Turbidity absorbance was utilized as an indirect measurement of the dose-dependent effect sucralose had on culture growth. Growth trends and patterns were analyzed to elucidate any possible impacts of sucralose. Sucralose demonstrated a dose-dependent attenuation of culture growth, especially in concentrations exceeding 6.29mM. At sucralose concentrations approximating Recommended Daily Allowance, results suggest no significant difference on growth rate and on peak absorbance values. Investigation into the metabolic processes hampered by the sweetener would be the next step to understand gut microbes response to sucralose exposures. These results should stimulate reconsideration of sucralose consumption as a dietary aid without knowing its full impact on the human gut microbiome. Additionally, high concentrations of sucralose may have therapeutic applications in addressing antibiotic resistant pathogens such as Klebsiella species.

Author Disclosure Block:

R.T. Kalyanam: None. **L.S. Manly:** None. **D.T. Ha:** None. **M. Osmani:** None. **V.S. Volante:** None. **C.B. Coughlin:** None.

Poster Board Number:

FRIDAY-646

Publishing Title:

A Food-Grade Antimicrobial Biopolymer Transiently Perturbs the Community Structure of the Murine Gut Microbiome

Author Block:

X. You, J. Einson, C. Lopez-Pena, M. Song, H. Xiao, D. J. McClements, D. A. Sela; Univ. of Massachusetts, Amherst, MA

Abstract Body:

Dietary components guide the establishment and maintenance of microbial communities and functions within the distal gastrointestinal tract. Antimicrobial compounds are ubiquitous in food systems, though it is unknown to what extent these molecules interact with endogenous gut microbiota once ingested. In this study, we investigated the influence of food-grade biopolymers (i.e. ε-polylysine, pectin, and ε-polylysine-pectin complexes) on composition of the murine microbiota. Accordingly, CD-1 mice were fed biopolymers incorporated into a high-fat diet over a 9-week time course. Microbial phylogenetic diversity present in fecal extracts were ascertained by high-throughput amplicon sequencing of the 16s rRNA gene. In addition, community function was predicted from relative abundances of taxa using PICRUSt. Interestingly, dietary ε-polylysine transiently altered the gut microbiome prior to restoration of the initial microbial population structure observed at the end of the feeding trial. Thus ε-polylysine shifts the murine gut microbiome *in vivo* with the community exhibiting an adaptive response over the course of the feeding trial. Moreover, when the cationic ε-polylysine is associated with an anionic polymer this perturbation is mitigated. Finally, the absolute population structure of these murine gut microbial communities was observed to be sex-dependent, but did not alter the response trajectory to exogenous biopolymers.

Author Disclosure Block:

X. You: None. **J. Einson:** None. **C. Lopez-Pena:** None. **M. Song:** None. **H. Xiao:** None. **D.J. McClements:** None. **D.A. Sela:** None.

Poster Board Number:

FRIDAY-647

Publishing Title:

Effect of Repetitive Applications of an Alcohol-based Nasal Antiseptic on the Nasal Vestibular Microbiota

Author Block:

E. F. Mongodin[1], L. Hittle[1], P. Nasipuri[2], E. W. Spannhake[2]; [1]Inst. for Genome Sci., Baltimore, MD, [2]Johns Hopkins Bloomberg Sch. of Publ. Hlth., Baltimore, MD

Abstract Body:

Interest in the substitution of non-specific non-antibiotic preparations to reduce nasal carriage of *Staphylococcus aureus* (SA) and other human pathogens is increasing. However, the effect of these broad-spectrum agents on bacterial reduction and subsequent re-growth is not well understood. This study evaluated the effects of repetitive applications of an alcohol-based antiseptic on the bacterial load and composition of the nasal microbiota.SA-positive subjects underwent daily applications of an alcohol-based antiseptic over a 3-day period. Swabs from the anterior nares were collected on the morning (pre) and evening (post-treatment) of each day and on the morning of day 4. Samples were cultured to assess CFU counts. DNA was also extracted, followed by 16S rRNA gene PCR amplification, sequencing on Illumina MiSeq and analyses using the QIIME software.Multi-day applications of alcohol antiseptic resulted in marked and significant reduction in total CFU counts on each treatment day (Table 1). The median decrease in nasal bacterial load on mornings 2-4 was 46%, 60% and 74% respectively, and reached statistical significance on day 4. Comparative analyses of the Gram(+) and Gram(-) bacterial composition showed little changes in bacterial richness (Chao1) and diversity (Shannon) after each treatment. Beta-diversity analyses also revealed minimal bacterial composition differences between time points, with samples primarily clustering by subjects. The relative abundance of the top 15 resident bacterial organisms remained mostly unaltered in response to ethanol application and during re-growth.The use of an ethanol-based nasal antiseptic resulted in effective broad spectrum decrease of total bacterial load in the nasal vestibule and did not lead to shifts in the nasal microbiota over a multi-day treatment regimen.

Subjects	**DAY 1**		**DAY 2**		**DAY 3**		**DAY 4**
	Baseline (A.M.)	**P.M.**	**A.M.**	**P.M.**	**A.M.**	**P.M.**	**A.M.**
A	15,486	-56.8 %	-76.4 %	-94.5 %	-63.0 %	-90.8 %	-63.2 %
B	7,063	-90.0 %	-51.1 %	-90.5 %	-56.8 %	-91.1 %	-44.7 %
C	63,657	-73.2 %	43.1 %	-89.2 %	-46.7 %	-98.1 %	-69.7 %
D	416,571	-99.1 %	-29.6 %	-94.5 %	-42.7 %	-99.4 %	-90.5 %

E	115,657	-99.1 %	-90.2 %	-99.1 %	-78.4 %	-98.9 %	-78.2 %
F	932,571	-99.6 %	-71.5 %	-91.7 %	-73.3 %	-99.6 %	-97.1 %

Table 1. Changes in total bacterial load after repetitive applications of an alcohol-based nasal antiseptic. Baseline: total CFUs ; other time points: % change from baseline.

Author Disclosure Block:

E.F. Mongodin: None. **L. Hittle:** None. **P. Nasipuri:** None. **E.W. Spannhake:** None.

Poster Board Number:

FRIDAY-648

Publishing Title:

A Metagenomic Approach to Understanding the Effect of Sugar on the Functional Composition of Gut Microbiome of Drosophila Melanogaster

Author Block:

S. Bhatnagar[1], J. A. Chandler[2], J. M. Lang[1], J. A. Eisen[1], A. Kopp[1]; [1]Univ. of California Davis, Davis, CA, [2]Univ. of California Berkeley, Berkeley, CA

Abstract Body:

The gut microbiome of a host has been correlated with many important functions that are required for the health of host. *Drosophila melanogaster* is a model organism, whose gut microbiome is extensively studied to uncover essential roles. There are multiple factors that play a role in determining the community structure of the microbiome, such host genetics and the environment. Diet is one such important determinant of gut microbiome composition. It is a source of nutrients for the microbiome as well as a reservoir of microbes that the host can sample from. In *D. melanogaster*, diet has been implicated as an important factor in bacterial and yeast community structure. These studies have looked only at diet-correlated differences in taxonomic compositions of the communities. Here we present a metagenomic framework to go beyond the taxonomy and elucidate the role of diet in shaping the functional potential of the gut microbiome in *Drosophila melanogaster*.We reared a colony of *D. melanogaster* on Bloomington diet and split the population onto two different nutrient media, yeast-rich and sugar-rich. For these two treatments, fecal swabs and dissected intestines were collected every day, for four days. An SSU-RNA and metagenomic libraries from these samples were compared to identify the similarities in the microbial community's structure and function. We identified specific taxa with differential abundance that responded to the divergence in diet. These differences were observed in both metagenomic and SSU-RNA libraries. For the same samples, a change in functional potential was also discerned as observed through changes in gene abundances in the metagenomic libraries. Using shotgun metagenomics of fecal swabs, we are able to identify the functional capability of the *Drosophila* microbiome using a non-invasive and cost-effective technique. This technique is easily transferable to other insects and non-laboratory populations, allowing the unexplored functional diversity of host-associated microbiome to be identified.

Author Disclosure Block:

S. Bhatnagar: None. **J.A. Chandler:** None. **J.M. Lang:** None. **J.A. Eisen:** None. **A. Kopp:** None.

Poster Board Number:

FRIDAY-649

Publishing Title:

Commensal Oral Bacteria as Probiotics against *Porphyromonas gingivalis*

Author Block:

P. Balani[1], J. Scott[1], L. Hu[2], M. Duncan[1]; [1]The Forsyth Inst., Harvard Sch. of Dental Med., Cambridge, MA, [2]Tufts Univ., Boston, MA

Abstract Body:

The aim of this study was to identify effective probiotics against a model bacterial system and its target genes and metabolic pathways. Periodontitis is a complex polymicrobial disease affecting up to 40% of the US population (1), but the molecular interactions between species in this community are poorly understood. *Porphyromonas gingivalis (Pg*), an oral anaerobe, is associated with the etiology of periodontitis. We previously reported that non-pathogenic commensal bacteria can modulate *Pg* virulence (2), warranting further study of the mechanisms underlying such interspecies interactions. We screened a library of 300 oral commensal *Streptococcus* and *Actinomyces* strains as potential probiotics that inhibit growth of *Pg*. Of the five different non-pathogenic oral species isolated from healthy adults and children, 70% of the *Streptococci* and 10% of *Actinomyces* species completely inhibited the growth of *Pg*. Supernatants of the inhibitory strains were tested for probiotic activity against growth of *Pg* biofilms. Several *Streptococcus oralis* strains produced an inhibitory activity only after co-culture with *Pg*, suggesting communication between the organisms whereas *Actinomyces naeslundii* produced inhibitory compounds that were byproducts of growth. To systematically identify Pg genes that render it sensitive to the inhibitors, we constructed a *Pg* Transposon (Tn) insertion library to be challenged by coculture with the probiotic strains or their inhibitory metabolites. We expect that among the challenged survivors, *Pg* Tn-mutants defective in inhibitor target genes or pathways will be predominantly represented. These mutants will be identified by Tn-seq (transposon sequencing) analysis. The results of this study will increase our understanding of the molecular mechanisms of probiotics and will allow us to determine the *in vivo* role of probiotics in preventing/eliminating periodonatal disease.

Author Disclosure Block:

P. Balani: None. **J. Scott:** None. **L. Hu:** None. **M. Duncan:** None.

Poster Board Number:

FRIDAY-650

Publishing Title:

Comparative Analysis of Antiseptic Perturbations and Skin Microbial Community Dynamics

Author Block:

A. SanMiguel, J. Meisel, J. Horwinski, Q. Zheng, E. Grice; Univ. of Pennsylvania, Philadelphia, PA

Abstract Body:

Antimicrobial drugs are commonly employed to inhibit the growth of pathogenic microorganisms. These treatments are rarely narrow in spectrum, and act upon a range of bacterial species in our commensal microbiota. Only recently have these effects been fully appreciated, as previous research has relied on isolated, culture-based systems. While informative, these studies cannot account for the complex growth environments of most microbial communities, and fail to evaluate the dynamics of previously uncultured bacteria. This is especially relevant when considering the skin surface, where antiseptics are used with the expectation of complete bacterial sterilization. Indeed, many of these treatments boast efficacy rates greater than 99% when used against pathogenic microorganisms in artificial culture studies. Despite these results, few studies have explored the impact of antisepsis on resident, non-pathogenic bacteria. By using hairless mice as a model system and culture-independent 16S rRNA gene sequencing, we report a minimal and conserved effect of antiseptics on resident bacterial populations. We also describe a potential impact of these drugs on transient bacteria such as *Staphylococcus* species. These results were confirmed by human studies in which limited, personalized effects of antiseptic treatment were observed on skin bacterial communities over time. This includes separate biogeographic regions of the skin including the forearm and upper back. In all, these results suggest that skin bacterial inhabitants represent a relatively stable community at the population level, but that particular residents may vary in an individualized manner. It also supports the notion that antimicrobial drugs, while potent *in vitro*, may have more modest effects in the context of resident bacterial populations at the skin surface.

Author Disclosure Block:

A. SanMiguel: None. **J. Meisel:** None. **J. Horwinski:** None. **Q. Zheng:** None. **E. Grice:** None.

Poster Board Number:

FRIDAY-651

Publishing Title:

Wgs Smrt Sequencing of Patient Samples from a Fecal Microbiota Transplant Trial

Author Block:

C. Heiner[1], S. Oh, 94025[1], R. Hall[1], C. Staley[2], M. Hamilton[3], A. Khoruts[4], C. Kelly[5], L. Brandt[6], M. Sadowsky[3]; [1]PacBio, Menlo Park, CA, [2]Univ. of Minnesota, St Paul, MN, [3]Univ. of Minnesota, St. Paul, MN, [4]Univ. of Minnesota, Minneapolis, MN, [5]Brown Univ., Providence, RI, [6]Montefiore Med. Ctr., New York, NY

Abstract Body:

Background: Fecal samples were obtained from human subjects in the first blinded, placebo-controlled trial to evaluate the efficacy and safety of fecal microbiota transplant (FMT) for treatment of recurrent *C. difficile* infection. Samples included pre-and post-FMT transplant, post-placebo transplant, and the donor control; samples were taken at 2 and 8 week post-FMT. Sequencing was done on the PacBio Sequel System, with the goal of obtaining high quality sequences covering whole genes or gene clusters, which will be used to better understand the relationship between the composition and functional capabilities of intestinal microbiomes and patient health. **Methods:** Samples were randomly sheared to 2-3 kb fragments, a sufficient length to cover most genes, and SMRTbell libraries were prepared using standard protocols. Libraries were run on the Sequel System, which has a throughput of hundreds of thousands of reads per SMRT Cell, adequate yield to sample the complex microbiomes of post-transplant and donor samples.**Results:** Here we characterize samples, describe library prep methods and detail Sequel System operation, including run conditions. Descriptive statistics of data output (primary analysis) are presented, along with SMRT Analysis reports on circular consensus sequence (CCS) reads generated using an updated algorithm (CCS2). Final sequencing yields are filtered at various levels of predicted accuracy from 90% to 99.9%. Previous studies done using the PacBio RS II System demonstrated the ability to profile at the species level, and in some cases the strain level, and provided functional insight.**Conclusions:** These results demonstrate that the Sequel System is well-suited for characterization of complex microbial communities, with the ability for high-throughput generation of extremely accurate single-molecule sequences, each several kilobases in length. The entire process from shearing and library prep through sequencing and CCS analysis can be completed in less than 48 hours.

Author Disclosure Block:

C. Heiner: None. **S. Oh:** None. **R. Hall:** None. **C. Staley:** None. **M. Hamilton:** None. **A. Khoruts:** None. **C. Kelly:** None. **L. Brandt:** None. **M. Sadowsky:** None.

Poster Board Number:

FRIDAY-652

Publishing Title:

Microbial Interactions in a Model Consortium of the Infant Intestinal Microbiome

Author Block:

A. Ovalle, F. Pinto, **D. Garrido**; Pontificia Univ. Católica de Chile, Santiago, Chile

Abstract Body:

The intestinal microbiome is a complex assembly of microorganisms, that impacts host physiology and health in several ways. The infant gut microbiome starts to develop at birth, where microbes from the fecal and vaginal microbiome of the mother come in contact with the infant and start a colonization process dominated by facultative anaerobes followed by strict anaerobes, especially *Bifidobacterium*. Diet is one the major factors shaping the composition of this community in the first years of life, with clear differences in microbiome composition during breast or formula feeding. It is not known how infant gut microbes respond to other members and how this interaction is shaped by dietary compounds. In this study we aimed to study microbial interactions in a model infant microbiota using a representative culture media containing fructooligosaccharides (FOS), a prebiotic commonly used in infant formula. We defined a model consortium of the infant gut microbiome, consisting of four representative species: *B. longum* subsp. *infantis* (Bi), *Lactobacillus acidophilus* (La), *Escherichia coli* (Ec) and *Bacteroides vulgatus (Bv)*. Using experimental design and surface response analysis, we modified a previously reported chemically defined media (ZMB-1), including variables such as partially degraded proteins, presence of bile salts, and pH of 5.5. This optimized media was used to evaluate the levels of each member of the consortium when given FOS as the sole carbon source. Experiments were performed as monoculture, and co-culture with every other member of the consortium. Bacterial levels were assessed every 12 h during 72 h by qPCR. We found that Bi always dominated each co-culture, supporting the prebiotic effect of FOS, and that Bv and Ec outnumbered La. Interestingly, when accounting for the amount of cells in co-culture over monoculture, this ratio was always higher for Bi, indicating that this bacterium achieves higher cell densities when growing with other members of the consortium than alone. In contrast, Bv numbers were always lower during co-culture than in monoculture. This suggests that while Bi enjoys the benefit of company in a commensalist interaction with other members of the microbiome, Bv instead displays a more competitive relationship with other bacteria. These results indicate that different ecological forces shape the infant gut microbiome and should be considered.

Author Disclosure Block:

A. Ovalle: None. **F. Pinto:** None. **D. Garrido:** None.

Poster Board Number:

FRIDAY-653

Publishing Title:

What Is in That Soiled Diaper: A Study on Enumeration and Characterization of *bifidobacterium* and Other Gut Bacteria from an Infant

Author Block:

T. W. Frazer, M. Santos; The Univ. of North Carolina at Pembroke, Pembroke, NC

Abstract Body:

The genus of bacteria *Bifidobacterium* contains over 50 known species with new species and subspecies still being discovered and heavily researched today. *Bifidobacteria* populations in newborns and infants are of particular interest, as these bacteria are among the initial colonizers of the infant gut and comprise the overwhelming majority of the infant's intestinal/gut microbiome. The positive influence of *Bifidobacteria* on infant health has long been noted as the various species within this genus have been shown to prevent allergic diseases, aid in breast-milk digestion, as well as inhibit potentially pathogenic microbes The study attempted to enumerate and characterize the antimicrobial effects of *Bifidobacteria* by collecting fresh fecal samples from soiled diapers during the first eight weeks of an infant's life. The fecal samples were collected using nitrogen enriched culture collection/transport device, were diluted using tryptic soy broth, and plated onto agar media selective for *Bifidobacteria*. The plates were incubated for 48 hours at 37C with and without oxygen. Standard plate counts and optical density readings were used to enumerate bacteria from the fecal sample. *The* antagonistic effects of *Bifidobacterium breve* against the pathogens *Escherichia coli*, *Clostridium difficile*, and *Candida albicans* were tested using the diffusion disk method. Preliminary anaerobic counts showed *Bifidobacteria* numbers were low in the first three days of life and then increased exponentially thereafter within the next 8 weeks to become the major type of microbe in the infant gut. Disk assays showed *B. breve* inhibited all three of the potential pathogens used in the study, while there was no inhibition between *E.coli* and *C. albicans*. The researchers plan to validate the bacterial counts and the identity of the other non-*Bifidobacterium* bacterial colonies from the frozen fecal samples during the months of January and February 2016.

Author Disclosure Block:

T.W. Frazer: None. **M. Santos:** None.

Poster Board Number:

FRIDAY-654

Publishing Title:

Strain-level Metagenomic Analysis Implicates Uropathogenic *E. coli* in Necrotizing Enterocolitis and Death in Preterm Infants

Author Block:

D. V. Ward[1], M. Scholz[2], M. Zolfo[2], D. H. Taft[3], K. R. Schibler[3], A. Tett[2], N. Segata[2], A. L. Morrow[3]; [1]Univ. of Massachusetts Med. Sch., Worcester, MA, [2]Univ. of Trento, Trento, Italy, [3]Cincinnati Children's Hosp. Med. Ctr., Cincinnati, OH

Abstract Body:

Background: Necrotizing enterocolitis (NEC) afflicts approximately 10% of extremely preterm infants with high fatality. One in fourteen infants born prior to 32 weeks of gestation or less than 1500 grams develop NEC, and nearly one-third of NEC cases are fatal. The etiology of NEC is not confidently established, but appears to result from a hyperinflammatory response to the gut microbiota. Inappropriate bacterial colonization with Enterobacteriaceae is implicated but no specific pathogen has been identified. We sought to apply metagenomic approaches to characterize, at the species and strain level, Enterobacteriaceae that may indicate risk for NEC.**Methods:** Metagenomic shotgun sequence data was generated from 144 preterm infants (less than 30 weeks gestational age) and 22 term infants (greater than 37 weeks gestational age) with stool samples collected between days 3 to 22 of life. Novel analysis methods were developed to subtype *E. coli* species present in the infant gut microbiome.**Results:** Metagenomic analysis indicated that function subtypes of *E. coli*, defined by species-specific accessory gene content were associated with development of NEC and death as NEC-outcome. Accessory gene content and metagenomic multi-locus strain typing indicated that risk was associated with uropathogenic *E. coli* strain types (UPEC).**Conclusions:** Colonization by UPEC is a risk factor for development of NEC and (ii) UPEC correlated with death as an outcome. These findings are the first to associate UPEC with NEC and provide a foundation for further investigating the epidemiology of NEC.

Author Disclosure Block:

D.V. Ward: None. **M. Scholz:** None. **M. Zolfo:** None. **D.H. Taft:** None. **K.R. Schibler:** None. **A. Tett:** None. **N. Segata:** None. **A.L. Morrow:** None.

Poster Board Number:

FRIDAY-655

Publishing Title:

Diversity and Variability of the Dominant Enterococci in Human Stool Samples

Author Block:

C. Del Rio-Ramos, L. A. Ríos-Hernández; Univ. of Puerto Rico, Mayaguez Campus, Mayaguez, Puerto Rico

Abstract Body:

The intestinal flora of humans is known to harbour a multitude of microorganisms. One important genus is *Enterococcus*, while it only composes less than 1% of the total gut flora, the ability to harbour a wide array of virulence factors and antibiotic resistance grant it medical importance. However, not much is known about how the species composition behaves in the human gut. In order to fill this gap, the focus of our study was to analyse and compare the dominant enterococci population of two subjects via daily stool sampling for a period of 7 consecutive days. A total of 140 Enterococci per subject were isolated and subjected to antibiotic resistance assays and molecular analysis. Molecular analysis allowed the comparison of isolates at the species level, presence of virulence factors and ultimately at the strain level. Total enterococci population was around 10^6 to 10^8 for both subjects, however species composition and dominance were different. In subject #1, the dominant enterococci vary daily but was limited to *E. faecium* and *E. faecalis*, with two transitions in seven days. Of the two species, *E. faecalis* was the most virulent with vancomycin resistance and gelE virulence factor for 3 days while *E. faecium* only had *gelE*. In subject #2, *E. faecalis* was the only dominant species during the sampling period. Although species dominance was consistent, the strains and the frequency of virulent factors among the dominant species were different and higher than in subject #1. Although Enterococci were always present at similar densities, the dominant population changed daily. One subject had a higher frequency of strains with virulence factors, gelE and esp, while the other had a wider range of antibiotic resistances, vancomycin being the most notable. Screening for colonization of virulent strains during hospital admittance might not safeguard the patient's health because of the high strain transition frequency.

Author Disclosure Block:

C. Del Rio-Ramos: None. **L.A. Ríos-Hernández:** None.

Poster Board Number:

FRIDAY-656

Publishing Title:

Microbial Transformation of *Populus* Secondary Metabolites By Microbiome Isolates

Author Block:

D. A. Pelletier, J. Morrell-Falvey, A. N. Bible, G. B. Hurst, K. Chourey, M. Denney, K. Kertesz, A. Zinkle, C. Timm, T-Y. Lu, N. Engle, T. Tschaplinski, M. Doktycz; Oak Ridge Natl. Lab., Oak Ridge, TN

Abstract Body:

Populus is a widely studied model woody plant species and a potential cellulosic feedstock for biofuels. These trees host a wide variety of microbial associates within their roots and rhizosphere and thus serve as a useful model to study interactions between plants and microorganisms. One of the defining characteristics of *Populus* is the production of secondary metabolites known as higher-order salicylates (HOS), which are involved in host defense and signaling mechanisms. We are interested in determining the influence of these HOS on the host-microbiome composition and physiology. We have isolated and characterized a number of bacteria from genera (*Pseudomonas*, *Sphigobium*, *Rhizobium*, *Bacillus*, *Pantoea*, *Duganella*, *Burkholderia*, *Caulobacter*, *Streptomyces*, *Bradyrhizobium*, *Rahnella* and *Varivorax)* prevalent in the endosphere and rhizosphere compartments of *Populus* roots and obtained whole genome sequence data. Here we describe physiological characterization of these bacterial isolates with the goals of determining the ability of these strains to transform salicin and other HOS extracted from *Populus* tissues, and measuring the sensitivity and resistance of these bacterial strains to HOS compounds and their degradation products. We present proteomics, metabolomics and comparative genomics data from select strains that have led to identification of potential mechanisms for transformation of the HOS. We hypothesize that the complex nature of these HOS metabolites and their degradation products may be a mechanism for modified plant-bacteria signaling, and may facilitate microbial cross feeding in which has been observed in bacterial co-cultivation experiments. This analysis provides initial insight into the prevalence and diversity of *Populus* microbiota capable of transforming HOS, potential transformation mechanisms, and interactions between microbiome community members.

Author Disclosure Block:

D.A. Pelletier: None. **J. Morrell-Falvey:** None. **A.N. Bible:** None. **G.B. Hurst:** None. **K. Chourey:** None. **M. Denney:** None. **K. Kertesz:** None. **A. Zinkle:** None. **C. Timm:** None. **T. Lu:** None. **N. Engle:** None. **T. Tschaplinski:** None. **M. Doktycz:** None.

Poster Board Number:

FRIDAY-657

Publishing Title:

A Meta-Analysis of Obesity and the Bacterial Microbiome: Is There Common Ground?

Author Block:

M. A. Sze, P. D. Schloss; Univ. of Michigan, Ann Arbor, MI

Abstract Body:

Background: There have been numerous studies investigating the role of the bacterial microbiome on obesity. The evidence in mouse and human studies show the powerful effect that the microbiome could have in obesity. Yet, two recent meta-analyses on different data sets suggest otherwise (Finucane et al. 2014 and Walters et al. 2014). We build on these two studies by incorporating all data sets with body mass index and microbiome data and add additional data from controls in the Great Lakes and New England (GLNE) cohort. By harnessing the increased power provided by combining multiple cohorts we investigate whether there are community or population-level signals that would allow us to differentiate people of varying body mass index (BMI).**Methods:** A total of 10 datasets were/are being analyzed (GLNE (n=172), Human Microbiome Project (HMP) (n=256), Ross et al (n=63), Escobar et al (n=30), MetaHit -Danish only (n=85), Nam et al (n=18), Turnbaugh et al (n=281), Goodrich et al (n=525), Zupancic et al (n=202), and Yatsunenko et al (n=528)). For each dataset we calculated the Shannon diversity, Bacteroidetes/Firmicutes ratio (B/F), and generated Random Forest models using individual operational taxonomic units to differentiate individuals based on BMI or obesity categories based on BMI. We also included demographic data for each subject to determine whether these could be used to strengthen the associations. We used an RE model to generate relative risk (RR) and confidence intervals (CI) for a pooled analysis across studies.**Results:** No difference was found between non-obese and obese groups for Shannon diversity and B/F ratio ($P > 0.05$). There was no significant increase in RR of obesity in high versus low diversity groups (RR = 1.08 (CI 0.92-1.27)). Analyzing the data together, there was a significant loss of diversity in the overweight versus obese group ($P=0.01$). Using continuous and categorized BMI data, each dataset had factors that predicted non-obese from obese with a high degree of accuracy (out of bag area under the curve, 0.71-0.95). The majority of variables that were predictive in a single dataset were not predictive in more than two datasets. Ruminococcaceae (4/7 datasets) and Shannon diversity (3/7 datasets) were the exception to this.**Conclusion:** These data suggest the lack of a consistent taxonomic signal that is associated with variation in a person's BMI.

Author Disclosure Block:

M.A. Sze: None. **P.D. Schloss:** None.

Poster Board Number:

FRIDAY-658

Publishing Title:

Dynamic Community Structure Of The Necrobiome Has The Potential To Aid Postmortem Interval Determination

Author Block:

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Abstract Body:

Little is known about the postmortem microbiome (necrobiome) in cadavers, particularly the microbial structure of the microflora residing within the human ecosystem, and their associations with decomposition stages. Recent work suggests that these postmortem bacterial communities are surprisingly dynamic. We describe how the microbiome of a living host changes and transmigrates within the body after death, linking the microbiome of the living to the postmortem necrobiome. These changes have demonstrated promise as usable evidence in criminal investigations for estimating the time since death occurred (postmortem interval). We investigated the postmortem microbial community structure in a mouse model along with the transmigration of the bacterial species present in a highly controlled setting which minimized outside microbial influence not associated with the microbiota. Immunocompetent mice (N=60) were inoculated intranasally with *Staphylococcus aureus*-RFP and *Clostridium perfringens* to introduce controlled aerobic and anaerobic communities, respectively, that have the ability to break down host tissue through protease production. Thirty of the inoculated mice were immediately surface sterilized with a bleach solution following sacrifice, in order to disrupt the influence of the external microbiota. DNA was isolated and purified from tissue samples starting at 1 hour and ending at 7 days postmortem, for use in library preparation and whole genome shotgun sequencing. Sequencing results were analyzed to identify changes in community structures associated with different stages of decomposition. The results from this study could provide insight into how commensal bacterial populations colonize, proliferate, and transmigrate throughout the host following death. Data obtained from this study contributes to our ongoing investigations that aim to identify novel methods that could be used in the field of forensic science to precisely measure time since death.

Author Disclosure Block:

Z. Burcham: None. **J. Pechal:** None. **J. Bose:** None. **E. Benbow:** None. **C. Schmidt:** None. **H. Jordan:** None.

Poster Board Number:

FRIDAY-659

Publishing Title:

Mapping the Gastrointestinal Microbiota of a Human Cadaver

Author Block:

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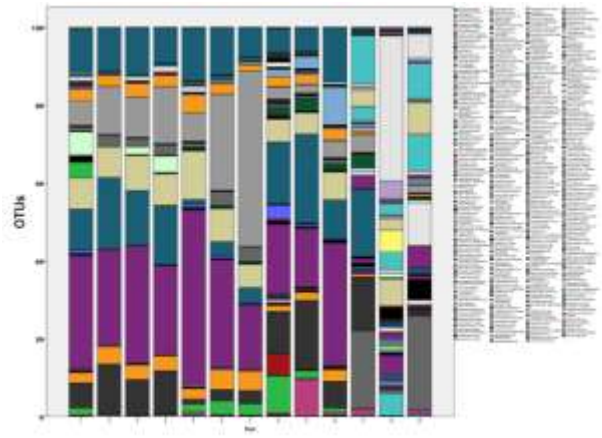
Abstract Body:

Background: Fresh frozen cadavers offer the opportunity to investigate the resident flora of the human gastrointestinal tract as the donated body is quickly frozen in saline to preserve tissue integrity.**Methods:** 13 fecal/mucus samples were collected from the stomach to the descending colon, DNA was extracted and bacterial amplicons from the V1-V3 hypervariable region of the 16S rRNA gene were sequenced using Ion Torrent DNA Sequencing and reads were clustered into operational taxonomic units (OTUs, 3% distance) and taxonomically classified using the Green Gene database.**Results:** The stomach region revealed OTUs with the lowest diversity and similar to the microbiota of the distal duodenum. There was a steady increase in the populations of *Lactobacillus casei*, *Bacillus sp.* and *Kocuria rosea* throughout the small intestine with a dramatic decrease towards the proximal end as diversity increased. The diversity of the ascending, transverse and descending regions of the colon were drastically distinct and highly diverse (Figure 1).**Conclusion:** Mapping the gastrointestinal tract of the human gut revealed distinct eubacterial communities relative to locations and structures within the gastrointestinal tract.



Author Disclosure Block:

J. Coffman: None. **M. Sanchez-Gonzalez:** None.

Poster Board Number:

FRIDAY-660

Publishing Title:

Using the Human Necromicrobiome to Estimate the Postmortem Interval

Author Block:

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Abstract Body:

The microbiome is the community of microbes that live in, on, and around the human body. In this project, we explore postmortem changes in this community. One goal of this work is to establish new tools for calculation of the postmortem interval (PMI) to aid death investigations. In the first phase of this project, we compared the bacterial communities on both living and deceased subjects to determine whether or not we could identify consistent differences among the microbial communities. We were successful and identified many microbial taxa, particularly genera and species, that allow us to characterize a sample as having come from a decomposing body. In phase two, we are focused solely on cadavers, and how the microbiome changes through the course of decomposition. Specifically we collected swab samples from the nostrils and external ear canals of four cadavers at the Anthropology Research Facility at the University of Tennessee at Knoxville over 4-6 weeks of decomposition. We then extracted DNA from these samples and performed 16S metagenomic sequencing using the miSeq platform from Illumnia. We then identified several dozen bacterial taxa that show a consistent pattern of change in all four bodies. We used these to construct a statistical algorithm with which to predict the PMI and tested it on eight test subjects. The results are both striking and encouraging. We conclude that the postmortem microbiome will be a successful tool in establishing PMI and will present our proof-of-concept study of how it might be done.

Author Disclosure Block:

D.D. Trinidad: None. **N.H. Lents:** None. **S. Guzman:** None. **J. Parziale:** None. **A. Lerer:** None.

Poster Board Number:

FRIDAY-661

Publishing Title:

Dominant *Bifidobacterium* and *Lactobacillus* Species in Patients with Non-alcoholic Fatty Liver Disease

Author Block:

C. Erdogdu[1], M. Y. Cirak[2], T. Karakan[2]; [1]Hacettepe Univ., Ankara, Turkey, [2]Gazi Univ., Ankara, Turkey

Abstract Body:

Background: Identification of *Lactobacillus* and *Bifidobacterium* species in certain conditions is of critical importance due to their health promoting effects. Here we hypothesized that patients with NAFLD and healthy microflora may have some distinct characteristics in terms of dominant species. Despite the many advantages of next generation sequencing, plasmid library construction still have some superiorities since it makes possible to determine the bacteria in species level. However the disadvantage of this method is being very time consuming and its high cost. In order to determine the *Lactobacillus* and *Bifidobacterium* microflora in species level we choose the metagenomic approach by constructing library from pooled and amplified patients and control stool samples which may be a good approach to analyze high number of samples with a cost-effective strategy.**Methods:** A total of 49 NAFLD patients and 35 control subjects stool DNA samples were pooled within each group in the same concentration. The DNA pools were amplified with specific primers for *Lactobacillus* and *Bifidobacterium* and ligated into PCR 2.1 TA cloning vector. After transformation E.coli clones were cultured in Luria-Bertani Agar containing 100 mg/L ampicillin. Approximately 50 colonies for each group were randomly selected and sequenced.**Results:** All sequences obtained showed more than 98% sequence identity. The most dominant Lactobacillus species was *L. ruminis* in both patients (44.6%) and healthy controls (50%). However *L. reuteri* which was the second dominant *Lactobacillus* member in patients group (21.4%) was not observed in healthy controls. Different from patient microflora healthy microflora contains *L. sakei* and *L. helveticus*. *L.acidophilus* could not be determined in both groups. Patient and healthy microflora has *B. adolescentis* and *B.bifidum* as dominant species. The main difference is patient group has 7.7% of B. infantis which could not be determined in healthy controls.**Conclusion:** Sample pooling before plasmid library construction is a successful cost-effective method for determining dominant species in a community. The dominant *Lactobacillus* and *Bifidobacterium* species seem to be different between healthy controls and NAFLD patients which promote the idea that the certain species may be unique for the disease or health situation.

Author Disclosure Block:

C. Erdogdu: None. **M.Y. Cirak:** None. **T. Karakan:** None.

Poster Board Number:

FRIDAY-662

Publishing Title:

Gut Microbiota in Patients with Non-Alcoholic Fatty Liver Disease

Author Block:

C. Erdogdu[1], M. Y. Cirak[2], T. Karakan[2]; [1]Hacettepe Univ., Ankara, Turkey, [2]Gazi Univ., Ankara, Turkey

Abstract Body:

Background: The effect of the gut microbiota on several diseases is becoming increasingly important issue. Since there is a close relationship between gut and liver via portal vein, the liver is consistently exposed to bacterial products such as endotoxin. The composition and quantification of microbiota members may be important for gut dysbiosis and subsequent bacterial translocation which may be the major cause of non-alcoholic fatty liver disease (NAFLD). In order to support this hyphothesis we performed qPCR analysis in stool of patients with NAFLD and healthy controls.**Methods:** The stool samples from 52 patients with NAFLD and 38 healthy controls have been collected. NAFLD has been proven by biopsy in 43 of the patients. 16S rRNA qPCR assay has been performed for quantification of *Akkermansia muciniphila, Faecalibacterium prausnitzii, Bifidobacterium spp, Lactobacillus spp, Bacteroides fragilis* group and *Enterobacteriaceae*. Serum IL-6, TNF-α, hs-CRP and **endotoxin levels were assessed.Results:** *A. muciniphila* and *B. fragilis* group were found significantly lower in patients with NAFLD ($p=0.003$ and $p=0.001$; respectively). As expected, the Enterobacteriaceae family members were found to be significantly higher in patients group ($p<0.001$). In consistent with the higher Enterobacteriaceae abundance in NAFLD patients, elevated serum endotoxin levels were also determined. TNF-α and IL-6 levels were not significantly different, however patients have 3 fold higher hs-CRP which is a well-known marker of inflammation. Multiple regression analysis has performed in order to adjust for BMI and gender. A. muciniphila was still significantly lower and Enterobacteriaceae levels were significantly higher in patients group after adjusting for BMI and gender ($p=0.0221$, $p=0.0186$; respectively).**Conclusion:** NAFLD patients were characterized with higher Enterobacteriaceae levels, lower *A. muciniphila* and *B. fragilis* group levels in our study cohort. Elevated endotoxin levels and inflammation are also supporting our hypothesis.

Author Disclosure Block:

C. Erdogdu: None. **M.Y. Cirak:** None. **T. Karakan:** None.

Poster Board Number:

FRIDAY-663

Publishing Title:

Microbial Occupation of Cecal Crypt Microniches Depends on Host Conditions

Author Block:

A. Zaborin, J. Defazio, S. K. Hyoju, M. Krezalek, **O. Y. Zaborina**, J. C. Alverdy; Univ. of Chicago, Chicago, IL

Abstract Body:

Background: It has been demonstrated that cecal crypts represent a specific microniche that is normally colonized by commensal bacteria providing the host with sustained tonic stimulation. Here we hypothesize that physiological stress in the form of surgical injury can disturb the cecal crypt microbiome providing an opportunism for colonization by pathogens. **Methods:** We used a mouse model in which mice undergo a short period of pre-operative starvation, antibiotic administration (IM cefoxitin) and a 30% hepatectomy that mimics the process of elective surgery. When mice were intestinally inoculated with a pathogen community (PC- *Serratia marcescens, Klebsiella oxytoca, Enterococcus faecalis*, and *Candida albicans*) isolated from the stool of a critically ill patient, mortality rates approached 60-80%. Five groups of mice were studied: untreated controls, starvation + antibiotics (SA), hepatectomy alone (H), starvation + antibiotics + hepatecomy (SAH), and starvation + antibiotics +hepatectomy + PC inoculation (SAHPC). Mice were sacrificed at 48 hours and cecal crypts were analyzed by scanning electron microscopy, hematoxylin and eosin staining, and fluorescent *in situ* hybridization using bacterial universal probe and probes specific for each member of pathogen community.**Results:** Cecal crypts in untreated mice showed dense filling with bacteria. Hepatectomy alone did not influence the crypt microbiota. The SA treatment demonstrated that 20-25% of crypts were empty and the remaining crypts were less packed. Full SAH treatment dramatically decreased crypt microbiota with upto 60% of crypts completely empty and the remaining crypts sparsely occupied by bacteria (n=5 mice, 3 cecal sections per mouse, 4000 crypts total, $p<0.01$ compared to control). SAHPC group displayed crypts sparsely colonized by the three bacterial pathogens with predominance of *S. marcences.* No *C. albicans* was detected in crypts. **Conclusions:** Crypt microbiota are not stable structures and can be readily disturbed during the process of major surgery. Empty crypts may create opportunism for pathogens to colonize with profound implications for disease.

Author Disclosure Block:

A. Zaborin: None. **J. Defazio:** None. **S.K. Hyoju:** None. **M. Krezalek:** None. **O.Y. Zaborina:** None. **J.C. Alverdy:** None.

Poster Board Number:

FRIDAY-664

Publishing Title:

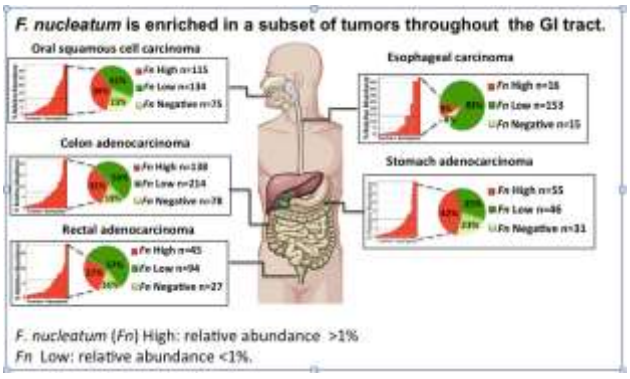
Pan Gi Analysis of *F. nucleatum* Colonized Human Tumors

Author Block:

S. Bullman, C. Pedamallu, M. Meyerson; Dana-Farber Cancer Inst., Boston, MA

Abstract Body:

Studies by our group have implicated an over abundance of Fusobacterium nucleatum (Fn) in association with colorectal cancer1,2. Noting that Fn is primarily an oral pathogen, providing a source of entry to the digestive tract, we set out to determine if Fn colonizes tumors of the gastrointestinal (GI) tract, from the mouth to the rectum via our computational subtraction pipeline, PathSeq3. Our analysis of whole genome and RNAseq data from over 2,000 human tumors from TCGA cohorts demonstrates Fn is associated with a subset of tumors throughout the GI tract. With this we reveal a conserved microbial signature characterized by the enrichment of anaerobic bacteria and depletion of aerobic organisms in tumors that are colonized with Fn. We observe Fn to be significantly enriched in tumors of stomach adenocarcinoma in comparison to matched normal tissue. Together these findings may suggest a Fn specific tumor microenvironment in a subset of tumors along the GI tract. Furthermore, we have comprehensively characterize Fn-associated tumors by correlating host mutation status, transcriptome and epigenetic landscapes, along with clinical information, to reveal site-specific and site-shared features of Fn associated tumors. Comparison of host gene expression signatures between Fn “high” and Fn “low/negative” tumors reveals a hypoxic inflammatory signature in the presence of Fn. We hypothesize that (i) particular Fn strains are associated with GI cancers and (ii) this occurs within a susceptible host population. As such, an ongoing focus of or work, via whole genome analysis of cancer and non-cancer associated Fn strains, is to determine if genomic features are shared specifically between tumor isolates, revealing “high-risk” strains.



Author Disclosure Block:

S. Bullman: None. **C. Pedamallu:** None. **M. Meyerson:** None.

Poster Board Number:

FRIDAY-665

Publishing Title:

Sources of DNA Contamination in a Study of Human Oral Microbial Communities

Author Block:

N. M. Davis, D. M. Proctor, D. A. Relman; Stanford Univ., Stanford, CA

Abstract Body:

Background: Sample contamination by exogenous microbial DNA is an important but understudied problem in sequence-based human microbiome compositional analysis. Low-biomass samples are of particular concern, as contaminant sequences from the laboratory environment, reagents, and other samples can obscure biologically meaningful findings. **Methods:** We collected oral mucosa samples (MS) from 7 distinct sites in each of 3 healthy adults daily for 29 days (n=609), and another set of the same 7 MS once a week in the same period (n=105). We extracted genomic DNA from 25 blank swabs and 5 no-template extraction control samples (EC) alongside biological samples. After PCR-amplifying the V4 region of the 16S rRNA gene using barcoded primers, amplicons were sequenced using Illumina MiSeq. A total of 16,272,135 paired-end, high-quality reads were analyzed.**Results:** Sample type (MS vs. EC) contributed most to the explained variance in Bray-Curtis distances between samples (PERMANOVA: $F=108$, $R^2=0.14$, $P=0.001$). 86 contaminant operational taxonomic units (OTUs) were classified as either oral cross contaminants or environmental contaminants using a k-Nearest Neighbors (k-NN) classifier trained on OTU sequence relative abundances and amplicon yield (PicoGreen dsDNA assay). Classifications were consistent with known contaminating environmental taxa like *Methylobacterium komagatae* and with known members of the oral microbiota like *Haemophilus parainfluenzae*. A pairwise, between-sample correlation matrix showed that removal of 76 putative environmental contaminants resulted in 12 of 30 EC becoming less distinct from MS.**Conclusions:** Our results point to multiple sources of contamination, including the laboratory environment and reagents, and to a lesser extent, biological samples processed alongside EC. Differences in OTU relative abundances across samples help to distinguish cross contaminant OTUs from environmental OTUs, which may allow a classifier like k-NN to identify environmental sequences for removal. A remaining question is exactly which laboratory sources (e.g. surfaces vs. reagents) give rise to environmental sequences. We will address this question by developing a physical model for the study of sample processing.

Author Disclosure Block:

N.M. Davis: None. **D.M. Proctor:** None. **D.A. Relman:** None.

Poster Board Number:

FRIDAY-666

Publishing Title:

Determining the Optimal Primers for Studying Microeukaryotic Diversity of the Human Gut

Author Block:

T. A. Auchtung, A. K. Nash, M. C. Wong, N. J. Ajami, J. F. Petrosino; Baylor Coll. of Med., Houston, TX

Abstract Body:

Microeukaryotes are an understudied component of the human gut microbiome — in healthy adults, fungi and protists compose typically 0.01 - 1% of the genetic material. They have been shown to be present at abnormal levels in some diseases. The extent of this diversity is infrequently examined, and it is often unclear whether the detected microeukaryotes are active components that have colonized the gastrointestinal tract, or are simply passing through with consumed food or saliva.To gain insight into the microeukaryotic diversity of the gut, we are amplifying, sequencing, and analyzing ribosomal internal transcribed spacer (ITS) and 18S rRNA genes that are useful for identifying fungi, and other microeukaryotes, respectively. As an initial step, it was necessary to determine which of the many published ITS primer sets was best for use with MiSeq 2x300 sequencing of fecal samples. The most important criteria were which primers had the best amplification efficiency, and which the broadest and least biased taxonomic coverage. We tested those commonly used to target ITS1 (ITS1F and ITS2R) and ITS2 (ITS3F and ITS4R), the published winner of another fungal primer set comparison, ITS2-targeting ITS86F and ITS4R, and as a control, the broad fungal 18S rRNA gene-targeting 775F and 1121R.DNA extracted from five children's fecal samples, five adult fecal samples, and a mock community were used as template in PCR with each the of four primer sets. When the 11 PCR-amplified samples were run on an agarose gel, there was a detectable band from 5/11 for ITS1F-ITS2R, 8/11 for ITS3F-ITS4R and ITS86F-ITS4R, and 9/11 for 775F-1121R. No primer set yielded consistently greater alpha diversity. ITS3F-ITS4R and ITS86F-ITS4R amplicons had no major taxonomic differences, but neither detected *Rhodotorula* (Sporidiobolales). ITS1F-ITS2R missed *Malassezia*, and mock community members *Cryptococcus neosporans* and *Aspergillus fumigatus* completely.Based on the above results, ITS3F-ITS4R was chosen for use in our large scale fungal analysis projects. This ITS primer set, in concert with 18S rRNA gene primers, are now yielding insights into the diversity, activity, and functions of the microeukaryotic portion of the human gut.

Author Disclosure Block:

T.A. Auchtung: None. **A.K. Nash:** None. **M.C. Wong:** None. **N.J. Ajami:** None. **J.F. Petrosino:** None.

Poster Board Number:

FRIDAY-667

Publishing Title:

Bioinformatics Workflow for Metagenomics Data Analysis

Author Block:

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Abstract Body:

Background: Next generation sequencing allows for comprehensive analysis of metagenomics samples. Nowadays researchers can examine the microbial species composition from a variety of environmental resources, such as gut and fecal microbiomes, soils, air, various water resources, etc. The existing data analysis tools are not always easy to use and have limited functionalities.**Methods:** We used the CLC Microbial Genomics Module, that supports the metagenomics analyses in CLC Genomics Workbench. The sequencing data were passed through QC, trimming, and alignment to Greengenes OUT reference database. The primary output of the clustering, tallying and taxonomic assignment processes is an OTU table, listing the abundances of OTUs in the samples under investigation viewable through a number of intuitive visualization options. Our secondary analyses included estimations of alpha and beta diversities, in addition to the statistical tests for differential abundance.**Results:** Here we present the analysis results from publically available chicken gut microbiome data. The samples were extracted from two different parts of the intestines (ilea and caeca) from chicks at different ages (7, 14, 21 and 42 days-old). In our analysis, we present the alpha diversity, the bar charts with different level of taxonomy, and the PCoA analysis. Different visualizations options and easy switches between the aggregate taxonomy units significantly speedup the analyses and promote the decision making.**Conclusions:** The described metagenomics workflow allowed for comprehensive analysis of metagenomics data in a user friendly environment.

Author Disclosure Block:

M. Matvienko: None. **S. Prince:** None. **M. Miyamoto:** None.

Poster Board Number:

FRIDAY-668

Publishing Title:

Metatranscriptome Analysis of Gnotobiotic Mice Harboring the Altered Schaedler Flora During Epithelial Injury Mouse Model of Colitis-associated Cancer

Author Block:

A. Proctor, G. J. Phillips; Iowa State Univ., Ames, IA

Abstract Body:

Background: Colorectal cancer (CRC) is a common type of cancer in both men and women, and individuals with inflammatory bowel disease (IBD) are at a greater risk of developing CRC. Both IBD and CRC are associated with dysbiosis of the gastrointestinal (GI) microbiota. Conventional mice harbor hundreds of species of bacteria in their GI tract, making associations between bacterial taxa and host functions difficult. Using Altered Schaedler Flora (ASF) mice, colonized with only 8 species of bacteria, we can more precisely evaluate how the GI microbiota responds to the GI environment. **Methods:** Gnotobiotic C3H:HeN mice harboring the ASF, as well as conventional C3H:HeN mice, were split into 4 groups each: control, dextran sodium sulfate (DSS) only, azoxymethane (AOM) only, and AOM+DSS. Mice in the AOM groups were given a single intraperitoneal (IP) injection of AOM on day 1, while the control and DSS only groups received an IP saline injection. Throughout a 10-week period, mice in DSS groups were treated with 2% DSS in drinking water for 7 days, with 2 week restitution between treatments. Fecal samples were taken throughout the study. After 10 weeks, mice were euthanized and cecal, colon and fecal pellets were collected, as well as colon tissue and tumors or abnormal lesions. Total RNA was extracted from fecal pellets from ASF mice after the last DSS treatment. RNA was pooled in the control, DSS only, and AOM+DSS groups for library preparation and sequencing of cDNA on the Illumina HiSeq2500 platform. **Results:** All DSS treated mice had shorter colon lengths at necropsy. Gross analysis of the GI tract of conventional mice revealed 30% of the animals had cancerous lesions. In contrast, ASF mice showed no lesions associated with AOM+DSS. Transcriptome analysis of fecal pellets from the ASF mice revealed differences in metabolic pathways associated with both inflammation and carcinogens. **Conclusion:** Our unique gnotobiotic mouse model has allowed us to detail the changes in metabolic pathways of the resident GI community with respect to GI health of the host. The observation that ASF mice displayed no lesions suggests the limited ASF community lacks important triggers of tumorigenesis found in a conventional GI community.

Author Disclosure Block:

A. Proctor: None. **G.J. Phillips:** None.

Poster Board Number:

FRIDAY-669

Publishing Title:

Metabolic Functionality and Interaction of the Altered Schaedler Flora Captured *In Vitro* and *In Silico*

Author Block:

G. Kolling[1], M. Biggs[1], G. Medlock[1], T. Moutinho, Jr.[1], J. Swann[2], H. Lees[2], J. Papin[1]; [1]Univ. of Virginia, Charlottesville, VA, [2]Imperial Coll., London, United Kingdom

Abstract Body:

Background: The Altered Schaedler Flora (ASF) is a model microbial community with both *in vivo* and *in vitro* relevance. Because the ASF has primarily been used to standardize gnotobiotic mice, very little is known about it—particularly as a dynamic, interacting community. This project is the first to characterize the ASF community *in vitro*, independent of a murine host.**Methods:** We performed a comprehensive, functional genetic analysis of all eight species. Furthermore, we developed a chemically-defined medium that supports growth of seven of the eight ASF members. Finally, to elucidate the metabolic capabilities of each ASF species—and the potential for competition and cross feeding—we performed a spent media screen and analyzed the results through dynamic growth measurements and unbiased metabolomics.**Results:** We found that cross-feeding is relatively rare (20 of 1512 possible cases), but is enriched between ASF356 and ASF519. We identified many cases of emergent metabolism (334 of 1512 possible cases), where metabolite consumption or biosynthesis was dependent on interspecies interactions. We validated metabolomics-based predictions by designing minimal media which supports the growth of ASF519.**Conclusions:** These data will inform efforts to understand ASF dynamics and spatial distribution *in vivo*, to design pre- and probiotics that modulate relative abundances of ASF members, and will be essential for validating computational models of ASF metabolism. Furthermore, this novel framework for characterizing microbial communities can be beneficially applied to many other communities of interest. Well-characterized, tractable model communities such as the ASF enable research which will translate into improved human health.

Author Disclosure Block:

G. Kolling: None. **M. Biggs:** None. **G. Medlock:** None. **T. Moutinho:** None. **J. Swann:** None. **H. Lees:** None. **J. Papin:** None.

Poster Board Number:

FRIDAY-670

Publishing Title:

Identification of ABC-JK2, a Small Molecule Inhibitor of Staphylococcal Biofilm Formation

Author Block:

Y. Yoshii[1], K-i. Okuda[1], S. Yamada[1], M. Nagakura[1], S. Sugimoto[1], T. Nagano[2], T. Okabe[2], H. Kojima[2], Y. Mizunoe[1]; [1]The Jikei Univ. Sch. of Med., Tokyo, Japan, [2]The Univ. of Tokyo, Tokyo, Japan

Abstract Body:

Background: *Staphylococcus aureus* is a major causative pathogen of hospital- and community-acquired infections. Especially, methicillin-resistant *S. aureus* (MRSA) is a serious global threat in recent years. Moreover, *S. aureus* attaches to medical device surfaces and forms multilayered communities of bacteria called biofilms. Since biofilm bacteria exhibit much greater resistance to antibiotics than planktonic bacteria, there are still no effective therapeutic agents for combatting biofilm-related infections. In this study, based on the concept that compounds specifically targeting biofilm formation would not exert high selective pressure for resistance, we aimed to identify compounds that inhibit biofilm formation by *S. aureus* without affecting bacterial growth.**Methods:** Biofilms were formed on 96-well flat-bottomed polystyrene plates in the absence or presence of compounds, and were subsequently quantified by the conventional crystal violet-staining method. A high-throughput screening has been employed using 50,000 compounds with characteristic structures stocked at Drug Discovery Initiative of The University of Tokyo. Effects of the hit compounds on metabolic profile, gene expression profile, and cell morphology were evaluated by metabolomic analysis, microarray/quantitative real-time PCR, and thin-sectioning transmission electron-microscopy (TEM) observation, respectively.**Results:** One of the hit compounds, named anti-biofilm-compound JK2 (ABC-JK2), inhibited biofilm formation of several strains of S. aureus including MRSA and Staphylococcus epidermidis at IC_{50} ranging from 12.0 to 22.5 μM. ABC-JK2 decreased intracellular levels of glycolytic metabolites and increased the expression of genes related to peptidoglycan biosynthesis and hydrolysis in *S. aureus*. In addition, aberrant morphologies of *S. aureus* with thick cell walls and abnormal rippled septa were observed in the presence of ABC-JK2.**Conclusions:** Overall, these results indicate that ABC-JK2 inhibits staphylococcal biofilm formation presumably by affecting glycolysis and quality control of cell wall. These findings might lead to innovative drug development for preventing and curing staphylococcal biofilm infections.

Author Disclosure Block:

Y. Yoshii: None. **K. Okuda:** None. **S. Yamada:** None. **M. Nagakura:** None. **S. Sugimoto:** None. **T. Nagano:** None. **T. Okabe:** None. **H. Kojima:** None. **Y. Mizunoe:** None.

Poster Board Number:

FRIDAY-671

Publishing Title:

Decoupling of Alginate Polymerization from C-Di-GMP Population in *Pseudomonas aeruginosa*

Author Block:

Sh. Ghods, B.H.A. Rehm, **M. Moradali**; Massey Univ., Palmerston North, New Zealand

Abstract Body:

The exopolysaccharide, alginate, is a virulence factor produced by the ubiquitous opportunistic human pathogen *Pseudomonas aeruginosa*. Alg8 and Alg44 are inner membrane proteins and subunits of the multiprotein complex directly involved in alginate polymerization. Activation of polymerization is positively regulated through binding of the secondary messenger c-di-GMP to the cytoplasmic PilZ domain of membrane anchored Alg44. Here, site-specific mutagenesis informed by an *in silico* model of Alg8-$PilZ_{Alg44}$ was applied to analyze the role of individual amino acid residues in the activation mechanism. The three amino acid residues H323, T457 and E460 were identified as critical for Alg8 in order to respond to c-di-GMP mediated activation while replacement of either H323 or E460 led to c-di-GMP mediated inhibition. Alanine substitution of these residues led to decoupling of alginate synthesis from cellular level of c-di-GMP.

Author Disclosure Block:

M. Moradali: None.

Poster Board Number:

FRIDAY-672

Publishing Title:

Comparative Genetic Analysis of Biofilm Formation by *Pseudoalteromonas* from Deep-Sea Sediment versus from Surface Seawater

Author Block:

X. Cai , P. Wang, B. Li, **X. Wang**; South China Sea Inst. of Oceanology, GuangZhou, China

Abstract Body:

Biofilm is a common lifestyle of bacteria in aquatic ecosystem, including in the in nutrient-deprived deep-sea sediment. Whole genomic studies have revealed genetic features of deep sea bacteria. However, the features and molecular basis of biofilm formation in deep-sea sediment bacteria have rarely been characterized. Two closely related *Pseudoalteromonas* strains, *Pseudoalteromonas* sp. SM9913 (SM9913) derived from deep-sea sediment and *Pseudoalteromonas* sp. SCSIO 11900 (11900) derived from surface seawater, can form both pellicles at the liquid-air interface and submerged biofilms at the liquid-solid interface. They are compared at genomic and gene expression levels during biofilm formation. Compared to SM9913 planktonic cells, genes for growth, cell division and DNA repair were down-regulated during pellicle formation. The lateral flagellar gene cluster and chitinase genes, which located in genomic islands and presented only in SM9913 but not in 11900, were all up-regulated in SM9913 pellicle, suggesting that this strain is capable of utilizing horizontally acquired genes to gain advantages in nutrient acquisition on the deep-sea sediment surface in pellicle lifestyle. Additionally, genes encoding extracellular serine proteases and alkaline phosphatases were highly up-regulated in both strains during the initial stage pellicle formation. The enzymes encoded by *melA* and *hmgA* play key roles in the generation and degradation of homogentisic acid, which can further lead to the production of extracellular pyomelanin. *melA* was substantially induced during SM9913 pellicle formation, and its deletion resulted in a decrease in biofilm formation. In SM9913 pellicle formation, expression of *melA* at a higher level relative to *hmgA* continually, that led to excess homogentisic acid production. Conversely, in 11900, expression of *melA* was relatively lower compared to *hmgA* during the initial stage, and it was enhanced repression of *hmgA* transcription relative to *melA* in the mature stage that resulted in homogentisic acid accumulation. These results suggest that although the same set of genes are present in these bacteria residing in different niches (surface water *vs*. deep-sea), they can be differentially regulated at the RNA levels in response to stress.

Author Disclosure Block:

X. Wang: None.

Poster Board Number:

FRIDAY-673

Publishing Title:

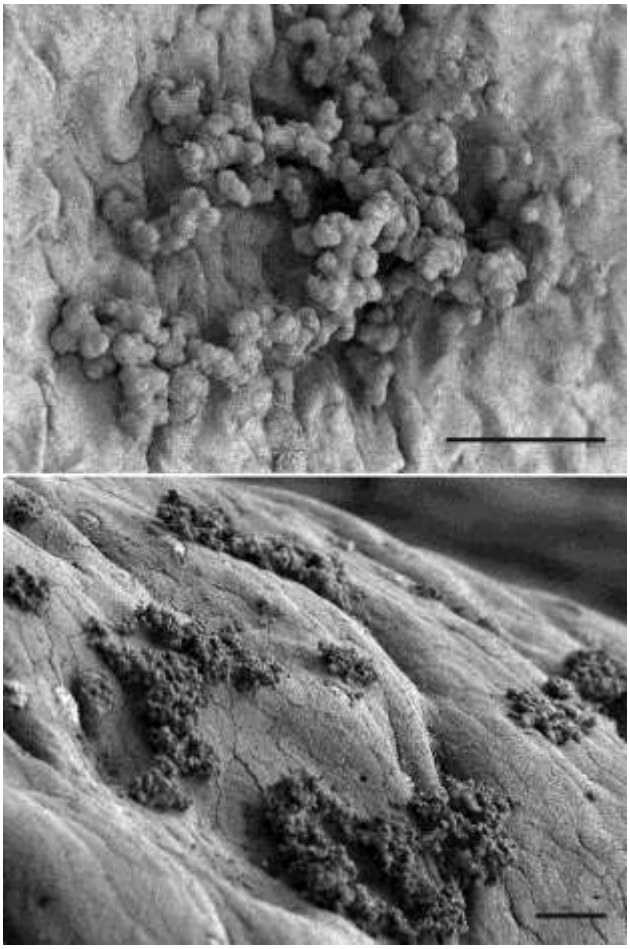
***Enterococcus faecalis* Biofilms Reveal Conservation of the Extracellular Matrix Morphology between Multiple *In Vitro* and *In Vivo* Animal Model Systems**

Author Block:

A. M. T. Barnes[1], K. L. Frank[2], J. L. Dale[1], Y. Chen[1], D. A. Manias[1], K. E. Greenwood Quaintance[3], P. C. Kashyap[3], R. Patel[3], G. M. Dunny[1]; [1]Univ. of Minnesota, Minneapolis, MN, [2]Uniformed Services Univ. of the Hlth.Sci., Bethesda, MD, [3]Mayo Clinic, Rochester, MN

Abstract Body:

Enterococcus faecalis biofilms are complex microbial communities associated with a surface and encased in a self-derived extracellular matrix (ECM). The matrix is a dynamic developmental structural and is compositionally complex, containing proteins, nucleic acids, and polysaccharides. Here we report work using several complementary light and electron microscopy techniques examining *E. faecalis* biofilm formation in three animal models: rat osteomyelitis, rabbit endocarditis (top), and a germ-free murine model of gut colonization (bottom). In all three models several unusual features were conserved: 1) discrete *E. faecalis* biofilm microcolonies were distributed over the host tissue without evidence of confluent growth days-to-weeks post-infection; 2) cells were covered in an ECM that is morphologically indistinguishable from that seen *in vitro*; 3) there was little to no evidence of an inflammatory host response in any of the *in vivo* model systems. In the rabbit endocarditis model, biofilm microcolonies were also notable for attachment to the host endothelium in the absence of preexisting gross damage, as well as an apparent lack of platelet involvement in initial microcolony development. Finally, pooled competition assays in the murine gut model demonstrated no correlation via sequencing of genes up- or down-regulated in the *in vivo* system and the *in vitro* CDC biofilm reactor highlighting the importance of evaluating mutant fitness in animal model systems. (Bars = 10 μm)



Author Disclosure Block:

A.M.T. Barnes: None. **K.L. Frank:** None. **J.L. Dale:** None. **Y. Chen:** None. **D.A. Manias:** None. **K.E. Greenwood Quaintance:** None. **P.C. Kashyap:** None. **R. Patel:** None. **G.M. Dunny:** None.

Poster Board Number:

FRIDAY-674

Publishing Title:

Characterization of the *Vibrio cholerae* Norspermidine-Preferential ABC-type Transporter PotABCD1

Author Block:

E. A. Villa[1], B. Sanders[2], R. Sobe[1], C. Wotanis[1], A. Rutkovsky[3], E. Karatan[1]; [1]Appalachian State Univ., Boone, NC, [2]Virginia Tech, Blacksburg, VA, [3]The Med. Univ. of South Carolina, Charleston, SC

Abstract Body:

Vibrio cholerae, an intestinal pathogen and the causative agent of cholera, is capable of prolonged persistence in aquatic environments in part through formation of biofilms. During biofilm formation, cells aggregate and become encased within a self-produced matrix. Biofilms can provide protection from environmental stressors and unfavorable conditions, and have been implicated in *V. cholerae* pathogenesis by providing protection from the acidity of the stomach. A variety of environmental signals are involved in the switch from the planktonic cell state into the biofilm. Polyamines, small, cationic hydrocarbons that are found in virtually all cells, are one such group of signals. The *V. cholerae* genome encodes a putative polyamine ABC-type transporter, PotABCD1. PotA is annotated as the ATPase, PotB and PotC as the transmembrane channel-forming proteins, and PotD1 as the periplasmic substrate-binding protein. Previously, our lab demonstrated a role of PotD1 in the uptake of the polyamines spermidine and norspermidine. In this work, we aimed to characterize the role of the remaining components of the transport system in polyamine uptake, and subsequent effects on biofilm formation. Through cellular polyamine extraction and HPLC analysis, we show that PotA, PotB, and PotC are all required for uptake of both norspermidine and spermidine, and that this system has a preference for norspermidine. Furthermore, deletion of any gene results in increased biofilm formation in a *Vibrio* polysaccharide-dependent manner. Finally, because bacterial genes involved in a common function are often found in operons, we confirm cotranscription of all *pot* genes. Together, our work indicates that the entire system must function as a whole to facilitate polyamine uptake, which then mediates the biofilm phenotype through an unidentified, VPS-dependent mechanism. This work not only establishes PotABCD1 as the first norspermidine transporter ever reported in any species, but also further elucidates the role of polyamines in *V. cholerae* biofilm formation.

Author Disclosure Block:

E.A. Villa: None. **B. Sanders:** None. **R. Sobe:** None. **C. Wotanis:** None. **A. Rutkovsky:** None. **E. Karatan:** None.

Poster Board Number:

FRIDAY-675

Publishing Title:

Implications Of Down Regulation Of Rcsa And Rcsa-Regulated Colanic Acid Biosynthesis Genes In Increased Acid Sensitivity And Enhanced Curli And Biofilm Production In Enterohemorrhagic *escherichia Coli* O157:H7

Author Block:

V. K. SHARMA; Natl. ANIMAL DISEASE CENTER, AMES, IA

Abstract Body:

Enterohemorrhagic *Escherichia coli* O157:H7 strain 86-24, originally linked to a disease outbreak in the western USA in 1982, exhibits acid resistance as indicated by its ability to survive exposure to acidic conditions (pH2.5) for several hours. The strain 86-24 is a poor biofilm producer which correlates with the production of low levels of curli fimbriae as assessed by Congo red binding assays, electron microscopy, and the levels of curlin (major structural protein of curli) expression using Western blotting. This parental strain 86-24, however, can produce variants that are highly acid sensitive but show increased biofilm formation and enhanced Congo red binding (CR^+), the latter correlating with increased curli expression. Transcriptomic analysis of the CR^+ variant revealed significantly reduced expression of genes associated with acid resistance, colanic acid production, but enhanced expression of genes encoding flagellar motility compared to the low Congo red binding (CR^-) parental strain. The expression of *rcsA*, which encodes a positive regulator (RcsA) of genes for colanic acid biosynthesis, was highly down regulated in the CR^+ variant. RcsA has been shown to serve as an auxiliary to the RcsB of the Rcs phosphorelay system in controlling colanic acid biosynthesis and expression of genes governing flagellar motility. In this study, we demonstrated that while *rcsA* was required for colanic acid biosynthesis and flagellar motility, findings that are in agreement with the other published reports, the *rcsA* gene did not negate the high levels of curli expression and biofilm formation in the CR^+ variant. RcsB reportedly regulates the expression of acid resistance in *E. coli* by interacting with GadE, which is the major regulator of genes required for the high level acid resistance. Whether RcsA has any role in controlling acid resistance through cooperation with RcsB or some other factors is not yet fully understood.

Author Disclosure Block:

V.K. Sharma: None.

Poster Board Number:

FRIDAY-676

Publishing Title:

The *Burkholderia thailandensis*-Encoded MarR Homolog, BifR, Is Redox-Sensitive And Represses Genes Related To Biofilm Formation

Author Block:

S. Fuentes, A. Gupta, A. Grove; Louisiana State Univ., Baton Rouge, LA

Abstract Body:

B. thailandensis locus *BTH_I0541-0542* encodes an EmrB family drug resistance transporter and a MarR family transcription factor named BifR. This operon is divergently oriented to *ecsC*. EcsC has been annotated in *Pseudomonas* as a LasA protease, but its function in *Burkholderia* is unknown. This gene locus is also conserved in *B. cenocepacia*, a serious pathogen of patients with cystic fibrosis (CF). The protease might likewise contribute to the success of *Burkholderia* in the lungs of patients with CF. *B. cenocepacia* forms biofilm, making it less sensitive to antibiotics. BifR has one cysteine per monomer, and SDS-PAGE analyses showed that it is oxidized by ROS. BifR exists as a dimer in the reduced state but as a dimer of dimer in the oxidized state, which is particularly efficiently induced by copper-mediated oxidation. DNA binding studies showed that BifR binds two adjacent palindromes in the *emrB-bifR* promoter. An excess of DNA separates BifR dimers such that one protein dimer binds to one DNA molecule, a binding mode in which protein oxidation is not observed. Mutation C104A significantly reduced binding affinity, yielding a K_d ten times higher than that of WT protein. This suggests that the mutation produces structural changes that are communicated to the DNA recognition helices. *In vivo,* BifR repressed both *ecsC* and *emrB* expression as evidenced by increased expression in a *bifRΔ* strain. This repression was better in the presence of H_2O_2. An *emrB-bifRΔ* gene disruption mutant showed increased biofilm formation when compared to wild type *B. thailandensis*, suggesting that EmrB exports a biofilm dispersal agent. Biofilm formation was also increased in *bifRΔ*, but comparatively less than in *emrB-bifRΔ.* We suggest that BifR links cellular redox state and biofilm formation by differentially repressing genes associated with biofilm formation in its reduced and oxidized states.

Author Disclosure Block:

S. Fuentes: None. **A. Gupta:** None. **A. Grove:** None.

Poster Board Number:

FRIDAY-677

Publishing Title:

Characterization of Key *H. pylori* Regulators Identifies a Role for Arsrs in Biofilm Formation

Author Block:

S. L. Servetas[1], B. M. Carpenter[1], J. J. Gilbreath[1], K. P. Haley[2], J. A. Gaddy[2], D. S. Merrell[1]; [1]Uniformed Services Univ. of the Hlth.Sci., Bethesda, MD, [2]Vanderbilt Univ., Nashville, TN

Abstract Body:

*Helicobacter pylor*i causes chronic infection of the gastric mucosa. In order to colonize and persist within this inhospitable niche, *H. pylori* must be able to rapidly respond to changing conditions. Despite this, *H. pylori* encodes few regulatory proteins. The well characterized regulators, Fur (ferric uptake regulator), NikR (nickel response regulator), and ArsR (the response regulator associated with the ArsRS acid response two component system), are paramount to the success of this pathogen. While numerous studies have examined these regulators individually, little is known about their combined effect. Thus, to study the interactions of these key regulatory proteins, a series of isogenic mutants was constructed in the commonly used lab strain, G27. This series includes single, double, and triple deletion strains of each regulator. Growth curve analysis under standard laboratory conditions revealed no outstanding growth defects of the strains. However, an interesting growth phenotype was noted in strains lacking ArsS; after 20 hours of growth, strains lacking ArsS formed large aggregates and a biofilm-like substance at the air liquid interface. Based on this observation, we further analyzed the biofilm-like phenotype in our isogenic mutant strains. In addition to the aforementioned collection, a non-phosphorylatable ArsR D52N mutant strain was also constructed. Phenotypic analysis using crystal violet assays and scanning electron microscopy showed that strains lacking ArsS or containing the ArsRD2N mutation formed significantly more biofilm than wild type G27. Furthermore, the ΔFur and ΔNikR single mutant strains appeared to form biofilms more slowly than wild type G27. Further characterization of the repertoire of regulatory mutants is ongoing and includes characterization of biofilm related properties such as adherence, auto-aggregation, and motility. Preliminary data suggest more pronounced aggregation and an increase in adherence in ΔArsS and ArsRD2N mutant strains as compared to wild type. Conversely, no overt differences in motility have been observed.

Author Disclosure Block:

S.L. Servetas: None. **B.M. Carpenter:** None. **J.J. Gilbreath:** None. **K.P. Haley:** None. **J.A. Gaddy:** None. **D.S. Merrell:** None.

Poster Board Number:

FRIDAY-678

Publishing Title:

Adaption Of *porphyromonas Gingivalis* To *streptococcus Gordonii* Observed Using Time-Coursed Rna-Seq

Author Block:

E. L. Hendrickson[1], R. Lamont[2], D. Miller[2], M. Hackett[1]; [1]Univ. of Washington, Seattle, WA, [2]Univ. of Louisville, Louisville, KY

Abstract Body:

The current view of dental infections is one where, while the proximal cause of the disease may be a pathogen such as *Porphyromonas gingivalis* (Pg), pathogenesis occurs in the context of a multi-species microbial community. Colonization of Pg involves attachment to the antecedent oral community inhabitant *Streptococcus gordonii* (Sg). A combination of Pg and Sg thus provides a simple model of potentially pathogenic oral biofilms. To explore the effect of Sg on Pg biofilms we used RNA sequencing to examine the transcriptome of Pg across the first six hours after shifting from planktonic to sessile in both the presence and absence of Sg. Two or three biological replicates were taken at 5, 30, 120, 240, and 360 minutes for each condition. Comparisons were made using DESeq2 to calculate *p*-values adjusting to *q*-values to account for multiple hypothesis testing. Significant differences were called at a *q*-value cutoff of 0.001 and had an absolute $\log_2$ fold difference of greater than 0.5. Most of the changes compared to planktonic controls were similar for both Pg alone and PgSg, increased levels for ribosomal protein, translational machinery, amino acid energy metabolism, and stress protein transcripts and decreased levels for polysaccharide biosynthesis. However, in the presence of Sg most of the changes were significantly closer to those observed for planktonic cells than for Pg alone. It appeared that the changes needed for Pg to shift to a sessile lifestyle, as would occur during the early stages of biofilm formation, were more extensive without Sg, implying significant support from Sg for Pg biofilms. One exception was oxidative stress protein transcripts. These showed higher increases with Sg presumably due to Sg peroxide production.

Author Disclosure Block:

E.L. Hendrickson: None. **R. Lamont:** None. **D. Miller:** None. **M. Hackett:** None.

Poster Board Number:

FRIDAY-679

Publishing Title:

The Role Of Quorum Sensing In Biofilm Formation Of The Antibiotic Resistant Gram-Negatives

Author Block:

S. Baho, S. Samarasinghe; De Montfort Univ., Leicester, United Kingdom

Abstract Body:

Background: Quorum sensing (QS) represents a mechanism for multicellular behavior in bacteria and known to regulate virulence and biofilm formation. Urinary tract infection (UTI) represents one of the most public health problems that affect women worldwide. The primary causing agent of this disease is *Escherichia coli* and, to a less extent, *Klebsiella.* Biofilm formation is one of the major health problems due to its difficulty to eradicate. Most studies focus on seeking for new antibiotics to treat biofilms but very little focus on the bacterial communication signals. In our work we are trying to find the relationship between biofilm formation and QS virulence factors genes. This work primarily has analyzed the susceptibility profiles and Biofilm formation ability of Gram-negative UTI causing pathogens. **Methods:** Double Disk Diffusion testing was used to confirm whether the strains are Extended Spectrum Beta Lactamases (ESBL) or Carbapenamases. PCR assay was used for confirmation that the strains has the antibiotic resistant gene. The amount of biofilm formation at different time points was determined using Biofilm Formation Assay (Tissue Culture Plate Assay; TCP). **Results:** Phenotypic characterization using Double Disk Diffusion testing confirmed that *K. pneumoniae* SHV-18, *E.coli* CTX-M-15 and *E.coli* TEM-3 are ESBL, and *K .pneumoniae* NDM-1, *K. pneumoniae* KPC-3 and *E.coli* IMP-type are carbapenemases. Whereas genotypic screening using PCR assay confirmed that *K. pneumoniae* OXA-48 belongs to carbapenemases, in addition for further confirmation of the above strains. Finally, TCP assay showed that amount of biofilm produced varies according to the bacterial growth stage at 6, 12, 24 and 48 hours of growth and culture condition (static or shaking). **Conclusions:** Our results demonstrated that quantity of biofilm produced is affected by the strain type, growth stage and culture conditions. We also demonstrated that double disk diffusion tests and molecular detection are useful for determining the ESBL and Carbapenemases strains. Further work is underway to determine the relationship between QS and biofilm formation and how they contribute to antibiotic resistance.

Author Disclosure Block:

S. Baho: None. **S. Samarasinghe:** None.

Poster Board Number:

FRIDAY-680

Publishing Title:

Genotypic And Biofilm Profiles Of *propionibacterium Acnes* Isolated From Pacemakers Without Clinical Signs Of Infection

Author Block:

K-i. Okuda[1], S. Yamada[1], S. Sugimoto[1], T. Iwase[1], M. Sato[2], C. Sato[2], Y. Mizunoe[1]; [1]The Jikei Univ. Sch. of Med., Tokyo, Japan, [2]Natl. Inst. of Advanced Industrial Sci. and Technology (AIST), Tsukuba, Japan

Abstract Body:

Background: Development of biofilms on medical devices often causes biofilm-associated infections. There is little information about biofilm formation on devices without clinical signs of infection. In this study, colonization of bacteria on the surfaces of cardiac pacemakers explanted from patients without clinical evidence of infection was consecutively analyzed. Additionally, genotypic and biofilm profiles of the isolated bacteria were investigated. **Methods and Results:** Blood agar plates were stamped with explanted pacemakers, and were subsequently incubated anaerobically at 37°C for 7 days. As a result, 8 of the 31 pacemakers were culture-positive. *Propionibacterium acnes* was isolated from 7 of the pacemakers and *Staphylococcus hominis* was isolated from 1 of the pacemakers. The *P. acnes* isolates were categorized into 5 different sequence types (STs) including ST2 (JK5.3 and JK6.2), ST4 (JK18.2), ST53 (JK17.1), ST69 (JK12.2 and JK13.1), and novel ST (JK19.3) by multi locus sequence typing. *In vitro* biofilm formation assay using microtiter plates revealed that all of the *P. acnes* isolates excepting JK6.2 form biofilms depending on glucose supplemented into the culture medium. Next, effects of enzymes on biofilm formation of the isolates were investigated. We used DNase I, RNase A, proteinase K, and dispersin B which degrade DNA, RNA, protein and poly-N-acetyl glucosamine, respectively. DNase I completely inhibited biofilm formation by all of the *P. acnes* isolates tested, on the other hand, susceptibilities to RNase A, proteinase K, and dispersin B were different among the isolates. High-resolution observation of nanostructures in the biofilms by transmission electron microscopy and atmospheric scanning electron microscopy visualized cytoplasmic components leakage along with cell lysis and fiber structures connecting cells. **Conclusions:** *P. acnes* was isolated from pacemakers without clinical signs of infection at high frequency (23%). Biofilm forming capacities of the *P. acnes* isolates and biochemical properties of the biofilms were different among strains regardless of the STs, however, extracellular DNA was suggested to be a factor commonly involved in biofilm formation of diverse strains of *P. acnes*.

Author Disclosure Block:

K. Okuda: None. **S. Yamada:** None. **S. Sugimoto:** None. **T. Iwase:** None. **M. Sato:** None. **C. Sato:** None. **Y. Mizunoe:** None.

Poster Board Number:

FRIDAY-681

Publishing Title:

Interaction Of Flagellin-Homologous Proteins With Eps And Its Effect On Biofilm Formation Of *vibrio Vulnificus*

Author Block:

Y-C. Jung, M-A. Lee, K-H. Lee; Sogang Univ., Seoul, Korea, Republic of

Abstract Body:

A pathogenic bacterium *Vibrio vulnificus* exhibits an ability to form biofilm, of which initiation is dependent upon swimming motility by virtue of a polar flagellum. The filament of its flagellum is composed of multiple flagellin proteins, FlaA, B, C, and D. The ORFs putatively encoding flagellin-homologous proteins (FHP) are also present in the *V. vulnificus* genomes. Although these FHPs are not involved in filament formation and cellular motility, they were found to be well-expressed and secreted to extracellular milieu through the secretion apparatus for the flagellins. In the extrapolymeric matrix (EPM) of *V. vulnificus* biofilms, significant amounts of FHPs were detected. The major components of its EPM are constituted with diverse extracellular polysaccharides, such as LPS, CPS, and EPS. Thus, the role of FHPs in biofilm formation was investigated using recombinant FHPs. Exogenous addition of FHPs to the FHP-defective mutants resulted in recovery of biofilm formation up to the wild-type level, and the added FHPs were predominantly incorporated in biofilm matrix. The same positive effect of FHP-addition on biofilm formation was apparent in biofilm formation by mutants defective in LPS or CPS. In contrast, biofilm formation by a mutant *V. vulnificus* defective in EPS biosynthesis was not influenced by addition of FHPs. These results raised a possibility that FHPs specifically interact with EPS within the biofilm matrix. To examine whether FHPs directly interact with EPS, EPS fractions were prepared and treated to the resins bound by recombinant FHPs or flagellin. Analyses of the eluted fractions of protein-conjugated resins showed the significantly more EPS was retrieved from the FHP-resins than the flagellin-resins. Taken together, this study demonstrates that FHPs are crucial in biofilm formation of *V. vulnificus*, via directly interacting with EPS that are known to be one of the major factors essential for biofilm formation.

Author Disclosure Block:

Y. Jung: None. **M. Lee:** None. **K. Lee:** None.

Poster Board Number:

FRIDAY-682

Publishing Title:

A Rodent-Specific Two-Component System Plays a Key Role in Biofilm Formation of *Lactobacillus reuteri* 100-23

Author Block:

R. M. DUAR, X. B. Lin, M. Bording-Jorgensen, E. Wine, M. G. Gänzle, J. Walter; Univ. of Alberta, Edmonton, AB, Canada

Abstract Body:

Lactobacillus reuteri is a gut symbiont of vertebrates that has diversified into host-specific phylogenetic lineages. The ability of *L. reuteri* to form biofilms in the murine forestomach is a key determinant of host-adaptation. Comparative genomic analysis identified a rodent-specific two component system (TCS) lr70529-30 (Frese et al. 2011). This project aimed to determine the ecological significance of lr70529-30 in gut colonization. For this purpose, isogenic deletion mutants of the histidine kinase (HK) lr70529 and the response regulator lr70530 were generated from a rodent isolate *L. reuteri* 100-23. Forestomach biofilms were quantified by confocal laser scanning microcopy (CLSM), measuring the pixel area in images where bacterial cells were stained with propidium iodide. Cell adherence to tissue culture dishes was determined by optical density and matrix morphology examined in Filmtracer™-stained biofilms visualized by CLMS. Disruption of the response regulator significantly impaired (p <0.0001) biofilm development *in vitro* and reduced biofilm by 50% in gnotobiotic mice. In contrast, disruption of the histidine kinase resulted in more robust biofilms (p = 0.001) than the ones formed by the wild type *in vitro,* without significantly influencing biofilm formation *in vivo.* Current experiments are ongoing to determine the mechanisms by which two proteins organized in the same TCS exert highly different effects on biofilm formation. Overall, our findings demonstrate that lr70529-30 TCS is involved in regulatory pathways driving biofilm formation in *L. reuteri* 100-23. Functional characterization of these pathways will contribute to our understanding of the molecular mechanisms underlying host-adaptation of rodent isolates and will offer new insights into the strategies governing symbiotic bacterial adaptation to the vertebrate gut.

Author Disclosure Block:

R.M. Duar: None. **X.B. Lin:** None. **M. Bording-Jorgensen:** None. **E. Wine:** None. **M.G. Gänzle:** None. **J. Walter:** None.

Poster Board Number:

FRIDAY-683

Publishing Title:

Regulation of EPS Production and Biofilm Formation by Synergistic Interation of DctD and IIA^{Glc}

Author Block:

S. Kang, H. Park, K-J. Lee, J-A. Kim, M-A. Lee, K-H. Lee; Sogang Univ., Seoul, Korea, Republic of

Abstract Body:

A previous study showed that the mutant strains of *Vibrio vulnificus* defective in the components of carbon-PTS exhibited decreased ability to form biofilms (Kim *et al*., 2007 Mol. Microbiol. 63:559) To further investigate the role of glucose-PTS in biofilm formation, a mutant defective in IIA^{Glc} (*crr* mutant) was examined in its ability to form biofilm. The mutant was severely impaired in biofilm formation due to minimal biosyntheses of exopolysaccharides (EPS), which are essentially required for the maturation of biofilm structure. In the absence of IIA^{Glc}, the expression of the gene clusters for EPS biosynthesis (EPS-clusters) were significantly decreased. To elucidate how the transcription of the EPS-clusters was regulated by IIA^{Glc}, a transcription factor was isolated from a pool of the proteins bound to the recombinant IIA^{Glc}. DctD, one of the sigma N-dependent transcription factors, was identified as an activator for the EPS-clusters' expression. Deletion of *dctD* gene in *V. vulnificus* resulted in reduced levels of EPS production and biofilm formation. The abilities of a *dctD*/*crr* double-mutant to produce EPS and to form biofilm were almost the same as those of single mutants, suggesting that IIA^{Glc} and DctD act together in inducing the EPS-clusters. Two forms of IIA^{Glc}, a phospho- and a dephospho-IIA^{Glc} (d-IIA^{Glc}), have been shown to interact with diverse proteins. Both *in vitro* and *in vivo* assays for protein-protein interaction showed that d-IIA^{Glc} was able to specifically bind DctD. *In silico* analysis of nucleotide sequences revealed the presence of the DctD-binding consensus sequence in the upstream regions of EPS-clusters. Binding affinity of DctD to these upstream regions was increased when d-IIA^{Glc} was included in the binding assay. *In vivo* expression of the EPS-clusters were induced when cells entered the mid-exponential phase, at which the cellular contents of IIA^{Glc} and DctD reached the maximal levels. Therefore, this study demonstrates that the EPS-clusters' transcription is highly induced by the d-IIA^{Glc}-DctD complex and then the resultant EPS accumulation facilitates the maturation of *V. vulnificus* biofilms.

Author Disclosure Block:

S. Kang: None. **H. Park:** None. **K. Lee:** None. **J. Kim:** None. **M. Lee:** None. **K. Lee:** None.

Poster Board Number:

FRIDAY-684

Publishing Title:

Bacterial Extracellular DNA Contributes to *Streptococcus mutans* Biofilm Formation on the Damaged Valve in the Experimental IE Rat Model

Author Block:

C-J. Jung, J-S. Chia; Coll. of Med., Natl. Taiwan Univ., Taipei, Taiwan

Abstract Body:

Background: Host factors like platelets enhance biofilm formation of oral commensal streptococci for inducing infective endocarditis (IE), but the bacterial components contribute directly to biofilm formation *in vivo* is still not clear. We demonstrated earlier that the autolysin Atl contributes to the colonization of *Streptococcus mutans* on injure heart valves in an experimental endocarditis rat model, but the role of Atl in the biofilm formation *in vivo* is unknown.**Methods and Result:** By using confocal laser scanning confocal analysis, we found that *atl*-defective mutant strain significantly reduced the ability to form biofilm *in situ* along with the loss of extracellular DNA (eDNA) embedded inside bacterial floes. In accordance with the maturation and autolysin activity of the Atl, the amount of eDNA released or biofilm formed by *S. mutans in vitro* was also enhanced dose-dependently by plasma and calcium ions and was significantly reduced in *atl*-deficient strain. In addition, an Atl-like protein (All) also modulates bacterial eDNA release contributing bacterial biofilm formation.**Conclusions:** Our data suggested that Atl and All-mediated eDNA release contributes directly to *S. mutans* biofilm formation *in vivo* to induce IE.

Author Disclosure Block:

C. Jung: None. **J. Chia:** None.

Poster Board Number:

FRIDAY-685

Publishing Title:

Identification of a Novel Regulator Controlling *Staphylococcus epidermidis* Biofilm Formation in the Presence of Serum

Author Block:

A. Both[1], C. Wiechmann[1], N. Hector[1], H. Büttner[1], M. Christner[1], C. Heinze[1], P. Valentin-Weigand[2], H. G. Rohde[1]; [1]Univ. Clinic of Hamburg-Eppendorf, Hamburg, Germany, [2]Univ. of Vet. Med. Hannover, Hannover, Germany

Abstract Body:

Background: *Staphylococcus epidermidis* is a leading cause of implant associated-infections, and biofilm formation is thought to significantly contribute to the species' success in this clinical setting. Previous work has shown that the presence of serum induces biofilm formation in some *S. epidermidis* strains. **Hypothesis:** The aim of this study was to characterize serum-induced *S. epidermidis* biofilm formation in more detail, and to identify genes contributing to biofilm assembly under this growth condition.**Results:** PIA-producing *S. epidermidis* reference strain 1457 formed strong biofilms in TSB as well as TSB containing 50 % serum. In contrast, *S. epidermidis* 1585 was biofilm-negative in TSB, but became biofilm-positive in the presence of serum. Screening of a Tn*917* transposon mutant bank derived from *S. epidermidis* 1585 identified four mutants exhibiting a biofilm-negative phenotype after growth in serum. Sequencing of Tn-flanking chromosomal regions showed that all mutants carried insertions within an open reading frame encoding for a so far uncharacterized transcriptional regulator, preliminarily referred to as serum response regulator (Srr). Growth in serum induced *srr* expression in *S. epidermidis* 1585, indicating that Srr is relevant for adaptation to this condition. *In trans* complementation of *srr* fully rescued the biofilm positive phenotype, unambiguously linking the biofilm-negative phenotype with inactivation of *srr*. Further analysis of 1585*srr*::Tn917 identified changes in AtlE- and Embp-production. Consistent with phenotypic assays, these changes indicate that *srr* is crucial for expression of mechanisms involved in adherence as well as intercellular adhesion and biofilm accumulation. **Conclusion:** This is the first systematic approach to identify genes relevant to serum-induced biofilm formation. *srr* is a novel regulator, influencing biofilm accumulation through pleiotropic effects on adherence properties and intercellular adhesion. In depth analysis of regulatory circuits and functional molecules depending on *srr* expression will provide important insights into mechanisms allowing *S. epidermidis* to adopt to hostile environments to cause device-related infections.

Author Disclosure Block:

A. Both: None. **C. Wiechmann:** None. **N. Hector:** None. **H. Büttner:** None. **M. Christner:** None. **C. Heinze:** None. **P. Valentin-Weigand:** None. **H.G. Rohde:** None.

Poster Board Number:

FRIDAY-686

Publishing Title:

Co-cultivation Models Reveal Xip to be an Intercellular Communication Molecule for *Streptococcus mutan*s

Author Block:

J. Kaspar, A. Reyes, R. A. Burne; Univ. of Florida, Gainesville, FL

Abstract Body:

Streptococcus mutans, the etiological agent of human dental caries, displays complex behaviors in response to short hydrophobic peptides, often called pheromones. Development of genetic competence is initiated via activation of transcription of an alternative sigma factor, encoded by *comX*, by the *comX*-inducing peptide (XIP) and the transcriptional regulator ComR. It has been proposed that XIP may function in an autocrine-like manner to control onset of competence, and not as a quorum sensing molecule. Here, we designed a co-culture system that utilizes a *comS*-overexpressing strain (*comS* encodes the precursor for XIP) that is marked with a dsRed fluorescent protein, in combination with an otherwise-wild-type strain of *S. mutans* carrying a *gfp* gene fused to the *comX* promoter (PcomX::*gfp*). The two strains were simultaneously inoculated in a co-culture biofilm system and *comX* expression was monitored over time using a Synergy 2 plate reader. In addition, at selected time points the biofilms were observed using confocal microscopy, or dispersed and subjected to analysis by flow cytometry to obtain quantitative data at the single cell level. Our results showed that the co-culture system could be used to measure and monitor XIP-dependent signaling in maturing biofilms and 1) there was evidence of co-operation and influences on fitness between the donor (*comS* overexpresser) and responder (PcomX::*gfp*); 2) differences in spatial organization of the donor and responder within the biofilm were evident; and 3) growth in sucrose, which allows for the production of an adhesive exopolysaccharide matrix, as opposed to glucose, had a profound influence on the distribution and proportion of responsive cells (GFP-positive). Notably, as the proportions of sucrose to glucose were increase, the positive feedback from intracellular XIP signaling (autoactivation of PcomX::*gfp*) increased. This study clearly demonstrates that XIP can signal between cells, but importantly it also highlights the unusual dynamics of XIP signaling within biofilm populations, both at the single cell and population levels, and how environmental factors can influence intercellular communication in a way that may modify the virulence and persistence of the organism.

Author Disclosure Block:

J. Kaspar: None. **A. Reyes:** None. **R.A. Burne:** None.

Poster Board Number:

FRIDAY-687

Publishing Title:

Arginine Treatment Re-engineers Oral Microbial Community *In Vitro* and Prevents Sucrose-induced Ph Drop

Author Block:

L. Cen[1], **M. Agnello**[1], J. S. McLean[2], X. He[1]; [1]Univ. of California, Los Angeles, Los Angeles, CA, [2]Univ. of Washington, Seattle, WA

Abstract Body:

Background: The human oral cavity is home to a complex microbial community. Dysbioses of the oral microbiota can lead to diseases such as dental caries and periodontitis. Dietary sucrose increases the risk of dental caries through induction of acid production by acidogenic bacteria, which in turn promotes demineralization of tooth enamel and further selects for acidogenic and aciduric bacterial species. Arginine has been shown to induce alkali production in oral bacteria, which may inhibit caries formation. The aim of the current study was to evaluate the response of an *ex vivo*-derived oral bacterial community to pre-treatment with arginine and subsequent sucrose exposure. **Methods:** *In vitro* oral microbial communities were generated by inoculating pooled saliva collected from 6 dental patients into specialized media developed in our laboratory (SHI media) that has been shown to preserve up to 80% of the species diversity present in the original inoculum. Cultures were grown anaerobically overnight, and subsequently grown as biofilms in 24-well plates in chemically-defined media at a starting pH of 5.5 or 7. Arginine (75mM) was added at time 0, followed by the addition of 0.5% sucrose after 0, 3, 6, 12, 20, 48, or 54 h. At each time point, pH was measured and biofilms were collected for 16S sequencing and GC-MS metabolomics analysis. Results were compared to media-only, sucrose-only, and arginine-only controls. **Results:** The addition of sucrose at time 0 led to a pH drop to 4.5 after 3h regardless of starting pH (5.5 or 7). pH did not drop in biofilms pre-incubated with arginine 6, 8, 12, 20 or 48 hours prior to the addition of sucrose (pH=8 at 48 h). 16S sequencing revealed a shift in the microbial community structure of biofilms pre-incubated with arginine, specifically an increase in arginine-metabolizing species and a decrease in acid-producing *S. mutans*. GC-MS analysis revealed an increase in arginine secondary metabolites by the arginine-treated biofilms, indicating active uptake and metabolism. **Conclusion:** Treatment with arginine re-engineers the oral microbial community by selecting for less acid-producing bacteria, leading to protection against the sucrose-induced pH drop seen in non-treated biofilms. This suggests that arginine may potentially be used as a method of caries prevention.

Author Disclosure Block:

L. Cen: None. **M. Agnello:** None. **J.S. McLean:** None. **X. He:** None.

Poster Board Number:

FRIDAY-688

Publishing Title:

The Role of Fatty Acids in Biofilm Formation by *Streptococcus mitis*

Author Block:

C. O'Brien, A. Higgins, D. Lenehan, P. Donovan, S. McCarthy, C. Ward, **J. Mitchell**; Univ. Coll. Dublin, Dublin, Ireland

Abstract Body:

Streptococcus mitis is a member of the normal human oral microflora. However it can make the transition from a harmless commensal to a pathogen and is a leading cause of infective endocarditis and bacteremia in immunocompromised individuals. The ability of *S. mitis* to form biofilms is a major virulence factor in the pathogenesis of these infections and may contribute to the resistance of *S. mitis* to many commonly used antibiotics. The aim of this study was to identify the factors, both genetic and environmental, that influence the ability of the organism to form biofilm. We firstly assessed the role of genes involved in temperate phage replication on the ability of *S. mitis* to form biofilm. These genes were not found to influence the biofilm forming capacity of *S. mitis* strains tested. Following this several growth conditions that *S. mitis* encounters in the oral environment were tested for their ability to influence the biofilm forming capacity of *S. mitis*. Growth of *S. mitis* in glucose promoted biofim formation, while NaCl, ethanolamine and ethanol all inhibited biofilm formation. Each of these growth conditions were found to have a distinct effect on autolysis of *S. mitis*.We then tested the impact of a panel of fatty acids for their ability to influence biofilm formation by *S. mitis*. Growth of *S. mitis* in the presence of eicosapentanoic acid (EPA) and docosahexanoic acid (DHA), which are found in fish oils, were found to have an inhibitory effect on biofilm formation whereas arachadonic acid promoted biofilm formation. The other fatty acids tested did not significantly impact biofilm formation. These results indicate that EPA and DHA represent a starting point to develop compounds to target biofilm formation and potentially infections by this increasingly antibiotic resistant pathogen.

Author Disclosure Block:

C. O'Brien: None. **A. Higgins:** None. **D. Lenehan:** None. **P. Donovan:** None. **S. McCarthy:** None. **C. Ward:** None. **J. Mitchell:** None.

Poster Board Number:

FRIDAY-689

Publishing Title:

Gyia Is an Sos- and Heat-Shock Induced DNA Gyrase Inhibitor in *Escherichia coli*

Author Block:

Yunxue Guo, Xiaoxiao Liu, T. Wood[1], **X. Wang**[2]; [1]Dept. of Chemical Engineering, University Park, PA, [2]South China Sea Inst. of Oceanology, Guangzhou, China

Abstract Body:

DNA gyrase introduces negative supercoils into DNA, coupling the reaction to ATP hydrolysis. DNA gyrase inhibitors induce relaxation of negative supercoiled DNA by inhibiting gyrase activity, and this relaxation is required for transcription, replication, repair and recombination. Here we demonstrate that chromosomally-encoded YfbC from *Escherichia coli* blocks the formation of a negative supercoiled plasmid DNA, and the activity of GyiA is enhanced by Mg^{2+} and it is ATP independent. Overproduction of GyiA causes extensive filamentous growth. GyiA interacts with the DNA gyrase subunit GyrA but not GyrB. We propose to rename YfbC as GyiA (Gyrase Inhibitor A). Additionally, deletion of *gyiA* greatly decreases cell viability during the SOS response and heat shock stress, and *gyiA* expression is induced upon the SOS response. Notably, different from the previously identified gyrase inhibitor gene *gyrI*, *gyiA* expression is not induced by the stationary phase sigma factor RpoS, but it is induced by heat shock sigma factor RpoH via direct binding to the promoter region. Furthermore, deletion of *gyiA* increased swimming motility and early biofilm formation, indicating an important role of GyiA during biofilm formation. Hence, we show GyiA is a DNA gyrase inhibitor in *E. coli* and it is beneficial to cells when they encounter SOS response and heat shock stress to which bacteria must adapt.

Author Disclosure Block:

T. Wood: None. **X. Wang:** None.

Poster Board Number:

FRIDAY-690

Publishing Title:

***Pseudomonas fluorescens* Integrates Environmental Information Across a Large C-Di-Gmp Network**

Author Block:

K. M. Dahlstrom[1], A. Collins[1], C. E. Harty[1], T. Gauvin[1], J. Rudd[1], C. Greene[2], G. A. O'Toole[1]; [1]Geisel Sch. of Med. at Dartmouth, Hanover, NH, [2]Univ. of Pennsylvania, Perelman Sch. of Med., Philadelphia, PA

Abstract Body:

Cyclic diguanylate (c-di-GMP) is a near-universal second messenger used by bacteria to control a large array of cellular decisions. Proteins called diguanylate cyclases (DGCs) produce c-di-GMP while phosphodiesterases (PDEs) degrade it, and any given bacterial species may contain up to several dozen DGCs and PDEs. To dissect the involvement of DGCs and PDEs in a given cellular process, previous studies have often focused on a single laboratory medium to assay the desired phenotype. While these efforts have associated many DGCs with cellular functions, they have left many more as orphan enzymes, without an obvious regulatory role. This raises two important questions: what is the role of these additional DGCs/PDEs, and how does the cell sort the signals from a large c-di-GMP network into meaningful outputs?To address these questions, our lab studies the c-di-GMP network in *Pseudomonas fluorescens*. *P. fluorescens* has several dozen potential DGCs, but only four were previously found to impact biofilm formation in our standard group medium. We hypothesized that when tested under other conditions, the function of more DGCs would be elucidated. To this end, we made deletion or insertion mutations of all known DGCs, PDEs, and c-di-GMP receptors of *P. fluorescens*. We utilized a systems-level approach to test each of our 53 mutant strains for biofilm formation in 192 carbon source conditions, examine transcript levels in a subset of these conditions, and test each member of the network for interaction with other members of the network using a bacterial two-hybrid assay. Our findings indicate that nearly half of DGCs act on biofilm formation through a series of nodes at the inner-membrane organized around effector proteins where physical interaction is utilized. Further, although several classes of organic molecules emerged as promoters or inhibitors of biofilm formation, few molecules of the same class utilized the same DGCs or PDEs indicating a surprising lack of redundancy among the dozens of enzymes. Collectively, our results support a model whereby bacteria may utilize a large c-di-GMP network in order to exquisitely regulate important life-style decisions, and that environmental context is a critical factor in organizing functional nodes of DGCs.

Author Disclosure Block:

K.M. Dahlstrom: None. **A. Collins:** None. **C.E. Harty:** None. **T. Gauvin:** None. **J. Rudd:** None. **C. Greene:** None. **G.A. O'Toole:** None.

Poster Board Number:

FRIDAY-691

Publishing Title:

The Role of Serine Metabolism in *Bacillus subtilis* Biofilm Formation

Author Block:

J. Greenwich, G. Di Cecco, Y. Chai; Northeastern Univserity, Boston, MA

Abstract Body:

Bacillus subtilis is a Gram-positive bacterium used to study biofilm formation. It has recently been shown that serine plays a role in biofilm formation in *B. subtilis*, but the exact mechanism has yet to be determined. Under serine starvation, translation of SinR, the master biofilm repressor, is decreased. The *sinR* gene has an above average number of TCN serine codons (N stands for A,G,T,C) and ribosomes have been shown to preferentially pause on TCN serine codons under serine starvation, relative to AGT and AGC codons. Small changes in SinR levels can have drastic effects on the genes it regulates, suggesting that this decreased translation rate may partially regulate the transition to biofilm formation.We hypothesize that *B. subtilis* is using serine as a sensor of its overall nutrient state, and that low serine levels indicate low nutrient availability, triggering biofilm formation. Because exogenous addition of serine to a culture delays biofilm formation, the next step was to assess the effect of manipulating intracellular serine levels. An increase in intracellular serine levels led to decreased biofilm formation. Overexpression of *serA*, the key structural gene in serine biosynthesis, as well as deletion of the two-protein serine dehydrogenase complex (encoded by *sdaAA/sdaAB*) reduces biofilm formation relative to wild type in biofilm-inducing media based on a transcriptional reporter for *yqxM*, the gene encoding a protein component of the biofilm.Additionally, tRNA isoacceptor levels may be important in this regulation. The genes for the serine tRNA isoacceptors are downregulated in stationary phase relative to exponential phase, based on qPCR analysis. Overexpression of *trnD-ser* does not alter biofilm formation in the wild type strain, but does reduce biofilm formation in a hyper-biofilm producing strain. This supports the hypothesis that increased tRNA isoacceptors allows for increased read through and translation of the *sinR* transcript, leading to increased protein synthesis of the master regulator of biofilm formation. Taken together, these results suggest that *B. subtilis* uses serine as a signal for its overall nutrient availability. This simple sensor allows bacteria to quickly adapt to their changing environments and provides new insights into bacterial physiology.

Author Disclosure Block:

J. Greenwich: None. **G. Di Cecco:** None. **Y. Chai:** None.

Poster Board Number:

FRIDAY-692

Publishing Title:

Designing of a Chimeric Protein for Specific Diagnosis of *Mycoplasma hominis* Infection

Author Block:

S. Saadat[1], R. Yousefi Mashouf[1], M. Y. Alikhani[1], **Z. Rikhtegaran Tehrani**[2]; [1]Hamadan Univ. of Med. Sci., Hamadan, Iran, Islamic Republic of, [2]Pasteur Inst. of Iran, Tehran, Iran, Islamic Republic of

Abstract Body:

Background: Molecular and serological diagnosis of heterogeneous and antigenic variable *Mycoplasma hominis*, a multi-potential pathogen, is usually a controversial issue. Designing of reliable recombinant antigens containing specific immunodominant epitopes selected from different proteins exposed on the bacterial membrane can be useful in immunoassay based methods. The aim of this study is prediction and rational selection of specific epitopes from p120 and p80 and designing of a chimeric protein based on a combinational algorithm. **Methods:** Antigenic epitopes was predicted by Immune Epitope Database and Analysis Resource using "Kolaskar and Tongaonkar Antigenicity Scale" and "Bepipred Linear Epitope Prediction" methods. The peptides with more than 80% similarity to species other than *M. hominis* were removed after BLASTP search at UniProt. The specific peptides were scored with weighted scoring based on different parameters including conservation, flexibility, hydrophilicity, length and position of epitope. The selected peptides were sorted and the specificity of the final protein was evaluated again. **Results:** Among 26 predicted epitopes, 11 peptides were nonspecific and excluded. The score of 15 specific peptides were calculated and eight of them with score of equal or more than 3.0 were selected. The final protein, with 247 amino acids, is composed of conserved, specific and highly antigenic epitopes. **Conclusions:** The inherent polymorphism of *M. hominis* makes its surface proteins unreliable targets for immunoassays. p120 is an abundant surface lipoprotein containing hyper-variable N-terminal region which is the target of human antibodies. p80, a membrane anchored protein, has an N-terminal cytoplasmic signal peptide and can be released after cleavage. The proposed algorithm used in this study increases the probability of finding of conserved, specific and immunoreactive epitopes within p120 and p80 proteins. Such peptides will increase the sensitivity and specificity of diagnosis methods based on immunoassay. We plan to experimentally evaluate the efficiency of this chimeric protein in diagnosis of individuals infected by *M. hominis*.

Author Disclosure Block:

S. Saadat: None. **R. Yousefi Mashouf:** None. **M.Y. Alikhani:** None. **Z. Rikhtegaran Tehrani:** None.

Poster Board Number:

FRIDAY-693

Publishing Title:

Computational Identification and Characterization of Promiscuous T Cell Epitope on Extracellular Protein 85B of *Mycobacterium* spp. for Peptide-Based Subunit Vaccine Design

Author Block:

M. Hossain, M. Wakayama; Ritsumeikan Univ., Kusatsu, Japan

Abstract Body:

Tuberculosis (TB) is a re-emerging disease that remains one of the leading causes of morbidity and mortality in humans. Bacille Calmette Guerin (BCG) vaccine for TB become complicated and in trouble more in TB endemic countries, particularly for pulmonary TB patients. We herein conducted an in silico study to identify and characterize a suitable T cell epitope, which might be an efficient target for epitope vaccine against Mycobacterium spp.We have assessed all the antigenic proteins of *Mycobacterium* spp recorded to date in Protegen database with Vaxign server and found that extracellular protein antigen 85B (Ag85B) was superior among all others. Analysis with NetMHCII 2.2 server and NetCTL 1.2 servers for identification of T cell epitope predicted an epitope 181-QQFIYAGSLSALLDP-195. These servers predicted that the epitope could bind with at least 13 major histocompatibility complex (8 MHC II and 5 MHC I molecules) and it revealed promiscuous nature of epitope. We increased the accuracy of our analysis by using multiple prediction servers for each DRB allele. Analysis with bioinformatics tools revealed that this epitope was highly stable. To validate the identification of the epitope with the computer aided programs, the *in silico* approaches were applied to identify the epitope on a positive control protein, an influenza viral antigen nucleoprotein peptides that has successfully used as peptide vaccine. We also analyzed HLA frequency of high burden TB endemic countries for the respective allele in the allele frequency database and found epitope has covered almost all TB endemic areas of populations. Finally, molecular docking simulation showed that epitope could bind with the binding groove of MHC II and MHC I molecules with numerous significant hydrogen bonds. Molecular docking analysis showed that the epitope had distinctive binding patterns to all DRB1, A and B series of MHC and presented almost no polymorphism in their binding site.Physico-chemical, alleles coverage and binding properties analyzed in this study revealed that the polypeptide '181-QQFIYAGSLSALLDP-195' is the novel epitope. This epitope is ~90% identical in all the non-identical Ag85B sequences available in the protein database. Therefore, Ag85B might be the universal candidate to produce efficient peptide or epitope- based vaccine for TB.

Author Disclosure Block:

M. Hossain: None. **M. Wakayama:** None.

Poster Board Number:

FRIDAY-694

Publishing Title:

Recognition of Supercoil Handedness by *Mycobacterium tuberculosis* Gyrase

Author Block:

R. Ashley[1], T. Blower[2], J. Berger[3], N. Osheroff[4]; [1]Vanderbilt Univ. Sch. of Med., Nashville, TN, [2]Durham Univ., Durham, United Kingdom, [3]Johns Hopkins Univ. Sch. of Med., Baltimore, MD, [4]VA Tennessee Valley Hlth.care System, Nashville, TN

Abstract Body:

The coiled structure of DNA creates numerous topological issues. Transcription and replication require the strands to be unwound, resulting in significant DNA overwinding ahead of the moving forks. Recombination and replication also create knots and tangles that must be removed for proper chromosome segregation. Type II topoisomerases resolve these issues by creating a transient double-strand break in DNA, passing another segment through the break, and religating the cut strands. The hallmark of this process is the cleavage complex, in which the cut DNA is covalently attached to the enzyme. Bacterial type II enzymes are the targets of quinolones, which are highly effective broad-spectrum antibacterials. *Mycobacterium tuberculosis* encodes one type II topoisomerase, gyrase. Gyrase is a unique enzyme that can actively underwind DNA to facilitate access to the genome. As quinolone use to treat tuberculosis increases, it is critical to understand how DNA topology affects gyrase activity and its interactions with these drugs. Thus, we investigated the ability of *M. tuberculosis* gyrase to distinguish between overwound (positively supercoiled) and underwound (negatively supercoiled) DNA. Gyrase maintained 3-4-fold lower levels of cleavage complexes on overwound DNA than it did on underwound DNA in the absence or presence of quinolones. However, the enzyme established cleavage-religation equilibria on both isoforms at equal times. Gyrase bound positively supercoiled DNA more tightly than it did negatively supercoiled molecules and could only form stable interactions with underwound DNA if a divalent cation was present. Finally, gyrase rapidly and processively removed positive supercoils to generate relaxed DNA, but introduced negative supercoils much more slowly and distributively. These results indicate that gyrase is uniquely adapted to work on positively supercoiled DNA. We propose that its critical function is to quickly and safely remove positive supercoils to maintain genome accessibility while minimizing the risk of permanent double-strand breaks.

Author Disclosure Block:

R. Ashley: None. **T. Blower:** None. **J. Berger:** None. **N. Osheroff:** None.

Poster Board Number:

FRIDAY-695

Publishing Title:

Analyzing Novel Components Of Developmentally-Associated Genome Segregation For Streptomyces Coelicolor

Author Block:

M. Hasipek, R. Dedrick, **J. R. McCormick**; Duquesne Univ., Pittsburgh, PA

Abstract Body:

There are five known components of the partition (*par)* system needed for developmentally-associated chromosome segregation in *Streptomyces coelicolor*: the centromere-like *parS* sites and ParA, ParB, ParJ and Scy. Chromosome segregation in bacteria generally involves a ParA ATPase and centromere-binding ParB complexes. The purpose of this study was to characterize the function of a ParA homologue ParH, investigate interactions between ParH with known segregation proteins and screen a genomic library to find novel interacting proteins.A *parH* null mutant was isolated and strains containing ParH-EGFP, and ParB-EGFP in a *parH* strain were constructed and analyzed by fluorescence microscopy. Site-directed mutagenesis of *parH* was used to change a conserved lysine residue (K99E) in the ATPase Walker A box. To determine if ParH interacts with known segregation proteins, an *E. coli* bacterial two-hybrid system was used. Plasmids carrying *parH*, *parH* variants, and target genes fused with T25 and T18 fragments of *cyaA* and were screened on MacConkey indicator plates. A random genomic library of *S. coelicolor* was constructed to screen for novel ParH interacting proteins using ParH as bait.In aerial hyphae of the Δ*parH* mutant, 4% of spores were anucleate compared to 24% for a *parA* null mutant. In predivisional aerial filaments, ParH-EGFP occasionally localized into a bright band or bands of fluorescence in apical compartments or as increased diffuse fluorescence toward the tip in these filaments. As judged by two hybrid analyses, ParH interacts with itself and ParB. However, the Walker A motif K99E mutation in ParH and N-terminal in-frame deletion in ParH impaired the two-hybrid interaction between ParH and ParB. No evidence was obtained to indicate there was a heterodimer interaction between ParH with ParA. By screening a random genomic library, the highly conserved actinobacterial signature protein HaaA (ParH and ParA-associated protein A) was found as a novel interacting partner of ParH. Interestingly, HaaA, also interacts with ParA in a bacterial two-hybrid system. This pattern of interaction was also conserved for homologs of *Mycobacterium smegmatis* and *Corynebacterium glutamicum.* Preliminary characterization indicates that a *haaA*-null mutant has no overt segregation defect, but exhibits a slight developmental defect.

Author Disclosure Block:

M. Hasipek: None. **R. Dedrick:** None. **J.R. McCormick:** None.

Poster Board Number:

FRIDAY-696

Publishing Title:

Elucidating the Role of Rhomboid Proteases in *Streptomyces coelicolor*

Author Block:

N. Carmona, R. Argawal, W. E. Nieves, L. Lara, M. Trujillo; Queensborough Community Coll., Bayside, NY

Abstract Body:

Background: Cell membranes are controlled borders with the outside world and serve as organizers of cell signaling, metabolic pathways and ultra-structure assembly. Proteases that reside within the cell membrane play an important role in regulating these events but it is still not clear how intramembrane proteases function. Rhomboids are intramembrane proteases found in all forms of life that have been implicated in human diseases. *Streptomycetes* are gram positive soil bacteria that produce secondary metabolites with biological activities. The production of these natural products relies on a cell signaling system, not fully understood, that integrates environmental, physiological and stress signals. We propose rhomboid proteases play a role in these signaling mechanisms.**Methods:** Bioinformatics tools were used to identify rhomboid genes in *Streptomycetes*. Reverse transcription showed that four rhomboid genes from *Streptomyces coelicolor*, the model organism for this genus, were transcribed under normal growth conditions. A *Providencia stuartii* rhomboid mutant (XD37.A) was complemented with rhomboid A (SCO3855) and D (SCO2139) from *S. coelicolor*. Furthermore, a SCO3855 knock out (KO) was constructed using CRISP technology. Additionally a plasmid with SCO3855 under the *erm* promoter was conjugated into *S. coelicolor*. The phenotypes of the constructs are being characterized. Currently, SCO3855- green fluorescent protein fusions are being constructed.**Results:** Five families of putative rhomboid genes were identified in *Streptomycetes*. SCO3855 fully complemented XD37.A demonstrating this is a functional rhomboid enzyme; it can cleave the substrate of aarA, the rhomboid enzyme from *P. stuartii*. SCO2139 only partially complemented XD37. A proving this enzyme has a different substrate. Preliminary results suggest that the deletion and/or overexpression of SCO3855 affects sporulation in *S. coelicolor*.**Conclusion:** Of the five families of rhomboid genes in *Streptomycetes*, two (A and D) are highly conserved. A and D play different biological roles as shown by the complementation experiments with XD37.A. Initial results from deletion and overexpression of SCO3855 support our hypothesis that rhomboids are involved in cell communication as it is well known that sporulation is influenced by external and internal signals.

Author Disclosure Block:

N. Carmona: None. **R. Argawal:** None. **W.E. Nieves:** None. **L. Lara:** None. **M. Trujillo:** None.

Poster Board Number:

FRIDAY-697

Publishing Title:

Biochemical Characterization of Two Aldoxime-forming, Flavin Dependent *n*-Hydroxylases Involved in Phosphonocystoximic Acid Biosynthesis

Author Block:

M. N. Goettge, J. P. Cioni, K-S. Ju, W. W. Metcalf; Univ. of Illinois Urbana-Champaign, Urbana, IL

Abstract Body:

Phosphonocystoximic acid (PnCys) and its hydroxylated congener (hPnCys) are two recently discovered natural products produced by *Streptomyces* sp. NRRL WC-3744 that contain an unusual oxime moiety. A related organism, *Streptomyces* sp. NRRL S-481 produces only PnCys. Bioinformatic analyses identified a novel FAD-NAD(P)H-dependent *N*-hydroxylase that we hypothesized would install this oxime moiety during biosynthesis. The putative flavin-dependent *N*-hydroxylase protein is found in both organisms and is significantly longer in length (750 vs. 450 amino acids) than known oxime-forming flavin-dependent *N*-hydroxylases. We previously showed that formation of hPnCys involves an α-ketoglutarate-dependent dioxygenase that converts 2-aminoethylphosphonic acid (AEPn) to 1-hydroxy-2-AEPn (HAEPn). Notably, this enzyme is absent from *Streptomyces sp.* S-481, which only produces PnCys. Thus, we expected that AEPn and HAEPn would be the substrates for the putative N-hydroxylases, while the enzyme from WC-3744 would show a preference for HAEPn and the enzyme from S-481 would preferentially oxidize 2AEPn. To test these hypotheses, both enzymes were purified after expression in *Escherichia coli*. UV-Vis spectroscopy was used to follow the consumption of the required nicotinamide cofactor, NADPH, allowing kinetic studies to identify the cofactor and substrate preferences of each enzyme. NMR spectroscopy and mass spectrometry were used to follow substrate consumption and product formation, allowing identification and assignment of the products, including *E* and *Z* aldoxime isomers and a nitro-containing product. Both enzymes catalyzed NADPH-dependent oxime formation with both AEPn and HAEPn as substrates, while the S-481 *N*-hydroxylase also showed activity with phosphonoalanine. As expected, the S-481 *N*-hydroxylase preferentially oxidized AEPn, while the preferred substrate for the WC-3744 *N*-hydroxylase is HAEPn, with k_{cat}/K_M values *ca.* 100 fold higher for the preferred substrate. Accordingly, these unique enzymes are members of a novel subclass of oxime-forming *N*-hydroxylases involved in the formation of the oxime moiety during PnCys biosynthesis.

Author Disclosure Block:

M.N. Goettge: None. **J.P. Cioni:** None. **K. Ju:** None. **W.W. Metcalf:** None.

Poster Board Number:

FRIDAY-698

Publishing Title:

Design and Application of Novel Multicatalytic Cellulases for Improved Microbial Degradation of Cellulosic Biomass

Author Block:

K. O. Duedu[1], C. E. French[2]; [1]Univ. of Hlth.& Allied Sci., Ho, Ghana, [2]Univ. of Edinburgh, Edinburgh, United Kingdom

Abstract Body:

Background: In naturally occurring cellulose degrading microbes, bioconversion is catalyzed by a battery of enzymes with different catalytic properties. We have reported construction and characterization of an endoglucanase-exoglucanase fusion protein (CxnA) which improves microbial degradation of cellulosic biomass.**Methods:** In order to investigate the capabilities of recombinant multimeric fusion proteins in improving cellulosic biomass conversion, we constructed six additional fusions of cellulases from *Cellulomonas fimi* (endoglucanases CenA, CenB and CenD, exoglucanase Cex, and β-glucosidase) and *Cytophaga hutchinsonii* (cellodextrinase CHU2268). The fusions were made using flexible proline-threonine linkers. Enzyme activity was tested by expressing fused and non-fused devices in *Escherichia coli*. All fusions retained catalytic activity of both parental enzymes. To investigate the benefits of fusion, Citrobacter freundii ATCC8090 was transformed with either fused or non-fused enzymes and cultured with cellulose blotting papers (Whatman® GB003, 0.8 mm, 300 gsm or Ford 428 mill 0.2 mm, 148gsm) as main carbon source.**Results:** Cells expressing the fusions of Cex with CenA or CenD reproducibly showed higher growth than cells expressing non-fused versions, as well as more rapid physical destruction of paper. The opposite was observed for the other combinations. A newly constructed Cex/CenA fusion (CxnA2) which contains two CBMs degraded the GB003 paper faster than the previously reported Cex/CenA fusion (CxnA1) which contains only one CBM and led to improved cell growth.**Conclusions:** Our results demonstrate that use of fusion proteins can improve biomass conversion in vivo, and could potentially substitute cloning of multiple enzymes and improve product yield. Our procedure for generating fusion proteins from existing libraries of constructs is simple and may be useful in other applications.

Author Disclosure Block:

K.O. Duedu: None. **C.E. French:** None.

Poster Board Number:

FRIDAY-699

Publishing Title:

Deciphering the Mechanism of Flavoprotein Mediated Tellurite Reduction

Author Block:

F. Arenas[1], M. Arenas[2], C. Muñoz[1], C. Vasquez[1], J. Vargas[1], F. Cornejo[1]; [1]Univ. de Santiago de Chile, Santiago, Chile, [2]Univ. de Talca, Talca, Chile

Abstract Body:

Tellurite is extremely toxic to most organisms at very low concentrations. However, tellurite can be enzymatically reduced to its elemental non-toxic form in a reaction coupled to NAD(P)H oxidation (Tellurite Reductase activity, TR), which is described as a mechanism of resistance. In the present work, different proteins with TR activity were evaluated *in silico* by using various databases of protein classification, showing that the majority belong to the "FAD/NAD(P) fold binding domain" (flavoprotein family), are related by the PFAM structural domains PF00070, PF02852 and PF07992, and all have cysteine residues at their active site. Bioinformatic analysis identified six *E. coli* flavoproteins (TrxB, AhpF, YkgC, GorA, E3 and NorW) which were characterized. All have NADH- and/or NADPH-dependent TR activity *in vitro*, an optimum pH of 8-9, a temperature of 42°C. The generation of tellurium-derived nanostructures from the *in vitro* and *in vivo* TR activity of these enzymes was evaluated. To date, the amino acids involved in reducing TeO_3^{-2} are unknown. Using E3 as a model, the importance of cysteine residues in these enzymes was tested by using specific inhibitors and site-directed mutation of C45A. The role of FAD was analyzed by removing and incubating E3 with FAD and by mutating residue E354K in the FAD binding domain. Finally, mutant H322Y was constructed, since this amino acid is directly involved in binding NADH. In all cases tested, TR activity was affected, allowing to infer that residues C45, H322 and E354 are key for the TR activity of these enzymes.

Author Disclosure Block:

F. Arenas: None. **M. Arenas:** None. **C. Muñoz:** None. **C. Vasquez:** None. **J. Vargas:** None. **F. Cornejo:** None.

Poster Board Number:

FRIDAY-700

Publishing Title:

Impact of Thioredoxin System Deletion Mutations on Sulfur Metabolism and Heavy Metal Reduction in Sulfate Reducing Bacteria

Author Block:

E. L. W. Majumder[1], G. M. Zane[1], T. R. Juba[1], K. B. De Leon[1], S. D. Smith[1], G. P. Butland[2], M. W. W. Adams[3], A. P. Arkin[2], P. D. Adams[2], J. D. Wall[1]; [1]Univ. of Missouri, Columbia, MO, [2]Lawrence Berkeley Natl. Lab, Berkeley, CA, [3]Univ. of Georgia, Athens, GA

Abstract Body:

Thioredoxins are small proteins that facilitate reduction of other proteins and small molecules in cells with a dithiol/disulfide exchange. Thioredoxins are thought to be ubiquitous in nature and have established roles in dealing with oxidative stress, redox signaling and supplying electrons for nucleotide synthesis. While well characterized in plants and animals, the role(s) of thioredoxins in anaerobes and particularly Sulfate Reducing Bacteria have not been well established. The sulfate reducing bacterium (SRB) *Desulfovibrio vulgaris* Hildenborough (DvH) is an obligate anaerobe that has the ability to reduce Uranium (VI) to Uranium (IV) and has been shown methylate mercury. DvH does not have glutathione, common in eukaryotes, and relies on thioredoxin as the main low molecular weight sulfide compound. A phylogenetic analysis showed that only 14 of 38 phyla of bacteria and archaea have glutathione, but all 38 have type I thioredoxin. DvH has two thioredoxins and four thioredoxin reductases in the genome. All six thioredoxin system genes are expressed *in vivo,* but with different fitness values. Recent work by Li and Krumholz (Ecotoxicology **23**(4): 726-733. 2014.) has implicated a non-canonical thioredoxin as necessary for the uranium reduction process. Five of the six single and multiple deletion mutants of the thioredoxins and thioredoxins reductases have been generated and are being characterized with growth curves and uranium reduction assays. We will also test these strains for oxygen sensitivity, effect of thioredoxin inhibitors and screen various electron acceptors and donors. Thioredoxin deletion mutants will also be generated in the *Desulfovibrio desulfuricans* ND132 strain capable of mercury methylation and be assayed for growth and methylation of mercury. Tagged copies of thioredoxins and thioredoxin reductases will be overexpressed on *Escherichia coli,* purified and assayed for direct interactions with metals, small molecules or other proteins. These results will begin to elucidate the function of the thioredoxin system in SRB.

Author Disclosure Block:

E.L.W. Majumder: None. **G.M. Zane:** None. **T.R. Juba:** None. **K.B. De Leon:** None. **S.D. Smith:** None. **G.P. Butland:** None. **M.W.W. Adams:** None. **A.P. Arkin:** None. **P.D. Adams:** None. **J.D. Wall:** None.

Poster Board Number:

FRIDAY-701

Publishing Title:

An Intestinal Bacterium Degrades Carnitine by a Novel Pathway That Avoids Generation of Known Precursors to Proatherosclerotic TMAO

Author Block:

D. J. Kountz, L. Zhang, J. Krzycki; The Ohio State Univ., Columbus, OH

Abstract Body:

Background: Microbial degradation of quaternary amines, such as L-carnitine and glycine betaine, generates trimethylamine (TMA) in the intestines. Once in the bloodstream, liver enzymes oxidize TMA to TMAO, which promotes atherosclerosis and increases risk of stroke, heart attack, and death. We recently described MtgB, a glycine betaine:corrinoid methyltransferase that permits *Desulfitobacterium hafniense* to produce N,N-dimethylglycine, rather than TMA, from glycine betaine. MtgB is a member of the largely uncharacterized TMA methyltransferase (MttB) superfamily, members of which are found in the genomes of multiple intestinal isolates. Important active site residues are not conserved across the MttB/MtgB superfamily, which raises the possibility of substrate diversity beyond betaine. **Methods and Results:** By performing BLASTp searches that used MtgB as a query, we identified a candidate organism, *Eubacterium limosum* ATCC 8486. This organism is the type strain of a species that is found in the intestines of approximately 7% of individuals with western diets. We found *E. limosum* demethylated carnitine to norcarnitine with the production of acetic, butyric and caproic acids. Cell extracts from carnitine-grown cells exhibited significant carnitine:cob(I)alamin and carnitine:tetrahydrofolate methyltransferase activities, suggesting involvement of a corrinoid methyltransferase in carnitine catabolism. Proteomic data indicated that genes encoding an MttB homolog, a methyl-tetrahydrofolate:corrinoid methyltransferase homolog, and two corrinoid proteins were upregulated during growth on carnitine compared to growth on lactate. The MttB homolog (designated MtcB) was shown to be a carnitine:cob(I)alamin methyltransferase while the MetH homolog (MtcA) demethylates methylcob(III)alamin to produce methyl-THF. This novel corrinoid dependent pathway thus fuels both oxidative and reductive branches of the Wood-Lundahl pathway for generation of acetyl-CoA. **Conclusions:** These data have revealed the first such pathway that is both directly and indirectly incapable of generating TMA, and raise the possibility that benign and potentially detrimental pathways may compete for quaternary amines in the human intestine.

Author Disclosure Block:

D.J. Kountz: None. **L. Zhang:** None. **J. Krzycki:** None.

Poster Board Number:

FRIDAY-702

Publishing Title:

Mutation Network Based Understanding of Pleiotropic and Epistatic Effects of Double Mutations on *Enterococcus faecalis* FMN-Dependent Azoreductase

Author Block:

H. Chen[1], J. Sun[1], O. Kweon[1], J. Jin[1], G-X. He[2], X. Li[2], C. E. Cerniglia[1]; [1]Natl. Ctr. for Toxicological Res./U.S.FDA, Jefferson, AR, [2]Univ. of Massachusetts Lowell, Lowell, MA

Abstract Body:

Background: AzoA from *E. faecalis*, a homodimeric FMN-dependent NADH-preferred azoreductase, cleaves the azo bonds (R-N=N-R) of diverse azo dyes. A recently reconstructed mutation network of AzoA has systematically explained the pleiotropic effects of the 13 residues on the functional interaction among the three substrates (FMN, NADH, methyl-red). The residues, Arg-21 and Asn-121 have been considered as mutational hotspots with positive pleiotropic effects. Here we report the experimental network-based pleiotropic and epistatic mutational effects of double mutations at positions Arg-21 and Asn-121 on AzoA from *E. faecalis*.**Methods:** Site-directed mutagenesis; recombinant protein expression and purification; azoreductase assays; network analysis.**Results:** In addition to the previous R21G and N121A, six more mutants were made and characterized: two single mutants (R21K and N121A) and four double mutants (R21G/N121A, R21G/N121Q, R21K/N121A, R21K/N121Q). Using the K_m^{MR}, K_m^{NADH}, FMN, and relative specific activity of the mutants, we reconstructed an AzoA-mutational network, showing a general correlation between enzyme activity and connection distance. Single mutation of Arg-21 and Asn-121 with Lys and Gln, respectively, yielded mutant enzymes with properties very similar to those of the wild-type enzyme; they shared a very similar network connection in shape and distance. Out of the 4 double mutants, the two double mutants R21G/N121A and R21G/N121Q showed enhanced enzyme activity, whereas the other (R21K/N121A and R21K/N121Q) showed decreased enzyme activity. R21G/N121A and R21G/N121Q show apparently different network connection shapes with a relatively short distance, indicating some striking kinetic differences from the wild-type enzyme and the double mutants with decreased activity.**Conclusions:** Mutational network-based analysis of the double mutations not only proves the position Arg-21 as a hotspot with positive mutational effects but also suggests that rational control of the epistatic mutational interaction between the two residues is necessary for productive enzyme engineering of AzoA.

Author Disclosure Block:

H. Chen: None. **J. Sun:** None. **O. Kweon:** None. **J. Jin:** None. **G. He:** None. **X. Li:** None. **C.E. Cerniglia:** None.

Poster Board Number:

FRIDAY-703

Publishing Title:

YbtT is a Virulence-associated Siderophore Biosynthetic Repair Enzyme in Uropathogenic *E. coli*

Author Block:

S. I. Ohlemacher, J. P. Henderson; Washington Univ. Sch. of Med., St. Louis, MO

Abstract Body:

Iron limitation is an important aspect of the host innate immune response. Consequently, pathogens often produce multiple siderophores, or small molecule Fe(III) chelators, to acquire this essential nutrient. Uropathogenic *E. coli* (UPEC) can produce up to four chemically distinct siderophores: yersiniabactin (Ybt), enterobactin (Ent), salmochelin (Sal), and aerobactin (Aero). Ybt is unique because it is a metallophore that interacts with iron and copper. Both Ybt and Ent/Sal are produced by nonribosomal peptide synthetases (NRPS), a class of multimodular enzymes that use phosphopantetheine (ppant) cofactors to covalently attach substrates and intermediates to the enzymes. With multiple siderophore pathways operating inside a single bacterial cell, it remains unclear how biosynthetic integrity is maintained when the starting substrates are chemically similar. One possibility is the existence of a proofreading enzyme. YbtT is a thioesterase encoded amongst the Ybt biosynthetic genes that has homology to editing enzymes from other NRPS systems. We constructed a knockout of *ybtT* in UPEC and used mass spectrometry to directly observe a 90% reduction in Ybt production. Therefore, we hypothesized that YbtT removes incorrect substrates from the Ybt biosynthetic machinery to promote siderophore production. To identify YbtT binding partners, we performed a pull down with UPEC lysate. YbtT pulled down the two major Ybt biosynthetic proteins, HMWP1 and HMWP2, and did not pull down other siderophore biosynthetic enzymes. We next used fluorescence polarization to examine protein-protein interactions between YbtT and individual domains of HMWP1 and HMWP2. These studies revealed the importance of the ppant posttranslational modification for YbtT binding to carrier protein domains. Finally, we demonstrated that YbtT is an active thioesterase that can cleave a range of acyl groups from N-acetylcysteamine (SNAC) substrates, which mimic the native ppant. Together, the results of this study suggest a mechanism where YbtT binds HMWP1 and HMWP2 carrier protein domains, with significant engagement of the ppant, and hydrolyzes covalently attached, non-native small molecules. This activity may prevent incorrect substrates from acting as suicide substrates during Ybt biosynthesis. The elucidation of this mechanism could lead to novel methods of inhibiting Ybt biosynthesis.

Author Disclosure Block:

S.I. Ohlemacher: None. **J.P. Henderson:** None.

Poster Board Number:

FRIDAY-704

Publishing Title:

Siderophore Biosynthesis as a Model System for Dissecting the Role of Mbth-like Proteins in Natural Product Biosynthesis

Author Block:

R. A. Schomer[1], H. Park[2], M. G. Thomas[1]; [1]Univ. of Wisconsin-Madison, Madison, WI, [2]Massachusetts Inst. of Technology, Cambridge, MA

Abstract Body:

Nonribosomal peptides (NRPs) are a structurally diverse class of natural products that comprise a significant portion of our current medicinal arsenal. In nature, microorganisms have evolved to use these molecules for a wide range of physiological roles from nutrient acquisition to virulence. NRPs are constructed by nonribosomal peptide synthetases (NRPSs) that use a mechanistically repetitive and modular enzymology. NRPS enzymology has been extensively studied, but until recently it was unappreciated that many NRPSs are dependent on the presence of an MbtH-like protein (MLP) for function and solubility. This incomplete understanding of the role of MLPs in NRP biosynthesis implies there is more to be learned about the basic enzymology of NRPSs. Further investigation of MLPs may lead to an understanding of how to disrupt their function, opening up the possibility of using MLPs as new drug targets. Structural and genetic studies indicate that MLPs do not have a catalytic site, leading to the hypothesis that a complex protein-protein interaction between the NRPS and MLP is required. Using enterobactin biosynthesis in *E. coli* as a model system, we have identified several residues in the cognate MLP, YbdZ, required for *in vivo* metabolite production. Analysis of YbdZ variants and other noncognate MLPs *in vitro* and *in vivo* will be presented here. These data have led to the generation of hypotheses for how MLPs interact with NRPSs and enable optimal NRPS enzymology. A detailed understanding of what dictates functional MLP/NRPS interaction is essential to the successful engineering of NRPs for medicinal or other applications.

Author Disclosure Block:

R.A. Schomer: None. **H. Park:** None. **M.G. Thomas:** None.

Poster Board Number:

FRIDAY-705

Publishing Title:

Trimeric Spa47 is an Active Type Three Secretion System (T3SS) ATPase from *Shigella flexneri*

Author Block:

J. L. Burgess[1], H. B. Jones[1], P. Kumar[2], R. T. Toth, IV[2], C. R. Middaugh[2], E. Antony[3], **N. E. Dickenson**[1]; [1]Utah State Univ., Logan, UT, [2]Univ. of Kansas, Lawrence, KS, [3]Marquette Univ., Milwaukee, WI

Abstract Body:

Many Gram-negative pathogens, including *Shigella* spp., use conserved type three secretion systems (T3SS) as key virulence factors. The *Shigella* T3SS relies on an associated needle-like type three secretion apparatus (T3SA) which penetrates the host cell membrane and provides a unidirectional conduit for injection of effectors into host cells. A great deal is now understood about the complex structure of the *Shigella* T3SA, however, the specific mechanisms of formation, secretion activation, and regulation remain unclear. Sequence homology of the *Shigella* protein Spa47 to other known T3SS ATPases and its location within the sorting platform of the T3SA suggest that perhaps it is an ATPase responsible for providing the energy for T3SA formation and secretion. We have recently overcome the long standing hurdle of producing active recombinant Spa47 and report here the details of the intein fusion based purification protocol along with the first direct evidence that Spa47 is in fact a *bona fide* ATPase. Biophysical characterization of the recombinant Spa47 identified multiple discrete oligomeric species with the highest order representing a unique Spa47 trimer exhibiting >10 fold higher ATP hydrolysis activity over the monomeric form. This increase in activity by the oligomer is consistent with observations for other T3SS ATPases, but represents an active trimeric intermediate that is either unique to *Shigella* or has yet to be observed in other systems. Additionally, access to active recombinant Spa47 permitted investigation of the influence on Spa47 oligomerization and activity by several factors, including active site Walker motif residues, the protein N-terminus, and key interactions with other T3SS proteins. Together, these results identify Spa47 as a *Shigella* T3SS ATPase and suggest that its activity is linked to oligomerization, perhaps as a regulatory mechanism controlled through interaction with chaperone proteins such as MxiN. The *in vitro* characterization of Spa47 structure and function described here provides a strong platform for additional studies dissecting its role in virulence and identifies an attractive target for much needed anti-infective agents against *Shigella* spp.

Author Disclosure Block:

J.L. Burgess: None. **H.B. Jones:** None. **P. Kumar:** None. **R.T. Toth:** None. **C.R. Middaugh:** None. **E. Antony:** None. **N.E. Dickenson:** None.

Poster Board Number:

FRIDAY-706

Publishing Title:

Ribosome-Dependent Growth Inhibition by YhaV in *Escherichia coli*

Author Block:

J-H. Park, W. Choi; Korea Res. Inst. of BioSci. and Biotechnology, Cheongju-si , Chungbuk, Korea, Republic of

Abstract Body:

Background: Most of bacteria have toxins which inhibits cell growth and may lead to cell death when overproduced. They are located in one operon with their cognate antitoxins and neutralized in normal growing cells. Antitoxins are readily degradable under stress conditions, leading cytotoxicity. mRNA interferases are one of the well-known toxins which cleave cellular mRNAs. They are classified into two different types based on their dependence on ribosome. Here, we showed that YhaV binds to ribosomal subunits and it does not show its toxicity without ribosome.**Methods:** YhaV was overexpressed in *E. coli* deletion strain which PrlF(YhaV's antitoxin) and YhaV genes are deleted. DNA, RNA and protein synthesis were compared with deletion strain by incorporating [35S]methionine, [3H]thymidine, [a-32P]UTP , respectively. Northern blot analysis was carried out in ompA, ompF and lpp genes with YhaV incubation. Ribosome profiles fractions were detected by Western blotting. For identifying the ribosome dependence of YhaV toxicity, it was incubated with CAT expression vector in both prokaryotic and eukaryotic *in vitro* translation system.**Results:** Protein synthesis was almost completely inhibited within 5min after YhaV induction while DNA and RNA synthesis were not blocked, suggesting that YhaV is a general inhibitor of all protein synthesis. When the cellular mRNAs of *E. coli* carrying pBAD-YhaV were analyzed at different time points after induction of YhaV, intact mRNAs were observed only at the 0-time point, suggesting that YhaV has endoribonuclease activity and inhibits protein synthesis by cleaving mRNA. When measured cell-free protein synthesis using an *E. coli* T7 S30 extract system, the synthesis of CAT fusion protein was almost completely inhibited by higher concentration of YhaV. However, in eukaryotic *in vitro* translation, the synthesis of luciferase protein did not show any changes unlike MazF_bs, which is ribosome-independent and its higher concentration showed complete inhibition in this system.**Conclusions:** This study indicates that YhaV binds to ribosomal subunits and inhibits protein synthesis depending on ribosome like RelE toxin, which is different from ribosome-independent mRNA interferase, such as MazF. Therefore, we showed that YhaV is a ribosome-dependent toxin in contrast to previous study that concluded YhaV as a ribosome-independent toxin.

Author Disclosure Block:

J. Park: None. **W. Choi:** None.

Poster Board Number:

FRIDAY-707

Publishing Title:

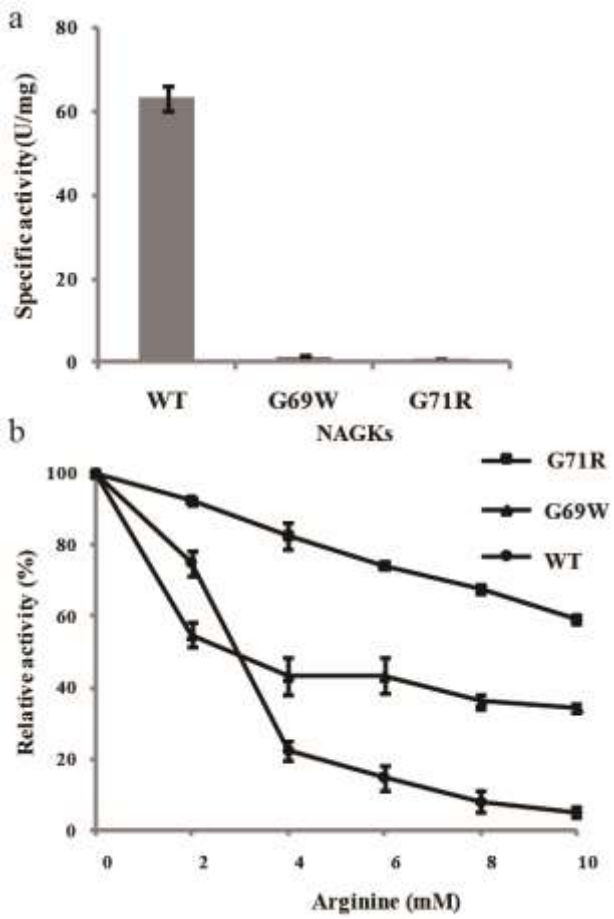
Mutational Analysis to Explore the Essential Residues for the Linkage Mechanism of the Remote Allosteric Regulation of NAGK

Author Block:

Y. Huang, Female, W. Ao, N. Zhao, S. Zheng; South China Univ. of Technology, Guangzhou, China

Abstract Body:

Background: *N*-acetyl glutamate kinase (NAGK) catalyzes the phosphorylation of the γ-COO^- group of *N*-acetyl-L-glutamate (NAG) by the γ-PO_4^{3-}group of ATP. The NAGK from *Corynebacterium glutamicum* (C.g NAGK) is allosterically inhibited by L-arginine, and is considered to be the key enzyme that regulates the production of L-arginine (Sakanyan et al. 1996). In pre-study, the docking results of the mutants indicated a linkage mechanism for the remote allosteric regulation of NAGK activity (Huang et al. 2015). In order to further explore the linkage mechanism, the amino acids in substrate binding region of NAG are mutated to explore the proposed signal transduction pathway of the feedback inhibition. **Methods:** The potential binding sites of substrates and the residues essential for the inhibition and catalysis are identified by homology modeling, molecular docking and site-directed mutagenesis. **Results**: The docking result shows that G69 and G71 directly interact with NAG and are closed to the β3-β4 hairpin. The mutants G71R and G69W have a severe impact on the catalytic activity, which only retain approximately 2.3% specific activity relative to that of wild-type CgNAGK (Fig. 1a). The wild-type CgNAGK, which is strongly inhibited by arginine, is half inhibited in 3.3 mM L-arginine. The mutants G69W and G71R can retain 34%, 59% activity in the presence of 10 mM L-arginine (Fig. 1b). Comparison the structure of G71R and G69W with the wild-type CgNAGK found that the β3-β4 hairpin is changed, which also indicate the residues G69 and G71 play an essential role in the signal transduction pathway of arginine inhibition. **Conclusions**: The amino acids G69 and G71 play an essential role in the catalysis of CgNAGK and the signal transduction pathway of the arginine inhibition.



Author Disclosure Block:

Y. Huang: None. **W. Ao:** None. **N. Zhao:** None. **S. Zheng:** None.

Poster Board Number:

FRIDAY-708

Publishing Title:

Structure-Function Analysis of Site-Specific Protease PRP: A New Target for Antibiotic Design in *Staphylococcus aureus* and Related Pathogens

Author Block:

E. A. Wall, A. L. Johnson, D. Peterson, G. E. Christie; Virginia Commonwealth Univ., Richmond, VA

Abstract Body:

Ribosomal protein L27 is a component of the eubacterial large ribosomal subunit that has been shown in *E. coli* to play a critical role in substrate stabilization during protein synthesis. In *Staphylococcus aureus* and other Firmicutes, L27 is synthesized with an N-terminal extension of nine amino acids that must be removed post-translationally to expose the conserved residues important for tRNA stabilization at the peptidyl transferase center. We identified the novel cysteine protease that performs this cleavage, Prp, and found that it represents a new clade of uncharacterized proteases, now designated as C108 in the MEROPS database. In this work, we demonstrate L27 cleavage to be essential in *S. aureus*; un-cleavable L27 could not complement an L27 deletion. A truncated variant of L27 beginning at the cleavage site also failed to complement, indicating an essential regulatory role for the cleavage event. L27 cleavage by Prp is thus a new target for development of antimicrobials against *S. aureus* and many related pathogens. We developed an assay based on the cleavage of a fluorogenic peptide derived from the conserved L27 motif, and found that the enzyme demonstrates tight binding and high substrate specificity. Molecular modeling and site-directed mutagenesis support a structural model in which rearrangement of a flexible loop upon binding of the peptide substrate is required for the active site to assume the proper conformation for catalysis. This research lays the foundation for the design of protease inhibitors that target this novel, essential pathway.

Author Disclosure Block:

E.A. Wall: None. **A.L. Johnson:** None. **D. Peterson:** None. **G.E. Christie:** None.

Poster Board Number:

FRIDAY-709

Publishing Title:

Detection of Small Molecule Effectors of Pyruvate Carboxylase via the Development of a Novel High-throughput Screen

Author Block:

B. Wyatt, M. St. Maurice; Marquette Univ., Milwaukee, WI

Abstract Body:

Pyruvate carboxylase (PC) is a metabolic enzyme that catalyzes the conversion of pyruvate to oxaloacetate (OAA)[1]. In most organisms, this is one of a few anaplerotic reactions that serve to replenish tricarboxylic acid cycle (TCA) intermediates[1]. Due to an incomplete TCA cycle in *Listeria monocytogenes,* PC is the exclusive anaplerotic input for generating OAA and has been shown to be vital for the bacterium's carbon metabolism and pathogenicity[2]. Despite the importance of PC in the central metabolism of most organisms, there are no known *specific* and *potent* small molecule regulators of PC available to study the enzyme[1]. We aim to find, develop, and characterize novel small molecule effectors of PC that will aid in understanding the structure, mechanism, and regulation of PC and that will ultimately lead to new chemical probes to study PC in its cellular context. A novel, fixed-time assay has been developed based on the reaction of OAA with Fast Violet B, which produces a colored complex with an absorbance maximum at 530 nm[3]. Results indicate that the fixed time assay is reproducible, sensitive and responsive to known effectors of *Rhizobium etli* PC, *Staphylococcus aureus* PC, *and L. monocytogenes* PC and has a strong potential to be amenable to high-throughput screening. Assays were optimized in a 96-well format and consist of three basic steps: 1) a reaction of PC with substrates 2) enzyme inactivation with EDTA, and 3) incubation with FVB prior to measuring the absorbance at 530 nm. Preliminary validation demonstrates that the assay is responsive to weak, nonspecific effectors of PC in a dose-dependent manner. Acetyl-CoA, an allosteric activator, increases PC activity and oxalate, a competitive inhibitor, decreases PC activity with excellent signal to background windows. Statistical measures indicate that this is an excellent assay for high-throughput screening with *Z*-factors of 0.85 for the activation assay and 0.87 for the inhibition assay. The assay has been validated with a plate uniformity assessment test and was used in an initial pilot screen to test the assay's response to 1,280 compounds. The pilot screen results indicate that the assay has the strong potential to find novel small molecule effectors of PC that can be developed into valuable research tools to study the enzyme in its cellular context and provide potential novel antimicrobial agents targeted against bacterial PC.

Author Disclosure Block:

B. Wyatt: None. **M. St. Maurice:** None.

Poster Board Number:

FRIDAY-710

Publishing Title:

Characterization of Dipeptidyl-Peptidase 4 from *Tannerella forsythia*

Author Block:

M. Nakasato[1], Y. Shimoyama[1], Y. Ohara-Nemoto[2], T. K. Nemoto[2], D. Sasaki[1], T. Yaegashi[1], S. Kimura[1]; [1]Iwate Med. Univ. Sch. Dent., Morioka, Japan, [2]Nagasaki Univ., Nagasaki, Japan

Abstract Body:

Tannerella forsythia is a Gram-negative anaerobic rod, and is a member of 'red complex species' the major causative organisms of chronic periodontitis in human. Since *T. forsythia* as well as *Porphyromonas gingivalis* is asaccharolytic, the proteolytic enzyme(s) including dipeptidyl-peptidases (DPPs) could be crucial for the growth. In this study, we determined the properties of DPP4 hydrolyzing the Pro^2-Xaa^3 bond from *T. forsythia* (TfDPP4) and compared them with those of *P. gingivalis* DPP4 (PgDPP4). The cell suspension of *T. forsythia* as well as *P. gingivalis* markedly hydrolyzed Gly-Pro-4-methylcoumaryl-7-amide (MCA), suggesting that *T. forsythia* possesses cell-associated functional DPP4 as PgDPP4 does. The optimal pH range for the cell-associated TfDPP4 was 7.5 to 8.0. In addition, the cell-associated TfDPP4 activity was decreased with NaCl in a concentration-dependent manner. TfDPP4 was composed of 722 amino acids with molecular masses of 81875 Da with 61.1% amino acid sequence homology to PgDPP4. The enzymatic properties of recombinant TfDPP4 (rTfDPP4) expressed in *Escherichia coli* were also compared to rPgDPP4. The rTfDPP4 preferentially hydrolyzed Gly-Pro-MCA and the DPP4 activity was comparable to that of rPgDPP4. The rTfDPP4 has an isoelectric point of 6.09 that was lower than that of rPgDPP4 by 1 unit, and showed the substrate specificity, and pH- and salt-dependences similar to rPgDPP4. In consequence, the present findings indicated that *T. forsythia* could possess functional DPP4 on the cell surface with very similar enzymatic properties to PgDPP4, which could be involved in the pathogenicity of *T. forsythia*, leading to the onset of human periodontitis.

Author Disclosure Block:

M. Nakasato: None. **Y. Shimoyama:** None. **Y. Ohara-Nemoto:** None. **T.K. Nemoto:** None. **D. Sasaki:** None. **T. Yaegashi:** None. **S. Kimura:** None.

Poster Board Number:

FRIDAY-711

Publishing Title:

A Mechanism of Glucose Tolerance of GH1 β-Glucosidases

Author Block:

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Abstract Body:

Background: β-Glucosidases are enzymes that hydrolyze β-glycosidic bonds to release non-reducing terminal glucosyl residues from glycosides and oligosaccharides, and thus have significant application potential in industries. However, most β-glucosidases are feedback inhibited by the glucose product, which restricts their application. Remarkably, some β-glucosidases of the glycoside hydrolase (GH) 1 family are tolerant to or even stimulated by glucose. The mechanisms of glucose tolerance and stimulation of the GH1 β-glucosidases is unclear yet. We supposed there might be other glucose binding sites and affecting mechanisms other than the active site and competitive inhibition. **Methods:** In this study, by comparing the primary and tertiary structures of two GH1 beta -glucosidases with distinct glucose dependence, some sites were mutated to investigate their relevance to the glucose effects, and the mutants were biochemically and structurally characterized to reveal the affecting mechanism of the sites. **Results:** Some mutants of a few sites at the entrance and middle of the substrate channel showed altered glucose dependence. Enzymatic kinetics analysis and ligand binding simulation of the wild-type proteins and mutants suggested that the geometry and relative binding affinity/preference of these sites to glucose modulates the glucose dependence. **Conclusions:** A mechanism was therefore proposed to interpret the glucose dependence of GH1 β-glucosidases : (1) Besides binding to the active site, glucose can bind to other sites along the substrate channel with varying affinities. (2) Glucose will inhibit the enzyme activity when it preferentially binds to the active site, while it will be tolerated when it prefers to bind to other sites. (3) Glucose bound to certain sites will enhance substrate cleavage activity through transglycosylation.

Author Disclosure Block:

Y. Xiao: None. **X. Zhang:** None. **Z. Fang:** None. **W. Yi:** None.

Poster Board Number:

FRIDAY-712

Publishing Title:

***Francisella tularensis* Ybex: Characterization of Homodimerization**

Author Block:

A. M. Hernandez, G. J. Nau; Alpert Sch. of Med. at Brown Univ., Providence, RI

Abstract Body:

Background: *Francisella tularensis*, the pathogen responsible for tularemia, has been classified as a category A biodefense agent by the CDC. *F. tularensis* is a facultative intracellular pathogen that blocks the proinflammatory response after invading macrophages, ultimately preventing the production of cytokines and chemokines. We have previously shown that FTL_0883/FTT_0615c is a *F. tularensis* gene important for virulence and evasion of the host immune response. Little is known about the function of YbeX. A crystal structure for YbeX has been recently generated in which two YbeX molecules are predicted to homodimerize. We investigated this the homodimerization as a means to understand YbeX's function, as well as its role in the pathogenesis of tularemia.**Methods:** The coding sequence of *ybeX* was subcloned into mammalian expression vectors to perform bimolecular fluorescence complementation (BiFC).**Results:** We are the first to show the homodimerization of YbeX using BiFC, confirming the crystal structure prediction. We created constructs expressing full length YbeX fused to fragments of the Venus fluorescent protein, and cotransfected them into 293Tcells. We observed fluorescence when YbeX-VN and YbeX-VC were cotransfected. We have also shown that the position of YbeX relative to the Venus fragment will determine the intensity of the fluorescence, with the most fluorescence signal observed when both subunits were fused to the amino termini of the Venus fragments. We have also shown that deletion of predicted YbeX domains decreases the function of the protein in bacteria, which is predicted to have an effect on YbeX homodimerization.**Conclusion:** YbeX homodimerization occurs within mammalian cells and may function as a molecular sensory of the host cell cytosol.

Author Disclosure Block:

A.M. Hernandez: None. **G.J. Nau:** None.

Poster Board Number:

FRIDAY-713

Publishing Title:

Consequences of Neocentromere Formation In *Candida albicans* on Chromosome Segregation Accuracy and Drug Sensitivity

Author Block:

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Abstract Body:

Genome stability requires accurate chromosome segregation via assembly of a functional kinetochore at the centromere. Defects in chromosome segregation accuracy can cause DNA damage and chromosome rearrangements as well as aneuploidy, an imbalance in the numbers of individual chromosomes. In cancer cells and fungal pathogens, aneuploidy is linked with the development of drug resistance. However, high levels of genome instability can also sensitize cells to certain drugs and environmental stresses. Here, we used *Candida albicans*, fungi with small, epigenetically-inherited centromeres, as a model system to study how alteration of centromere position affected chromosome segregation accuracy and sensitivity to several classes of chemotherapy drugs and antifungal drugs. We constructed multiple strains where the native centromere was deleted and a neocentromere, or new centromere, assembled at different locus on chromosome 5. We then measured chromosome loss rates using a counter-selectable marker (*URA3*) and SNP-RFLP to confirm homozygosis of the remaining chromosome. All characterized neocentromeres mediated chromosome segregation, but some loci had up to 50-fold higher chromosome loss rates than others. For some stress treatments, there was no difference in the response of strains with native centromeres and neocentromeres. Elevated temperature and treatment with nicotinamide, an inhibitor of histone deacetylase activity, increased chromosome loss rates in all strains tested. Interestingly, treatment with radicicol, an inhibitor of Hsp90, had a greater effect on chromosome loss rates in the tested neocentromere strain compared to the control strain. We are currently expanding these studies to additional neocentromere strains and to additional antifungal and chemotherapy drugs to determine if strains with elevated chromosome loss rates are sensitized to drug treatment and/or if they differ in the frequency of drug resistant isolates.

Author Disclosure Block:

L.S. Burrack: None. **A. Plemmons:** None. **A. Saha:** None. **B. Turman:** None. **J. Berman:** None.

Poster Board Number:

FRIDAY-714

Publishing Title:

Two Mitochondrial Matrix Proteases Act Sequentially in the Processing of Mitochondrial Peroxiredoxin Prx1p in *Saccharomyces cerevisiae*

Author Block:

F. Gomes, L. Netto; Univ. of São Paulo, São Paulo, Brazil

Abstract Body:

Mitochondrial peroxiredoxin (Prx1p) from *Saccharomyces cerevisiae* is a thiol-dependent peroxidase that catalyzes the reduction of mitochondrial hydrogen peroxide. Prx1p is synthesized in the cytosol as a precursor with a cleavable N-terminal targeting signal (presequence) and subsequently imported into mitochondrial matrix. The importing process of Prx1p into mitochondrial matrix involves two sequential cleavages catalyzed by mitochondrial processing peptidase (MPP), which removes the presequence, and octapeptidyl aminopeptidase 1 (Oct1p), which removes an octapeptide from the N-terminus of the precursor intermediate generated by MPP. The function of this second cleavage step is still poorly understood, but there is evidence that could be important to the stability protein according to the N-end rule of protein degradation. Thus, this study aimed assesses the role of the Prx1p cleavage by Oct1p toward protein stability and activity. To monitor Prx1p stability, we perform *western blot* analysis using isolated mitochondria from WT and *ΔOCT1* yeast cells incubated over an increasing period of time. We observed a faster degradation of the Prx1p in *ΔOCT1* mitochondria compared with mitochondria from WT strain. The *OCT1* reexpression under its endogenous promoter and terminator in the *ΔOCT1* background recovered the Prx1p degradation rate for levels similar as WT strain, indicating that only absence the Oct1p is responsible for faster degradation of Prx1p. However, the Prx1p cleavage by Oct1p does not significantly alter the peroxidase activity of Prx1p *in vitro*, as assessed by NADPH consumption assays using the recombinant proteins of Prx1p (representing the forms cleaved and not-cleaved by Oct1p). Our results show that processing of Prx1p-intermediate pre-protein by Oct1p leads to a stabilization of Prx1p after import into the mitochondria. This constitutes a protein quality control system that regulates Prx1p homeostasis and probably mitochondrial redox processes.

Author Disclosure Block:

F. Gomes: None. **L. Netto:** None.

Poster Board Number:

FRIDAY-715

Publishing Title:

Structural, Biochemical Characterization, and Evolutionary Origin of Alcohol/Aldehyde Dehydrogenases (Adhe) in *Entamoeba* Varieties

Author Block:

J. Leitao, A. Espinosa; Roger Williams Univ., Bristol, RI

Abstract Body:

Bifunctional alcohol/aldehyde dehydrogenase (ADHE) enzymes are essential for anaerobic respiration in protists and bacteria (i.e. *Entamoeba histolytica*, *Escherichia coli*, group-A-*Streptococcus*, *Pasteurella multocida*, *Clostridium perfingens*, *Clostridium difficile*). Structural and biochemical analysis of ADHE enzymes would contribute to the understanding of their evolution in prokaryotic and eukaryotic lineages. We have previously characterized the bifunctional *Entamoeba histolytica* alcohol/aldehyde dehydrogenase (EhADH2). Since EhADH2 is required for amoebic survival, it is an ideal target for the search of antiamoebic alternatives. Here we report significant features of the ADHE protein structure and function, which was isolated, cloned, and expressed from six *Entamoeba* varieties (*E. dispar*, *E. invadens*-IP-1, *E. invadens* VK, *E. moshkovskii* Snake, *E. moshkovskii* Laredo, and *E. terrapinae*). Alcohol and aldehyde dehydrogenase activities from each ADHE enzyme were analyzed. Six similar molecular weight ADHE proteins (2.7 kDa) were isolated, sequenced to determine the amino acid structure, and demonstrated consistency of evolutionary convergence within ADHE enzymes, which allowed *Entamoeba* varieties to adapt and survive under different environmental conditions. Considering that the evolutionary origin of the family of ADHE enzymes appears to be from diverse lineages, the purpose of this study is to examine the kinetic properties and efficiency of *Entamoeba* ADHE proteins. Concurrently, inhibitory studies using pyrazoline derivatives on *Entamoeba* growth by targeting its EhADH2 enzyme are pursued in our laboratory as a potential anti-amoebic strategy.

Author Disclosure Block:

J. Leitao: None. **A. Espinosa:** None.

Poster Board Number:

FRIDAY-716

Publishing Title:

Characterization of the Unique Phosphofructokinase in Malaria

Author Block:

A. Y. Gandhi, A. M. Guggisberg, A. R. Odom; Washington Univ. Sch. of Med., Saint Louis, MO

Abstract Body:

The methylerythritol phosphate (MEP) pathway for isoprenoid biosynthesis is a promising drug target for *Plasmodium falciparum*, the causative agent of severe malaria. Through a forward genetics approach, we identified that the enzyme PfHAD2 regulates isoprenoid biosynthesis in malaria by limiting the availability of metabolites to the MEP pathway. Laboratory strains containing a loss-of-function mutation at the *PfHAD2* locus (PfHAD2^{R157X}) demonstrate increased resistance to fosmidomycin (FSM), a specific inhibitor of the MEP pathway, as well as increased steady-state levels of MEP pathway metabolites. Furthermore, FSM sensitivity and MEP pathway metabolite levels are restored in PfHAD2^{R157X} strains possessing hypomorphic alleles of the unique malarial phosphofructokinase *PfPFK9* (PfPFK9^{T1206I}). Sequence similarity analysis groups PfPFK9 in a poorly characterized family of plant-like pyrophosphate-dependent phosphofructokinases (PP_i-PFKs).We hypothesized that PfHAD2 regulates substrate availability to the MEP pathway by controlling the availability of allosteric regulators to phosphofructokinase, the rate-limiting step of glycolysis. In accordance, we identified that purified recombinant PfHAD2 dephosphorylates the purine nucleotides *in vitro*, with highest specificity towards the glycolytic effectors AMP and GMP. To begin evaluating the allosteric regulation of PfPFK9, we used commercially available reagents to implement an assay that links phosphofructokinase activity to the loss of NADH (ε_{340} = 6220 M^{-1} cm^{-1}). We are currently using this assay to measure the specific PfPFK9 activity from wild type, PfHAD2^{R157X}, and PfHAD2^{R157X}/PfPFK9^{T1206I} strains in the presence and absence of AMP and GMP.This approach will identify if a direct regulatory link exists between PfHAD2, glycolysis, and isoprenoid biosynthesis in malaria. Since malaria glycolytic enzymes are distinct from the analogous host enzymes, such a relationship would suggest that the extensive libraries of PP_i-PFK inhibitors already validated in other parasitic protozoa (*Trypanosoma* and *Leishmania*) could be readily repurposed for malaria chemotherapy.

Author Disclosure Block:

A.Y. Gandhi: None. **A.M. Guggisberg:** None. **A.R. Odom:** None.

Poster Board Number:

FRIDAY-717

Publishing Title:

Anaerobic Metabolism of Choline by a Human Gut Bacterium

Author Block:

A. Martinez-del Campo, S. Bodea, C. Chittim, E. P. Balskus; Harvard Univ., Cambridge, MA

Abstract Body:

The human gut microbiota has an extensive set of metabolic capabilities that directly influence human health. These host-microbe metabolic interactions may be beneficial, but can also lead to the development of diseases, such as the case of microbial degradation of choline. Breakdown of this essential nutrient by gut bacteria into trimethylamine (TMA) and further oxidation by liver enzymes into trimethylamine N-oxide (TMAO) has been linked to multiple diseases, including cardiovascular disease, non-alcoholic fatty liver disease, and trimethylaminuria. We recently discovered the first gene cluster (*cut*) responsible for anaerobic choline utilization and also characterized the key enzyme encoded in this cluster that catalyzes choline breakdown to TMA and acetaldehyde, choline trimethylamine-lyase. Having identified these enzymes, we sought to genetically characterize the *cut* cluster from a human gut derived *Escherichia coli* strain. In this study we evaluated the ability of *E. coli* MS 200-1 to grow on choline minimal media either by fermentation or by anaerobic respiration using different terminal electron acceptors. In order to identify the essential genes for choline utilization we constructed knockout mutants of the *cut* cluster genes and analyzed their growth and TMA production phenotypes. Our results demonstrate that fumarate, nitrate, DMSO and TMAO are able to support anaerobic growth on choline, whereas *E. coli* grew poorly under fermentation conditions. Deletion of genes involved in choline transport, choline cleavage, acetaldehyde processing, and encapsulation into a bacterial microcompartment showed impaired growth and lower TMA production when compared to the wild-type strain. Overall, our study confirms the role of the *cut* genes during anaerobic choline metabolism and contributes to establishing a mode for choline metabolism by human gut bacteria.

Author Disclosure Block:

A. Martinez-del Campo: None. **S. Bodea:** None. **C. Chittim:** None. **E.P. Balskus:** None.

Poster Board Number:

FRIDAY-718

Publishing Title:

Characterizing the Fitness Landscape of Bacteriophage MS2 Virus-Like Particles

Author Block:

E. Hartman, M. B. Francis, D. Tullman-Ercek; Univ. of California, Berkeley, Berkeley, CA

Abstract Body:

Background: The bacteriophage MS2 is a well-studied virus that infects *Escherichia coli* and other members of the Enterobacteriaceae. During viral replication, 180 copies of a small Cap protein form an icosahedral capsid, encapsulating its RNA-based genetic information. This process can be mimicked by recombinantly expressing the MS2 coat protein in *E. coli*, such that virus-like particles are spontaneously formed. Researchers are investigating the utility of this highly ordered protein structure in drug delivery, *in vivo* imaging, and water filtration, among other applications. Here, we performed a structure-activity relationship study of the MS2 Cap protein to characterize the genetic landscape of assembly-competent virus-like particles (VLPs).**Methods:** We first generated a library of MS2 Cap protein mutants using error-prone PCR. In recombinant expression, the Cap protein multimerizes and encapsulates available RNA, including Cap protein mRNA. Chromatographic isolation selected for assembly-competent mutants of assembled MS2 VLPs, which were subsequently identified by sequencing the encapsulated RNA. Assembly-competent MS2 Cap protein variants were then individually expressed and characterized by transmission electron microscopy and size-exclusion chromatography.**Results/Conclusions:** These studies have identified multiple residues that can be mutated without disturbing the global structure of MS2 virus-like particles. Indeed, the MS2 Cap protein tolerated a surprising array of mutations—including glycine and proline mutations within regions with conserved secondary structure, a solvent-exposed tryptophan, and charge inversions. Tolerance varied by amino acid chemistry and position. This is the first in-depth biochemical analysis of this virus-like particle, and our study will inform future engineering efforts for all MS2 applications.

Author Disclosure Block:

E. Hartman: None. **M.B. Francis:** None. **D. Tullman-Ercek:** None.

Poster Board Number:

FRIDAY-719

Publishing Title:

Breaking Out: Exploring the Range of Susceptibility to Mycobacteriophage-Encoded Lysterases and Their Potential as Novel Antimicrobials

Author Block:

D. J. Barbeau, G. F. Hatfull; Univ. of Pittsburgh, Pittsburgh, PA

Abstract Body:

All double-stranded DNA bacteriophages encode a specialized set of proteins that allows their progeny phages to escape the current host cell at the end of the lytic cycle. At a minimum, this lytic cassette consists of a holin and an endolysin to destabilize the host's plasma membrane and peptidoglycan layer, respectively. This two-enzyme system is sufficient to break open most Gram-positive bacterial hosts, but the phages of bacteria with more complex cell walls often encode additional lysis proteins. For example, the mycobacteria have waxy, mycolic acid-rich cell walls that aid in their antibiotic resistance, and their phages—the mycobacteriophages—encode a lysterase (LysB) in order to efficiently overcome this barrier. These serine esterases have been shown to release mycolic acids from whole mycobacterial cells and purified cell wall components via thin layer chromatography of cells grown in the presence of a ^{14}C-carbon source. Our data also show that exogenous addition of purified lysterases has antimicrobial activity against liquid cultures of several bacteria in the Order Actinomycetales, including bacteria associated with disease and—surprisingly—bacteria that do not contain mycolic acids. These findings highlight our incomplete understanding of lysterase proteins and provide the opportunity to explore their use as antimicrobial agents. In addition to using killing assays to further test the range of LysB-susceptible bacteria, we have been performing preliminary experiments, including testing for human cell cytotoxicity and synergy between LysB and commonly used antibiotics, toward determining the therapeutic potential of LysB in *Propionibacterium* and *Mycobacterium* infections.

Author Disclosure Block:

D.J. Barbeau: None. **G.F. Hatfull:** None.

Poster Board Number:

FRIDAY-720

Publishing Title:

Integrative Biology of Mycobacteriophage for Better *Mycobacterium tuberculosis* Control Measures

Author Block:

J. XIE, X. FAN, S. YAN, R. WANG, Q. LI; Southwest Univ., Chongqing, China

Abstract Body:

Background: Tuberculosis, caused by *Mycobacterium tuberculosis*, remains a serious global public health threat largely due to the emergence of antimicrobial resistant strains, shortage of novel antibiotics, and synergy of HIV coinfection. Mycobacteriophage, the virus of Mycobacterium, holds great promise in improving the control of tuberculosis, including diagnostics, and therapeutics.**Methods:** Novel mycobacteriophages were isolated from China, the genome, transcriptome, proteome were determined, together with the transcriptome of the interacting phage and host mycobacteria. Significantly differential expression genes of phage or host were individually characterized by overexpression or deletion to confirm their function.**Results:** Novel mycobacteriophages have been isolated from China soil. Their genomes have been defined. The transcriptome and proteome of interacting Mycobacteriophage SWU1 with *M.smegmatis* were determined. Bioinformatic analysis showed that TCA cycle genes, pentose phosphate pathway, nucleotide metabolism, and metal ions transporter were involved in the interaction.**Conclusions:** The integrative biology of Mycobacteriophage and host is robust and high throughput way to depict the interaction, and pinpoint the weakest point of Mycobacterium for better antibiotics targeting.

Author Disclosure Block:

J. Xie: None. **X. Fan:** None. **S. Yan:** None. **R. Wang:** None. **Q. Li:** None.

Poster Board Number:

FRIDAY-721

Publishing Title:

The Analysis of Toxicity by *Gene 52* of Mycobacteriophage Fruitloop

Author Block:

C-C. Ko, G. F. Hatfull; Univ. of Pittsburgh, Pittsburgh, PA

Abstract Body:

The sequenced genome collection of mycobacteriophages—viruses that infect the genus of *Mycobacterium*—has grown to more than 1000 genomes to date. These genomes are highly diverse, and the majority of the encoded genes' functions are unknown. As a gateway to exploring the unknown, a selection of mycobacteriophage genes were over-expressed in *Mycobacterium smegmatis* mc^2155—a relative of tuberculosis-causing bacteria—to identify those that are detrimental to bacterial growth. Of the 202 genes tested, 21 are toxic to mycobacteria with additional 24 being inhibitory. Using this approach, *gene 52* of mycobacteriophage Fruitloop was identified as a toxic mycobacteriophage gene.To understand how the protein product of Fruitloop *gene 52* (gp52) kills *M. smegmatis*, a co-immunoprecipitation assay was performed followed by mass spectrometry analysis to identify Fruitloop gp52's interacting target(s). As a result, Wag31 of *M. smegmatis*—an essential protein involved in cell shape and cell wall integrity—was identified as a target. Interestingly, over-expression of Fruitloop gp52 results in a change in mycobacterial cell morphology from rod to round, a phenotype similar to that seen when Wag31 is depleted in mycobacteria. Moreover, over-expressing Wag31 allows mycobacteria to survive in the presence of Fruitloop gp52. Further analysis through the isolation of non-toxic Fruitloop gp52 mutants revealed that gp52's V45, I70, and W71 residues are important for its toxicity.Why does mycobacteriophage Fruitloop encode a protein that interacts with the cell wall synthesis machinery of its host? Further elucidating the phage-encoded toxic proteins' mechanisms will advance our knowledge about host-phage interactions. In addition, it also creates opportunities to discover new anti-tuberculosis drug targets at a time when drug-resistant tuberculosis is becoming a severe concern.

Author Disclosure Block:

C. Ko: None. **G.F. Hatfull:** None.

Poster Board Number:

FRIDAY-722

Publishing Title:

Using Rnaseq and Riboseq to Understand Mycobacteriophage L5 Genetics

Author Block:

R. M. DEDRICK, G. F. Hatfull; Univ. of Pittsburgh, Pittsburgh, PA

Abstract Body:

Few in-depth genome-wide profiles of phage gene expression, translation and protein abundance have been performed, but should provide insights into bacteriophage biology genetics. Although expression patterns are established in a small number of well-characterized *Escherichia coli* phages, little is known within the broad array of highly diverse phages of other hosts. Although 1007 completely sequenced mycobacteriophage genomes have been collected, only two have been characterized by RNAseq to determine gene expression patterns. With the exception of *E. coli* bacteriophage lambda, no other phage has been evaluated using ribosome profiling. Here we describe the transcription, translation and protein profiles for L5, a temperate phage that infects *Mycobacteria smegmatis* and is classified into cluster A (subcluster A2). We have used RNAseq to determine the expression patterns of L5 in early (30min) and late (2.5hrs) lytic growth, as well as the L5 lysogen. Ribosome profiling was completed during late lytic growth using methods previously published by Ingolia *et al*., 2012 and total protein abundance was determined using MS/MS. There are distinct gene expression patterns in L5 with transcription of the right arm of the genome occurring in early infection and the virion structural genes in the left arm of the genome during late infection. The immunity repressor, as expected, characterizes the transcriptional profile of the L5 prophage. Surprisingly, all three expression profiles contain a highly expressed 500bp region at the extreme right end of the genome. Ribosome profiling during late lytic growth reveals that ribosomes are actively translating mRNA from genes transcribed in the RNAseq data and the ribosomes are protecting this highly expressed region. Bioinformatics and MS/MS indicate that this region does not produce a protein product and we predict that it is a ncRNA. Further analysis of this possible ncRNA indicate that it is essential for lytic growth of the phage and is toxic to the host, as well as *E. coli*, unless co-expressed with the L5 immunity repressor. This is just one example of insights derived using "-omics" approaches in mycobacteriophages.

Author Disclosure Block:

R.M. Dedrick: None. **G.F. Hatfull:** None.

Poster Board Number:

FRIDAY-723

Publishing Title:

A Capsular Polysaccharide-Dependent *Campylobacter jejuni* Phage Depends on Intracellular Host O-Glycosylation Biosynthetic Enzymes for Infection

Author Block:

J. C. Sacher, M. A. Javed, C. M. Szymanski; Univ. of Alberta, Edmonton, AB, Canada

Abstract Body:

Background:*Campylobacter jejuni* Pse enzymes encode an O-glycosylation system involved in flagellin modification needed for motility and virulence. Although protein glycosylation systems are increasingly identified in bacteria, interactions between bacteriophages and these systems have scarcely been studied. We have observed that the NCTC 12673 *C. jejuni* phage, although capsular polysaccharide-specific, also depends on intracellular O-glycosylation enzymes.**Methods:**We isolated a spontaneous NCTC 12673 phage variant, "MutC" from a plaque obtained following infection of *pseC* mutant cells by NCTC 12673. We sequenced the genomes of MutC and wild type (WT) phage using Illumina MiSeq. I-TASSER server was used for protein structure and function prediction.**Results:**We found that WT phage was unable to plaque on *C. jejuni pseC* or *pseH* (early gene) mutants, yet plaquing was observed on *pseG, pseI* and *pseF* (late gene) mutants. These results suggest that the phage may require the glycan intermediate produced by PseH, a UDP-linked altropyranose. We identified MutC as a variant able to infect all *pse* mutants. Genomic comparisions of WT and MutC uncovered a frameshift mutation in a polyG tract in the hypothetical gene *gp116*. This resulted in a predicted functional MutC Gp116 product, but a truncated WT product. Previous analysis of an NCTC 12673 phage variant detected Gp116 peptide fragments (22% coverage), suggesting it is a structural protein. BLAST showed that *gp116* is conserved in only 4 other *C. jejuni* phage genomes. Interestingly, I-TASSER showed multiple hits involving glycan-binding proteins.**Conclusion:***C. jejuni* phage NCTC 12673 depends on O-linked protein glycosylation for infection. We have identified a candidate phage protein, Gp116 that could help compensate for the loss of *pse* gene products and restore phage infection. The phage appears to be able to switch Gp116 expression, a mechanism not previously described for *Campylobacter* phages. Future directions will involve purifying $Gp116^{MutC}$ in order to elucidate its function. We also plan to express $gp116^{MutC}$ in *C. jejuni* cells to determine whether it can rescue WT phage infectivity. Understanding the mechanism by which MutC circumvents dependence on *C. jejuni* O-glycosylation could lead to better understanding mechanisms of phage predation.

Author Disclosure Block:

J.C. Sacher: None. **M.A. Javed:** None. **C.M. Szymanski:** None.

Poster Board Number:

FRIDAY-724

Publishing Title:

Photorhabdus Virulence Cassettes (Pvcs) - Novel Phage Tail-Like Mechanisms and Their Role in Streptomyces

Author Block:

M. Vladimirov, S. Mak, J. R. Nodwell, A. R. Davidson; Univ. of Toronto, Toronto, ON, Canada

Abstract Body:

Interactions of bacteria with bacteriophages through evolution has led to the integration of many viral genes into bacterial genomes. In certain cases, incomplete phage genomes can be maintained by bacteria thanks to a novel gain of function. Examples of phage derived mechanisms which contribute to bacterial fitness include the *P. aeruginosa* R-type pyocin and the Type VI secretion system. A new class of contractile phage tail-like genetic elements was recently discovered in *Photorhabdus luminescens* and *Serratia entomophila* and linked to insecticidal activity. These elements were therefore termed PVCs - Photorhabdus Virulence Cassettes. Following extensive annotation of phage-like genes in bacterial genomes we discovered that conserved genetic clusters homologous to PVCs were found in numerous prokaryotes from all phyla, including Gram-negative and Gram-positive bacteria, as well as in several Archaea. Interestingly, PVCs appeared to be highly conserved in *Streptomyces* and some members of the genus contain several PVC clusters in a single genome, however their role remains unknown. The knockout of the tail sheath coding gene, one of the key structural components of the PVC in *S. coelicolor* resulted in a reduction in antibiotic production and negatively affected the ability of *S. coelicolor* to kill other bacterial strains and yeast. We suggest that PVCs could represent the first case of a phage-tail like machinery which constitutes a novel putative secretion system in Gram-positive bacteria.

Author Disclosure Block:

M. Vladimirov: None. **S. Mak:** None. **J.R. Nodwell:** None. **A.R. Davidson:** None.

Poster Board Number:

FRIDAY-725

Publishing Title:

Characterization and Genome Analysis of Novel Broad Host Range Bacteriophages Infecting Opportunistic Human Pathogens, *Klebsiella* and *Cronobacter sakazakii*

Author Block:

K-J. Yuk[1], E-A. Park[1], S. Ryu[2], J-H. Lee[1]; [1]Inst. of Life Sci. and Resources, KyungHee Univ., Yongin, Korea, Republic of, [2]Res. Inst. of Agriculture and Life Sci., and Ctr. for Food and Bioconvergence, Seoul Natl. Univ., Seoul, Korea, Republic of

Abstract Body:

Klebsiella is a well-known opportunistic human pathogen infecting primarily immunocompromised individuals underlying diseases including diabetes mellitus or chronic pulmonary obstruction. To control this pathogen, novel phages PKO111 and PKP126 were isolated from various environment. Interestingly, their host range tests showed that these phages infect both *K. oxytoca*, and *K. pneumoniae* as well as even *Cronobacter sakazakii*, suggesting that they are broad host range phages. TEM observation revealed that PKO111 and PKP126 belong to the family *Myoviridae* and *Siphoviridae*, respectively. In addition, these phages showed high stability under various temperature (-20 to 65°C) and pH (3 to 11) conditions. Challenge assay showed that PKO111 and PKP126 inhibit their host strains in 3 log and 2 log reductions, respectively. Their complete genome sequences reveled, PKO111 genome has 168,758-bp DNA length with GC content of 39.39% containing 291 ORFs and 16 tRNA gene, and PKP126 genome has 50,934-bp DNA length with GC content of 50.37% containing 78 ORFs and no tRNA gene. Subsequent phylogenetic analysis of phage major capsid proteins from various *K. oxytoca*, *K. pneumoniae*, and *C. sakazakii* showed the presence of three different phylogenetic clusters. Interestingly, each cluster was associated with phage morphology and genome size (Cluster I including PKO111, *Myoviridae* with ~170-kb; Cluster II including PKP126, *Siphoviridae* with ~50-kb; Cluster III, *Podoviridae* with ~40-kb), suggesting that phages in each cluster may have been evolved from different ancestor. These phages may be good candidates for biocontrol agents against *Klebsiella* and probably for natural food preservatives against *C. sakazakii*.

Author Disclosure Block:

K. Yuk: None. **E. Park:** None. **S. Ryu:** None. **J. Lee:** None.

Poster Board Number:

FRIDAY-726

Publishing Title:

Characterization and Genomic Study of a Novel Broad Host Range Bacteriophage Hy03 Infecting Both *Escherichia coli* O157:H7 And *Shigella flexneri* Reveal Its Potential as a Biocontrol Agent in Foods

Author Block:

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Abstract Body:

Escherichia coli O157:H7 is one of the most severe food-borne pathogen, which infects even at a very low dose. It causes diarrhea, hemolytic uremic syndrome, and hemorrhagic colitis. To control this pathogen, bacteriophage HY03 infecting *E. coli* O157:H7 was isolated from a poultry fecal sample. The morphology was observed with transmission electron microscopy (TEM) and it belongs to the *Myoviridae* family. Interestingly, host range test of HY03 showed that it can inhibit the growth of *E. coli* O157:H7 as well as *Shigella flexneri*, suggesting that it is a broad host range phage. The phage stability test was performed and showed that it is generally stable under various stress condition of pH (3 to 11) and temperature (-20 to 60^{o}C). One-step growth curve analysis showed that eclipse/latent periods are 10 and 20 min and burst size is 10 at a multiplicity of infection (MOI) 0.001. Complete genome sequencing of HY03 by 454-GS FLX revealed that the genome consists of a 170,770 bp-length DNA with GC content of 35.3% containing 292 ORFs. Functional classification of the annotated ORFs showed that three major functional categories including structure and packaging, tails, and host lysis contain 16, 10 and 1 genes, respectively. Food applications of HY03 using fresh edible lettuce were conducted with a clinical isolate of *E. coli* O157:H7 (ATCC 43890) and a food isolate (ATCC 43895), revealing 2 log reduction by the phage HY03 in food environments. In conclusion, the phage HY03 could be useful for development of a novel biocontrol agent or a novel natural food preservative against *E. coli* O157:H7 and even *S. flexneri* in foods.

Author Disclosure Block:

Y. Kim: None. **E. Park:** None. **S. Ryu:** None. **J. Lee:** None.

Poster Board Number:

FRIDAY-727

Publishing Title:

Rna-Seq Analysis of Enterohemorrhagic *Escherichia coli* O157:H7 Strains Showing Different Levels of Shiga Toxin 2 Production

Author Block:

A. Allué Guardia, B. Rusconi, F. Sanjar, S. S. K. Koenig, M. Eppinger; Univ. of Texas at San Antonio, San Antonio, TX

Abstract Body:

Background: Shiga toxin (Stx) production is the major virulence hallmark of enterohemorrhagic *Escherichia coli* (EHEC) of the O157:H7 serotype. Human disease severity and progression to HUS is a direct result of the mobilization of Stx-converting bacteriophages and coherent toxin production. A high level of heterogeneity in Stx prophage architecture and toxin production capabilities has been observed, however the specific bacterial host and phage loci and pathomechanisms that define and control the strains' individual Shiga toxin virulence state are largely unknown. A substantial number of Stx phage genes are annotated as hypothetical genes of unknown function and it is unknown if these are expressed**.** To investigate pathomechanisms of elevated Stx2 production we used an RNA-Seq approach to characterize the global transcriptomes of different *E. coli* O157:H7 with varying Stx production capabilities, cultivated under basal and Ciprofloxacin inducing conditions. **Methods:** Transcriptomes for eight strain pairs were generated on the Illumina HiSeq platform. Profiled strains produce equal levels of Stx2 and are phylogenetically distant, or vice versa, are closely related strains with varying Stx2 production levels. Sequence reads were comprehensively analyzed using CLC Bio Workbench and visualized with MeV Viewer. **Results:** Our analyses allowed us to test for confounding chromosomal and phage borne factors of increased Stx production. We found significant isolate-specific differences in the global expression profiles, likely associated to the limited yet very important plasticity in these isolates affecting Stx production. We further identified regulatory circuits driven by phage and chromosomal genes that were coordinately expressed along with Stx2 and other virulence factors. **Conclusion:** This study highlights the importance of comparative transcriptomics for identifying loci that are directly or indirectly involved in Stx-phage mediated pathogenesis. Understanding the underlying pathomechanisms and regulatory circuits that define high Stx2 producers through RNA-Seq improves risk assessment and provides targets for the development of alternative anti-stx2 strategies.

Author Disclosure Block:

A. Allué Guardia: None. **B. Rusconi:** None. **F. Sanjar:** None. **S.S.K. Koenig:** None. **M. Eppinger:** None.

Poster Board Number:

FRIDAY-728

Publishing Title:

Characterization of Proteins in Cbk Phage Lysate

Author Block:

K. Wilson, B. Ely, C. Callahan; Univ. of South Carolina, Columbia, SC

Abstract Body:

Bacteriophage CbK is the best studied representative of the most common type of bacteriophage that infects *Caulobacter crescentus*. The CbK 216 kb genome is estimated to code for 338 proteins. However the function of the majority of these proteins has not been determined. A comparison of the CbK genome to the genomes of 11 closely related phages revealed that only 108 of the protein-coding genes were present in all 12 phages. However the function of the majority of these proteins has not been determined. Therefore, we initiated a study of the proteins contained in the phage particles to identify those genes that code for structural proteins. During CsCl gradient purification of the CbK phage particles, we noticed an additional band of material that had a density that was less than 1.5 g/ml and therefore contained no DNA. Electron microscopy of this material revealed that it contained phage tails, empty phage heads, phage heads containing spiral structures, and globular particles. SDS-PAGE followed by trypsin digestion and MALDI mass spectrometry allowed us to identify the major proteins in this fraction by comparison of the peptide molecular weights to those predicted from the genome sequence. This analysis revealed the presence of homologues to a rIIb-like protein, a transcription termination factor Rho protein, and a nicotinate phosphoribosyl transferase protein in addition to phage head and tail proteins. The presence of these proteins suggested that CbK may form a DNA replication that complex aids in the replication of this virus within the host cell similar to that previously observed for the E. coli bacteriophage T4. Efforts are underway to separate the globular structures from the phage heads and tails so that the proteins involved in each of these structures can be identified.

Author Disclosure Block:

K. Wilson: None. **B. Ely:** None. **C. Callahan:** None.

Poster Board Number:

FRIDAY-729

Publishing Title:

Cloning and Sequencing of the *Flavobacterium johnsoniae* Bacteriophage Cj1

Author Block:

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Abstract Body:

Bacteriophage Cj1, which infects motile cells of the gliding bacterium *Flavobacterium johnsoniae*, was one of several bacteriophage isolated from ponds and streams around Madison, WI in the 1970s. There is no published sequence data for bacteriophage that infect *F. johnsoniae*; therefore, a shotgun cloning and sequencing approach was used to obtain bacteriophage genome sequence. A plate lysate method was used to grow bacteriophage Cj1 in *F. johnsoniae*. Bacteriophage genomic DNA was isolated by protease digestion, phenol-chloroform extraction, and ethanol precipitation. Single digests of bacteriophage genomic DNA were performed with *Apo*I, *Dra*I, *Eco*RI, *Nsi*I, *Rsa*I, and *Xba*I, and the resulting DNA fragments were ligated into pBluescript KS+. Cloned DNA was introduced into *E. coli* DH5α by heat-shock. From 39 plasmids, approximately 15 kb of bacteriophage Cj1 DNA has been sequenced, but the genome has not been completely sequenced. Analysis of the bacteriophage Cj1 sequence identified 20 open reading frames that would encode proteins that are greater than 50 amino acids in size. Six of the predicted protein sequences (predicted sizes of 390, 286, 346, 126, 51, and 68 amino acids, respectively) have amino acid identity to other proteins based on standard Protein BLAST searches: Orf1 (34% amino acid identity to RecA); Orf2 (36% amino acid identity over 108 residues to a single-stranded DNA binding protein); Orf4 (33% amino acid identity over 132 residues to a peptidoglycan-binding protein), Orf5 (37% amino acid identity over 82 residues to muramidase); Orf12 (38% amino acid identity over 40 residues to TonB); and Orf 15 (55% amino acid identity over 66 residues to deoxyuridine 5'-triphosphate nucleotidohydrolase). Based on protein database similarity, the proteins Orf4 and Orf5 may be involved in releasing bacteriophage from the host cell and proteins Orf1, Orf2, and Orf15 may be involved in bacteriophage DNA replication. Of the fourteen remaining predicted proteins, nine had amino acid identity to hypothetical proteins in the database while five had no significant similarity to any of the proteins in the database. The predicted proteins of each open reading frame and their identity to proteins in the database will be discussed. Further nucleotide sequencing and analysis will result in a better understanding of the genes and proteins found in bacteriophage Cj1 and potentially how the bacteriophage infects motile bacterial cells.

Author Disclosure Block:

M. Kempf: None.

Poster Board Number:

FRIDAY-730

Publishing Title:

Function and Evolution of the Cluster A Actinobacteriophage Genetic Circuit

Author Block:

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Abstract Body:

Temperate bacteriophages control their lifecycle by employing genetic circuits. These circuits coordinate the temporal expression of genes during infection, lysogeny, and superinfection immunity. Investigation of how phage genetic circuits function and evolve can provide deeper understanding of natural microbial biology as well as help improve the design of artificial circuits for synthetic biology.Over 350 genetically related, temperate phages that infect various hosts of the phylum Actinobacteria have been isolated. These Cluster A phages appear to harness a homologous genetic circuit to control transcription. In contrast to other highly characterized phage regulatory strategies, this circuit utilizes a single transcriptional repressor capable of recognizing a canonical operator site as well as dozens of other potential sites distributed throughout the genome, and *in vitro* it is capable of regulating two stages of transcription - initiation and elongation. However, we do not understand how repression occurs *in vivo*, what degree of selection is imposed on the position, frequency, or sequence of sites, and how this circuit evolves.To investigate how this circuit functions and to identify the evolutionary forces that shape it, we have employed a comparative genomics approach that leverages the natural variation and diversity present among Cluster A phages. Phylogenetic analyses have highlighted that the repressor gene appears to evolve differently than other highly conserved genes. Bioinformatic identification of the repressor's predicted binding sites reveals that while sites co-evolve with the repressor within each genome, they retain a conserved hexameric promoter element. Lastly, superinfection immunity assays have shown that small, incremental changes in the repressor genes and their predicted binding sites may have marked effects on the circuit's regulatory capability. The combination of these approaches has begun to elucidate the dynamics of this evolving circuit.

Author Disclosure Block:

T.N. Mavrich: None. **G.F. Hatfull:** None.

Poster Board Number:

FRIDAY-731

Publishing Title:

The Multi-component Antirestriction System of Coliphage P1

Author Block:

D. K. Piya, L. Vara, R. Young, J. J. Gill; Texas A&M Univ., College Station, TX

Abstract Body:

Bacterial type I restriction-modification (R-M) systems present a major barrier to foreign DNA entering the bacterial cell, by cleaving inappropriately modified DNA in a sequence-specific manner. To overcome this defense system, the temperate coliphage P1 encodes proteins that protect its DNA from host type I R-M systems. Previous work has shown that the P1 anti-restriction system was comprised of DarA and DarB (dar for *d*efense *a*gainst *r*estriction), virion-associated proteins that protect DNA against the *Eco*A, *Eco*B, and *Eco*K type I R-M systems. P1*darA*$^-$ has a reduced efficiency of plating (EOP) in *E. coli* strains encoding the *Eco*A, *Eco*B or *Eco*K R-M systems, while P1*darB*$^-$ has a reduced EOP in *E. coli* encoding the *Eco*B or *Eco*K systems. To examine the role of DarA and DarB in the P1 antirestriction system, isogenic P1 deletion mutants were used to reconstitute the restriction phenotypes associated with *darA* and *darB*. While P1(Δ*darA*) and P1(Δ*darB*) produced the expected phenotypes, deletions of adjacent genes *hdf* and *ddrA* also gave a *darA*-like phenotype and deletion of *ulx* produced a *darB*-like phenotype. Interestingly, disruption of *ddrB* caused the phage EOP to become approximately 10-fold higher on *Eco*B or *Eco*K strains, suggesting DdrB acts as a modulator of DarB. All of these restriction phenotypes could be complemented *in trans.* SDS-PAGE and mass spectrometry of CsCl-purified virions revealed that *darA, hdf, ddrA* and *ulx* mutants fail to package DarB in virions and *hdf* mutants fail to package DarA or DarB. Interestingly, virions of P1(Δ*ddrA*), with restriction sensitivity to *Eco*A, still contain DarA, indicating that DarA is necessary but not sufficient for protection of phage DNA against *Eco*A. A combination of isopycnic CsCl gradient centrifugation and transmission electron microscopy showed that *darA* and *hdf* mutants produced high proportions of small-headed virions, suggesting that the antirestriction system is also linked to the process of head morphogenesis. The P1 antirestriction system is more complex than previously realized and is comprised of multiple proteins including DdrA, DdrB, Hdf, and Ulx in addition to the previously described DarA and DarB.

Author Disclosure Block:

D.K. Piya: None. **L. Vara:** None. **R. Young:** None. **J.J. Gill:** None.

Poster Board Number:

FRIDAY-732

Publishing Title:

Altering the Speed of the Bacteriophage T4 Dna Packaging Motor

Author Block:

S. Lin, C. J. VanGessel, W. Tang, T. I. Alam, V. B. Rao; The Catholic Univ. of America, Washington, DC

Abstract Body:

Double-stranded DNA packaging in bacteriophage T4 and other viruses is a complex biological process that serves as an excellent model to understand fundamental mechanisms of ATP energy transduction into mechanical work (1). gp17, the packaging motor of bacteriophage T4, translocates T4 genome (171-kbp) into the prohead capsid via an ATP-driven process at a speed of up to 2,000 bp/s, the fastest reported to date (2). The speed of another phage packaging motor, one from phage phi29 that packages ~20-kbp genome, is only 100-bp/s. The speed of the motor may have evolved with the size of the genome, in order to efficiently generate an infectious virus in ~30 min phage life cycle. However, the mechanisms by which the speed of a motor is controlled are unknown in any system. We proposed that the hydrophobic and electrostatic environment in the vicinity of the catalytic glutamate (E256 of gp17) and the gamma-phosphate of the bound ATP might modulate the rate of activation of water molecule, which in turn could control the rate of ATP hydrolysis and the speed of the motor. Guided by structural analyses, we identified two amino acid residues, F259 and M195, in the vicinity of E256 and the gamma-phosphate of the bound ATP in gp17 that might influence the ATP hydrolysis rate. Combinatorial mutagenesis and recombinational rescue were performed at these residues; 138 mutants were screened for each mutant library, and about 130 different phenotypes were sequenced. We found that only aromatic amino acid and leucine substitutions were tolerated at F259 position. At M195 position hydrophobic amino acid substitutions were also tolerated, whereas polar residue substitutions resulted in null or cold-sensitive phenotypes. Interestingly, substitution with serine at M195 resulted in null phenotype, whereas threonine substitution gave cold-sensitive phenotype. Preliminary results showed that gp17 purified from the null and cold-sensitive phenotypes showed greatly reduced ATP hydrolysis and *in vitro* DNA packaging. These results suggest that modulating the hydrophobic environment in the vicinity of catalytic glutamate might be one mechanism to control the speed of the phage T4 DNA packaging motor.

Author Disclosure Block:

S. Lin: None. **C.J. VanGessel:** None. **W. Tang:** None. **T.I. Alam:** None. **V.B. Rao:** None.

Poster Board Number:

FRIDAY-733

Publishing Title:

Dissecting the Role of the Partition Factor Parf in TP228 Plasmid Segregation

Author Block:

G. Allison, Dr Daniela Barillà; Univ. of York, York, United Kingdom

Abstract Body:

Antibiotic resistance is one of the most significant health concerns for the public. Bacterial antibiotic resistance can occur for many reasons but the most common is the presence of drug resistant plasmids. Therefore understanding how these drug resistant plasmids are passed from one generation to the next is of high importance and could provide potential drug targets to combat antibiotic resistance. Low copy number plasmids, implicated in antibiotic resistance, utilise partition cassettes in order to ensure faithful segregation at cell division. The multidrug resistance plasmid TP228 is a low copy number plasmid that contains the *parFGH* partition cassette. *parH* is a *cis*-acting centromere-like site, ParG is a DNA binding protein and ParF is a Walker-type ATPase that is able to assemble into higher order structures upon ATP binding. Recent structural data has shown that ParF-ATP dimers interact with each other to form dimer-of-dimer units which create the building blocks of the filaments. This project dissects ParF and its non-specific DNA binding properties as well as looking at how ParF is able to form polymeric structures with the aim of gaining further insights into TP228 plasmid segregation.A range of *in vitro* biochemical assays have been used to study ParF DNA binding and assembly into higher structures. Conventional fluorescence and super resolution microscopy has been used to investigate the *in vivo* localisation of ParF. In this study mutants were constructed to disrupt both DNA binding and ParF self-assembly. Mutants constructed at monomer-monomer and dimer-dimer interfaces affect ParF assembly. Changes in residues in a proline-rich motif positioned at the monomer-monomer interface cause ParF to form stronger monomer-monomer interactions and to self-associate into high order structures in the absence of ATP. This stretch of residues is also important in the interaction between ParF and the binding partner ParG, as the mutants have been shown to have a weaker interaction with ParG. Surface exposed basic residues were identified as potentially being involved in ParF DNA binding and two informative mutants were studied using *in vitro* DNA binding assays. Based on the findings and super resolution microscopy images obtained, a new model for TP228 plasmid segregation is proposed.

Author Disclosure Block:

G. Allison: None.

Poster Board Number:

FRIDAY-734

Publishing Title:

Making Vital Connections: Probing Protein-Protein Interactions Underpinning the Segregation of a Multidrug Resistance Plasmid

Author Block:

C. Pennica, M. Barge, A. Leech, D. Barilla'; Univesity of York, York, United Kingdom

Abstract Body:

Bacterial resistance to antibiotics is becoming a global concern. Most of the antibiotics used to treat infections in the past are now obsolete because bacteria developed resistance, hence finding alternative treatments or targets is of fundamental importance. Antibiotic resistance is often encoded by low copy number plasmids, which require an active segregation system to be evenly distributed to the daughter cells at cell division.The segregation system under investigation is that of the TP228 plasmid. The plasmid, first identified in *Salmonella newport*, is responsible for multidrug resistance and has high stability *in vivo*. Segregation stability is conferred by the *parFGH* partition cassette, which encodes a Walker-type ATPase, ParF, a DNA-binding protein, ParG, and contains an upstream centromeric region, *parH*. It has been shown that ParG binds to *parH* in a sequence specific manner and that this interaction recruits ParF to the binding site. This complex, called segrosome, is the fundamental unit for plasmids segregation. Published data suggest that ParF works as a motor protein that drives sister plasmids towards opposite poles of the cell, using the nucleoid as a scaffold.The aim of the project is to probe the molecular interaction between the components of the TP228 segrosome. ParG is a dimeric protein, consisting of a folded C-terminal domain, involved in DNA binding, and two unstructured N-terminal tails. These flexible regions seem to be involved in ParF binding and in improving its ATPase activity. ParG tails are likely to acquire a specific fold upon binding the partner protein ParF. Alanine-scanning mutagenesis of the ParG N-terminal tail has highlighted a number of residues, whose change either abolishes or severely abrogates plasmid partition *in vivo*. Mutant ParG proteins were purified and microscale thermophoresis and surface plasmon resonance were used to quantify the interaction between ParF and ParG N-terminal mutants. These techniques are allowing us to determine which amino acids are directly involved in ParF recognition and which are involved in other steps of the segregation process. The results obtained so far suggest that specific amino acids are essential for ParF binding and mutations at these positions abolish the interaction completely. A model for the ParF-ParG interaction will be proposed and discussed.

Author Disclosure Block:

C. Pennica: None. **M. Barge:** None. **A. Leech:** None. **D. Barilla':** None.

Poster Board Number:

FRIDAY-735

Publishing Title:

Identification and Characterization of the Lipoprotein *N*-Acyl Transferase Gene in *Enterococcus faecalis*

Author Block:

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Abstract Body:

Approximately 1-5% of all bacterial proteins are lipoproteins, characterized by an N-terminal lipidated cysteine residue anchoring the globular domain to the surface of the cell membrane. Lipoproteins have a wide variety of cellular functions, including nutrient uptake, signal transduction, adhesion, and virulence. They also serve as potent ligands for Toll-like receptor 2 (TLR2), inducing a host innate immune response. Previously, lipoproteins were categorized as either diacyl or triacyl based on the absence or presence of an amide-linked fatty acid. In *E. coli*, addition of this *N*-acyl chain is catalyzed by the integral membrane protein lipoprotein *N*-acyl transferase (Lnt). Despite lacking an *lnt* sequence ortholog, recent structural analyses of lipoproteins from certain Gram-positive *Firmicutes* have demonstrated *N*-acylation [1], suggesting a unique lipoprotein biosynthetic pathway specific to these organisms. To determine the *Enterococcus faecalis* gene responsible for lipoprotein *N*-acylation, a genomic library was constructed and screened for Lnt-type activity. Our results revealed a single candidate protein demonstrating Lnt-type activity encoded by an unannotated sequence harboring a domain of unknown function. Genetic and functional characterization of this candidate protein will be presented.

Author Disclosure Block:

K.M. Armbruster: None. **T.C. Meredith:** None.

Poster Board Number:

FRIDAY-736

Publishing Title:

Elucidating The Role Of A Member Of The MbtH-Like Protein Superfamily In *Myxococcus xanthus*

Author Block:

K. Esquilin-Lebron, M. G. Thomas; Univ. of Wisconsin-Madison, Madison, WI

Abstract Body:

One way bacteria and fungi produce bioactive natural products such as antibiotics and siderophores is through the use of nonribosomal peptide synthetase (NRPS) multimodular assembly lines. Many NRPSs in bacteria require members of the MbtH-like protein (MLP) superfamily for their solubility and function. Although MLPs are known to interact with adenylation domains of NRPSs, the role MLPs play in NRPS enzymology has yet to be elucidated. MLPs are nearly always encoded within NRPS-encoding gene clusters. To date the only exemption to this rule is found in the Myxococcales order, where genes encoding members of the MLP superfamily are commonly found independently of NRPS-encoding gene clusters. For example, *Myxococcus xanthus* has fourteen NRPS-encoding gene clusters and only one MLP-encoding gene (MXAN_3118), which is not encoded within any of the NRPS-encoding gene clusters. These observations led to these hypotheses that MXAN_3118 may interact with one or more NRPSs, may play some NRPS-independent role, or plays some role in both NRPS enzymology and other physiological processes in *M. xanthus*. To investigate potential NRPS partners for MXAN_3118 I used a comparative genomics approach, leading to the identification of two candidate NRPS-encoding gene clusters. I investigated whether the solubility of any of these NRPSs is influenced by the presence of MXAN_3118 as observed for other MLP-NRPS partners. A cryptic cluster was identified to require MXAN_3118 for solubility of its NRPS components. This finding suggests that MXAN_3118 can functionally interact with NRPSs. Preliminary data suggests that MXAN_3118 is also required for adenylation domains function. We are currently optimizing conditions to identify substrate specificity to further understand the MLP/NRPS interactions in this system. Ongoing efforts to identify the associated metabolite are being pursued. *M. xanthus* NRPS-associated secondary metabolism have been extensively studied, here we identify and characterize the first MLP-dependent NRPS in the Myxococcales order. These findings will allow for the development of a model for the role of MXAN_3118 plays in the physiology of *M. xanthus* and other members of the Myxococcales order and will provide insights into the function of MLPs.

Author Disclosure Block:

K. Esquilin-Lebron: None. **M.G. Thomas:** None.

Poster Board Number:

FRIDAY-737

Publishing Title:

1D and 2D Gel Electrophoresis Analyzing of Recombinant PHB Proteins from New Identified *Bacillus cereus* tsu1 Strain

Author Block:

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Abstract Body:

One *Bacillus cereus* Strain was isolated from cellulose-agar plate at Tennessee State University, which named as tsu1 strain. The genomic DNA was extracted from the above-mentioned strain and sequenced (Cornell University) and assembled (Pittsburgh Supercomputer Center). Genome sequence of *Bacillus cereus* tsu1 was deposited in NCBI with accession No. JPYN00000000. The assembled DNA contigs were translated into proteins by NCBI Prokaryotic Genome Annotation Pipeline. Five Poly-3-hydroxybutyrate (PHB) synthesis proteins were identified. With Sudan black staining, this bacterium was found producing PHB. The physical properties of the tsu1-PHB were found to be comparable to the standard compounds from Sigma (catalog Number 363502) when analyzed with HPLC and thermal degradation temperature. The five PHA genes (PhaA- beta keto-thiolase, PhaP- Phasin protein, PhaB- acetoacetyl-coA reductase, PhaR PHB synthase subunit, PhaC_N synthase subunit) were cloned and expressed in *E.coli* using Champion pET Directional TOPO Expression Kit (Carlsbad, CA). The recombinant proteins were separated on 1-dimensional and 2-dimensional gels, and analyzed using in-gel trypsin digestion followed by mass spectrometry analysis.

Author Disclosure Block:

H. Li: None.

Poster Board Number:

FRIDAY-738

Publishing Title:

Structural and Biosynthetic Analysis of Fabrubactin, a Siderophore from *Agrobacterium fabrum* c58

Author Block:

V. Vinnik[1], H. Park[2], T. P. Wyche[3], F. Zhang[1], T. S. Bugni[1], M. G. Thomas[1]; [1]Univ. of Wisconsin-Madison, Madison, WI, [2]Massachusetts Inst. of Technology, Cambridge, MA, [3]Harvard Med. Sch., Boston, MA

Abstract Body:

The bioavailability of iron in aerobic environments at neutral pH is low due to the formation of insoluble oxidized iron complexes. To overcome this challenge many bacteria produce siderophores, which are soluble high affinity iron-chelating metabolites. Members of our lab previously identified a siderophore and the associated biosynthetic gene cluster from *Agrobacterium fabrum* strain C58. Here we present conditions for large-scale production and purification of this metabolite that allow for structure determination. Preliminary NMR data suggest the siderophore contains a heterocyclic moiety characteristic of the anachelin family of siderophores. We propose fabrubactin as the name for this siderophore. Our data suggest fabrubactin is made via hybrid polyketide synthase (PKS) and nonribosomal peptide synthetase (NRPS) enzymology. In addition to structural studies, the PKS and NRPS biosynthetic components were purified in order to assess substrate specificity. The enzymology and structure of fabrubactin will provide new insights into the formation of the heterocyclic moiety. These aspects of fabrubactin structure and biosynthesis will be discussed.

Author Disclosure Block:

V. Vinnik: None. **H. Park:** None. **T.P. Wyche:** None. **F. Zhang:** None. **T.S. Bugni:** None. **M.G. Thomas:** None.

Poster Board Number:

FRIDAY-739

Publishing Title:

Studying Hemagglutinin Protein Interactions in Botulinum Neurotoxin Complex

Author Block:

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Abstract Body:

Botulinum neurotoxin (BoNT), produced by *Clostridium botulinum*, is considered one of the most lethal poisons known to humankind. In C. *botulinum* serotype A1 strains, the neurotoxin is non-covalently bonded with several associated non-toxic proteins (ANTPs) such as NTNH and hemagglutinin (HA) proteins; forming a complex up to 900 kD in. The ANTPs in the complex protect the toxin from the acidic and proteolytic environment of the digestive tract as well as facilitate the absorption of BoNT across the intestinal epithelium. The gene organization that harbors the *bont* and *antp* has been well characterized and its association with mobile elements has been reported. However, it is not clear how the neurotoxin complex is formed at the protein level. In this study, we studied protein interactions within the toxin complex using size-exclusion chromatography (SEC), the Yeast Two-Hybrid System, and protein *in-vitro* studies. The SEC of the crude protein extract revealed that the protein complex assembles into two different-sized complexes: the 900kD LL and 500kD L. Further SEC analysis showed that the L complex rearranging itself gradually into the stable LL complex. Interactions of individual proteins were measured by the Yeast Two-Hybrid System. Among several positive interacting pairs, the strongest interaction occurred between HA17 and HA52. Mutation studies of HA52 indicated that the C-terminus was responsible for the binding with HA17. Protein *in-vitro* studies were also performed to provide further confirmation of the interaction between these two proteins. The optimal conditions facilitating the interactions are also investigated. Our data support the hypothesis of HA52 as a bridge between BoNT/NTNH and other HA proteins.Understanding how the neurotoxin complex is assembled will provide information leading to identify ways for preventative and therapeutic treatment of botulism.

Author Disclosure Block:

L. Ab Samad: None. **P. Lwin:** None. **W. Lin:** None.

Poster Board Number:

FRIDAY-740

Publishing Title:

Investigation of Protein Synthesis by Mycobacterial Alternative Ribosomes

Author Block:

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Abstract Body:

Many bacteria have at least one ribosomal protein with a homologous gene coding for an alternative ribosomal protein (AltRP). The question of whether or not the homologous ribosomal proteins offer distinct functionality to the mycobacterial ribosome was investigated using the bacteria *Mycobacterium tuberculosis* (*Mtb*) and *Mycobacterium smegmatis* (*Msm*), which possess four alternative ribosomal proteins arranged together in an operon. We use genetic manipulation, phenotypic analysis, and proteomics to investigate a role of mycobacterial AltRPs. In culture, mutants devoid of the AltRPs in both *Mtb* and *Msm* have a loss of clumping phenotype and exhibit an altered cell morphology, e.g., bulging of *Mtb* cells. Comparison of the proteomes from *Mtb* and *Msm* with mutants devoid of the AltRPs has identified that the loss of AltRPs causes a perturbation in the proteomic profile of the bacteria during zinc limitation, some of which may be involved in cell wall synthesis and explain the mutant phenotype. It was further observed that the *altRP* mutant of *Msm* has a distinct odor, suggesting overproduction of volatile metabolites. The mutant also has impaired biomass production in the late stationary phase when grown under low zinc conditions, compared to the wild type strain. In conclusion, our data suggest that pathogenic and non-pathogenic mycobacteria share a common mechanism of translational regulation, with zinc as a trigger for switching between two forms of ribosomes.

Author Disclosure Block:

A. Dow: None. **S. Prisic:** None.

Poster Board Number:

FRIDAY-741

Publishing Title:

The Alginate Lyase (AlgL) is Dispensable for Alginate Production by *Pseudomonas aeruginosa*

Author Block:

Y. Wang[1], M. F. Moradali[1], A. Goudarztalejerd[2], I. M. Sims[3], B. H. A. Rehm[4]; [1]Massey Univ., Palmerston North, New Zealand, [2]Bu-Ali Sina Univ., Hamadan, Iran, Islamic Republic of, [3]Victoria Univ. of Wellington, Lower Hutt, Wellington, New Zealand, [4]MacDiarmid Inst. for Advanced Materials and Nanotechnology, Massey Univ., Palmerston North, New Zealand

Abstract Body:

Alginates display exceptional physicochemical properties appropriate for numerous medical and industrial applications. However, if secreted by the opportunistic human pathogen *Pseudomonas aeruginosa*, it is an important virulence factor, especially during chronic lung infection of cystic fibrosis patients. Alginate is synthesized by a multi-protein complex spanning the inner and outer membranes and periplasm. Components of this apparatus are encoded within the alginate biosynthesis gene cluster which also encodes an alginate degrading enzyme AlgL - the function of which has been highly controversial. Here, we showed that deletion of *algL* gene doubled alginate yield, while enhancing epimerization and impairing O-acetylation of the polymer, without affecting polymer length distribution. However, inactivation of *algL* during planktonic mode completely abolished alginate biosynthesis. Together with our findings from mutual stability and protein-protein interaction experiments, we propose that AlgL is likely to be a free periplasmic protein dispensable for alginate production during growth on solid medium.

Author Disclosure Block:

Y. Wang: None. **M.F. Moradali:** None. **A. Goudarztalejerd:** None. **I.M. Sims:** None. **B.H.A. Rehm:** None.

Poster Board Number:

FRIDAY-742

Publishing Title:

Structural and Functional Insights into the ComD Histidine Protein Kinase, a Quorum-sensing Signaling Peptide Receptor of *Streptococcus mutans*

Author Block:

X-L. Tian, Y-H. Li, Oral Biofilm Ecology Group; Dalhousie Univ., Halifax, NS, Canada

Abstract Body:

Background: Quorum sensing activation by a signal peptide pheromone (CSP) in *Streptococcus mutans* depends on the membrane-associated histidine protein kinase receptor, ComD, which senses the signal and triggers the signaling cascade for bacteriocin production and other cell density-dependent activities. However, the mechanism of the signal recognition via the ComD receptor in this species is nearly unexplored.**Methods:** In this study, we investigated the membrane topology of the ComD protein using a dual *phoA-lacZ* reporter system. We then determined the roles of the membrane domain of the ComD in CSP recognition and quorum sensing activation.**Results:** We demonstrated that the membrane domain of the ComD receptor protein forms six transmembrane segments with three extracellular loops, loopA, loopB and loopC. By mutational analysis of these extracellular loops combined with luciferase report assays of two CSP-inducible promoters, P*cipB* and P*nlmAB*, we also demonstrated that both loopC and loopB are required for CSP recognition and quorum sensing activation, while loopA plays little role in CSP perception. A deletion or substitution mutation of four residues NVIP in loopC abolishes CSP recognition for quorum sensing activities. Western blotting detected all the mutant proteins in the membrane fractions of the mutant strains, suggesting that a deletion or mutation of these extracellular loops does not affect the insertion of the mutant proteins into the membrane. Consistent with these findings, the loopC and loopB mutants are completely or partially defective in bacteriocin production.**Conclusions:** We conclude that both loopC and loopB are required for forming the receptor and the residues NVIP of loopC are essential for CSP recognition and quorum sensing activation in *S. mutans*.

Author Disclosure Block:

X. Tian: None. **Y. Li:** None.

Poster Board Number:

FRIDAY-743

Publishing Title:

Structure-Based *In vivo* Functional Analysis of the *E. coli F*tsB-FtsL Cell Division Complex

Author Block:

D. Mahbuba, S. J. Craven, L. M. LaPointe, N. Rangarajan, J. C. Weisshaar, A. Senes; Univ. of Wisconsin-Madison, Madison, WI

Abstract Body:

Background: The FtsB/FtsL complex is a key component of the divisome of *Escherichia coli* and other bacteria. FtsB and FtsL interact with FtsQ and are essential to recruit other downstream proteins to the division site. Despite their importance, the function and the structural arrangement of FtsB/FtsL complex are still poorly understood. Previous evidence from *in vitro* experiments suggests that FtsB and FtsL associate to form a higher-order oligomeric complex. This interaction of FtsB-FtsL complex depends on both the transmembrane and the juxta-membrane coiled-coil regions.**Method:**To address how the structural features of the FtsB/FtsL complex participate in supporting its function, we present the results of a structure-based phenotype analysis of an extensive number of mutations in the transmembrane and coiled coil regions of the complex.**Result:**Despite the fact that the transmembrane domains of FtsB/FtsL form a stable complex *in vitro*, we found that mutations introduced in this region have only a mild effect in cell division. We also found that mutations in the linker region between the transmembrane and coiled coil domain of FtsL impair cell division, whereas similar mutations in FtsB have a milder effect. This is consistent with the hypothesized presence of a flexible linker between the two helical domains in FtsB.**Conclusion:**This work provides a foundation for interpreting the role of protein-protein association in regulating the biological activity of FtsL/FtsB complex and the overall structure-function relationship at the molecular level.

Author Disclosure Block:

D. Mahbuba: None. **S.J. Craven:** None. **L.M. LaPointe:** None. **N. Rangarajan:** None. **J.C. Weisshaar:** None. **A. Senes:** None.

Poster Board Number:

FRIDAY-744

Publishing Title:

Defining the Regulatory Role of Lipid Raft Micro-Domains in the T Cells Receptor Signaling Pathway

Author Block:

C. C. Osigwe, A. D. Levine; Case Western Reserve Univ., Cleveland, OH

Abstract Body:

We have previously reported that heterogeneity in the lipid composition of the plasma membrane plays a critical role in the T cell receptor (TCR) activation pathway. Our lab has shown that highly-ordered, densely packed lipids in raft-like micro-domains is required for the activation of this receptor complex. In particular, we have shown that the early, enzymatic events associated with TCR activation are dependent on the unique distribution of protein components of the TCR signaling complex with these lipid raft micro-domains. While our results support the importance of these lipid raft micro-domains in TCR signaling, no evidence is available to describe how these rafts are assembled and organized within the plasma membrane. The objective of our study is to define the regulatory role for lipid rafts on the TCR signaling pathway. The following techniques have been employed to investigate this objective. From whole blood, peripheral mononuclear cells (PBMCs) were isolated by Ficol-hypaque density separation. Purified T cells were expanded from PBMC culture by a nine day feeding with interleukin-2. The T cells were activated by cross-linking the TCR with anti-CD3/anti-CD28 co-stimulatory antibodies. Propagation of the signal was analyzed through immunoblot and phospho-flow cytometry analysis for a phospho-ERK response. Lipid raft composition, structure, and assembly will be probed with pre-designed fluorescently labelled phospholipids that selectively associate with distinct regions of the plasma membrane, labeling either a raft-like domain or non-raft domain. Localization of the specific fluorescently labelled phospholipids, balanced against the localization of protein components of the TCR signaling complex using fluorescently-tagged antibodies, is analyzed using confocal and deconvolution microscopy. The biological implications of this study will address how TCR engagement within the context of a lipid raft may lead to an immune response or immune tolerance.

Author Disclosure Block:

C.C. Osigwe: None. **A.D. Levine:** None.

Poster Board Number:

FRIDAY-745

Publishing Title:

The Transmembrane Domain of the *Staphylococcus aureus* ESAT-6 Secretion System Component EssB Interacts with the Integral Membrane Protein EssF

Author Block:

M. M. Ahmed[1], K. M. Aboshanab[2], Y. M. Ragab[3], K. A. Aly[1]; [1]Sinai Univ., North Sinai, Egypt, [2]Ain Shams Univ., Cairo, Egypt, [3]Cairo Univ., Cairo, Egypt

Abstract Body:

The *Staphylococcus aureus* ESAT-6 Secretion System (Ess) is a specialized machinery encoded by an 11 gene cluster, and dedicated to the secretion of several substrates into mammalian hosts. All 11 proteins coordinate substrate injection into the host cell, resulting in severe health complications. To gain insights into the *S. aureus* Ess pathway, we focused on a pivotal membrane component, termed EssB. EssB is highly conserved in many Gram-positive bacteria, and is a member of the Cluster of Orthologous Groups COG4499. In *S. aureus*, EssB is composed of extended N- and C-terminal stretches that are separated by a 23 amino acid transmembrane (TM) domain. Previous studies have found that EssB is required for toxin secretion into the extracellular environment, but without addressing the molecular events that mediate substrate translocation across the bacterial envelope. Here, a bacterial two-hybrid screen reveals that EssB strongly interacts with EssF, another integral membrane component of the Ess pathway. Further, we created 5 different EssB variants: 1- EssB C-terminal deletion (NTM), 2- EssB TM and C-terminal deletion (N), 3- EssB N-terminal deletion (TMC), 4- EssB N-terminal and TM deletion (C) and 5- EssB N-terminal and C-terminal deletion (TM). Results show that deleting the N- or the C-terminus of EssB, one at a time, did not abrogate EssB-EssF interaction, as long as the TM domain of EssB is present. Interestingly, retaining the TM domain and deleting both, the N- and C-termini of EssB still led to strong interaction with EssF, indicating that the TM domain is indispensable for interaction with EssF. The hydrophobic nature of the TM domain suggests that it may interact with one of the 6 TM domains found in EssF. This hypothesis has been validated by the failure of the only soluble 75 kDa domain of EssF to interact with EssB. Taken together, this study contributes to unveiling the membrane-associated events pursued by the *S. aureus* EssB towards toxin secretion, and will have an important impact on the development of novel treatment options.

Author Disclosure Block:

M.M. Ahmed: None. **K.M. Aboshanab:** None. **Y.M. Ragab:** None. **K.A. Aly:** None.

Poster Board Number:

FRIDAY-746

Publishing Title:

Fitness Costs of Disrupting the Glycine Riboswitch in *Bacillus subtilis*

Author Block:

N. E. Lea, A. M. Babina, M. M. Meyer; Boston Coll., Chestnut Hill, MA

Abstract Body:

The glycine riboswitch is a dual aptamer glycine-binding mRNA structure located in the 5' untranslated region of the *gcvT* operon transcript in many bacteria. The mRNA structure acts as a genetic switch that senses cellular levels of glycine. In *Bacillus subtilis,* glycine binds each riboswitch aptamer and allows full-length transcription of the downstream *gcvT* operon via the disruption of a rho-independent transcription terminator. The *gcvT* operon encodes enzymes involved in the glycine cleavage system, and thus the glycine riboswitch is proposed to maintain intracellular glycine levels and facilitate the use of glycine as an energy source. Additionally, the *gcvT* operon is important for glycine detoxification in environments in which high glycine levels can inhibit bacterial growth. The evolutionary conservation, biophysical dynamics, and functional constraints on the glycine riboswitch are particularly interesting. Previous studies propose that the two aptamers demonstrate cooperative binding of glycine, and the vast majority of natural examples of the riboswitch include dual aptamer structures indicating this attribute may be important for cellular fitness. However, recent data suggests that the cooperative binding is an artifact of the constructs used for *in vitro* studies. To examine this question directly, we measured the impact of several riboswitch point mutations on gene expression using a beta-galactosidase reporter assay. Point mutations to the glycine binding sites of each aptamer, the combination of these mutations, and point mutations to the proposed dimerization interface between the aptamers, result in significantly decreased expression of downstream genes in the presence of glycine, suggesting that the regulatory function of the riboswitch is eliminated by these mutations. Furthermore, we find that when integrated into the native locus within the *gcvT* operon, these mutations disrupt biofilm formation, cell motility, and overall cell growth in *B. subtilis* in high glycine environments. These results suggest that a functional dual aptamer glycine riboswitch is necessary for optimal cell fitness under specific environmental conditions.

Author Disclosure Block:

N.E. Lea: None. **A.M. Babina:** None. **M.M. Meyer:** None.

Poster Board Number:

FRIDAY-747

Publishing Title:

Two Small Regulatory Rnas, Omra and Omrb, Affect Type 1 Pilus Expression and Survival of *Escherichia coli* Within the Murine Urinary Tract

Author Block:

W. R. Schwan, I. Wu, C. Grosshuesch, D. Baumann; Univ. Wisconsin-La Crosse, La Crosse, WI

Abstract Body:

Background: Uropathogenic *Escherichia coli* (UPEC) is the number one cause of urinary tract infections in humans. The ability to survive in the high osmolality/acidic environment of the urinary tract is an important facet of *E. coli*. In this study, we have examined the roles two small regulatory RNAs (OmrA and OmrB) have on type 1 pilus expression and survival of *E. coli* within the murine urinary tract.**Methods:** Initially, *E. coli* K-12 strains with mutations in *omrA*, *omrB*, and *omrAB* were compared to the wild-type strain using reporter plasmids containing *fim::lacZ* fusions on single copy numbers plasmids. An *omrAB* double mutant was also created in the UPEC strain NU149. The NU149 *omrAB* double mutant was compared to the wild-type strain and the complemented mutant strain by PCR for the orientation of the *fimS* element and hemagglutination titer (HA) when grown in different *in vitro* environments. Furthermore, the NU149 *omrAB* mutant was inoculated into the urinary tracts of female Swiss Webster mice to assess whether the double mutation affected *E. coli* survival in the murine urinary tract.**Results**: Single mutations in *omrA* and *omrB* did not affect *fim* gene transcription in an *E. coli* K-12 strain. However, an *E. coli K-12 omrAB* double mutant demonstrated 1.7-fold greater *fimB* transcription and 3.2-fold greater *fimE* transcription compared to the wild-type strain when the *E. coli* were grown in pH 5.5 Luria broth containing 400 mM NaCl (pH 5.5 LB+). In an *omrAB* UPEC strain, the *fimS* element switched to being more phase OFF and had a lower HA titer compared to wild-type when the *E. coli* were grown in pH 5.5 LB+ medium. The NU149 *omrAB* strain had significantly lower viable counts compared to the wild-type strain within murine kidneys**Conclusion**: Deletion of the individual *omrA* and *omrB* genes had no effect on *fim* gene transcription. However, deletion of both *omrA* and *omrB* had a significant effect on *fimB* and *fimE* transcription, orientation of the *fimS* element, and UPEC survival in murine kidneys.

Author Disclosure Block:

W.R. Schwan: None. **I. Wu:** None. **C. Grosshuesch:** None. **D. Baumann:** None.

Poster Board Number:

FRIDAY-748

Publishing Title:

Hfq Modulates the Locus of Enterocyte Effacement of Enteropathogenic *Escherichia coli* by Means of the Magnesium-Responsive sRNA MgrR

Author Block:

S. Bhatt, T. Buerkert, J. Palmer, E. Storm, J. Ramirez, C. Xander; Saint Joseph's Univ., Philadelphia, PA

Abstract Body:

Enteropathogenic *Escherichia coli* (EPEC) infects host cells by destroying intestinal microvilli and leads to diarrhea. The locus of enterocyte effacement (LEE) is responsible for the observed symptoms. Therefore, regulators of the LEE are critical determinants of the pathogenic state of the bacterium and must be considered for the development of any measures to combat bacterial infections. Recent studies have shown that the LEE-encoded *grlRA* operon, which specifies the LEE repressor, GrlR, and the LEE-activator, GrlA, serves as a hub for multiple regulatory inputs. Whereas transcriptional regulation of the *grlRA* operon has been rigorously investigated, posttranscriptional control remains elusive. We used a reporter *E. coli* strain containing a *grlR'-'lacZ* translational fusion to screen for Hfq-dependent sRNAs regulating *grlR.* One such sRNA that was isolated was MgrR. Overexpression of MgrR reproducibly repressed the *grlR'-'lacZ* fusion. An extensive region of complementarity was evident between MgrR and the cloned region of *grlR,* suggesting that MgrR exerts its effect by direct base pairing. Predictably, a mutation in the base-pairing region of MgrR abolished its ability to repress *grlR.* Intriguingly, though, overexpression of MgrR, activated the cotranscribed downstream gene *grlA,* instead of repressing it. Thus, in summary, our results suggest that the Hfq-dependent sRNA, MgrR, base-pairs to the *grlRA* mRNA and destabilizes *grlR* but stabilizes *grlA.* In the future, we intend to use biochemical approaches including toeprinting assays to corroborate this hypothesis.

Author Disclosure Block:

S. Bhatt: None. **T. Buerkert:** None. **J. Palmer:** None. **E. Storm:** None. **J. Ramirez:** None. **C. Xander:** None.

Poster Board Number:

FRIDAY-749

Publishing Title:

The Srna Ryhb Represses the Lee Pathogenicity Island in Enteropathogenic *Escherichia coli* by Base-pairing to the *grlra* Mrna

Author Block:

M. Egan, J. Ramirez, C. Xander, S. Bhatt, Department of Biology, Department of Mathematics; Saint Joseph's Univ., Philadelphia, PA

Abstract Body:

Enteropathogenic *Escherichia coli*, or EPEC, is a diarrheal pathogen that infects infants in developing countries. EPEC belongs to the attaching and effacing (A/E) morphotype, because it directly binds to intestinal epithelial cells and destroys the cellular microvilli. The virulence of EPEC is attributed to its major pathogenicity island: the locus of enterocyte effacement (LEE). Currently, there are no vaccines against EPEC. The problem is only exacerbated by the emergence of multi-drug resistant strains of EPEC. Thus, understanding the regulation of the LEE is critical to develop effective ways to combat EPEC infections. The LEE responds to a host of environmental cues, with the majority of them targeting three LEE-encoded transcription factors: Ler, GrlR, and GrlA. Whereas transcriptional regulation of the LEE has been widely characterized, post-transcriptional regulation, including regulation by trans-encoded regulatory small RNAs (sRNAs), remains understudied. Most sRNAs directly base-pair to their target mRNAs, influencing the translation and/or stability of the mRNA. A subset of these sRNAs requires Hfq, a chaperone protein that assists in the finding and base-pairing of sRNAs to their target mRNAs. One such sRNA is RyhB. Data from beta-galactosidase assays and Western Blots suggest that Hfq and RyhB corepress the *grlRA* mRNA transcript. To better understand how RyhB exerts its effect on *grlRA*, we performed *in silico* analysis. Using IntaRNA, we identified regions of complementarity between RyhB and the ribosomal binding site in the 5' untranslated region (UTR) of the upstream gene *grlR* in *grlRA*. In order to confirm this prediction of direct base-pairing between RyhB and *grlRA*, we constructed a polynucleotide mutation in RyhB's seed region. This mutation completely abolished the ability of the mutant RyhB to base pair to and repress the *grlR'-'lacZ* fusion. Collectively, our results suggest that RyhB represses the LEE by directing base-pairing to the leader segment of the *grlRA* mRNA and preventing the expression of GrlR and GrlA. Consistent with this prediction, overproduction of *ryhB* completely abolished the expression of GrlA and the GrlA-regulated target *ler*. Future studies are aimed at elucidating the precise molecular mechanism of the RyhB-mediated regulation of the LEE.

Author Disclosure Block:

M. Egan: None. **J. Ramirez:** None. **C. Xander:** None. **S. Bhatt:** None.

Poster Board Number:

FRIDAY-750

Publishing Title:

The Catabolite-Responsive sRNA Spot42 Represses Indole Production by Directly Base-Pairing to the *tnaC-tnaA* Intergenic Region of enteropathogenic *Escherichia coli* (EPEC)

Author Block:

S. Bhatt, V. Jenkins, E. Mason, J. Ramirez; Saint Joseph's Univ., Philadelphia, PA

Abstract Body:

Enteropathogenic *Escherichia coli* bacteria (EPEC) is a major cause of diarrheal disease in developing countries. EPEC causes disease due to the presence of the pathogenicity island locus of enterocyte effacement (LEE). A number of non-LEE encoded regulators control the LEE. One such regulatory element is the *tnaCAB* operon. The *tnaCAB* operon specifies the genes *tnaC*, *tnaA*, and *tnaB*. *tnaA* encodes the enzyme tryptophanase which converts tryptophan to indole, ammonia, and pyruvate. Indole is a bifunctional molecule, which induces the LEE and also functions as a secreted exotoxin that kills the worm *C.elegans*. Thus, any regulator of the *tnaCAB* operon would contribute to the ability of EPEC to cause disease in the mammalian and worm model systems. We screened for sRNA regulators of the *tnaCAB* operon by overexpressing sRNAs in EPEC and measuring indole production. One sRNA, Spot42, completely abolished indole production. Computational analysis revealed a region of complementarity between Spot42 and the intergenic region of *tnaC* and *tnaA*. The predicted *tnaC-tnaA* intergenic base pairing region was fused to a reporter *lacZ* gene lacking its 5' UTR and some of the N terminal codons to generate a hybrid *tnaA'-'lacZ* translational fusion. Overproduction of Spot42 repressed this minimal *tnaA'-'lacZ* translational fusion. Presently, we have introduced mutations in the base-pairing region of Spot42 and compensatory mutations in *tnaC-tnaA* intergenic region to provide genetic evidence for direct interaction between the Spot42 and *tnaC-tnaA* intergenic region. Additionally, we we are exploring how overexpression of Spot42 affects EPEC pathogenesis in the *C. elegans* and mammalian models of infection.

Author Disclosure Block:

S. Bhatt: None. **V. Jenkins:** None. **E. Mason:** None. **J. Ramirez:** None.

Poster Board Number:

FRIDAY-751

Publishing Title:

Discovery and Validation of a Conserved S6:S18-Interacting RNA *cis*-Regulator That Inhibits Translation of *E. coli rpsF*

Author Block:

A. M. Babina, Y. Fu, M. W. Soo, K. Deiorio-Haggar, M. M. Meyer; Boston Coll., Chestnut Hill, MA

Abstract Body:

More than half of the ribosomal protein operons in *Escherichia coli* are regulated by structures within the mRNA transcripts that interact with specific ribosomal proteins to inhibit further protein expression. These RNA structures exhibit a great deal of variation in size and complexity and utilize different mechanisms to regulate gene expression, including inhibition of translation initiation or elongation and premature transcription termination. The majority of these RNA regulatory structures are specific to Gammaproteobacteria. However, we and others have recently identified a widely distributed putative mRNA structure preceding the operons encoding small subunit ribosomal proteins S6 (*rpsF*) and S18 (*rpsR*) via comparative genomic approaches. Examples of this RNA from both *E. coli* and *Bacillus subtilis* are shown to interact *in vitro* with an S6:S18 complex. Structural probing and nuclease protection assays verify the predicted RNA secondary structure, identify the protein-binding site, and reveal that the proposed binding site bears a strong resemblance to the S18-binding site within the 16S rRNA. We also demonstrate that in *E. coli*, this RNA structure regulates gene expression in response to the S6:S18 complex. β-galactosidase activity from a *lacZ* reporter translationally fused to the 5'UTR and first nine codons of *E. coli rpsF* is reduced fourfold when both S6 and S18 are overexpressed together, but not individually. Measurement of transcript levels shows that although reporter levels do not change upon protein overexpression, levels of the native transcript are reduced fourfold, suggesting that this mRNA regulator prevents translation and this effect is amplified on the native transcript by other mechanisms. This work emphasizes how experimental verification can translate computational results into concrete knowledge of biological systems. The S6:S18-interacting mRNA structure is a relatively unique example of RNA *cis*-regulators of ribosomal protein synthesis found in bacteria, as it is highly conserved throughout the bacterial world and interacts with a complex of secondary rRNA-binding proteins.

Author Disclosure Block:

A.M. Babina: None. **Y. Fu:** None. **M.W. Soo:** None. **K. Deiorio-Haggar:** None. **M.M. Meyer:** None.

Poster Board Number:

FRIDAY-752

Publishing Title:

Anaerobic Induction of FrsA Expression by the Oxygen-FNR-sRNA Regulatory Pathway

Author Block:

B-R. Jang, K-J. Lee, K-H. Lee; Sogang Univ., Seoul, Korea, Republic of

Abstract Body:

Fermentation respiration switch (FrsA) is an enzyme catalyzing a conversion of pyruvate to acetaldehyde and carbon dioxide. FrsA protein level was not detectable in *Vibrio vulnificus* cells grown under oxygen-rich condition, and thus the *in vivo* activity of pyruvate decarboxylation derived from FrsA was observed in the cells grown under oxygen-limited condition. To investigate the regulatory mechanism(s) for the anaerobic induction of FrsA expression and activity, its transcription was monitored using both *frsA*-transcription reporter and quantitative RT-PCR assays. However, no significant difference was observed in its transcription and the resultant transcripts in the cells grown under aerobic or anaerobic conditions. This result lead us to consider the specific regulation at the post-transcription level and to examine the involvement of sRNA in FrsA expression. A candidate regulatory sRNA for FrsA expression (Rsf), including the sequences complementary to the 5'-UTR of *frsA* mRNA, was identified in *V. vulnificus* genome. A northern blot revealed the presence of 350 nucleotide-long sRNA. Its regulatory role was examined via monitoring FrsA levels in the *rsf*-deleted mutant *V. vulnificus*. In the absence of *rsf* gene, the negative effect of oxygen on the cellular level of FrsA was abolished, and thus the *rsf* mutant exhibited high activity of pyruvate decarboxylation even under the aerobic condition. It was further determined the regulatory dependency of Rsf on oxygen in repressing FrsA expression. Expression of the *rsf* gene was repressed by a transcription factor FNR under anaerobic condition, whereas repression of *rsf* transcription by FNR was relieved in the presence of oxygen. Thus, this study demonstrates that the cellular content of FrsA is minimized during aerobic growth via repression of its expression by Rsf. This repression, however, is relieved under anaerobic condition via repression of the *rsf* transcription by FNR, resulting in higher levels of the cellular FrsA and the mixed-acid fermentative metabolisms.

Author Disclosure Block:

B. Jang: None. **K. Lee:** None. **K. Lee:** None.

Poster Board Number:

FRIDAY-753

Publishing Title:

***Neisseria meningitidis* Utilizes Twin Small Rnas (Twin Nmsrs) to Switch from Cataplerotic to Anaplerotic Metabolism**

Author Block:

Y. Pannekoek[1], R. A. G. Huis in 't Veld[1], K. Schipper[1], S. Bovenkerk[1], G. Kramer[2], M. Brouwer[1], D. van de Beek[1], D. Speijer[1], A. van der Ende[1]; [1]Academic Med. Ctr., Amsterdam, Netherlands, [2]EMBL, Heidelberg, Germany

Abstract Body:

Neisseria meningitidis is primarily a commensal of the human oropharynx that sporadically causes septicemia and meningitis. Meningococci adapt to diverse local host conditions, differing in nutrient supply, like blood and cerebrospinal fluid (CSF), by changing their metabolism and protein repertoire. We investigated whether small RNAs are used by the meningococcus to regulate gene expression leading to adaptation to nutrient variation. By whole transcriptome analyses two structurally nearly identical small RNAs (designated Neisseria metabolic switch regulators NmsR-A and NmsR-B) with 70% nt identity (twin NmsRs) were identified. Functionality of twin NmsRs was investigated using growth assays with a wild type strain (wt) a deletion strain (Δ*twin*), by complementation of Δ*twin* and by comparative proteomics of these variants. Overexpression of twin NmsRs impaired growth in nutrient poor medium and cerebrospinal fluid but not in nutrient rich medium and blood, suggesting a connection between the twin NmsR regulon and the stringent response. Ten proteins, functionally involved in the tricarboxylic acid cycle, with increased expression in Δ*twin,* were identified. By using a *gfp*-reporter system in *E. coli*, direct antisense interaction between 6 out of 10 of these potential mRNA targets and NmsR-A was confirmed. Regulation of target mRNA by twin NmRs was assessed by RT-qPCR in the variant meningococcal strains and confirmed for 4 targets (*prpB, prpC, gltA* and *sucC*). These results suggest that twin NmsRs are involved in regulating switches between cataplerotic and anaplerotic metabolism in this pathogen. The connection with the stringent response was further indicated by the differential expression of twin NmRs in a *relA* deletion versus wt strain (RT-qPCR). In conclusion: The identification of small regulatory RNAs of meningococci as regulators of general metabolic switches, adds an interesting twist to their versatile repertoire in bacterial pathogens.

Author Disclosure Block:

Y. Pannekoek: None. **R.A.G. Huis in 't Veld:** None. **K. Schipper:** None. **S. Bovenkerk:** None. **G. Kramer:** None. **M. Brouwer:** None. **D. van de Beek:** None. **D. Speijer:** None. **A. van der Ende:** None.

Poster Board Number:

FRIDAY-754

Publishing Title:

Non-Canonical Translational Regulation of *E. coli manXYZ* Operon by Small RNASgrS

Author Block:

S. Azam, C. K. Vanderpool; Univ. of Illinois, Urbana, IL

Abstract Body:

The canonical model of small regulatory RNA (sRNA) mediated translation regulation assigns different roles for the sRNA and the chaperone partner Hfq. According to this model, the sRNA forms a duplex near the mRNA Shine-Dalgarno (SD) region and directly competes with the incoming ribosome. Here, the sRNA is the primary regulator; it shields the SD region from the ribosome while the chaperone Hfq facilitates duplex formation between the sRNA and the target. Contrary to this well-studied model, there are examples where an sRNA base pairs with a non-SD region, *e.g.,* the coding region, and inhibits translation by a mechanism independent of mRNA degradation. Although several studies have reported examples of non-traditional base pairing mediated regulation, mechanistically, these are the least understood aspects of sRNA biology. The secondary glucose transporter *manXYZ*, is the only known polycistronic target of SgrS targetome and the mRNA possesses two binding sites for the sRNA SgrS. One site is located in the *manX* coding region and the second is in the *manX-manY* intergenic region. Preliminary findings based on a set of in vivo and in vitro studies suggest that *manX* translation is repressed in a 'role-reversal' fashion where the chaperone Hfq acts to directly to occlude ribosome binding. We anticipate that the outcome of this study will reveal the mechanistic details of the interplay among Hfq, sRNA and the translation initiation complex.

Author Disclosure Block:

S. Azam: None. **C.K. Vanderpool:** None.

Poster Board Number:

FRIDAY-755

Publishing Title:

Alba4'S Stage-specific Roles in Translational Repression and Active Translation in the Malaria Parasite

Author Block:

E. E. Munoz, M. F. Kennedy, M. M. Shipley, S. E. Lindner; Pennsylvania State Univ., University Park, PA

Abstract Body:

Translational repression, the blocking of mRNA translation by stabilization or degradation of mRNA, has been identified as a critical mechanism for the transmission and successful establishment of malarial infections. Interestingly, ALBA domain (acetylation lowers binding affinity)-containing proteins functionally bind RNA, and in Plasmodium, ALBA proteins associate with known translational repressors (e.g., DOZI/CITH). Thus, ALBA4 may also play a role in translational repression. To understand its role, we used reverse genetics to knock out ALBA4 and to append a C-terminal GFP-tag to determine its importance. We observe two major phenotypes at transmission points in the ALBA4KO parasite: 1) an increase in the number of exflagellating male gametes, and 2) a significant decrease in salivary gland sporozoite load due to a defect in sporozoite development. To determine ALBA4's mechanism-of-action we conducted immunoprecipitation/mass spectrometry (IP/MS) experiments and total RNA-seq to identify protein binding partners and transcripts that may be regulated by ALBA4. In gametocytes, we found that ALBA4 associates with translational repression machinery. However, in asexual stages ALBA4 associates with active translational machinery (e.g. elongation factors, ribosomal proteins). Excitingly, we also performed the first successful IP/MS of a protein complex from sporozoite stages. In oocyst-sporozoites, ALBA4 associates with yet another complex comprised of other ALBA proteins and putative RNA-binding proteins. Our RNA-seq data indicates that ALBA4 is involved in the regulation of transcripts of other RBPs (including Puf1), and transcripts important in gametocytes and early mosquito stage (e.g., MDV1, PSOP2, PSOP17, CelTOS, PLP2, p47, p48/p45). In the absence of ALBA4, we believe the exflagellation phenotype is a result of the deregulation of transcripts encoding kinases and cytoskeletal elements that are important for male gamete activation. Taken together, we propose that ALBA4 is an important player in RNA homeostasis through translational repression and translational efficiency, depending upon stage and complex composition. These data also suggest RBPs can be involved in multiple molecular mechanisms, highlighting that RNA homeostasis is a dynamic and intricate process.

Author Disclosure Block:

E.E. Munoz: None. **M.F. Kennedy:** None. **M.M. Shipley:** None. **S.E. Lindner:** None.

Poster Board Number:

FRIDAY-756

Publishing Title:

RNA Anti-Sense Expression System to Test Targets for Genetically Specific Therapy against Pathogenic Bacteria

Author Block:

V. Casas, S. R. Maloy; San Diego State Univ., San Diego, CA

Abstract Body:

Traditional broad-spectrum antibiotics suffer from high levels of resistance, and disruption of the host microbiome that can result in chronic secondary sequelae. New diagnostic methods allow rapid, precise identification of pathogens responsible for an infection, allowing the rationale use of narrow-spectrum antimicrobials. It is possible to design genome based antisense nucleotides that disrupt a critical genetic target present in a particular pathogen, but lacking in the microbiome. In addition, antisense nucleotides can be rapidly redesigned for new target specificity in response to development of resistance. The focus of our research was to lay the foundation for this new strategy, by first developing a system to test the concept of using antisense RNA to knock down expression of essential genes leading to inhibition of bacterial growth. The system could then be used to screen bacterial genes identified via bioinformatics as targets for use in antisense RNA therapy. The system needed to be inducible and tightly regulatable and produce a short transcript more likely to bind to its target RNA. We used a vector previously optimized for high expression of toxic proteins, carrying an *rrnB* terminator, and under control of the *Escherichia coli rhaTRS* promoter and regulatory genes (pVX128). Into pVX128, we cloned an antisense oligo for the RNA polymerase alpha subunit (*rpoA*) and transformed it into *E. coli*. Expression of the *rpoA* antisense construct was tightly regulated by growth in D-glucose or L-rhamnose. Upon induction, growth was consistently inhibited by 10x when compared to controls. Expecting to see greater growth inhibition, we hypothesized that the antisense transcript was being degraded by the bacteria's RNAse III enzyme responsible for degrading RNA/RNA duplexes. To test this hypothesis, we made an rnc-14::ΔTn10d deletion in the *E. coli* strain using P1 transduction and tested this strain with the antisense construct. The deletion of the RNAseIII function increased growth inhibition by 100-1000x, as compared to the controls and RNAseIII wild type strain. The RNAseIII mutant results suggest a multiplex approach to targeting essential genes as well as genes that degrade antisense RNA duplexes would yield greater growth inhibition. These results lay the groundwork for building an arsenal of adaptable antisense therapies that can stay ahead of a quickly evolving pathogen.

Author Disclosure Block:

V. Casas: None. **S.R. Maloy:** None.

Poster Board Number:

FRIDAY-757

Publishing Title:

Rpos Independent, Growth Phase Dependent Gene Expression in *Escherichia coli*

Author Block:

D. Sharma, S. Chiang, H. Schellhorn; McMaster Univ., Hamilton, ON, Canada

Abstract Body:

Global changes in gene expression in response to environmental cues is hallmark of bacterial adaptation. In Escherichia coli, the alternative sigma factor RpoS is important for adaptation to nutrient deprivation. While RpoS controls many genes in early stationary phase it is not clear if RpoS has an important physiological role during extended stationary phase or during periods of senescence. We found that levels of RpoS dependent transcripts are maximal during early stationary phase (OD600 = 1.5 - 2.0) and subsequently decline, suggesting that additional regulators may be important following early stationary phase adaptation. Other genes such as tryptophanase, which controls indole signalling, is induced in a growth phase dependent manner in both WT and rpoS backgrounds. We examined global changes in gene expression during extended incubation, up to 2 days, to examine expression profiles during post stationary phase in planktonic cells. Data indicates that mRNA levels of RpoS and its regulon members decrease to low levels after 48 h. Gene transcripts that are abundant during prolonged starvation are RpoS-independent and include those important in biofilm formation and those of cryptic prophage. These data are consistent with the idea that RpoS acts as a transient regulator important during initial stages of stress adaptation but less important during prolonged starvation or senescence and that the post stationary phase gene expression during extended incubation is characterized by a set of regulators/survival genes that are independent of RpoS.

Author Disclosure Block:

D. Sharma: None. **S. Chiang:** None. **H. Schellhorn:** None.

Poster Board Number:

FRIDAY-758

Publishing Title:

Influence of Natural Products and Heat Shock on Swarming and Gene Expression of Shiga-Toxin Producing *Escherichia coli*

Author Block:

C. Caballero, **J. Merino**, N. L. Heredia, S. Garcia; Univ. Autonoma de Nuevo León, San Nicolás, N.L., Mexico

Abstract Body:

Background: Shiga-toxin producing *Escherichia coli* (STEC) strains are pathogenic for humans and capable to causing severe illnesses such as bloody diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome. Heat and natural antimicrobials are being used to control STEC. This study analyzed the effect of heat and natural products on swarming and expression of virulence genes in strains of STEC (serotype O103, O26 and O111).**Methods:** Control strains were non-pathogenic *E. coli* and EHEC O157:H7. Citral, eugenol and hexanal were used alone or in combination with heat stress (HS, cultures from 37°C subjected to 46°C for 1h and then to 55 °C for 1h). Minimum bactericidal concentration and sub-inhibitory concentrations were determined. Swarming was determined in plates with 0.3% agar. Total RNA was extracted from O26 serotype and the cDNA synthetized for analysis of gene expression by qPCR, using GAPDH as housekeeping gene.**Results:** MBC against the five strains ranged from 0.9 to 1.3 mg/mL for citral, 0.58 to 0.73 mg/mL for eugenol and 2.2 to 2.5 mg / mL for hexanal. Natural products and heat sock significantly reduced cell viability. Eugenol and hexanal reduced swarming of O26 STEC by 16% and 14% respectively and O26 swarming was equal or greater than EHEC O157:H7. Eugenol w/wo HS decreased (0.5 to 0.4-fold) *flhC* gene (flagella) expression of STEC O26 compared to control without treatment. In a similar way, hexanal plus heat decreased 0.5 fold *flhC* expression. This decrement in *flhC* expression could explain reduction of swarming observed. Eugenol treatment decreased 0.2 fold expression of *rpoH* and *rpoE*; and hexanal plus HS reduced (0.6 fold) *rpoS*. Hovever, Citral w/wo HS, eugenol with HS and hexanal without HS overexpressed (1.8, 1.4, 1.2, 0.6 fold) *rpoS* gene. stx1 gene expression was reduced 0.4 and 0.2 fold after HS and citral plus HS respectively. Citral showed the higher decrease (42% of reduction compared with untreated control) in swarming mobility of STEC O26, followed by citral plus HS (30% of reduction).**Conclusions:** This study indicates that combined use of natural products and heat stress affects the growth and swarming motility and expression of genes involved in virulence.

Author Disclosure Block:

C. Caballero: None. **J. Merino:** None. **N.L. Heredia:** None. **S. Garcia:** None.

Poster Board Number:

FRIDAY-759

Publishing Title:

UV and Temperature Resistance in Phyllosphere Microbial Isolates

Author Block:

L. Posada, I. C. Acosta, C. A. Ruiz, C. Garcia, M. M. Zambrano; Corporacion Corpogen, Bogota, Colombia

Abstract Body:

Background The Paramo ecosystem in the Colombian Andes is exposed to environmental conditions that can be considered harsh for microorganisms, such as high UV exposure and daily shifts in temperature. We hypothesized that microbial isolates obtained from the phyllosphere of endemic *Espeletia sp.* plants could harbor unique adaptations that could be potentially useful in industrial, environmental or clinical settings.**Methods** We isolated 50 strains from the *Espeletia* sp. phyllosphere and characterized them morphologically and by sequencing the 16S rRNA gene for bacteria and the ITS region for fungi. Strains were screened for survival under high doses of UVC radiation, from 50 to 800 J/m^2, and tested for their capacity to survive at different temperatures (-20, 4, 45, 55°C). Selected strains were analyzed for their capacity to withstand temperature shifts between 37C° and -20°C. One strain resistant to high doses of UV radiation was selected for transcriptomic analysis using Illumina HiSeq.**Results** The isolates included 10 fungi, 26 Gram negative and 14 Gram positive bacteria. The majority of the strains survived poorly (<90%) when exposed to 50 J/m^2 radiation. Two S*phingomonas sp.* strains were able to resist 100 J/m^2, one *Pseudomonas sp.* strain resisted 50 J/m^2, and the yeast *Cryptococcus flavescens* survived 95% with 200J/m^2. The most resistant strain, *Deinoccocus swuensis*, showed >95% survival at 800 J/m^2; transcriptomic analysis of this strain is underway. Three *Bacillus sp.* strains were capable of resisting high temperatures (45 and 55C°), while 13 strains were able to resist freezing (-20ºC, >99% viability). Two of these strains, the yeast *Hannaella oryzae* and the bacterium *Burkholderia glathei*, were further analyzed and found to have > 94% survival when subjected to shifts of temperature between -20 and 37ºC.**Conclusions** The Paramo environment imposes selective pressure on plants and their associated microbiota, resulting in microorganisms capable of resisting high doses of UV radiation and rapid changes of temperature, conditions characteristic of this ecosystem. Both bacteria and fungi were identified in this study, some of which are promising in terms of understanding the mechanisms involved and their possible biotechnological potential.

Author Disclosure Block:

L. Posada: None. **I.C. Acosta:** None. **C.A. Ruiz:** None. **C. Garcia:** None. **M.M. Zambrano:** None.

Poster Board Number:

FRIDAY-760

Publishing Title:

Cold-Responsive Proteome and Transcriptome in a Psychrophilic Antarctic Bacterium *Psychrobacter* sp. PAMC 21119 at Subzero Temperature

Author Block:

H. Koh, J. Lee, S. Han, H. Park, S. Lee; Korea Polar Res. Inst., Incheon, Korea, Republic of

Abstract Body:

Background: Psychrophilic Antarctic *Psychrobacter* sp. PAMC 21119 was isolated from permafrost in Antarctica, and it grows and divides at -5°C. In this study, we investigated the transcriptomic and proteomic responses of this species to cold temperature by comparison approach of -5°C with 20°C to understand how extreme microorganisms survive under subzero condition. **Methods:** For transcriptome analysis was subjected to next-generation sequencing using an Illumina Hiseq. Proteome analysis employed two-dimensional electrophoresis (2-DE) and a 4800 MALDI TOF/TOF instrument (ABSCIEX). We subjected the differentially expressed transcripts and proteins to GO analysis and were classified into different categories using COG databases. Molecular pathways of differentially abundant proteins were analyzed using KEGG online resource. Some cellular metabolic activities were measured, including succinate dehydrogenase activity, intracellular ATP concentration, and proton concentration.**Results:** We found 2,906 transcript expression in all replicates from both temperature treatments, and 611 differentially expressed genes ($\geq$ 2 fold, $p < 0.005$) by RNA-seq. A total of 60 spots ($\geq$ 1.8 fold, $p<0.005$) showed differential expression on 2-DE and the proteins were identified by MS/MS. The most prominent up-regulated genes encoded proteins involved in metabolite transport, protein folding, and membrane fluidity. Proteins involved in energy production and conversion, and heme protein synthesis were down-regulated.**Conclusion:** This study has highlighted some of the strategies and different physiological states that *Psychrobacter* sp. PAMC 21119 has developed to adapt to the cold environment of Antarctica and has revealed strong candidate molecules involved in cold adaptation.

Author Disclosure Block:

H. Koh: None. **J. Lee:** None. **S. Han:** None. **H. Park:** None. **S. Lee:** None.

Poster Board Number:

FRIDAY-761

Publishing Title:

Characterization of *dnaK* Mutation in Persistence of *Staphylococcus aureus*

Author Block:

A. Loveland, V. K. Singh, N. R. Chamberlain; A. T. Still Univ. - Kirksville Coll. of Osteopathic Med., Kirksville, MO

Abstract Body:

Background: Persistence is a non-heritable bacterial characteristic that results in bacteria surviving toxic events via the alteration of gene expression. Numerous genes for stress response proteins have been studied and indicate the importance of these proteins in bacterial survival. The *dnaK* gene codes for a stress response protein called a heat-shock chaperone that can also prevent denaturation of vital proteins following exposure to other types of stress. The purpose of this study was to characterize the effects that a knockout *dnaK* mutation has on persister cell numbers in *Staphylococcus aureus* SH1000 (wild type, WT) when treated with antibiotics at several growth stages.**Methods:** Previous studies using multiple bacterial strains have suggested that cell size plays an important role in determining optical density, quantifying colony forming units per milliliter (CFU/ml), and treating cells with an appropriate amount of antibiotic. The optimal optical densities (OD_{600}) were determined for WT (0.25, 0.50, 0.75, 1.0, 1.5) and *dnaK* mutant (0.22, 0.37, 0.48, 0.70, 1.25). Two samples were obtained at the optimal OD_{600}: one sample was taken to determine the pre-treatment CFU/ml and another sample was taken to determine the post-treatment CFU/ml after being treated with either oxacillin (30 µg/ml) or erythromycin (30 µg/ml). Survival percentages were calculated and statistical analysis was completed using a Student's T-test.**Results:** In this study, we have determined that the *dnaK* mutant was smaller in size than the WT strain ($P < 0.0001$). When treated with oxacillin, the *dnaK* mutant exhibited similar survival to the WT strain at each optical density except at OD_{600} 0.75 (0.48 *dnaK* mutant equivalent). At OD_{600} 0.75 the *dnaK* mutant displayed a significantly lower percent survival (*P = 0.024*). When erythromycin was utilized as the antibiotic treatment, the *dnaK* mutant exhibited decreased survival relative to the WT at OD_{600} 0.75/0.48 (*P = 0.022*), 1.0/0.70 (*P = 0.006*), and 1.5/1.25 (*P = 0.043*).**Conclusions:** These findings suggest the importance of DnaK in bacterial persistence. However, its role in persistence is dependent on the antibiotic that is used for treatment as well as the optical density at which samples are harvested and treated. Further studies will focus on the effects seen with other classes of antibiotics.

Author Disclosure Block:

A. Loveland: None. **V.K. Singh:** None. **N.R. Chamberlain:** None.

Poster Board Number:

FRIDAY-762

Publishing Title:

Reexamining Persister Formation in *Escherichia coli*

Author Block:

Y. Shan, K. Lewis; Northeastern Univ., Boston, MA

Abstract Body:

Persisters are dormant variants that form a subpopulation of drug tolerant cells largely responsible for recalcitrance of chronic infections. Toxin/antitoxin modules have been linked to persister formation in *Escherichia coli*. The current standard model holds that nutrient stress causes stringent response, upregulation of ppGpp synthesis by RelA/SpoT, which causes activation of the Lon protease to degrade antitoxins; active toxins then inhibit translation, resulting in dormant, drug-tolerant persisters. We examined several stresses including stringent response and found that they all upregulate at least some of the interferase toxins. Two of the stresses, high salt and stringent response, induced persister formation, but unexpectedly, the upregulated toxins were not responsible for increased persister formation under these conditions. While a *relA/spoT* mutant formed less persisters, we trace this to primarily the lower cell density in a population of this strain. We similarly did not see an effect of deletion of the other components of the proposed model on persister formation, Lon and PPK/PPX. This suggested the need to reexamine the mechanism of persister formation. Sorting dim cells carrying a ribosomal promoter linked to degradable GFP (*rrnB* P1-GFP) enriches in persisters. *rrnB* P1 is repressed by ppGpp and activated by ATP. Enrichment of persisters is observed in the background of a *relA/spoT* mutation, suggesting that *rrnB* P1-GFP is a persister marker responding to ATP levels rather than ppGpp. Decreasing the level of ATP by treatment with arsenate causes drug tolerance, apparently due to inactivity of targets. We conclude that persisters are cells that stochastically lose ATP, possibly due to random errors.

Author Disclosure Block:

Y. Shan: None. **K. Lewis:** None.

Poster Board Number:

FRIDAY-763

Publishing Title:

Characterizing the Role That the Translational GTPase BipA Plays in Adaptation in *E. coli* MG1655

Author Block:

R. A. Bova, V. L. Robinson, D. R. Benson; Univ. of Connecticut, Storrs, CT

Abstract Body:

BipA, also known as TypA, is a conserved translational GTPase. It has been proposed to be involved in translation, ribosome biogenesis and assembly, tRNA modification, translocation, cell polarity, cell division and signaling events in a cell. BipA interacts with the 70S ribosome in a GTP-dependent manner and has been shown to have an altered association in the presence of (p)ppGpp and associate with the 30S subunit under certain conditions. Mutations in *bipA* seem not to affect normal balanced growth but do confer a variety of other phenotypes including loss of virulence or symbiotic competence, enhanced motility, growth at low temperatures and cell surface alterations. Nevertheless, few unified explanations for the observed phenotypes of *bipA* mutants are available. We tested the effect that various nutritional and physical transitions have on the balanced growth of *Escherichia coli MG1655* and its Δ*bipA* derivative. Conditions tested included sudden shifts in carbon and nitrogen sources, aerobic-anaerobic transition, temperature, detergents, salinity and oxidizing agents. Under the experimental conditions used, a lag in the adaptation to the new growth condition was consistently observed for *bipA* null mutants. After the lag, the specific growth rate of the two strains was the same. The lag could be complemented with a full-length *bipA* gene. We conclude that BipA is needed foradaptation to new conditions but is not necessary during normal cellular growth. We hypothesize that BipA can be considered an adaptational GTPase that interacts with the ribosome during environmental change.

Author Disclosure Block:

R.A. Bova: None. **V.L. Robinson:** None. **D.R. Benson:** None.

Poster Board Number:

FRIDAY-764

Publishing Title:

Opsins Inhibit Lycopene Elongase Enzymes in Halophilic Archaea

Author Block:

R. F. Peck; Colby Coll., Waterville, ME

Abstract Body:

Halophilic Archaea live in dynamic environments that undergo dramatic changes in osmolarity and nutrient availability. These organisms must rapidly change resource allocation to respond to current conditions. In *Halobacterium salinarum*, the light-driven proton pump bacteriorhodopsin is produced under low-oxygen conditions and catalyzes the conversion of light energy to usable cellular energy. Bacteriorhodopsin consists of an integral membrane protein, bacterio-opsin, and a covalently-bound retinal cofactor. The retinal cofactor is synthesized *de novo* in *H. salinarum* from carotenoid precursors including the 40-carbon lycopene. In the biosynthetic pathway of membrane-protective compounds called bacterioruberins, lycopene elongase (Lye) converts lycopene into a 50-carbon compound. Thus, Lye activity reduces lycopene available for retinal production. We previously identified a regulatory mechanism in which bacterioopsin inhibits Lye to promote retinal synthesis.To examine if this mechanism is found in organisms besides *H. salinarum*, we expressed homologs of bacterioopsin and Lye in *Haloferax volcanii*, a halophilic Archaea that lacks native opsins. Three archaeal and two bacterial opsins tested demonstrated no ability to inhibit *H. salinarum* Lye. However, our preliminary results indicated that expression of the *Haloarcula vallismortis* cruxopsin-3 inhibits the activity of Lye from the same organism. Correspondingly, *H. salinarum* bacterioopsin does not inhibit the *H. vallismortis* Lye. These results suggest that opsins specifically inhibit Lye from the same organism. Together, our findings raise the possibility that opsins and Lye co-evolved to allow efficient production of retinal when required for functional opsin-retinal complexes.

Author Disclosure Block:

R.F. Peck: None.

Poster Board Number:

FRIDAY-765

Publishing Title:

Induction of Gene Expression under Respiration-Inhibitory Conditions by the AldR Transcriptional Regulator in *Mycobacterium smegmatis*

Author Block:

J-A. Jeong, J-I. Oh; Pusan Natl. Univ., Busan, Korea, Republic of

Abstract Body:

Although *Mycobacterium* species are obligate aerobes, a gradual depletion of oxygen from their culture is known to lead to dramatic changes in gene expression. Expression of the *ald* gene encoding alanine dehydrogenase (Ald) was induced under oxygen-limiting conditions and in the presence of alanine, and the induction of *ald* expression was mediated by the AldR transcriptional regulator in response to alanine availability. It was also proposed that Ald is required for both utilization of alanine as a nitrogen source and growth under hypoxic conditions by maintaining the redox state of NADH/NAD^+. We have previously shown that hypoxic induction of *ald* results from increased intracellular levels of alanine in *Mycobacterium smegmatis* under hypoxic conditions. *M. smegmatis* contains two terminal oxidases, an aa_3-type cytochrome *c* oxidase and a cyanide-insensitive *bd*-type quinol oxidase. Using aa_3 cytochrome *c* oxidase (Δ*ctaC*) and *bd* quinol oxidase (Δ*cydA*) mutant strains grown aerobically, we demonstrated that the blocking of electron flux through the cytochrome *c* pathway of the respiratory electron transport chain led to induction of *ald* expression. Under respiration-inhibitory conditions such as the presence of cyanide, expression of the *ald* gene in the Δ*cydA* mutant was more strongly induced compared to that in the wild-type (WT) over the same concentration range of cyanide, implying that the extent of electron flow through the electron transport chain is inversely related to *ald* expression. The lower intracellular alanine levels in the Δ*ald* mutant than in the WT during hypoxic growth indicate that Ald catalyzes the reductive amination of pyruvate to L-alanine to recycle NADH to NAD^+ when oxygen becomes limiting. Consistent with the results, Δ*ctaC* mutant strain grown aerobically displayed an increase in both the ratio of NADH to NAD^+ and the cellular level of alanine compared to the WT. When *M. smegmatis* encounters respiration-inhibitory conditions, the reductive amination reaction by Ald is a way the bacterium recycles NADH to NAD^+, thereby increasing the intracellular level of alanine. These findings suggest that AldR senses not only alanine directly but also respiration-inhibitory signals indirectly to induce *ald* expression under respiration-inhibitory conditions.

Author Disclosure Block:

J. Jeong: None. **J. Oh:** None.

Poster Board Number:

FRIDAY-766

Publishing Title:

Cellular Responses of *Escherichia coli* to the Treatment of Extracellular Atp

Author Block:

J. Wu, C. Xi; Univ. of Michigan Sch. of Publ. Hlth., Ann Arbor, MI

Abstract Body:

Background: Extracellular adenosine-5'- triphosphate (eATP) is now well known a signaling molecule in animals and plants. Recent study show it promotes biofilm formation in bacteria on biotic and abiotic surfaces (Xi, C., et. al., 2010). Here we investigated how E. coli cells respond to the treatment of eATP. **Methods:** A whole-transcriptome analysis was conducted to reveal the differential gene expression induced by eATP. qPCR was used to confirm the results of the microarray assay. Traditional biochemical assays were used for detecting and quantifying the key intermediates identified by both assays. **Results:** Results showed that extracellular ATP impeded *Escherichia coli* swimming and swarming motility, promoted its production of enterobactin, and rendered its ability to resistant acid. The whole-transcriptome analysis and qPCR data demonstrated that eATP induced the expression of an array of genes involved in the above mentioned responses. The whole-transcriptome analysis also revealed that eATP promoted *E. coli* TCA and glyoxylate cycles by transporting and using exogenous amino acids, di-peptides as carbon resources, and produced critical biochemical intermediates (i.e. chorismate and indole) as important precursor and internal signaling molecule, respectively. **Conclusion:** These data suggest that extracellular ATP can alter metabolism pathways in bacteria by affecting cellular processes both at transcriptional level and metabolomics level, potentially playing a physiological role in transducing stress and pathogenicity.Keywords: extracellular adenosine-5'-triphosphate (eATP), signaling molecule, *Escherichia coli*, metabolism, TCA and glyoxylate cyclesReferences:Chuanwu Xi, Jianfeng Wu. 2010. dATP/ATP, a multifunctional nucleotide, stimulates bacterial cell lysis, extracellular DNA release and biofilm development. PLoS One. 5(10): e13355.

Author Disclosure Block:

J. Wu: None. **C. Xi:** None.

Poster Board Number:

FRIDAY-767

Publishing Title:

Regulation of Cellular Level of Fermentation Respiration Switch (FRSA) by IIA^{Glc} and DegQ

Author Block:

K-J. Lee, H-W. Yang, K-H. Lee; Sogang Univ., Seoul, Korea, Republic of

Abstract Body:

Enzymatic activity of FrsA catalyzing a conversion of pyruvate to acetaldehyde and carbon dioxide is required for the complete mixed-acid fermentation of glucose by *Vibrio vulnificus* under oxygen-limited conditions. Its activity is highly enhanced by a direct interaction with the dephospho-form of IIA^{Glc} (d-IIA^{Glc}). The cellular level of FrsA has been shown to be increased in cells grown in glucose-rich medium under anaerobic condition. Screening of *V. vulnificus* mutants by western analysis using anti-FrsA antibodies revealed that a mutant deficient in a gene encoding the protease homologous to the *Escherichia coli* DegQ contained higher FrsA than the wild type. Complementation of the mutant with the intact *degQ* gene resulted in decrease of FrsA in cells grown glucose-limited medium. To investigate whether FrsA is a substrate for DegQ, *in vitro* proteolysis reaction was reconstituted using recombinant proteins of FrsA and DegQ. SDS-PAGE analysis of the reaction mixtures showed that FrsA proteolysis by DegQ was apparent in a dose-dependent manner of added protease. Since the *in vivo* level of the cellular FrsA was influenced by glucose and FrsA exhibited a strong affinity to d-IIA^{Glc}, recombinant IIA^{Glc} was included in the *in vitro* proteolysis reaction. FrsA proteolysis by DegQ was inhibited by d-IIA^{Glc} in a concentration-dependent manner. The reaction mixture including the phospho-form of IIA^{Glc}, however, did not show any inhibitory effect on DegQ proteolysis. Therefore, this study demonstrates that the stability of FrsA protein is highly increased in cells growing in glucose-rich condition via evading the proteolytic attack by DegQ by binding with d-IIA^{Glc}. It further suggests that the increased levels of cellular content and enzymatic activity of FrsA under this condition would guarantee enhanced mixed-acid fermentation of glucose.

Author Disclosure Block:

K. Lee: None. **H. Yang:** None. **K. Lee:** None.

Poster Board Number:

FRIDAY-768

Publishing Title:

Molecular Engagements for Hyper Glucose-Tolerance of Acetic Acid Bacteria, *Tanticharoenia sakaeratensis* and *Asaia bogorensis*

Author Block:

H. Hadano[1], N. Okamoto[1], S. Takebe[1], K. Matsushita[2], **Y. Azuma**[1]; [1]Kindai Univ., Kinokawa, Japan, [2]Yamaguchi Univ., Yamaguchi, Japan

Abstract Body:

Acetic acid bacteria (AAB) form a divergent phylogenetic group, and two phylogenetically closed AAB, *Tanticharoenia sakaeratensis* and *Asaia bogorensis*, show a distinctive ability to grow in media containing 30% glucose. To clarify mechanisms for the hyper glucose-tolerance, comparative omics analyses, including genome DNA sequencing, transcriptome and proteome analyses, were performed. The results illustrated that the two AAB altered expressions of similar genes and systems under different conditions with low and high glucose concentrations. In both AAB gene expressions for glycolysis and pentose phosphate pathways decreased, and ones for antioxidant enzymes (such as superoxide dismutase (SOD) and peroxidase) and cytochrome o ubiquinol oxidase increased. On the contrary, the omics analyses indicated a variety uniqueness of each bacterium. For instance, under high glucose conditions, *T. sakaeratensis* repressed a main energy metabolism including NADH dehydrogenase (complex I) and induced gene expression of pyruvate decarboxylase, whereas *A. bogorensis* promoted expressions of more stress responsible genes involved in anti-oxidation, such as DNA starvation/stationary phase protection protein (Dps) and osmotically inducible peroxiredoxin OsmC. Because the complex I is a main superoxide producer, *T. sakaeratensis* deems to need less antioxidant enzymes than *A. bogorensis*. We herein propose that enzymes managing oxidative stresses are engaged in overcoming the high glucose stress, and the acquisitions of the glucose tolerance systems in the two AAB were evolutionally independent.

Author Disclosure Block:

H. Hadano: None. **N. Okamoto:** None. **S. Takebe:** None. **K. Matsushita:** None. **Y. Azuma:** None.

Poster Board Number:

FRIDAY-769

Publishing Title:

The Role of the Srna-encoded Small Protein Sgrt in the *Escherichia coli* Glucose-phosphate Stress Response

Author Block:

C. Lloyd, C. Vanderpool; Univ. of Illinois Urbana-Champaign, Urbana, IL

Abstract Body:

* SgrT is a 43-amino acid protein encoded within the small regulatory RNA (sRNA) SgrS; both counterparts are expressed in *Escherichia coli* under glucose-phosphate stress - a condition in which the accumulation of non-metabolizable phosphosugars (such as αMG) is bacteriostatic. SgrT and SgrS base pairing can independently mitigate glucose-phosphate stress but act through separate mechanisms; SgrS base pairing destabilizes the mRNA of the major glucose transporter PtsG and inhibits its synthesis, while SgrT inhibits its transport activity. Using the radiolabeled glucose analog αMG in transport assays, we found that SgrT exhibits a strong and speedy inhibition of stable preexisting PtsG transporters while SgrS base pairing only modestly inhibits tranport as an indirect result of PtsG translational repression. To compare SgrT and SgrS target specificities, we used induction of the stress response as a measure of transport via a transcriptional P_{sgrS}-*lacZ* fusion and found that while SgrT was able to inhibit uptake of the PtsG substrate αMG, it was unable to inhibit uptake of the analog 2DG, which is solely transported through ManXYZ - another mRNA target of SgrS riboregulation. The specific targeting of PtsG by SgrT was thought to be because the activator of PtsG, $EIIA^{Glc}$, performs inducer exclusion. Indeed, when SgrT-producing cells were grown in both lactose and αMG, SgrT overrode inducer exclusion and allowed the utilization of lactose as a carbon source, recovering faster than cells without lactose. In this way direct inhibition of PtsG activity indirectly allows cells to replenish depleted glycolytic intermediates expended by the glucose PTS to import non-metabolizable analogs, which is believed to be the root of the stress. We used an SgrT-regulated hybrid PtsG and SgrT-resistant PtsG V12F mutant to determine the membrane domain of PtsG is physiologically important for SgrT regulation, however further structure-function studies must be performed to understand the extent and mechanism of this interaction.

Author Disclosure Block:

C. Lloyd: None. **C. Vanderpool:** None.

Poster Board Number:

FRIDAY-770

Publishing Title:

The Effects Of Cell Density And Biofilm Formation On Melanin Production In A Novel *pseudomonas* Species

Author Block:

P. L. Lawrence, L. R. Aaronson; Utica Coll., Utica, NY

Abstract Body:

Pseudomonas sp. UC17F4 is a novel bacterial species that was isolated from the cutaneous flora of red-backed salamanders on the basis of its antifungal activity. One of the most interesting characteristics of this species is its ability to produce two different forms of the brown pigment, melanin. Eumelanin is a high MW molecule that is contained within the cytoplasm of the bacterial cell, while pyomelanin is a low MW secreted form. Studies in our lab have shown that melanin production in this bacterium is regulated by light exposure, and by the availability of iron and suitable carbon sources. We have recently observed that melanogenesis is dependent on high cell density and the development of biofilms. Cultures of UC17F4 were grown in either tryptic soy-yeast extract (TSYE) broth or in citrate-enriched Lawrence Minimal Medium (LMM). Eumelanin content was assayed by spectrophotometric analysis of cell lysates in 1% SDS. As culture densities increased, so did cellular melanin content, increasing 6-fold from the onset of stationary phase. Cultures inoculated at higher cell density also exhibited higher melanin content. Biofilm-forming cultures produced 5-fold higher levels of eumelanin than did planktonic broth cultures. Biofilm cultures were grown in 35 mm plates in both TSYE and LMM broth, and exhibited density-dependent melanin content. Similar results were observed in biofilms formed on nitrocellulose filters placed on LMM and TSYE agar plates. To further study the dependence of UC17F4 biofilm formation of melanogenesis, NaCl was added to cultures to inhibit the development of biofilms. Bacterial eumelanin content in the biofilm mass declined by 90% as NaCl concentration increased to 1% (w/v) compared to untreated controls. Treatment of cultures with Furanone 56, an inhibitor of quorum signaling in Pseudomonads, resulted in a 30% reduction in eumelanin content. The quorum signaling compound C12-homoserine lactone stimulates only a modest increase in melanogenesis. Supplementation of early exponential-phase cultures with cleared supernatants from 48 hr biofilm cultures resulted in up to a 2-fold increase in cellular melanin content. These data suggest that melanogenesis in *Pseudomonas* sp. UC17F4 is under the control of one or more signaling compounds. Ongoing studies are being conducted to isolate and characterize melanogenic signal compounds, and regulatory pathways in the bacterium.

Author Disclosure Block:

P.L. Lawrence: None. **L.R. Aaronson:** None.

Poster Board Number:

FRIDAY-771

Publishing Title:

Characterization of the Global Regulatory Network of *Trans*-Translation in *Pseudomonas aeruginosa*

Author Block:

B. Li[1], L. Silo-Suh[2], S-J. Suh[1]; [1]Auburn Univ., Auburn University, AL, [2]Mercer Univ. Sch. of Med., Macon, GA

Abstract Body:

Trans-translation, catalyzed by tmRNA, is a uniquely bacterial process for recycling ribosomes stalled on mRNA. To understand the regulation of tmRNA in *P. aeruginosa,* we previously isolated 18 transposon insertion mutants with altered expression of *ssrA,* the gene encoding for tmRNA. The site of transposon insertion in these mutants suggested that LPS modification and c-di-GMP metabolism may affect ribosome recycling. We also took a predictive approach based on the mutant phenotype and identified putative regulatory circuits between *trans*-translation and various global regulators of gene expression in *P. aeruginosa*. Our data indicated that tmRNA is required for expression of *rpoS, lasR, rhlR,* and *vfr*. Interestingly, RpoS, LasR, and RhlR were all required for full expression of *ssrA,* suggesting a complex regulatory circuit between *trans*-translation and these global regulators. In contrast, although Vfr was required for full expression of *ssrA, trans*-translation appeared to repress *vfr* expression. Since the nutritional alarmone (p)ppGpp mediates expression of *rpoS* and other genes which are coordinately controlled by LasR and RhlR in *P. aeruginosa*, we also assessed *ssrA* expression in both the *relA* mutant and *relA spoT* double mutant, which is severely impaired for accumulation of (p)ppGpp during nutritional stress. Surprisingly, we found that even though *ssrA* expression decreased by approximately 80% in the *relA* mutant, it only decreased by 30% in the *relA spoT* double mutant, suggesting that the level of cellular (p)ppGpp is a crucial factor for *ssrA* expression in *P. aeruginosa*. Moreover, our data from static biofilm assays with wild-type PAO1 and the *ssrA* mutant showed that the mutant forms approximately 40% more biofilm than the parent, indicating a role for tmRNA in *P. aeruginosa* biofilm formation. Finally, in order to study the overall effect of ribosome recycling in *P. aeruginosa,* we constructed a derivative of the *ΔssrA* mutant in which we can control the expression of *PA3990,* a putative ArfA encoding gene, to maintain the viability of the bacterium. Our previous attempts to construct *ΔssrA ΔPA3990* double mutants had been unsuccessful although the *ΔPA3990* mutant behaved similarly to the wildtype. These data support our hypothesis that PA3990 is a ArfA homologue.

Author Disclosure Block:

B. Li: D. Employee; Self; Auburn University. **L. Silo-Suh:** None. **S. Suh:** None.

Poster Board Number:

FRIDAY-772

Publishing Title:

Identification of Clpxp Substrates during Carbon Starvation

Author Block:

C. N. Peterson[1], N. Maragos[1], P. Ngo[2], T. Von Rosen[3]; [1]Suffolk Univ., Boston, MA, [2]Suffolk Univ.y, Boston, MA, [3]Max Planck Marine Microbiol. Inst., Bremen, Germany

Abstract Body:

Cellular protein concentrations reflect a balance between synthesis, degradation, and dilution by cell growth. Synthesis is routinely analyzed in high-throughput experiments, but fewer studies consider degradation, particularly under non-optimal growth conditions such as starvation. ClpXP is a central and conserved protease that degrades numerous proteins. Here, we modified the ClpXP protease trap assay to identify which proteins are degraded under glucose and no glucose conditions. Substrates involved in replication, cAMP hydrolysis, protein synthesis, pyrimidine synthesis and carbohydrate transport and metabolism were found to be associated with ClpXP during starvation. Conversely, substrates involved in the stress response, glycogen synthesis and pyrimidine metabolism were more likely to be degraded in glucose conditions. Almost all of these proteins had a canonical C or N terminal ClpX tag on them. Thus, the Clpx protease markedly remodels its substrate profile during starvation.

Author Disclosure Block:

C.N. Peterson: None. **N. Maragos:** None. **P. Ngo:** None. **T. Von Rosen:** None.

Poster Board Number:

FRIDAY-773

Publishing Title:

Isolation of Suppressors That Recover Cold-Sensitive Growth Defect Caused by *bipA* Deletion in *Escherichia coli*

Author Block:

E. Choi, J. Hwang, H-h. Lee, J. Park, Y-S. Seo; Pusan Natl. Univ., Busan, Korea, Republic of

Abstract Body:

Background: BipA (BPI-inducible protein A) protein is translational GTPase conserved in a large variety of bacterial species and structurally similar to other translational GTPases, including EF-Tu, EF-G, and EF4. In addition, *bipA* deletion mutant shows various phenotypes, indicating that it has global regulatory properties. These results have given rise to conjecture that BipA may play a role as regulatory protein controlling translation of target genes through interaction with the ribosome. Furthermore, recent structural studies show that BipA interacts with 50S ribosomal subunit and A-site tRNA. However, precise molecular mechanism of BipA in translation regulation is not completely understood.**Methods:** To investigate whether overexpression of other *E. coli* genes can recover the cold-sensitive growth defect observed in *bipA* deletion mutant, we constructed genomic library from *bipA* deletion strain and screened suppressors for the cold-sensitive growth phenotype of Δ*bipA* strain.**Results:** We isolated and identified two suppressors that were capable of restoring the Δ*bipA* mutant phenotype; RplT and YebC. RplT, ribosomal protein L20, is a component of 50S ribosomal subunit and it is assumed that L20 might be required for maintaining the 50S ribosomal subunit in the correct conformation for binding of aminoacyl-tRNA. YebC is putative transcriptional factor, but its precise role has not yet been addressed. Their overexpression also restore ribosome assembly defect at low temperature and sensitivity to antibiotics. In contrast, capsule formation at low temperature of Δ*bipA* strain didn't decrease, when each suppressor was overexpressed, indicating that rescues of growth defects of Δ*bipA* strain at low temperature have no correlation with capsule formation.**Conclusions:** Our findings suggest that BipA may regulate translation efficiency through stabilization of binding of aminoacyl-tRNA to ribosome binding and raise the possibility that YebC participates in translation via regulating transcription of component of ribosome or ribosome assembly factor.

Author Disclosure Block:

E. Choi: None. **J. Hwang:** None. **H. Lee:** None. **J. Park:** None. **Y. Seo:** None.

Poster Board Number:

FRIDAY-774

Publishing Title:

Testing the Composition of the *Bacillus subtilis* Stressosome Complex Using Co-Immunoprecipitation *In Vivo*

Author Block:

K. Lopez-Damian, C. Y. Bonilla; Gonzaga Univ., Spokane, WA

Abstract Body:

A bacterium's ability to cope with environmental stresses such as, pH, temperature and reactive oxygen species, determines its chances of survival. The stress response mechanisms used by pathogens such as *Staphylococcus aureus* and *Listeria monocytogenes*, are well conserved in non-pathogenic bacteria like *Bacillus subtilis*. Therefore, examining the stress response mechanism in *B. subtilis* will aid in understanding how bacteria, including pathogens, adapt and survive exposure to environmental stress. In *B. subtilis*, the stressosome is composed of RsbS, RsbT, and four RsbR homolog proteins, and acts as a signaling hub that integrates different environmental signals. We know that the kinase RsbT is inhibited by RsbS and RsbR proteins in the absence of stress. Releasing RsbT activates the SigB cascade leading to the up regulation of multiple genes to respond to the stress. Yet the reason for the four homologs and how they transduce the signal is not fully understood. The purpose of my project is to determine the composition of the *B. subtilis* stressosome *in vivo* in the presence of environmental stress in order to elucidate the mechanism of action of each RsbR homolog. When exposed to different stresses, the stressosome could respond by (1) changing the RsbR protein-protein interactions revealing the role of each homolog or (2) affirming their redundancy by requiring all RsbR proteins in order to multiply the signal and effectively respond. My approach was to tag the canonical RsbR gene, RsbRA, using a Myc tag at the endogenous locus. I verified insertion of the tag by sequencing and western blot; and analyzed the viability of the strain using a survival assay, which exposed growing the cells to Hydrogen Peroxide (5mM) before plating to allow recovery. The results indicated that the presence of the Myc tag impacted the function of the protein rendering the cells more sensitive than wild type cells. I plan to test the effect of smaller tags on RsbRA protein function using the survival assay. Once a tagged strain is found to be functional, stressosome composition will be analyzed by western blot and immune-precipitation to identify the protein-protein interactions or the ratio of each RsbR protein present in the complex.

Author Disclosure Block:

K. Lopez-Damian: None. **C.Y. Bonilla:** None.

Poster Board Number:

FRIDAY-775

Publishing Title:

Bacterioferritin Comigratory Protein (BCP) Plays a Protective Role against Thermal Stress in the Fission Yeast

Author Block:

S. H. Lee[1], **I. W. Ryu**[1], H. W. Lim[2], K. Kim[1], C. J. Lim[1]; [1]Kangwon Natl. Univ., Chuncheon, Korea, Republic of, [2]Shebah Biotech Co., Chuncheon, Korea, Republic of

Abstract Body:

The *Schizosaccharomyces pombe* structural gene encoding bacterioferritin comigratory protein (BCP) was previously cloned using the shuttle vector pRS316, and the recombinant plasmid was named pBCPlO. The *BCP*$^{+}$ mRNA level in the pBCPlO-containing yeast cells was significantly higher than that in the control yeast cells, indicating that the cloned gene is functioning. In this work, we tried to evaluate the protective role of BCP against high incubation temperatures using the BCP-overexpressing recombinant plasmid pBCP10. When the incubation temperature was shifted from 30°C to 37°C or 42°C, the *S. pombe* cells harboring the plasmid pBCPIO exhibited better growth at both 37°C and 42°C than the vector control yeast cells. They were identified to contain the lower reactive oxygen species (ROS) and nitrite content, an index of nitric oxide (NO), after the shifts to higher incubation temperatures, than the vector control cells. After the temperature shifts, changes in glutathione content, superoxide dismutase and glutathione peroxidase activities were also compared. In brief, the *S. pombe* BCP plays a protective role in the defensive response to thermal stress.

Author Disclosure Block:

S.H. Lee: None. **I.W. Ryu:** None. **H.W. Lim:** None. **K. Kim:** None. **C.J. Lim:** None.

Poster Board Number:

FRIDAY-776

Publishing Title:

Analysis of Orf19.7296p in the *Candida albicans* Osmotic Stress Response

Author Block:

R. Rodriguez, K. A. Conrad, E. C. Salcedo, J. M. Rauceo; John Jay Coll. of The City Univ. of New York, New York, NY

Abstract Body:

Fungal signaling pathways coordinate morphogenesis, biofilm formation, and stress adaptation. These processes are essential to the pathogenicity of *Candida albicans*, the most common human fungal pathogen. Here, we explore the role of plasma membrane protein Orf19.7296p in the osmotic stress response. We previously showed that *ORF19.*7296 transcription is significantly increased following acute cationic (1M NaCl) stress; however, Orf19.7296p function and localization following osmotic stress is unknown. We used fluorescence microscopy to examine the cellular localization of an Orf19.7296p-YFP fusion protein following exposure to 1M NaCl in yeast cells and hyphae. Surprisingly, our microscopic images revealed that Orf19.7296p-YFP formed punctate foci around the cell periphery. Also, Orf19.7296p was present in the vacuole; although we cannot rule out the possibility this observation may be a consequence of protein recycling. Quantitative analysis using flow cytometry revealed that the Median Fluorescence Intensity (MFI) of log phase cells treated with salt was statistically greater than untreated samples (*p*-value = 0.0008). Moreover, the MFI was higher in early and late stationary phase samples compared to log phase cells for salt-treated and untreated cells. Strikingly, we did not observe fluorescence in hyphae. We phenotypically characterized a homozygous *orf19.7296*Δ/Δ null mutant strain. We did not identify a growth defect when cells were exposed to various pH levels, hyperosmotic stress, cell wall stress, and plasma membrane stress. Also, we did not observe any defects in germ tube morphology or endocytosis. Collectively, our results show for the first time that Orf19.7296p forms stable puncta in the PM, is maximally expressed during stationary phase, and specifically localizes to yeast cells. Our future studies will investigate the extent that Orf19.7296p may associate with eisosomes, plasma membrane microdomains that form punctate foci. In fission yeasts, eisosomes were shown to mediate osmotic stress signaling.

Author Disclosure Block:

R. Rodriguez: None. **K.A. Conrad:** None. **E.C. Salcedo:** None. **J.M. Rauceo:** None.

Poster Board Number:

FRIDAY-777

Publishing Title:

Microwave Facilitation of Cryo-substitution

Author Block:

D. W. DORWARD; NIH, NIAID, Hamilton, MT

Abstract Body:

Cryo-substitution of vitrified biological samples is a premier process for preserving near-native morphology for electron microscopy. Typically frozen samples are immersed in fixatives and organic solvent below -80 °C, then warmed for hours to days to dissolve the ice and crosslink structures for embedding, sectioning, and examining. Although agitation can hasten processing, it involves enclosing samples and fixatives in vials at -195 °C, inserting the vials into pre-cooled aluminum blocks, and allowing the blocks to reach 20-23 °C on a shaker. Potential issues include vial rupture or leakage, uneven sample warming, unmeasured pressure changes and effects, and variable temperature ramping rates. To assess if microwave energy could facilitate the process, a commercial cryo-thermal platform was adapted for use in a microwave processor. The platform was configured to hold vented microwave sample vessels, and to use a controller, feedback sensors, liquid nitrogen, and heaters to regulate sample temperature between +/-100 °C for desired time periods. During processing, samples were moved to fresh reagents at desired time points and temperatures. Using the platform and 80 W of microwave power, frozen samples such as *Bacillus subtilis* or yeast were substituted, crosslinked, and dehydrated in 1 to 1.5 hr. Infiltration with low-temperature embedding resin added about 1 hr. ImageJ line profiles showed that contrast between adjacent structures in *B. subtilis* and yeast was roughly two-fold greater in cryo-substituted cells than in samples processed conventionally. Multiple processing runs showed less than +/- 1.5% deviation from mean temperatures (°K) at 33 two-minute time points from sample introduction to completion. Samples processed successfully with this system include Archaea, Eubacteria, protozoans, algae, ticks, and mammalian cells and tissues. Post-section immune labeling of *Borrelia burgdorferi* for NapA was plentiful and specific. Furthermore, 250 nm sections of cryo-substituted samples were ideal for light microscopy and electron tomography. Thus, cryo-substitution in microwave processors can dramatically increase fixation rate and throughput, providing stability and reproducibility, with considerable flexibility in types of samples, reagents, parameters, and applications.

Author Disclosure Block:

D.W. Dorward: None.

Poster Board Number:

FRIDAY-778

Publishing Title:

Alteration of Substrate Binding Site of Cel12a by Site-Directed Mutagenesis & Its Comparison with Wild-type Enzyme

Author Block:

A. S. Prajapati[1], V. A. Pawar[1], K. J. Panchal[1], B. R. Dave[1], D. H. Patel[2], R. B. Subramanian[1]; [1]Sardar Patel Univ., Anand, India, [2]P.D. Patel Inst. of Applied Sci., Anand, India

Abstract Body:

Global demand for energy has grown with the development of new industries, requiring constant improvement and search for new sources of energy. One of the challenges today is releasing the energy of glucose that nature has cleverly locked into lignocellulosic biomass. Lignocellulose can provide ample raw materials for biofuel production, via its hydrolysis and the fermentation of the released sugars. Currently, cellulolytic enzymes are often used to catalyse this step, particularly cellulases, which hydrolyse the β-1, 4 glycosidic linkages in cellulose. Even though many cellulases are being isolated, the efficiency of hydrolysis is very poor. Thus, there is a need to modify and improve this enzyme to make it potent, efficient and suitable for our own purposes. This could be done by altering the substrate specificity of enzyme which help to accommodate various substrates. Approaches like enzyme engineering, reconstitution of enzyme and bioprospecting for superior enzymes are gaining importance. Using Site-Directed Mutagenesis, the role of different amino acids within the catalytic domain of an enzyme can be studied. Mostly the aromatic residues of cellulase limit the size range of substrate accepted in the binding site. Five different aromatic residues of the substrate binding pocket of Cel12A were investigated by Homology modelling. These residues are highly conserved and play an important role in steric hindrances to substrate binding. The alteration of these residues may allow the enzyme to accommodate various substrates. All five residues substituted with alanine (A) through Polymerase Chain Reaction. Wild-type Cel12A and its mutated genes were cloned and overexpressed in BL21 and the proteins were analyzed by SDS-PAGE. A band with molecular weight of ~29 kDa was observed. Results of the site-specific mutation led to an alteration in substrate specificity from CMC to xylan. This indicates the effect of aromatic residue on substrate binding, catalytic process and overall catalytic efficiency, making it an interesting candidate for further studies.

Author Disclosure Block:

A.S. Prajapati: None. **V.A. Pawar:** None. **K.J. Panchal:** None. **B.R. Dave:** None. **D.H. Patel:** None. **R.B. Subramanian:** None.

Poster Board Number:

FRIDAY-779

Publishing Title:

Mutations in the Ribosome: A Story of Treatment, Resistance and Physiological Shifts

Author Block:

A. Jimenez-Fernandez[1], M. Brauer[1], E. Juskewitz[1], H. K. Johansen[2], S. Molin[1]; [1]Danmarks Tekniske Univ., Hørsholm, Denmark, [2]RigsHosp.et, Copenhagen, Denmark

Abstract Body:

RNA polymerase and ribosomes, composing the macromolecular synthesis machinery (MMSM), carry out the central processes of transcription and translation, but are usually seen as mechanical elements with no regulatory function. Extensive investigations of gene regulation and the high degree of evolutionary conservation of the cellular MMSM tend to support this view. However, under certain selective conditions the machinery itself may be targeted by adaptive mutations, which result in fitness increasing phenotypic changes. Here we investigate and characterize the role of ribosomal mutations in adaptive evolution.Several mutations in ribosomal genes have been identified in the genome analysis of nearly 700 *Pseudomonas aeruginosa* isolates from the airways of infected cystic fibrosis patients. Among these mutations we have repeatedly identified insertions, deletions and substitutions in specific ribosomal genes. Our goal is to elucidate the consequences of these mutations.Here we show the phenotypic characterization of mutations in three ribosomal proteins: L4, L6 and S1. Strains with mutations in L6 and S1 have reduced growth rate and an altered antibiotic resistance pattern, while none of these phenotypes are observed in L4 mutant strains. The selection for mutations in ribosomal protein genes is partly explainable by the antibiotic treatment of the patient as observed in the L6 case. But other mutations cannot be directly associated with antibiotic resistance.Clarification of the potential pleiotropic consequences of the specific mutations in ribosomal proteins is important for our understanding of biological evolution, and will have impacts on the design of new treatment strategies to combat microbial infections.

Author Disclosure Block:

A. Jimenez-Fernandez: None. **M. Brauer:** None. **E. Juskewitz:** None. **H.K. Johansen:** None. **S. Molin:** None.

Poster Board Number:

FRIDAY-780

Publishing Title:

Structural Analysis of Proteome Vulnerability to Oxidative Damage in *Deinococcus radiodurans*

Author Block:

R. L. Chang[1], J. W. Sher[2], D. A. Stork[1], P. A. Silver[1]; [1]Harvard Med. Sch., Boston, MA, [2]Yale Univ., New Haven, CT

Abstract Body:

Protein damage by reactive oxygen species (ROS) affects molecular function and cellular phenotype. The most critical protein damage by ROS is irreparable carbonylation of arginine, lysine, proline, and threonine sidechains (RKPT), leading to misfolding, aggregation, and proteolysis. Empirical data suggests intrinsic vulnerability to ROS varies with tertiary structure, but previous studies have been only in sequence space. This study investigates whether distribution of RKPT in 3D structures determines vulnerability of RKPT. Understanding properties underlying ROS sensitivity also enables study of protein oxidation phenotypes. We analyzed the structural proteome of the radioresistant bacterium *D. radiodurans* in comparison to a non-resistant bacterium. *D. radiodurans* is especially robust to ROS exposure from irradiation; this may be due in part to evolved intrinsic protection of key proteins.Structures were obtained for all encoded proteins in *D. radiodurans*, *E. coli*, and for a training set with experimentally measured carbonylated sites (CS). PDB structures were supplemented by homology modeling using I-TASSER and ProtMod. The CS data paired with structures includes 419 unique proteins and 840 CS. RKPT content, relative reactivity, depth, solvent exposure, and interatomic distances were analyzed as potential determinants of carbonylation. Pairwise comparison of *D. radiodurans* and *E. coli* structural proteomes was enabled by mapping orthologs by sequence and functional annotation.Our findings show site depth strongly correlates with CS. Solvent exposure is not a strong determinant. Structure-derived reactivity of RKPT agrees with sequence-based, except that threonine is more reactive than previously thought. Proximity of CS may owe to progressive unfolding of proteins exposing natively buried sites. Proteome-wide comparisons of RKPT distributions in *D. radiodurans* and *E. coli* only showed a slightly more buried trend in *radiodurans*; however, certain *radiodurans* proteins exhibit far less surface RKPT than their *E. coli* orthologs, most notably 30S ribosomal protein S13 and 50S ribosomal protein L4. This finding supports that proteins involved in translation, required for recovery from widespread ROS damage, are intrinsically protected from ROS in *radiodurans* and may be key to radioresistance.

Author Disclosure Block:

R.L. Chang: None. **J.W. Sher:** None. **D.A. Stork:** None. **P.A. Silver:** None.

Poster Board Number:

FRIDAY-781

Publishing Title:

Differences in Heat Susceptibility of Human Norovirus Strains is Predicted by Docking and Molecular Dynamics Simulations

Author Block:

M. Moore, B. Mertens, B. Bobay, L-A. Jaykus; North Carolina State Univ., Raleigh, NC

Abstract Body:

Background: Environmental stresses, chemical disinfectants and physical processes impact human norovirus infectivity, frequently by destroying capsid integrity. Recent data suggest variability in the resistance of different noroviruses to these treatments, but the molecular basis for such differences has not been well investigated. Ligand binding assays combined with ligand docking and molecular dynamics (MD) simulations have potential to be used to explain the nature of differential norovirus capsid stability. The purpose of this study was to apply docking and MD approaches to select norovirus strains to facilitate prediction of strain-specific differences in heat susceptibility.**Methods:** The major capsid proteins (VP1) of GII.4 Houston (HOV), GII.4 Sydney (SYV), and GII.2 Snow Mountain (SMV) were constructed using MODELLER v10.1 based upon previously reported crystal structures. MD simulations of the VP1 were performed using the GROMACS 4.4.5 package with a 100 ns simulation. A 3D structure of aptamer M6-2 was generated using the MC-Fold/MC-Sym webserver and docked to each of the VP1 proteins using HADDOCK to compare to aptamer M6-2 binding to the VLPs. Predicted strain-based differences were evaluated using aptamer and receptor binding assays in addition to dynamic light scattering (DLS) and transmission electron microscopy (TEM).**Results:** MD simulations predicted HOV to be much more heat labile than SMV or SYV as it had a lower number of P-to-P-domain hydrogen bonds and higher total solvent accessible surface area. Further, the degree of secondary structure change in the receptor/aptamer binding area of the VP1 was more dynamic for HOV compared to SMV and SYV. Docking analysis revealed similar residues for M6-2 binding to HOV, SMV, and SYV. Binding assays confirmed MD simulation predictions, as HOV had a significantly ($p < 0.05$) lower melting temperature ($68.9 \pm 1.1^{o}C$) than SMV ($75.0 \pm 0.8^{o}C$) and SYV ($73.1 \pm 0.4^{o}C$) using M6-2. Receptor binding, DLS, and TEM also confirmed this prediction.**Conclusions:** This is the first report describing norovirus GII.4 strain-based heat susceptibility differences and the molecular basis for such differences. Combinations of the reported receptor binding and *in silico* methods can be useful in predicting and identifying differences in norovirus strain-based resistance to inactivation strategies.

Author Disclosure Block:

M. Moore: None. **B. Mertens:** None. **B. Bobay:** None. **L. Jaykus:** None.

Poster Board Number:

FRIDAY-782

Publishing Title:

Molecular Basis for Pyranopyridine Inhibition of Gram-Negative Antibiotic Efflux Pumps

Author Block:

H. Sjuts[1], A. V. Vargiu[2], S. M. Kwasny[3], S. T. Nguyen[3], H-S. Kim[4], X. Ding[3], A. R. Ornik[1], P. Ruggerone[2], T. L. Bowlin[3], H. Nikaido[4], **K. M. Pos**[1], T. J. Opperman[3]; [1]Goethe-Univ. Frankfurt am Main, Frankfurt am Main, Germany, [2]Univ. of Cagliari, Cagliari, Italy, [3]Microbiotix, Inc., Worcester, MA, [4]Univ. of California Berkeley, Berkeley, CA

Abstract Body:

Background: The *Escherichia coli* AcrAB-TolC efflux pump is the archetype of the Resistance-Nodulation-cell Division (RND) exporters from Gram-negative bacteria. Overexpression of RND-type efflux pumps is a major factor in multidrug resistance (MDR), which makes these pumps important antibacterial drug discovery targets. However, the development of potent efflux pump inhibitors has been hindered by the lack of structural information for rational drug design.**Methods:** Because the AcrB efflux pump is an integral membrane protein with a polyspecific binding behavior, co-crystallization with drugs has been challenging. To overcome this obstacle, we have engineered and produced a soluble version of AcrB (AcrBper), highly congruent in structure with the periplasmic part of the full-length protein, and that is capable of binding substrates and potent inhibitors. Here, we describe the molecular basis for pyranopyridine-based inhibition of AcrB using a combination of cellular, X-ray crystallographic, and molecular dynamics (MD) simulations studies.**Results:** The pyranopyridines bind within a phenylalanine-rich cage that branches from the deep binding pocket of AcrB, where they form extensive hydrophobic interactions. Moreover, the increasing potency of improved inhibitors correlates with the formation of a delicate protein- and water-mediated hydrogen bond network.**Conclusions:**These detailed insights provide a molecular platform for the development of novel combinational therapies using efflux pump inhibitors for combating multidrug resistant Gram-negative pathogens.

Author Disclosure Block:

H. Sjuts: None. **A.V. Vargiu:** None. **S.M. Kwasny:** None. **S.T. Nguyen:** None. **H. Kim:** None. **X. Ding:** None. **A.R. Ornik:** None. **P. Ruggerone:** None. **T.L. Bowlin:** None. **H. Nikaido:** None. **K.M. Pos:** None. **T.J. Opperman:** None.

Poster Board Number:

FRIDAY-783

Publishing Title:

Structure-Function Relationships of S46 Peptidases from Non-Fermenting Gram-Negative Rods

Author Block:

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Abstract Body:

Background: Opportunistic infections caused by antimicrobial resistant bacteria are becoming serious threats in many countries. Many opportunistic pathogens are Non-Fermenting Gram-Negative Rods (NFGNR). Some of these pathogens utilize protein or peptide as carbon or energy source. Especially, Dipeptide production is an important in the peptide metabolism, because of an inner membrane preferentially transports "dipeptide" rather than an "amino acid". Dipeptidyl peptidases (DPPs) have a key role in the dipeptide production in periplasm. Moreover, Growth retardation was observed in DPPs gene disruptant of *Porphyromonas gingivalis* (Pg) (Nemoto *et al., JBC* 2011). Major DPPs in periplasm are consists of DPP4, POP (Proryl Oligo Peptidase), DPP7 and DPP11. These DPPs liberate a dipeptide from N-terminus of peptides according to their specificity in the P1 position (Explanation of their specificity: DPP4 (Pro or Ala), POP (Pro or Ala), DPP7 (Hydrophobic or Basic), DPP11 (Acidic)). DPP7, DPP11 belong to Clan SC S46, and others belong to Clan SC S9. Whereas The S9 peptidases are widely distributed from prokaryotes to eukaryotes, The S46 peptidases are peculiar to some anaerobic gram-negative bacteria. **Methods:** Crystallizations of PgDPP11 and *Psedoxanthomonas mexicana* WO24 DAP BII (PmDAP BII : bacterial DPP7) were performed with the hanging drop vapor diffusion method and counter-diffusion method under a micro-gravity environment in ISS "kibo". Initial phases of the PmDAP BII and PgDPP11 were determined by Single-wavelength Anomalous Diffraction (SAD) method and Multi-wavelength Anomalous Diffraction (MAD) method, respectively. **Results**: We determined the structures of PgDPP11 and PmDAP BII, but with different substrate specificity. These structures describe the peptide digestion and substrate recognition mechanisms in S46 peptidases. **Conclusions:** These structure-function studies could be useful for the Structure Based Drug Design of novel antibiotics for NFGNR which have S46 peptidases. **References:** Sakamoto *et al., Sci. Rep.* 4977 (2014), *Sci. Rep.* 11151 (2015).

Author Disclosure Block:

S. Roppongi: None. **Y. Suzuki:** None. **I. Iizuka:** None. **K. Inaka:** None. **H. Tanaka:** None. **M. Yamada:** None. **K. Ohta:** None. **T. Nonaka:** None. **W. Ogasawara:** None. **N. Tanaka:** None. **Y. Sakamoto:** None.

Poster Board Number:

FRIDAY-784

Publishing Title:

Inhibition of the Central Quorum-Sensing Transcription Factor LuxO

Author Block:

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Abstract Body:

Quorum sensing (QS) is a mechanism of bacterial cell-cell communication that enables bacteria to assess cell population density and control collective behaviors. QS relies on the production, release, accumulation, and detection of signal molecules called autoinducers. In pathogenic vibrios, including *Vibrio cholerae*, the accumulation of autoinducers at high cell density triggers repression virulence factor production and biofilm formation. At low cell density, in the absence of autoinducers, transmembrane receptors phosphorylate the AAA+ ATPase transcriptional regulator LuxO. LuxO~P activates expression of genes required for virulence and biofilm formation. At high cell density, autoinducer binding inhibits the kinase activity of the receptors leading to de-phosphorylation and subsequent inactivation of LuxO. This event terminates QS-controlled transcription of biofilm and virulence genes. Xray crystallography has revealed the structure of LuxO and, therefore, the native mechanism of regulation in the central protein of the QS circuit. Natural negative regulation occurs through competitive inhibition of the ATPase activity via the linker domain that connects the regulatory receiver and the catalytic central domain. Mutational analysis of key interacting residues in both domains of LuxO have constitutively activated the protein in support of this structure-based mechanism. Interestingly, crystals of the central domain bound to a specific LuxO-inhibitor, AzaU, revealed a similarly competitive mechanism, mirroring the natural regulation.

Author Disclosure Block:

A. Hurley: None. **H. Boyaci Selcuk:** None. **F. M. Hughson:** None. **B.L. Bassler:** None.

Poster Board Number:

FRIDAY-785

Publishing Title:

Non-Canonical Mode of De-Repression by Phage Antirepressor

Author Block:

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Abstract Body:

DNA-binding repressors negatively regulate a transcription of many genes, and de-repression is necessary for the desired gene expressions. Thus, several mechanisms for a dissociation of repressor from the operator have been studied. Previously, we identified an antirepressor-mediated de-repression system from a *Salmonella* temperate phage SPC32H. Here, we present a non-canonical mode of de-repression, as revealed by the crystal structure of homo-tetramer of truncated repressor (Rep) and hetero-octameric complex of the Rep and cognate antirepressor (Ant). Instead of competing with the Rep for the DNA-binding site, Ant binds in a tetrameric form to the C-terminal domains of two Rep dimers, then bind to N-terminal domains of Rep dimers as well. This resulted in a release of two Rep dimers from the operators. Mutational analysis with size-exclusion chromatography and gel retardation assay suggested that Rep Phe187 plays an important role in the DNA binding via Rep tetramerization and Asp36, Arg40, and Lys46 are critical for the DNA binding. Inability of Rep mutants in the gene repression was also revealed in an *in vivo* bioluminescence reporter assay. Intriguingly, Rep F187A mutant, which existed as the dimer form only, hardly bound to DNA even at high concentration, suggesting that the dimer pairs for DNA-binding are contributed by different dimers of the Rep tetramer. Ant residues (Arg37, Asn58, and Tyr76) that positioned at the interfaces between Rep and Ant in the Rep-Ant complex were also assessed in their function both *in vitro* and *in vivo*; Ant mutants showed reduced binding (K_d > 100 μM) to the Rep, and consequently, exhibited a decreased prophage induction from host *Salmonella* in the presence of an induction agent. Based on these results, we propose a non-canonical mode of de-repression where the DNA-binding Rep was reversibly inactivated by the binding of cognate Ant that collapsing the Rep tetramer.

Author Disclosure Block:

M. Kim: None. **H. Kim:** None. **S. Son:** None. **H. Yoon:** None. **Y. Lim:** None. **J. Lee:** None. **J. Chun:** None. **H. Na:** None. **Y. Seok:** None. **K. Jin:** None. **Y. Yu:** None. **S. Kim:** None. **S. Ryu:** None. **H. Lee:** None.

Poster Board Number:

FRIDAY-786

Publishing Title:

Structural Studies of the Bile Salt Hydrolase from *Lactobacillus salivarius*

Author Block:

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Abstract Body:

Background: The bile salt hydrolase (**BSH**) is a gut bacterial enzyme that negatively influences host fat digestion and energy harvest. BSH enzyme activity functions as a gateway reaction in the small intestine by deconjugation of conjugated bile acids. Extensive gut microbiota studies have suggested that BSH is a key mechanistic microbiome target for developing novel non-antibiotic feed additives to improve food animal production and for designing new measures to control obesity in humans. However, research on BSH is still in its infancy, particularly in terms of structural basis of BSH function, which has hampered development of BSH-based strategies for improving human and animal health.**Methods:** The C-terminal His-tagged BSH from *Lactobacillus salivarius* NRRL B-30514 was purified for protein crystallization and structural analysis. The predicted functionally important residues were subjected to site-directed mutagenesis; the activity and substrate specificity of the mutated BSH enzymes were evaluated.**Results:** The crystal structure of the *L. salivarius* BSH was obtained at a resolution of 1.90 Å, which revealed this BSH as a member of the N-terminal nucleophile hydrolase superfamily. Two BSH molecules packed perfectly as a dimer in one asymmetric unit. Comparative structural analysis of *L. salivarius* BSH identified residues critical for catalysis and substrate specificity. Replacement of the residue Cys 2 with Ser abolished activity of the *L. salivarius* BSH, confirming that Cys 2 plays an essential role in BSH catalysis. In addition, the Tyr-24-Phe amino acid substitution dramatically changed substrate specificity for the *L. salivarius* BSH.**Conclusions:** This study revealed the first crystal structure of the BSH from lactobacillus species, the major BSH producer in the intestine. Our findings also provided insights into structural basis of BSH activity, which is important for future translational research by modulating *in situ* BSH activity.

Author Disclosure Block:

F. Xu: None. **W. Geng:** None. **X. Hu:** None. **J. Lin:** None.

Poster Board Number:

FRIDAY-787

Publishing Title:

Studies on the Unfolding of a Global Staphylococcal Virulence Regulator with a Flexible C-Terminal End

Author Block:

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Abstract Body:

Background: SarA, a dimeric protein, acts as a global virulence regulator in *Staphylococcus aureus*. The single-domain structure or the folding-unfolding mechanism of this regulator has not been verified or demonstrated.**Method:** To determine the folding-unfolding mechanism and the domain structure of SarA, a recombinant SarA (rSarA) has been studied using various *in vitro* tools.**Results:** Our limited proteolysis data indicate that rSarA is a single-domain protein with a flexible C-terminal end. rSarA appears to unfold by different mechanism in the presence of different denaturant. While guanidine hydrochloride (GdnCl)-induced unfolding of this protein occurred via the generation of two dimeric intermediates, urea-induced unfolding of rSarA proceeded sequentially through the production of a dimeric and a monomeric intermediate. The structures and the surface hydrophobicity of the intermediates differed and also varied notably from those of native rSarA. Of the intermediates, the GdnCl-generated intermediates possessed a molten globule-like structure. In comparison with the native rSarA, the intermediate generated at low GdnCl concentration carries a compressed shape, whereas, other intermediates acquire an enlarged shape. The thermal unfolding of rSarA, unlike chemical-induced unfolding, was completely irreversible in nature.**Conclusion:** We have confirmed the single-domain structure of SarA and shown that its C-terminal end is relatively flexible in nature. In addition, we have provided clues about the folding-unfolding mechanism of this global virulence regulator.

Author Disclosure Block:

K. Sau: None. **A. Mahapa:** None. **S. Mandal:** None. **S. Sau:** None.

Poster Board Number:

FRIDAY-788

Publishing Title:

The Domain-Connecting Helix of a Mip-Like Peptidyl-Prolyl *cis-trans* Isomerase is Critical for Its Structure, Function, Stability and Shape

Author Block:

S. Sau, S. Polley; Bose Inst., Kolkata, India

Abstract Body:

Background: FKBP22, an *Escherichia coli*-encoded peptidyl-prolyl *cis-trans* isomerase, shares substantial identity with the Mip-like virulence factors. Two domains of the Mip-like proteins are joined by a protease-sensitive α-helix. While their N-terminal domains are involved in dimerization, their C-terminal domains carry catalytic site and bind inhibitors like rapamycin and FK506. Very little is known about the roles of the domain-connecting helix on the structure, function, stability and shape of any Mip-like protein.**Method:** To describe the roles of the domain-connecting helix in Mip-like proteins, a recombinant FKBP22 (rFKBP22) and its three point mutants I65P, V72P and A82P have been studied using multiple probes. Each mutant carries a Pro substitution mutation at a specific location in the helix.**Result:** We have demonstrated that the three mutants differ not only from each other but also vary from rFKBP22 in structure and activity. Urea-induced equilibrium unfolding of mutants occurred via the formation of at least one intermediate. Conversely, rFKBP22 was unfolded by a two state mechanism in the presence of urea. Besides, the stabilities of the mutants, particularly I65P and V72P, were considerably different from that of rFKBP22. However, mutations little affected the rapamycin binding affinity of rFKBP22. Of the mutants, I65P was not only associated with the maximum levels of structural/functional loss but also dissociated partially in solution. Our computational studies have indicated that there is a severe collapse of the shape in I65P due to the abnormal movement of its C-terminal domains.**Conclusion:** Our data have demonstrated that the domain-connecting α-helix is vital for the Mip-like proteins.

Author Disclosure Block:

S. Sau: None. **S. Polley:** None.

Poster Board Number:

FRIDAY-789

Publishing Title:

Assessment of Knowledge and Practice of Laboratory Personnel Regarding General Safety Rules in Microbiology Department of Private Laboratories in Karachi-Pakistan

Author Block:

U. Khattak, **Z. Fatima**, J. Zaheer, Z. Zahid, N. H. Ali; Dow Univ. of Hlth.Sci., Karachi, Pakistan

Abstract Body:

Background: Safety is an essential component of working of any laboratory. The infections caused by micro organisms can be prevented by using general safety rules in microbiology laboratory. This study was conducted to determine the knowledge and practice of laboratory personnel regarding general safety rules in microbiology laboratories and to identify the safety measures taken by them to prevent the spread of infection.**Method:**This was a comparative study which used a standardized questionnaire to survey knowledge, attitude and practice of laboratory personnel regarding general safety rules working in microbiology departments of different private laboratories in Karachi-Pakistan. Approximately 100 laboratory personnel were assessed on the basis of 14 questions.**Results:**Regarding knowledge, approximately 70% of laboratory personnel knew the very important issues related with laboratory safety like discarding of tested samples, importance of safety cabinets and protective devices etc. On the other hand the attitude towards practice, only 50% to 60% of the laboratory personnel follow safety rules i.e. wearing gloves and lab coats, washing hands properly, decontaminating their working bench before and after the work. Reporting of any spill or accident to laboratory supervisor was not practiced by any of them except only few. Avoidance of eating and drinking in laboratory is only practiced by 20%.**Conclusion:**Our research showed the deficit in the awareness of safety precautions amongst laboratory personnel and demonstrates that knowledge and practice of safety rules is unsatisfactory. The importance of training regarding practice of general safety rules needs emphasis. Efforts should be made to increase knowledge of laboratory personnel regarding spread of infection and its cure because if the laboratory personnel is not be safe from the infection than how can the quality is maintained.

Author Disclosure Block:

U. Khattak: None. **Z. Fatima:** None. **J. Zaheer:** None. **Z. Zahid:** None. **N.H. Ali:** None.

Poster Board Number:

FRIDAY-790

Publishing Title:

Implementation of Biosafety Program Audit for Infectious Diseases in Laboratories of Clevb at 2015

Author Block:

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Abstract Body:

On an annual basis , laboratory staff should be asked to rate the four primary controls of biosafety and justify each rating, so at the two last months in 2015, the biosafety committee of CLEVB has carried biosafety program audit to the four primary controls of biosafety programs in laboraties for persons that dealing with infectious agent. These four primary controls include Engineering , standard operating procedures (sops), personal protective equipment (PPE) and administration. The objective of this surveillance is monitore the professionality and technical capacity of the employees towards the biosafety programs. According to biosafety scoring system used to provide a grading scale for each primary control of biosafety, approximately 65%, 30%, 4%, 1% and 0% of the participants studied were given excellent , good, fair , poor and unacceptable scale. It was significantly that the workers in the different department promising in the implementation of biosafety measures through the programmed plan. Close analysis also indicated that there were significant differences participants in justify between the grade provided for each primary control of biosafety.

Author Disclosure Block:

S.-.-.-. El Mahdy: None.

Poster Board Number:

FRIDAY-792

Publishing Title:

Impact of Biorisk Management Training at National Public Health Laboratories

Author Block:

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Abstract Body:

Background: Over the past few years the National Laboratories of Kenya and Uganda have conducted a series of biorisk management trainings. The goal of this project was to measure the knowledge and skill retention from these trainings.**Method/Approach**œ BRM Trainingœ Approval from administrationœ Drafted a tool and shared with colleaguesœ Shared it with mentors **Results:** Specific implementation gaps ere noted in :Segregation and Decontamination Training AnalysisPersonal Protective Equipment (PPE) Training AnalysisBiosafety Cabinets Training Analysis **Overall Conclusion:** Overall Conclusion and Next Stepsœ Training being utilizedœ Favourable attitudeœ More application through refresher training **Next steps (mitigation)**œ Future trainings to be reviewed to provide more opportunities for practice/applicationsœ Review participants selection criteria to enroll more participants from lab facilities with BSCsœ Follow ups to identify gaps contributing to poor application of skills and knowledge in waste management and BSC useoverall value of Biorisk Management Training (BRM) on the proper use of Personal Protective Equipment (PPEs)? **Conclusion for Quantitative data**œ Generally the objectives were metœ Participants obtained support

Author Disclosure Block:

K.K.N.N. Yatich: None. **M.N.N.N. Umuro:** None. **J.N.N.N. Nkodyo:** None. **S.N.N.N. Aisu:** None.

Poster Board Number:

FRIDAY-793

Publishing Title:

Validation of Autoclave Parameters for Sterilization of Medical Waste on a Bio-Containment Unit

Author Block:

B. C. Ellis, K. Dionne, B. Garibaldi, M. Reimers, N. Parrish; The Johns Hopkins Hosp., Baltimore, MD

Abstract Body:

Background: Successful autoclave-based decontamination of medical waste depends on a number of parameters including sterilization temp, cycle type/time, and load configuration. The type of material, size of the load, and the way it is packed relative to the chamber size has a direct effect on adequate sterilization.**Methods:** Manufacturer default parameters were tested by running simulated loads with assessment of sterility. Loads consisted of items expected from a patient room such as saturated linens, protective personal equipment/dry trash, and liquids (0.1L to 1L). Both rapid (3 hr) and standard (48 hr) biological indicators (*Geobacillus stearothermophilus*) were placed in the center of each load to assess sterility. Various cycle types were tested: liquid, gravity and vacuum along with varied ancillary settings including purge, run, and dry times, and temp. Additional variables were tested including the type/number of bags, bag closure, and the use of a solidifying agent to absorb excess liquids. In total, 19 different load-run configurations were tested including unique cycle programs developed as a result of this study.**Results:** 84% (16/19) of the runs performed using factory default settings failed. The most difficult loads to sterilize were those containing saturated linens which required a vacuum cycle to achieve adequate sterilization. Liquids and other trash required different cycles/settings. Optimized cycle parameters required separation of load types into standard waste, saturated linens, and liquids. For linens and standard waste, vacuum cycle parameters were modified from factory default settings by maximizing the pre-vac steps, decreasing the psi differential, and increasing the time to 60 min (linens) or 30 min (standard waste). Liquids (0.1L to 1L) were sterilized using a 120 min cycle at 253°F on a liquid setting.**Conclusions:** This study demonstrates that use of factory default settings for sterilization of medical waste in the absence of validation using simulated loads risks release of infectious waste. Since every load differs in composition and density, it is critically important to simulate expected waste with concomitant assessment of sterility using the proper biological indicators. This is especially important in high-level, bio-containment facilities where BSL-3 or 4 level pathogens may be encountered.

Author Disclosure Block:

B.C. Ellis: None. **K. Dionne:** None. **B. Garibaldi:** None. **M. Reimers:** None. **N. Parrish:** None.

Poster Board Number:

FRIDAY-794

Publishing Title:

Preliminary Biorisk Group Categorization of Animal *Pathogens* Handled by Clevb

Author Block:

M. A. Saad, N. Mahmoud, A. Ali, M. Hashim; Central Lab for Evaluation of Vet Biologics, Cairo, Egypt

Abstract Body:

Subject: Biosafety steering committee had been developed a Preliminary biorisk group categorization of the virulent and zoonotic animal pathogens which currently used in routine and research works in the laboratory animal facilities.**Materials and Methods:** -Challenge viruses of FMD, RVF, sheep Pox, Rabies, HPAI, H5N1 and NDV. -Challenge bacteria of brucella species, pasteurella multocida species and salmonella species. -Biorisk groups 1, 2 and 3. -A scores estimated for each pathogens using parameters of virulency, mode of transmission, route of infections, infectious dose, natural hosts range, zoonosis, transmissibility, occurrence of insect vector, carrier, stability, availability of prophylaxis and treatment, endemicity and economic impact.**Results and conclusions:** -The out-finding was categorized the challenge pathogens of FMD, RVF, B.meletensis, HPAI, H5N1, ND and Pasteurella multocida under biorisk group(3) with scores ranged between 25/36 - 24/36. -Other pathogens were categorized under biorisk group(2). -It is recommended to conduct all challenge tests with the biosafety level 3 in CLEV-B animal facilities.

Author Disclosure Block:

M.A. Saad: None. **N. Mahmoud:** None. **A. Ali:** None. **M. Hashim:** None.

Poster Board Number:

FRIDAY-795

Publishing Title:

Improvement of Biosafety Measures for Applying Challenge Test in Evaluation of *Rift Valley Fever* & *Rabbies Vaccines*

Author Block:

M. A. Saad, F. Fouad, A. Ali, M. Hashim; Central Lab for Evaluation of Vet Biologics, Cairo, Egypt

Abstract Body:

Rift Valley Fever and *Rabbies* Viruses are highly dangerous and infectious viruses which are transmitted from animals to humans either by insects or sharp needles or biting by infected animals.CLEVB has the authority to Evaluate Veterinary Vaccines in Egypt .The master test for Evaluating most of vaccines is by applying challenge test using virulent viruses; for evaluation of *Rift valley fever* and *rabbies* vaccine challenge test was applied in mice. In Evaluation of inactivated *Rift Valley* vaccine 5 dilution of *RVF* tested vaccine (1/1 - 1/5 - 1/25 - 1/125 and 1/625 ,and one group kept as control) are inoculated intraproteanial in 5 groups each of 10 mice, and after boastring dose one week apart all groups are inoculated with virulent *Rift valley* virus for Evaluation of inactivated *Rift Valley* vaccine by a titer of 10^3 Log10 MLD 50, to calculate the ED50 for Evaluated Vaccines .In evaluation of inactivated *Rabbies* Vaccine 4 groups each of 10 mice were inoculated intraproteanial with 4 dilution from inactivated *Rabbies* Vaccine (1/5 - 1/10 - 1/50 - ,and 1/250) after one week apart boastring dose were inoculated as the first inoccupation and challenged 2 weeks apart with *virulent CVS* with a titer of 10^3 mice LD50 ,both *Rift valley* virus and *Rabbies* are zoonotic disease which will cause a fatal infection for human ,and all of these tests are done in an ordinary mice cages but we improved the process by using BSL3 mice cages and wearing the Proper PPE ,and all these is done inside a highly secured isolated room with insect proof and supplied with insects zapper to protect the Staff ,workers ,and environment from these dangerous infectious viruses. Training programs are regularly held for the staff and workers responsible for that.

Author Disclosure Block:

M.A. Saad: None. **F. Fouad:** None. **A. Ali:** None. **M. Hashim:** None.

Poster Board Number:

FRIDAY-796

Publishing Title:

Cell-Based Reference Material for Qpcr: Stability Study

Author Block:

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Abstract Body:

Microbial quantification and detection face a series of practical and technological challenges including those related to the nature of the samples involved in the analysis (e.g., matrix from clinical and environmental samples). Despite efforts to improve methods, confidence in the measurements is lacking, especially for measurements made at the point of need or point of care where results are used to inform critical decision-making (e.g. biothreat detection). To address the need for measurement confidence, we are developing a reference material based on whole cells as a low-risk surrogate to challenge nucleic acid-based detection technology workflows, including sampling, DNA extraction, and detection. We stably inserted DNA sequence External RNA Control Consortium-00095 (ERCC-00095 from NIST SRM 2374) into a *Saccharomyces cerevisiae* strain to convey specificity. Feasibility as a reference material for quantitative polymerase chain reaction (qPCR) was demonstrated previously via interlaboratory study. Currently, we report the on-going stability study of a dry-format of the material by measuring cell number, cell viability, and DNA integrity as a function of time (up to 4 months) and temperature in Celsius (-20, 4, 20, 50). These conditions represent deviations that might occur during shipping or storage and help establish shelf-life. Preliminary results suggest acceptable cell number stability, with no statistically significant change in cell number at any temperature over time. In contrast, ~90 % loss in cell viability was observed at 50 °C after just 30 days, while other temperatures had no viability change, indicating 50 °C should be avoided to maintain viability. DNA integrity is currently being assessed by qPCR and pulsed field gel electrophoresis. Overall, this engineered yeast holds promise to support measurement assurance for the analytical process of nucleic acid-based detection technologies, encompassing the method, equipment, and operator, to increase confidence in microbial detection results.

Author Disclosure Block:

S. Da Silva: None. **N. Lin:** None.

Poster Board Number:

FRIDAY-797

Publishing Title:

Improvement of Biosafety Measures in Evaluation of *Fowl Pox & Newcastle* Vaccines Using Challenge Test

Author Block:

A. Hussein, A. Ali; Central Lab for Evaluation of Vet Biologics, Cairo, Egypt

Abstract Body:

Pox is a common viral disease of domestic birds(chickens, turkeys, pigeons and canaries) caused by a group of avian *pox* viruses which are members of the genus *Avipox* viruses of the *poxivirdae*-It is a slow spreading disease characterized by the development proliferative of discrete nodular proliferative skin lesions o the non-feathered parts of the body (cutaneous form) or fibrino-necroric proliferative lesions in the mucous membranes of the upper respiratory tract, mouth and esophagus (diphtheritic form).-In contaminated environments, aerosols transmission occurs by feathers and dried scabs containing *pox* virus particles causing both cutaneous and respiratory infection.-Control of fowl and pigeon pox is carried out vaccination with modified live *pox* virus vaccines.-Challenge 3 weeks later after vaccination of chickens by scarification of the opposite wing with the local Egyptian virulent strain of fowl pox viruses in dose 10^3 EID_{50}/dose, the birds should be observed for 14 days.-Newcastle Disease is an infectious of domestic poultry and other bird species with virulent ND virus. It is world-wide problem that presents primarily as an acute respiratory disease, but depression, nervous manifestations, or diarrhea, may be the predominant clinical form.-A suitable challenge strain for assessment has been stressed. For live vaccines of *Newcastle* virus, the method recommended involves the vaccination of 10 or more SPF or other fully susceptible birds.After 14-25 days, each vaccinated bird and ten control birds are challenged intramusculary with at least 10^4 EID50 (50% egg infectious dose) of *ND* challenge virus, challenge birds are observed for 14 days, at least 90% of the control birds must develop clinical signs and die within 6 days of Newcastle disease.-Evaluation of *Fowl.Pox and Newcastle Vaccines* were done in an ordinary chicken cages, but now improvement in the quality control of the challenge virus is done at chicken isolators to improve and protect the environment from the challenge virus and chickens from the complication by poor environment and other infections.

Author Disclosure Block:

A. Hussein: None. **A. Ali:** None.

Poster Board Number:

FRIDAY-798

Publishing Title:

Bio-Risk Assessment Of Salmonella Food Poisoning As Public Health Threat In The Egyptian Hatcheries And Table Eggs

Author Block:

S. S. -. -. Salama, 1993; Central Lab for Evaluation of Vet Biologics, Cairo, Egypt

Abstract Body:

Introduction: Salmonellosis is one of the most important food poisoning organisms. There are many different kinds of these Salmonellae but *Salmonella typhimurium* and *Salmonella enteritidis* are the most common types. The CDC estimates that 1.4 million cases of salmonellosis occur annually. Because many milder cases are not diagnosed or reported, the actual number of infections may be 30 or more times greater. The situation of this problem not clearly known in Egypt specially source of contamination because the food poisoning may be noticed in children, adult and people with impaired immune systems are the most likely to have severe infections.So, It is important to map and assess salmonella bio-risk hazard along the poultry value chain in Egypt. This study focuses on investigation of salmonella infection in hatcheries as an intermediate node along the production chain and table egg farms.**Methods:** A total of 910 swap samples were taken from different places in different 5 hatcheries including storage room, fertile eggs, Setters, hatcheries, chicks and from workers. Another 420 table egg samples were collected from 5 different farms before distribution. Samples subjected to bacteriological isolation, biochemical identification and molecular confirmation.**Results:** Generally isolation rate of salmonellae was 41% but between workers and in table eggs were 32% 37% respectively. The isolated serotypes varied between S. enteritidis, S. typhimurium, S. Gallinarum , S. paratyphi, S. pullorum and S. Montevideo indicating a horrible situation from the public health hazardous point of view.**Conclusion:** Assessment of current situation based on isolation rate and the type of isolated salmonellae reflect a scary situation of salmonellosis in hatcheries node and table egg farms especially among workers and in table eggs. This requires awareness, development and application of biosecurity and bio-risk management campaigns to get rid of this problem to avoid public health hazards.

Author Disclosure Block:

S.S.-.-. Salama: None.

Poster Board Number:

FRIDAY-799

Publishing Title:

Update on Influenza Rapid Testing Course and Pandemic Preparedness Course for Clinicians and Utilization of Course E-Resources

Author Block:

L. O. Williams[1], S. Chitavi[2], B. Longo[2]; [1]CDC, Atlanta, GA, [2]The Joint Commission, Oakbrook Terrace, IL

Abstract Body:

Background: An online influenza rapid testing course for clinicians, "Strategies for Improving Rapid Influenza Testing in Ambulatory Settings (SIRAS)" was developed and launched in 2012 by The Joint Commission (TJC) and the CDC. The course was restructured in 2013 to four 30-minute modules. (http://www.jointcommission.org/siras.aspx). In response to SIRAS participants' feedback on training needs, an influenza pandemic preparedness course was developed and launched in Apr. 2015 and restructured in Oct. 2015 into two shorter segments: 1) pandemic planning and response, and 2) laboratory testing and patient management/treatment. Specimen collection videos https://www.youtube.com/playlist?list=PLNQfL_CJ36fK08KEPjxu1ZKJn7GuFtn-N. , and other e-resources are offered. Recent utilization data are presented. SIRAS is being translated into Spanish.**Methods**: Courses were developed and restructured into shorter modules based on participants' feedback. E-resources were offered, and unique webpages for easy access were designed. Courses are updated annually and relaunched in Oct. Social media and TJC communication channels are used for marketing. Course utilization data are compiled annually and e-resource utilization is tracked cumulatively.**Results**: There were 5,864 visitors to the SIRAS webpage and 1,615 enrollments from Oct. 2014 - Sept. 2015. There were 1,722 visitors to the pandemic course webpage and 608 course enrollments since it was launched in Apr. 2015 - Sept. 2015. Since updating and relaunch in Oct. 2015, there have been 477 enrollments in SIRAS and 571 enrollments in the pandemic course, representing upward trends. The SIRAS course CE issuance rate increased to 82% (Oct. - Dec. 2015) compared with 34% during the first year and 48% last year. There were 2,345 downloads of Infographics; 10,325 views of the RIDT post on TJC's "AmBuzz" blog, and 133,291 views of specimen collection videos through Dec. 2015. Satisfaction rates were 98% for SIRAS and 94% for the pandemic course.**Conclusions:** Clinicians have welcomed the ongoing opportunity for continued education in influenza testing and preparedness. Annual updating of courses before the onset of influenza season attracts increased usage of e-resources and enrollments. Specimen collection videos, and other course tools are increasingly popular.

Author Disclosure Block:

L.O. Williams: None. **S. Chitavi:** None. **B. Longo:** None.

Poster Board Number:

FRIDAY-800

Publishing Title:

Ecological Niche Modeling of *Dermacentor marginatus* in Georgia

Author Block:

I. Burjanadze[1], J. Blackburn[2], I. Kracalik[3], S. Chubinidze[4], P. Imnadze[1], N. Tsertsvadze[1], G. G. Chanturia[1]; [1]Natl. Ctr. for Disease Control and Publ. Hlth.(NCDC), Tbilisi, Georgia, [2]Spatial Epidemiology and Ecology Res. Lab, Dept. of Geography, Univ. of Florida, Gainesville,, FL, [3]Spatial Epidemiology and Ecology Res. Lab, Dept. of Geography, Univ. of Florida, Gainesville, FL, [4]Richard G. Lugar Ctr. of the Natl. Ctr. for Disease Control and Publ. Hlth.(NCDC), Tbilisi, Georgia

Abstract Body:

Dermacentor marginatus, also known as the ornate sheep tick, is the principal arthropod vector of many pathogens which are a major cause of diseases as in humans and animals. Transmission of many tick-borne diseases, including tularemia, are highly associated with this *Ixodidae* tick in Georgia. The purpose of this study was to describe spatial and epidemiological characteristics of *D.marginatus* helping lead to identification of its ecological niche in Georgia.The fieldwork data obtained from multiple projects was used to predict respective distributions of *D.marginatus* using the ecological niche modeling system Genetic Algorithm for Rule-Set Production (GARP). We used five variables to construct models at both resolutions: annual mean temperature, annual temperature annual range, annual precipitation, precipitation of wettest month, and precipitation of driest month. The accuracy of the current distribution was then quantified through the use of accuracy metrics, which utilized the 25% testing data that was withheld from the modeling experiment.The BioClim current scenario received an area under curve (AUC) score of 0.749, SE=0.063; Z=7.36. The model had a total omission of 20.0% and average omission of 23.0% meaning that 100.0% of the independent (testing) locality data were predicted correctly by at least one model and 77.0% of the independent locality data were predicted correctly by all models in ten best subsets. An average commission was 27.94% and total commission 18.62%.The 102 spatially unique points of *D. marginatus* were randomly divided into 75% (n=76) training set used for modeling and 25% (n=26) testing set for model prediction.Using ecological niche modeling strategies in conjunction with spatial clustering techniques could help in identifies areas of concern. The results of the study will help to guide the sampling efforts of future studies of tick-borne diseases focusing with the dissemination of *D. marginatus* in Georgia.

Author Disclosure Block:

I. Burjanadze: None. **J. Blackburn:** None. **I. Kracalik:** None. **S. Chubinidze:** None. **P. Imnadze:** None. **N. Tsertsvadze:** None. **G. G. Chanturia:** None.

Poster Board Number:

FRIDAY-801

Publishing Title:

Protective Capabilities of Bacterial Outer Membrane Vesicles to Improve Encapsulated Enzyme Stability

Author Block:

N. J. Alves, K. B. Turner, S. A. Walper; Naval Res. Lab., Washington, DC

Abstract Body:

Background: Utilizing common molecular biology techniques, researchers have been able to manipulate bacteria to produce enzymes for use in diverse industries. However, maintaining enzyme stability for many real world applications remains a long standing problem requiring the use of chemical additives as well as strict storage and transportation protocols. By programming the bacteria to both produce and package enzymes within outer membrane vesicles (OMV) we sought to improve enzyme stability for use in difficult enzyme applications. **Methods:** *E. coli* was utilized to develop a phosphotriesterase (PTE) packaging scheme in which a synthetic SpyTag/SpyCatcher (ST/SC) linkage was incorporated into the PTE-SC and a ST modified membrane anchoring protein (OmpA-ST) to facilitate packaging of PTE within OMV. OMV were isolated by ultra-centrifugation and enzyme stability was assessed utilizing paraoxon as a substrate post prolonged storage at elevated temperatures, iterative freeze-thaw cycles, and lyophilization. Enzyme activity was also assayed across a range of environmentally collected water samples. **Results:** Under all storage conditions the OMV encapsulated PTE demonstrated improved stability when compared to free PTE. The stability enhancement was particularly notable at elevated temperatures after 14 days of storage at 37C retaining 34.5% activity compared to 0.3%, post lyophilization 64.2% compared to 1.8%, and post four freeze-thaw cycles 92.7% compared to 27.7% for OMV packaged PTE compared to free PTE, respectively. Lyophilized samples were also utilized in long term remediation assays as well as in paraoxon contaminated environmental samples collected from: pond, building downspout, pavement puddle, and dirt puddle sources. **Conclusions:** Our findings demonstrate that packaging PTE within bacterial OMV provides for a supportive micro-environment that maintains enzyme activity across all storage conditions tested. This technique can be adapted for use with alternate enzymes and proteins for environmental remediation, pharmaceutical delivery, and industrial applications.

Author Disclosure Block:

N.J. Alves: None. **K.B. Turner:** None. **S.A. Walper:** None.

Poster Board Number:

SATURDAY-001

Publishing Title:

A Simple Pipeline to Assess Genetic Diversity Between Bacterial Genomes

Author Block:

J-F. Pombert, I. Veseli, Y. Sun, R. R. Butler, III; Illinois Inst. of Technology, Chicago, IL

Abstract Body:

Calculating the number of single nucleotide polymorphisms (SNPs) and insertions/deletions (indels) between distinct genomes is an important component of the assessment of their genetic diversity. The gold standard approach used to generate this information consists of mapping short accurate high-throughput sequencing reads from one or more species against a reference genome, from which variants are called. This approach works well when short sequencing read data are available, however sequenced genomes deposited in public repositories do not always include the reads that were used to generate consensuses. Moreover, the newest long read technologies from Pacific Biosciences and Oxford Nanopore, both increasingly used to sequence bacterial genomes, produce fragments that often exceed 20 kb. While one can align genomes and/or long reads against each other, the results are often suboptimal when the investigated samples feature a large amount of syntenic differences, as the presence of chromosomal reorganization can cause the mapping to fail. An elegant solution to this problem is to deconstruct the complete genomes or long reads into shorter fragments, a shotgun approach, and to use these smaller synthetic reads as input for mapping. We have implemented this approach as an easy-to-use portable Perl script named SSRG.pl (available on the lab's GitHub page), and have integrated it with BWA and VarScan, well-known read mapping and SNP/indel calling tools. Test cases indicate that the results obtained with the synthetic reads are similar in every way to those obtained from genuine illumina reads, with the same advantages and caveats, and can therefore be reliably used as substitute when analyzing haploid genomes. Using this pipeline, we are able to compare SNPs and indels between any two bacterial genomes available in GenBank.

Author Disclosure Block:

J. Pombert: None. **I. Veseli:** None. **Y. Sun:** None. **R.R. Butler:** None.

Poster Board Number:

SATURDAY-002

Publishing Title:

Intein-based Evolutionary Insights Into the Mycobacterial Phenotypic Network (Mpn) in the Genus Mycobacterium

Author Block:

O. Kweon, S-J. Kim, D. Bae, J. Chon, J. Sutherland, C. Cerniglia; NCTR, Jefferson, AR

Abstract Body:

Background: The bacterial genus *Mycobacterium* is of great interest in the medical and biotechnological fields. We previously generated a practical mycobacterial compendium using phylogenetic, genomic, pan-genomic, and phenotypic information and then reconstructed a mycobacterial phenotypic network (MPN). The systematic mycobacterial information allowed a new approach called Network Based Functional Pan-Genomics (NBFPG) to build connections between genomic dynamics and phenotypic evolution. In this study, we update the mycobacterial compendium and the MPN with newly genome-sequenced mycobacteria and use inteins (protein splicing elements), as indicators of gene flow to identify possible evolutionary trajectories in the genus *Mycobacterium*.**Methods**: Pan-genomic analysis using EDGAR; sequence alignment, clustering, and phylogeny using T-Coffee; mycobacterial genomes and intein from JGI/IMG and InBase.**Results**: Initially, we updated the mycobacterial compendium and the MPN with 50 newly completed genome-sequenced mycobacteria, giving a total of 77 strains of mycobacteria. The updated compendium and MPN provide the enhanced pleiotropic and epistatic evolutionary resolution of nine representative mycobacterial phenotypes according to the genomic dynamics. No noticeable alterations in the scale-freeness and connection strength were observed in the updated MPN. A bottom-up survey identified several types of intein from the proteins (RecA, GyrA, DnaB, and SufB), existing sporadically in the phylogeny. Integration of the distribution and phylogenetic signals of the inteins into the MPN revealed possible trajectories of gene transfer. The strong connection, from the PAH-degrading strains in the free-living mycobacterial group to obligately intracellular *M. leprae* strains via the nontuberculous mycobacteria (NTM) in the facultatively host-associated mycobacterial group, suggests a plausible evolutionary trajectory in the genus *Mycobacterium*.**Conclusion**: MPN-based integration of the intein-centric evolutionary signals enhances evolutionary resolution at the network level, which allows the more powerful NBFPG to fill the knowledge gaps between genome and phenome in the genus *Mycobacterium.*

Author Disclosure Block:

O. Kweon: None. **S. Kim:** None. **D. Bae:** None. **J. Chon:** None. **J. Sutherland:** None. **C. Cerniglia:** None.

Poster Board Number:

SATURDAY-003

Publishing Title:

Gene-by-Gene Diversity in Across Bacterial Genomes of Various Species, Including *M. tuberculosis* and *Neisseria*

Author Block:

S. Hauck; Univ. of Oxford, Oxford, United Kingdom

Abstract Body:

Background: With the rise of high throughput whole genome sequencing, thousands of genomes for epidemiologically important bacterial species have become available. While these genomes are on occasion of poor quality, in combination they are a useful resource for exploring the diversity and selective pressures acting on a species at a gene-by-gene level.**Materials & Methods:** Whole genomes from public archives such as the Sequence Read Archive (SRA) were added to the PubMLST database, following assembly via a Velvet optimiser pipeline. This database runs the BIGSdb (Bacterial Isolate Genome Sequence database) software, which allows for genome annotation based on a set of reference sequences that defines genes, and an iterative scanning and definition process for new alleles of those genes. Measures of diversity were then calculated from the length, count of unique alleles, and ratio of variable sites in each defined locus, using the mean for the genome as the point of comparison.**Results:** This method was first applied to a data set of 7681 *Mycobacterium tuberculosis* genomes, using the nearly four thousand genes in the H37Rv annotation as the reference. This identified both conserved and diverse genes, which were respectively often coding for the information pathway and for surface-bound products. Genes in the ESAT-6 family appeared to be genetically diverse but functionally conserved, suggesting perhaps two stages of evolution with different pressures. The distribution of synonymous mutations indicated that the vast majority of the *M. tuberculosis* genes are under purifying selection, while genes in *Neisseria meningitidis* showed an evenly distributed level of selection, and *N. gonorrhoeae* showed many positively selected genes in a background on mild purifying selection.**Conclusion:**The availability of thousands of genomes and fully annotated reference genomes, together with population annotation software, allows for genes to be explored as "gene pools" that can be compared in order to reveal the evolutionary forces which act of them. This method allows to simple and rapid evaluation of gene targets across an entire genome using a large number of isolates to avoid sampling bias. This method also allows for a more rigorous choice of molecular markers to be made, as it can take into account the diversity or lack thereof of the potential target.

Author Disclosure Block:

S. Hauck: None.

Poster Board Number:

SATURDAY-004

Publishing Title:

Comparative Transcriptomics of Enterotoxigenic *Escherichia coli* Isolates in Response to Bile

Author Block:

J. R. Sistrunk[1], J. Sahl[2], F. Qadri[3], J. Fleckenstein[4], D. Rasko[1]; [1]Univ. of Maryland, Baltimore, Baltimore, MD, [2]Translational Genomics Res. Inst., Flagstaff, AZ, [3]Intl. Ctr. for Diarrhoeal Disease Res., Mohakhali, Bangladesh, [4]Washington Univ. in St. Louis, St. Louis, MO

Abstract Body:

Background: Enterotoxigenic *Escherichia coli* (ETEC) is a non-invasive, diarrheagenic pathovar of *E. coli* that causes 40 million cases of Travelers' Diarrhea every year and results in an annual 500,000 deaths worldwide. ETEC virulence is characterized by the production of plasmid-encoded enterotoxins that typically lead to a profuse watery diarrheal illness. However, ETEC has been isolated from study participants exhibiting a range of clinical presentations including severe and mild diarrhea, and even asymptomatic colonization. Host products, such as bile salts, that the bacterium encounters along the human gastrointestinal tract are thought to induce expression of ETEC virulence genes that facilitate colonization and disease. **Methods:** RNA-Seq was used to elucidate the transcriptional responses of five clinically diverse ETEC isolates in response to bile salts. Two severe ETEC isolates, including prototype H10407, a mild, and an asymptomatic isolate were grown in LB and DMEM media supplemented with 3% sodium choleate prior to total RNA extraction. The transcripts were sequenced and mapped to each isolates' genome qRT-PCR validation on 25 additional ETEC isolates was used for validation. **Results:** RNA-Seq revealed differential expression of both isolate-specific and shared genes in response to bile salts. Interestingly, colonization factor genes encoding CFA/I fimbriae were only up regulated in the non-prototype severe ETEC isolate, while significantly down regulated in all other isolates. However, motility genes were up regulated in the isolates that saw colonization genes down regulation. qRT-PCR screening in 25 additional ETEC isolates demonstrated that neither clinical presentation nor *E. coli* phylogroup were predictors of the transcriptomic response to bile. **Conclusions:** All four ETEC isolates exhibited variable transcriptomes in response to bile. The transcriptional regulation of ETEC colonization and motility genes suggest presence of diverse regulatory networks in ETEC. Continued investigation of global transcriptional responses may further reveal differences in the pathogenic potential within the ETEC pathovar.

Author Disclosure Block:

J.R. Sistrunk: None. **J. Sahl:** None. **F. Qadri:** None. **J. Fleckenstein:** None. **D. Rasko:** None.

Poster Board Number:

SATURDAY-005

Publishing Title:

Comparative Transcriptomics of *Acinetobacter* Species Exposed to Multiple Classes of Antimicrobials

Author Block:

T. H. Hazen[1], S. C. Daugherty[1], A. C. Shetty[1], K. Abolude[1], J. K. Johnson[2], A. Harris[2], D. A. Rasko[1]; [1]Inst. for Genome Sci., Baltimore, MD, [2]Univ. of Maryland, Baltimore, MD

Abstract Body:

Acinetobacter baumannii and other disease-associated *Acinetobacter* species have become increasingly linked with hospital-acquired infections over recent decades making them a pathogen of increasing concern. In recent years, high-throughput sequencing has been used to characterize the genomic and transcriptomic diversity of *Acinetobacter* isolates associated with healthcare facilities. However, much remains unknown regarding the number and different types of transcriptional pathways involved in the development of antimicrobial resistance. In the current study, we used RNA sequencing (RNA-Seq) to characterize the global transcriptional responses of nine genomically-diverse *Acinetobacter* isolates upon exposure to four different classes of antimicrobials (carbapenem, aminoglycoside, polymyxin, fluroquinolone), and a general stress inducing agent (NaCl). Phylogenomic analysis of the nine *Acinetobacter* isolates analyzed in this study demonstrated that four were *A. baumannii* and belonged to the global clone 2, three others were part of the *A. calcoaceticus-A. baumannii* (ACB) complex, and the remaining two isolates were an *A. radioresistans* and an unidentified *Acinetobacter* species. These nine *Acinetobacter* isolates were obtained from a cohort study that investigated the distribution of antimicrobial resistant bacteria among patients receiving treatment at one of the intensive care units (ICUs) of the University of Maryland Medical Center. RNA-Seq analysis demonstrated there was diversity in the number and types of protein-encoding genes that were transcriptionally-altered in each *Acinetobacter* isolate following exposure to antimicrobials or to NaCl. The total number of genes that exhibited significant differential expression (DE) following exposure to an antimicrobial compared to growth without the antimicrobial ranged from 0-916 depending on the isolate and species. Among the genes that exhibited increased expression following exposure to antimicrobials were hypothetical proteins, transcriptional regulators, and translocators. These findings provide insight into the genes that are part of the global transcriptional responses of *Acinetobacter* to different antimicrobial agents.

Author Disclosure Block:

T.H. Hazen: None. **S.C. Daugherty:** None. **A.C. Shetty:** None. **K. Abolude:** None. **J.K. Johnson:** None. **A. Harris:** None. **D.A. Rasko:** None.

Poster Board Number:

SATURDAY-006

Publishing Title:

Comparative Genomic Analysis of Genetic Exchange among Bacteria Species

Author Block:

M. J. Soto-Giron, L. M. Rodriguez-R, K. T. Konstantinidis; Georgia Inst. of Technology, Atlanta, GA

Abstract Body:

Horizontal gene transfer, mediated by homologous recombination (gene replacement or conversion), contributes to diversification, genomic evolution, and ecological adaptation of bacterial species, including pathogens. Some bacteria are thought to follow a clonal structure model while others present a recombining mixture of diverse genotypes. The increased number of sequenced genomes makes the robust investigation of population diversification and recombination rates now possible. In this study, we propose an alternative method to estimate the rates of genetic exchange using the number of observed shared genes relative to the number of identical genes expected by chance based on the average amino acid identity (AAI) between genome pairs. We applied this method to several bacterial species with distinct ecologies and found that the expected level of recombination was robust and agreed with previous reports detecting close to zero recombination rates for obligatory and intracellular bacteria such as *Buchnera aphidicola*. However, the contribution of recombination to bacterial diversification was more similar than previously thought in generalist organisms such as *Neisseria meningitidis* and *Campylobacter jejuni*. We analyzed the population dynamics and recombination signatures of *C. jejuni*, a pathogen associated with foodborne infections, using isolates from distinct hosts and regions of the world (n >200). Our results suggested a cosmopolitan population clustered mainly in four clades with pervasive recombination among the clades (e.g., recombination to mutation ratio was 3.6). Among the 133 core genes with recombination signals, we identified genes related to resistance to bacitracin, erythromycin, and amikacin antibiotics as well as virulence factors associated with serum resistance, invasion of epithelial cells, and adhesion. The transmission of antibiotic-resistant genes requires more attention to control the emergence of multi-resistance genotypes and the exchange of virulence factors may increase the virulence of recipient strains.

Author Disclosure Block:

M.J. Soto-Giron: None. **L.M. Rodriguez-R:** None. **K.T. Konstantinidis:** None.

Poster Board Number:

SATURDAY-007

Publishing Title:

Automatic Text Mining of Prokaryotic Phenotypic Characters Using Micropie

Author Block:

L. R. MOORE[1], J. Mao[2], C. Blank[3], M. Ackerman[1], H. Cui[2]; [1]Univ. of Southern Maine, PORTLAND, ME, [2]Univ. of Arizona, Tucson, AZ, [3]Univ. of Montana, Missoula, MT

Abstract Body:

The study of the evolution of microbial traits requires both phylogenetic as well as phenotypic trait information (also called phenomics). Next generation sequencing has enabled high throughput (meta)genomic analyses, but collecting phenotypic information, either *de novo* or from published taxonomic literature, to create large taxon-character matrices is still tedious and time-consuming. We are creating a natural language processing (NLP) tool, Microbial Phenomics Information Extractor, or MicroPIE, that enables faster collection of microbial phenomic information from published literature. MicroPIE takes taxonomic descriptions in XML files as input and extracts more than 50 types of qualitative and quantitative microbial characters, such as Cell Shape, Organic Substrates Utilized, and Temperature Optimum. The main extraction steps are: 1) splitting paragraphs into sentences; 2) predicting the characters described in the sentences by using automated classifiers; and 3) extracting character values from the sentences by applying a variety of NLP methods. We provided MicroPIE with a library of microbial-specific terms derived from ~1000 taxonomic descriptions from the Archaea, Bacteroidetes, Cyanobacteria, and Mollicutes as a starting point, but have also developed an ontology (MicrO) of prokaryotic phenotypic and metabolic characters. MicrO is organized using a formal logical framework and will be incorporated into a future version of MicroPIE to assist with character identification and extraction, to facilitate identification of trait synonyms used in prokaryotic taxonomic descriptions, and to populate character matrices with higher-level character states. Matrices generated by the MicroPIE prototype are being compared automatically and manually to a hand-generated, gold standard matrix of 111 taxa with >4000 character values, and preliminary results of precision and recall are very promising (>80% for 38% of characters so far). MicroPIE will be open-source once it is released. The matrices extracted using MicroPIE are in a format that can be combined with phylogenomic trees for conducting phylogenetic comparative analyses to test evolutionary hypotheses.

Author Disclosure Block:

L.R. Moore: None. **J. Mao:** None. **C. Blank:** None. **M. Ackerman:** None. **H. Cui:** None.

Poster Board Number:

SATURDAY-008

Publishing Title:

The Effect of History on Evolution of *Desulfovibrio vulgaris* Hildenborough Under Elevated Temperature

Author Block:

M. L. Kempher[1], R. Song[1], A. Zhou[1], G. M. Zane[2], S. M. Kosina[3], T. R. Northern[3], J. D. Wall[2], A. P. Arkin[3], J. Zhou[1]; [1]Univ. of Oklahoma, Norman, OK, [2]Univ. of Missouri, Columbia, MO, [3]Lawrence Berkeley Natl. Lab., Berkeley, CA

Abstract Body:

The contributions of history, adaptation, and chance to evolution have long been debated. Adaptation and natural selection have been regarded as the most important influences on evolution. However, prior historical events may place constraints on or promote the appearance of certain mutations. In order to further investigate the influences of history on evolution, three groups of *Desulfovibrio vulgaris* Hildenborough (*Dv*H) with varying evolutionary histories were propagated in the laboratory under elevated temperature (41°C) for 1,000 generations. The three groups included six populations that were started from the Ancestor (An) with no prior evolution and named An-T 1 – 6, six populations that had been previously propagated for 1,200 generations under control conditions (LS4D medium, 37°C) and named EC-T 1 – 6 (EC: Evolved Control), and six populations that had been previously propagated for 1,200 generations under elevated salt conditions (LS4D medium, 37°C, +100 mM NaCl) and named ES-T 1 – 6 (ES: Evolved Salt). All 18 populations were then propagated in LS4D without additional salt but at an elevated temperature (41°C). Phenotypic changes and relative fitness of each population were then assessed using growth analysis (growth rate, lag phase time, and maximum OD). Each population was re-sequenced to map the mutations that had accumulated. Prior evolution under high NaCl provided some cross-protection to heat stress. The ES population had a slight advantage at 41°C compared to the EC and An population. However, after 1,000 generations at 41°C the advantage was minimized. Additionally, the EC-T populations had increased growth at elevated salt compared to the EC population, providing further evidence that an adaptation to either heat or salt provided cross-protection for the other. At 46°C, a temperature previously determined to be lethal to the ancestor, the populations with evolutionary history had slightly increased growth rate and final OD_{600} compared to the An populations. Direct head-to-head competitions between the evolved populations and the wild-type ancestor are currently ongoing. A direct measurement of fitness will be important in determining the role of chance, adaptation, and history on evolution.

Author Disclosure Block:

M.L. Kempher: None. **R. Song:** None. **A. Zhou:** None. **G.M. Zane:** None. **S.M. Kosina:** None. **T.R. Northern:** None. **J.D. Wall:** None. **A.P. Arkin:** None. **J. Zhou:** None.

Poster Board Number:

SATURDAY-009

Publishing Title:

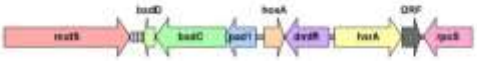
Genomic Characterization of the *mutS-rpoS* region of *Cronobacter* Species

Author Block:

E. E. Jackson, S. J. Forsythe; Nottingham Trent Univ., Nottingham, United Kingdom

Abstract Body:

Background: The *mutS-rpoS* genomic region has been identified as a highly variable and evolving via horizontal gene transfer. Genes in this region may contribute to virulence and within the *Cronobacter* genus, 3 of the 7 species have been linked to human illnesses. This work was undertaken to examine the *mutS-rpoS* region in *Cronobacter* to determine its role in the virulence of certain species. **Methods:** The genomes of 136 strains, covering six *Cronobacter* species were examined and sequences were extracted using Artemis. All genomes are available at http://www.pubmlst.org/Cronobacter. Phylogenetic networks of the region were constructed using SplitsTree to visualize possible recombination events.**Results:** All *Cronobacter* species and strains contained the same seven genes between *mutS* and *rpoS* (Figure 1). These genes include phenolic and aromatic acid decarboxylases (*bsdC, bsdC, pad1*), possibly related to the association of *Cronobacter* with plants. The region also includes genes encoding a drug efflux pump and two transcriptional regulators. Phylogenetic networks showed recombination events prior to the divergence of the *Cronobacter* spp. No recombination events were observed between strains of different species after divergence and limited recombination was observed between strains of the same species.Figure 1. Gene organization of the *mutS-rpoS* genomic region of *Cronobacter*



Conclusions: The *mutS-rpoS* genomic region of *Cronobacter* spp. is consistent among the species, despite that only 3 species have been linked to human illnesses. The *mutS-rpoS* region does not contain genes directly related to virulence, but some may be related to environmental survival. Very little recombination has occurred in this region, likely due the clonal nature of the *Cronobacter* genus.

Author Disclosure Block:

E.E. Jackson: None. **S.J. Forsythe:** None.

Poster Board Number:

SATURDAY-011

Publishing Title:

Phylogenetic Analysis of Electrically Conductive Pili of *Geobacter* Species

Author Block:

D. E. Holmes[1], Y. Dang[2], D. R. Lovley[2]; [1]Western New England Univ., Springfield, MA, [2]Univ. of Massachusetts Amherst, Amherst, MA

Abstract Body:

Studies have shown that electrically conductive pili (e-pili) can enable long-range electron transfer to insoluble minerals, to other cells, and through electrically conductive biofilms. Most studies of e-pili have focused on Fe(III)-respiring bacteria from the genus *Geobacter*, *G. sulfurreducens* and *G. metallireducens*. The e-pilin gene from both of these organisms is significantly shorter (60-90 aa) than type IV pilA from other organisms (>120 aa), and is accompanied by a gene (pilA-C) located directly downstream that codes for a small protein composed of beta sheets and a transmembrane domain. In order to evaluate the phylogenetic conservation of e-pilin and pilA-C genes among the order Desulfuromondales and other Fe(III) respiring bacteria, 96 different genomes were analyzed. Most (79%) of the bacteria in the order Desulfuromondales possess e-pilin genes, however, this gene was present in only 2 out of 72 Fe(III)-respiring bacteria from other taxonomic orders suggesting that most Fe(III)-reducing bacteria rely on mechanisms other than e-pili for electron transport to Fe(III). Further sequence analysis suggested that both the e-pilin and pilA-C gene is undergoing positive selection among the genus *Geobacter*. It has been speculated that the shorter pilus monomer permits tighter packing of aromatic amino acids, and possibly other amino acids, in a manner that promotes electron conduction along the length of the pili. It has been shown that e-pili are required for direct interspecies electron transfer (DIET) in defined co-cultures. Therefore, further studies were done on metagenomic and metatranscriptomic libraries assembled from environments where DIET is likely to occur: anaerobic digesters, ANME-dominated subsurface sediments, and rice paddy subsurface sediments. While the majority of e-pilin sequences from the bioreactor and rice paddy samples were most similar to *G. sulfurreducens*, e-pilin sequences from another metal respiring bacterium (*Desulfobacterium autotrophicum*) were predominant in ANME-1 dominated sediments. This study provided insight into the evolution of this unique electron transfer mechanism and identified molecular signatures that are diagnostic for DIET that can greatly aid the study of this process *in situ*.

Author Disclosure Block:

D.E. Holmes: None. **Y. Dang:** None. **D.R. Lovley:** None.

Poster Board Number:

SATURDAY-012

Publishing Title:

The Evolution Of The Amoa, Pmoa And Bmoa Genes Measured By Ka/Ks Ratios

Author Block:

E. LAU, D. P. Lukich, T. Le; West Liberty Univ., West Liberty, WV

Abstract Body:

Background: The copper-containing membrane-bound monooxygenase (CuMMOs) enzyme family consists of enzymes that facilitate the oxidation of methane (CH4) (catalyzed by particulate methane monooxygenase, pMMO) and ammonia (NH3/NH4) (catalyzed by ammonia monooxygenase, AMO), in both aerobic and anaerobic methanotrophs and nitrifiers, respectively, and the oxidation of C2-10 alkanes (catalyzed by particulate butane monooxygenase, pBMO). Despite catalyzing different substrates, pMMO, AMO and pBMO form catabolic enzymes related structurally and evolutionarily to each other, and they are encoded by the genes pmoA, amoA and bmoA, respectively. It has been suggested that oxygen-dependent methane and ammonia monooxygenases evolved from a substrate-promiscuous ancestor after horizontal transfer(s) into new hosts, which eventually became methanotrophs and nitrifiers. However, no extensive studies have been conducted on molecular selection and adaptation of the beta-peptide structure of pmoA, amoA and bmoA gene products, which form transmembrane polypeptides, and on selective pressures acting on them and their evolutionary history. **Methods:** In this study, over 80 near-complete pmoA, amoA and sequences of known methanotrophs and ammonia oxidizers and single bmoA gene from Nocardioides sp. CF8 from database were downloaded and analyzed to assess whether the combined amoA, pmoA and bmoA gene tree is congruent to the 16S rRNA gene tree for methanotrophs and ammonia oxidizers. The coding gene was separated into 15 segments and pairwise Ka/Ks values were calculated to assess the evolutionary and selection pressure exerted on these taxa in each segment, based on the hypothesis that they evolved from a methanotrophic ancestor, as well as calculate Ka/Ks values between members of each family. **Results:** Our analyses indicated a high level of negative (or purifying) selection between and amongst all taxa. Ka/Ks values were highest between methanotroph pmoA and the anammox or Ammonia oxidizing Archaea (AOA). **Conclusions:** Overall, these analyses demonstrate that the pmoA and amoA genes are under purifying selection within the pmoA and amoA clusters, and supports the hypothesis that one of these genes likely arose from the other via horizontal gene transfer.

Author Disclosure Block:

E. Lau: None. **D.P. Lukich:** None. **T. Le:** None.

Poster Board Number:

SATURDAY-013

Publishing Title:

Bacteria Form Distinct Ecological Groups That May Impact Nutrient Cycling and Dead Zone Formation in the Chesapeake Bay

Author Block:

E. Sakowski, S. Abraham, G. Kim, S. Preheim; Johns Hopkins Univ., Baltimore, MD

Abstract Body:

The Chesapeake Bay is vital habitat for numerous fish and invertebrate species but is subject to anoxic conditions and dead zone formation during the summer that can result in mass mortalities of these organisms. The development of these dead zones is intimately tied to bacterial metabolism and nutrient cycling, fueled largely by nitrogen and phosphorous pollution. However, high-resolution characterizations of the spatiotemporal distributions of bacterial groups and their role in nutrient cycling and dead zone formation in the Bay are lacking. In this study, bacterial communities were characterized by 16S rRNA amplification and sequencing at a single site in the Chesapeake Bay known to experience anoxia in the summer. Water samples were collected at 1m intervals from surface to bottom in June, July, and August 2015 to capture bacterial communities before and during anoxia formation. Overall, water sample bacterial communities segregated by sample month and depth and formed 11 clusters by hierarchical clustering (4 June clusters, 3 July clusters, 4 August clusters). The most abundant OTUs were present in all 11 clusters at varying relative abundances but belonged to one of four groups defined by OTUs with correlated relative abundance profiles, suggesting these OTUs may be interacting. Groups were specifically associated with one or more of the 11 month- and depth-specific clusters. One group consisted of OTUs that were associated with all four June clusters where dissolved oxygen remained above 2 mg L^{-1} (hypoxia). Two groups were associated with the oxygenated waters in the upper two July and August clusters, respectively, while one group was most abundant in July and August anoxic water clusters. Surprisingly, these OTU groups were composed of similar taxonomic lineages, as 17 taxonomic families representing on average 57% of the cluster communities were present in all four groups. This indicates that related OTUs may have different ecological impacts as a result of specific ecological interactions, and inferences of metabolism and distribution based on taxonomic lineage may be misleading. Ultimately, understanding the impact of specific OTU interactions on community distribution, metabolism, and dead zone development will be crucial to developing better models of dead zone formation and strategies to mitigate their impact in the Chesapeake Bay.

Author Disclosure Block:

E. Sakowski: None. **S. Abraham:** None. **G. Kim:** None. **S. Preheim:** None.

Poster Board Number:

SATURDAY-014

Publishing Title:

Abundance and Diversity of Nitrifying Microbes in Sediments Impacted by Acid Mine Drainage

Author Block:

B. Ramanathan, J. D. Sackett, T. Roane, A. C. Mosier; Univ. of Colorado Denver, Denver, CO

Abstract Body:

Background: Extremely acidic and metal-rich acid mine drainage (AMD) waters can have severe toxicological effects on aquatic ecosystems. AMD was shown to completely halt nitrification, which plays an important role in transferring nitrogen to higher organisms and in mitigating nitrogen pollution. **Methods:** We evaluated whether AMD differentially impacts three groups of microorganisms involved in nitrification: ammonia-oxidizing archaea (AOA), ammonia-oxidizing bacteria (AOB), and nitrite-oxidizing bacteria (NOB). Sediment and water were collected from AMD-impacted aquatic sites during June and August/September 2013 and 2014 in the Iron Springs Mining District (Ophir, Colorado). Many of the sites were characterized by low pH (<5), low dissolved oxygen concentrations (<6 mg/L), and high metal concentrations. Community sequencing based on the 16S rRNA gene revealed the presence of AOA (*Nitrososphaera* and *Nitrosopumilus*), AOB (*Nitrosomonas*), and NOB (*Nitrospira*) at multiple AMD-impacted sites. The overall abundance of AOA, AOB and NOB were examined using quantitative PCR (qPCR) amplification of the *amoA* and *nxrB* functional genes and 16s rRNA genes. **Results:** The total gene copy numbers across the 2013 and 2014 samples ranged from $2x10^3$-$4.9x10^7$ archaeal *amoA* copies/µg DNA, $7.3x10^5$-$7.7x10^7$ *Nitrospira nxrB* copies/µg DNA and $1.5x10^3$-$5.3x10^5$ AOB 16s rRNA copies/µg DNA. Overall, AOA were found to be more abundant than AOB and NOB in most of the sample sites across 2013 and 2014. Archaeal *amoA* genes were detected in sediments with pH as low as 3.2. Preliminary analyses showed no significant correlation between gene abundance and the measured environmental parameters (pH, temperature, DO and heavy metal levels). **Conclusion:** These findings extend our understanding of the relationship between AMD and freshwater nitrifying microbes and provide a platform for further research.

Author Disclosure Block:

B. Ramanathan: None. **J.D. Sackett:** None. **T. Roane:** None. **A.C. Mosier:** None.

Poster Board Number:

SATURDAY-015

Publishing Title:

Ecology Of Ammonium Oxidizing And Nitrite Oxidizing Microorganisms In Namibian Coastal Upwelling Zone At Different Depths

Author Block:

E. LAU[1], C. H. Frame[2], E. J. Nolan, IV[1], Z. W. Dillard[1], M. A. Kinker[1], D. P. Lukich[1], N. E. Mihalik[1], K. E. Yauch[1], S. Waychoff[1]; [1]West Liberty Univ., West Liberty, WV, [2]Univ. of Basel, Basel, Switzerland

Abstract Body:

Background: The upwelling of nutrient-rich South Atlantic waters (as part of the Benguela current) along the Namibian coast sustains some of the highest rates of primary production in oceans, which involves important steps in the Nitrogen cycle. However, the ecology of nitrifying microbes in this ecosystem is not well-studied. **Methods:** Using deep multiplex sequencing of (~450bp) 16S rRNA amplicons, complemented by salinity, temperature, dissolved oxygen, Photosynthetically Active Radiation (PAR), fluorescence and N2O isotope concentration measurements, we profiled the microbiota at 10m, 25m, 100m, 130m, and 250m depths. We assessed the diversity and abundance of Ammonia oxidizing Archaea (AOA), Ammonia oxidizing Bacteria (AOB), and nitrite oxidizing Bacteria (NOB), which oxidize ammonium (NH4+) or ammonia (NH3) to nitrite (NO2−), and nitrite to nitrate (NO3−).**Results:** Our data indicate that the AOA or anammox (anaerobic oxidation of ammonium by Archaeal ammonia oxidizing microbes) are the dominant nitrifying microbes at 25m and below, where dissolved oxygen levels in the upwelling seawater becomes depleted. Their abundances and diversity far exceed that of other nitrifying bacteria.**Conclusions:** This study supports previous reports on the abundance of anammox cells, and biomarker lipids that indicate that anammox bacteria are responsible for significant losses of fixed nitrogen in this ecosystem, as well as other oxygen-depleted upwelling seawaters.

Author Disclosure Block:

E. Lau: None. **C.H. Frame:** None. **E.J. Nolan:** None. **Z.W. Dillard:** None. **M.A. Kinker:** None. **D.P. Lukich:** None. **N.E. Mihalik:** None. **K.E. Yauch:** None. **S. Waychoff:** None.

Poster Board Number:

SATURDAY-016

Publishing Title:

Physiological Study of Ammonia-Oxidizing and Nitrite-Oxidizing Enrichment Cultures from a Freshwater System

Author Block:

A. Boddicker, N. Deevers, A. C. Mosier; Univ. of Colorado Denver, Denver, CO

Abstract Body:

The oxidation of ammonia into nitrite and nitrate (i.e., nitrification) is thought to be a critical, rate-limiting step in the removal of nitrogen pollution from freshwater systems. Nitrification is thought to be performed by four major groups of microorganisms: ammonia-oxidizing archaea (AOA) and bacteria (AOB), nitrite-oxidizing bacteria (NOB), and the newly discovered comammox bacteria that completely oxidize ammonia to nitrate. Very few representatives of these microorganisms have been cultured in the lab and even fewer have been completely isolated. The organisms are difficult to isolate due to their slow growth and competition with heterotrophic contaminants, but more cultivation will lead to a more in-depth understanding of nitrification biochemistry and capability. Here, we describe seven enrichment cultures from the South Platte River basin in Colorado. Functional gene PCR and Illumina MiSeq sequence analyses showed all seven cultures contain an NOB belonging to the *Nitrobacter* genus. Three of the cultures also contain an AOB belonging to the betaproteobacteria phylum, creating co-cultures capable of oxidizing ammonia all the way to nitrate. Nitrite production and consumption in the enrichment cultures has been monitored for more than one year. A growth curve was calculated for one of the AOB/NOB co-cultures showing the production and subsequent consumption of the intermediate nitrite. Several of the enrichment cultures were grown at different nutrient levels to determine optimal growth conditions and response to nutrient pollution. Future work will include in depth physiological and genomic characterization. These findings will aide in the understanding of the diversity and abilities of freshwater nitrifying organisms.

Author Disclosure Block:

A. Boddicker: None. **N. Deevers:** None. **A.C. Mosier:** None.

Poster Board Number:

SATURDAY-017

Publishing Title:

Nitrogen Cycle Evaluation (Nice) Chip to Quantify and Sequence All Functional Genes for the N Cycle Simultaneously

Author Block:

M. Oshiki[1], T. Segawa[2], **S. Ishii**[3]; [1]Natl. Inst. of Technology, Nagaoka Coll., Nagaoka, Japan, [2]Natl. Inst. of Polar Res., Tokyo, Japan, [3]Univ. of Minnesota, St. Paul, MN

Abstract Body:

Background: Various microorganisms play key roles in the Nitrogen (N) cycle. Quantitative PCR and PCR-amplicon sequencing of the N cycle functional genes allows us to analyze the abundance and diversity of these microbes in various environmental samples. However, analysis of multiple target genes can be cumbersome and expensive. Large sequence variations present in some of the target genes require the use of multiple primer sets, which further increases the time, labor, and cost required for the analysis. PCR-independent analysis such as metagenomics and metatranscriptomics is useful to identify the key players for the N cycle; however, it becomes expensive when we analyze multiple samples and try to detect N cycle functional genes present at relatively low abundance. Here, we present the application of microfluidic chip technology to quantify and prepare sequencing libraries targeting all functional genes for the N cycle (i.e., nitrification [*amoA*, *hao*], denitrification [*napA*, *narG*, *nirK*, *nirS*, *norB*, *nosZ*], dissimilatory nitrate reduction to ammonium [*nrfA*], anaerobic ammonia oxidation [*hzs*], nitrogen fixation [*nifH*]) as well as taxon-specific 16S rRNA gene markers.**Methods:** In the N cycle evaluation (NiCE) chip, 48 qPCR assays could be run for 48 samples each (a total of 2304 reactions) per run. The resulting amplicons were recovered from the chip and used for MiSeq sequencing. The DNA samples from pure and artificially mixed bacterial cultures and environmental samples were used as the test samples along with the standard plasmid DNA mixtures.**Results:** Similar results were obtained by NiCE chip and conventional PCR assays. We could obtain the quantitative information of each gene by monitoring the amplification curve in real time. In addition, results obtained by NiCE chip followed by MiSeq sequencing were similar to those obtained by MiSeq done without using NiCE chip.**Conclusions:** The NiCE chip can provide high-throughput format to quantify and prepare sequencing libraries for all functional genes in the N cycle. This tool should advance our ability to explore N cycling in various environments.

Author Disclosure Block:

M. Oshiki: None. **T. Segawa:** None. **S. Ishii:** None.

Poster Board Number:

SATURDAY-018

Publishing Title:

Bacterial Siderophores in the Underworld: Siderophore Production by Cave Bacteria in Carlsbad Caverns National Park

Author Block:

T. R. Duncan, D. E. Northup, M. Werner-Washburne; Univ. of New Mexico, Albuquerque, NM

Abstract Body:

Determining how iron is acquired by subsurface bacteria remains an unanswered question that would shed important light on how bacteria live in caves. Surface bacteria use iron acquisition molecules, such as siderophores, to acquire iron. Siderophores have been found in many oligotrophic (low nutrient) environments, which include the open ocean, deep subsurface soil, lakes, and polar ice, and give clues to the nature of siderophores in caves. Microbes in oligotrophic caves have to rely on highly efficient mechanisms to collect essential nutrients, including iron. We hypothesize that cave bacteria synthesize and use siderophores to acquire critical iron needed for cellular processes. We investigated potential siderophore production in Lechuguilla and Spider Caves in Carlsbad Caverns National Park. We subcultured media inoculated with cave secondary minerals in Spider and Lechuguilla Caves, obtaining 170 bacterial isolates. These isolates were tested with chrome azurol sulfate (CAS) assay for siderophore production, with Arnow's and $FeCl_3$ assay for siderophore-type, and a subset of 18 pure isolates were identified by 16S rRNA gene sequencing. The majority (60/170) of cave isolates tested Cas-positive and Cas-slightly positive at 11-25 days. The siderophore-type assays revealed that the majority of siderophores made were hydroxamate-positive and potential hydroxamate-positive and catechol-negative. 16S rRNA gene sequencing of 15 siderophore-positive isolates revealed members of α-, β-, γ-*Proteobacteria*, *Bacteroidetes*/*Chlorobi* group, *Actinobacteria*, and *Firmicutes*. The presence of isolates that produce siderophores supports our hypothesis that cave bacteria are using siderophores to acquire iron. These studies will shed light on the production of siderophores in oligotrophic subsurface environments.

Author Disclosure Block:

T.R. Duncan: None. **D.E. Northup:** None. **M. Werner-Washburne:** None.

Poster Board Number:

SATURDAY-019

Publishing Title:

Nitrate-dependent Iron Oxidation by an Autotrophic, Moderately Acidophilic *Geobacter* sp. Feam09 Isolated from Unsaturated Soil

Author Block:

O. M. Healy[1], J. Souchek[1], A. Heithoff[1], B. LaMere[1], C. L. Anderson[1], D. Pan[1], W. H. Yang[2], W. L. Silver[2], S. C. Fernando[1], K. A. Weber[1]; [1]Univ. of Nebraska-Lincoln, Lincoln, NE, [2]Univ. of California-Berkeley, Berkeley, CA

Abstract Body:

Iron (Fe) is the fourth most abundant element in the Earth's crust and plays a significant role controlling geochemistry in soils, sediments, and aqueous systems. As part of a study to understand microbially-catalyzed iron and nitrogen biogeochemical cycling in soils, an iron reducing isolate, strain FeAm09, was isolated from Fe-rich soils collected from a tropical forest (Luquillo Experimental Forest, Puerto Rico). Taxonomic analysis of the 16S rRNA gene sequence placed strain FeAm09 in the genus *Geobacter*. Characterization of the optimal growth conditions revealed that strain FeAm09 is a moderate acidophile with an optimal growth pH of 5.0 with little to no growth above a pH of 5.5 and below a pH of 4.5. Strain FeAm09 was isolated under Fe(III) reducing conditions and is capable of growth using soluble Fe(III), Fe(III)-NTA, as well as insoluble Fe(III) oxides (synthetic ferrihydrite) as terminal electron acceptors with H_2 as the electron donor. Strain FeAm09 was capable of coupling growth to the oxidation of solid-phase Fe(II) coupled to the biological reduction of nitrate to ammonium (Fe-DNRA). Dinitrogen (N_2) gas was also formed (denitrification) in equal molar amounts to ammonium. The draft genome sequence of FeAm09 supports the reduction of nitrate to nitrite (*napB*, *napD*) and subsequent nitrite reduction to ammonium (*nrfB*). Genes responsible for reduction of nitrous oxide reductase are absent, thus suggesting production of N_2 was an abiotic nitrite reduction reaction. These results indicate that the Fe-DNRA is microbially catalyzed. Carbon dioxide fixation was verified through assimilation of ^{14}C-labelled CO_2 into biomass under Fe(III) reducing and nitrate-dependent Fe(II) oxidizing conditions. The rTCA cycle was identified (*citB*, *frdB*, *frdC*, *fumB*, *icdI*, *korA*, *korB)* as the only known CO_2 fixation pathway in FeAm09. Together, these data describe the first acidophilic, autotrophic *Geobacter* species capable of nitrate-dependent Fe(II) oxidation. Additionally, the ability of FeAm09 to reduce nitrate to ammonium as the sole nitrate reduction pathway present a model organism that can be used to study the simultaneous biological and abiotic reduction of reactive intermediates such as nitrite.

Author Disclosure Block:

O.M. Healy: None. **J. Souchek:** None. **A. Heithoff:** None. **B. LaMere:** None. **C.L. Anderson:** None. **D. Pan:** None. **W.H. Yang:** None. **W.L. Silver:** None. **S.C. Fernando:** None. **K.A. Weber:** None.

Poster Board Number:

SATURDAY-020

Publishing Title:

Iron Oxidation Rates of Diverse Neutrophilic Iron-Oxidizing Bacteria Across a Range of Oxygen Concentrations

Author Block:

J. W. Cohen[1], R. A. Barco[2], J. P. Beam[2], D. Emerson[2], P. Girguis[1]; [1]Harvard Univ., Cambridge, MA, [2]Bigelow Lab. for Ocean Sci., East Boothbay, ME

Abstract Body:

Although neutrophilic microaerobic iron-oxidizing bacteria (FeOB) were some of the first chemolithoautotrophic organisms discovered, their ecophysiology remains poorly understood. Most FeOB require Fe^{2+} as an electron donor to respire O_2, yet the abiotic oxidation of iron proceeds rapidly under atmospheric O_2 concentrations. As such, it has been posited that FeOB predominate in environments with opposing gradients of Fe^{2+} and O_2, where O_2 concentrations are sufficiently low that FeOB out-compete abiotic reactions.[1] While it is apparent that FeOB compete with abiotic oxidations, there are few iron oxidation rate measurements across a range of oxygen concentrations. Here we are studying three marine FeOB in liquid culture across a range of environmentally relevant sub-atmospheric oxygen concentrations using zero-valent iron as a ferrous iron source. We use a non-invasive optical oxygen sensor to monitor headspace oxygen concentration in sealed vials over time. Biological iron oxidation rates derived from oxygen consumption rates were markedly faster than abiotic controls for all strains tested, showing that - at sub-atmospheric oxygen conditions - FeOB likely outcompete abiotic oxidations. Ongoing efforts will examine additional strains to ascertain if this trend remains true across a diversity of FeOB. Differences in iron oxidation rates between strains may reflect adaptations to different environments, as well as diversity in FeOB iron oxidation complexes. These rate measurements expand our knowledge of the biogeochemistry of one of the most common elements in the Earth's crust, and underscore the role of FeOB in global iron cycling.

Author Disclosure Block:

J.W. Cohen: None. **R.A. Barco:** None. **J.P. Beam:** None. **D. Emerson:** None. **P. Girguis:** None.

Poster Board Number:

SATURDAY-021

Publishing Title:

Oxidation of Cytochrome 583 Is Rate-Limiting When *Acidiplasma aeolicum* Respires Aerobically on Iron

Author Block:

R. Blake, II, K. Hunter, T-F. Li, R. Painter; Xavier Univ. of Louisiana, New Orleans, LA

Abstract Body:

Very little is known about the electron transfer reactions that occur during aerobic respiration on soluble iron by *Acidiplasma aeolicum*, an archaeal member of the Euryarchaeota phylum. We monitored electron transfer reactions among colored cytochromes in intact archaeal cells using an integrating cavity absorption meter (ICAM) that permitted the acquisition of accurate absorbance data in suspensions of intact cells that scatter light. The aerobic iron respiratory chain of *Ap. aeolicum* was dominated by the redox status of an abundant cellular cytochrome that had an absorbance peak at 583 nm in the reduced state. Intracellular cytochrome$_{583}$ was reduced within the time that it took to mix a suspension of the archaea with soluble ferrous iron at pH 1.5 and 45° C. Steady state turnover experiments were conducted where the initial concentrations of ferrous iron were less than or equal to that of the oxygen concentration. Under these conditions, the initial absorbance spectrum of the bacterium observed under air-oxidized conditions was always regenerated from that of the bacterium observed in the presence of Fe(II). The kinetics of aerobic respiration on soluble iron by intact *Ap. aeolicum* conformed to the Michaelis-Menten formalism, with values for V_{max} and K_M of 310 ± 4 nmol/min and 2.0 ± 0.1 × 10^{-4} M, respectively. The reduced intracellular cytochrome$_{583}$ represented the Michaelis complex whose subsequent oxidation appeared to be the rate-limiting step in the overall aerobic respiratory process. The velocity of formation of ferric iron at any time point was directly proportional to the concentration of the reduced cytochrome$_{583}$. Further, the integral over time of the concentration of the reduced cytochrome was directly proportional to the total concentration of ferrous iron in each reaction mixture. These kinetic data obtained using whole cells were consistent with the hypothesis that reduced cytochrome$_{583}$ is an obligatory steady state intermediate in the iron respiratory chain of this bacterium. The ability to conduct direct spectrophotometric studies under noninvasive physiological conditions represents a new and powerful approach to examine the extent and rates of biological events *in situ* without disrupting the complexity of the live cellular environment.

Author Disclosure Block:

R. Blake: None. **K. Hunter:** None. **T. Li:** None. **R. Painter:** None.

Poster Board Number:

SATURDAY-022

Publishing Title:

Comparative Microbial Analysis of an Endangered Desert Fish Habitat, Devils Hole, and a Manmade Replica

Author Block:

D. Huerta[1], J. Sackett[2], S. Hamilton-Brehm[2], B. Kruger[2], D. P. Moser[2]; [1]Univ. of Nevada Las Vegas, Las Vegas, NV, [2]Desert Res. Inst., Las Vegas, NV

Abstract Body:

Devils Hole (DH), NV, a water-filled tectonic cavern administered by Death Valley National Park, USA, is the only habitat for the critically endangered Devils Hole pupfish (*Cyprinodon diabolis*). In 2013, the Ash Meadows Fish Conservation Facility (AMFCF) was completed, with the aim of establishing a "backup" population of *C. diabolis*. This facility was painstakingly designed to duplicate the dimensions, microclimate, and water chemistry of DH; however, microbial biogeochemistry has not been compared. We assessed planktonic and sedimentary microbial and microalgal populations, as well as water chemistry, from paralleled locations in DH and the AMFCF to assess how successfully the replica duplicates the natural habitat.Planktonic cells were obtained by filtration (0.2 mm) and sediment was collected by push coring. A total of 21 samples were collected, DNA was extracted, and 16S rRNA gene libraries (V4) prepared on the Illumina platform. Statistical tests included alpha diversity and principal component analysis.While the major ion chemistries of DH and the AMFCF were nearly identical, microbial community compositions between sites were quite distinct. At the AMFCF, major planktonic microbial phyla included *Verrucomicrobia* (~41% of sequences) and *Proteobacteria* (~30%); while DH planktonic communities were dominated by *Planctomycetes* (~30%) and *Cyanobacteria* (~20%).Alpha diversity calculations indicate that DH planktonic communities were more diverse than the AMFCF; with OTU richness in DH being nearly twice that of the AMFCF. Principal component analysis of abundance-weighted UniFrac distances revealed environment-specific clustering of samples. Conversely, the sediment-associated microbial communities clustered together regardless of which location they were collected from (DH or the AMFCF).The findings from this study may be beneficial for the ongoing efforts to reestablish a reserve population of *C. diabolis*. The differences in microbial community composition between the two sites, despite extreme efforts to create identical habitats, could potentially indicate significant differences in the environmental nutrient cycling and primary productivity between the sites.

Author Disclosure Block:

D. Huerta: None. **J. Sackett:** None. **S. Hamilton-Brehm:** None. **B. Kruger:** None. **D.P. Moser:** None.

Poster Board Number:

SATURDAY-023

Publishing Title:

Patterns of Mutation in Acid-Evolved Strains of *Escherichia coli*

Author Block:

S. Penix, A. S. Gonzales, A. He, K. Creamer, D. Camperchioli, M. W. Clark, P. Basting, J. L. Slonczewski; Kenyon Coll., Gambier, OH

Abstract Body:

In order to colonize the human gut, the bacterium *Escherichia coli* contends with the acidic environment of the stomach. We previously conducted a 2000-generation laboratory evolution experiment of *E. coli* under moderately acidic conditions (buffered at pH 4.6-4.8), which is at the low end of its growth range. Eight isolates, chosen based on increased growth rates compared to the ancestor, were sequenced and analyzed using the breseq computational pipeline. Each isolate acquired several selected mutations, including strains that had insertions in the *cadC* region. Each sequenced isolate also had one mutation in a subunit of the RNA polymerase holoenzyme (*rpoB* or *rpoC*). Replacement of each of these mutant alleles with the ancestral sequence resulted in minimal fitness decreases in acid. The majority of the acid-evolved isolates showed loss of function of lysine and arginine decarboxylases, which was surprising given that these genes are preferentially expressed under acidic conditions. We hypothesize that the buffered system would make it energetically unfavorable to upregulate decarboxylases, because the reaction would fail to neutralize the buffered acid. Similarly, some of the strains showed a loss of GABA production, suggesting that glutamate decarboxylase, an extreme-acid survival system, was selected against during experimental evolution in acid. Consistent with this observation, the isolates in which GABA is not produced were unable to survive in extreme acid (pH 2) under conditions in which the ancestral strain does survive. In contrast, the isolates retaining GABA production have moderate extreme acid survival levels compared to the ancestral strain. We find evidence of varied phenotypes among the acid-evolved strains, suggesting alternative pathways to adaptive success. However, convergence in RNAP mutations and loss of acid-stress mechanisms suggests the potential predictability of evolutionary outcomes under acidic conditions.

Author Disclosure Block:

S. Penix: None. **A.S. Gonzales:** None. **A. He:** None. **K. Creamer:** None. **D. Camperchioli:** None. **M.W. Clark:** None. **P. Basting:** None. **J.L. Slonczewski:** None.

Poster Board Number:

SATURDAY-024

Publishing Title:

Human Immunodeficiency Virus Evolvability is Affected by Synonymous Nucleotide Recoding

Author Block:

M. A. Martinez, A. Jordan-Paiz, S. Franco, M. Nevot; IrsiCaixa, Badalona, Spain

Abstract Body:

Background: Human immunodeficiency virus type 1 (HIV-1) populations, like other RNA viruses, are described as a closely related mutant spectra or mutant clouds termed viral quasispecies. Mutant cloud composition can impact virus evolvability, fitness and virulence. The influence of codon choice in population diversity and evolvability of RNA viruses remains poorly explored.**Methods:** We compared the development of HIV-1 resistance to protease inhibitors (PIs) of wild-type (WT) virus and a synthetic virus (MAX) carrying a codon-pair re-engineered protease sequence with 38 (13%) synonymous mutations. WT and MAX viruses replicated indistinguishably in MT-4 cells or PBMCs. To explore the evolvability of the codon pair re-coded protease, WT and MAX viruses were subjected to serial passages with the selective pressure of PIs [atazanavir (ATV) and darunavir (DRV)].**Results:** After the same number of successive passages in MT-4 cells in the presence of PIs, WT and MAX viruses developed phenotypic resistance to PIs (IC50 14.63±5.39 nM and 21.26±8.67 nM, for ATV; and IC50 5.69±1.01µM and 9.35±1.89 for DRV, respectively). Sequence clonal analysis showed the presence, in both viruses, of previously described resistance mutations to ATV and DRV. However, a different resistance variant repertoire appeared in the MAX virus protease when compared to WT. The G16E substitution was only observed in the WT protease while the L10F, L33F, K45I, G48L and L89I substitutions were only detected in the re-coded MAX protease population. The influence of the G48L mutation, which is extremely rare *in vivo*, on viral fitness was explored and the results obtained will be discussed.**Conclusions:** The differences in the mutation pattern that emerged after PIs treatment suggested that WT and MAX virus proteases occupy different sequence spaces. A particular sequence space can delineates the evolution of its mutant spectra.

Author Disclosure Block:

M.A. Martinez: None. **A. Jordan-Paiz:** None. **S. Franco:** None. **M. Nevot:** None.

Poster Board Number:

SATURDAY-025

Publishing Title:

Environmental Stress Selects Functional Quorum Sensing System in *Pseudomon asaeruginosa*

Author Block:

R. García Contreras[1], P. Castañeda Tamez[1], M. Saucedo-Mora[1], T. Maeda[2], T. K. Wood[3]; [1]Natl. Autonomous Univ. of Mexico, Mexico City, Mexico, [2]Kyushu Inst. of Technology, Kitakyushu, Japan, [3]Pennsylvania State Univ., State College, PA

Abstract Body:

Background: Quorum sensing (QS) is widespread in bacteria, including *Pseudomonas aeruginosa*, responsible for 10% of nosocomial infections. QS allows the coordination of gene expression for the production of virulence factors like exoproteases, phenazines, and siderophores that are secreted to the environment, therefore these compounds are public goods available for all the individuals of the population. Those individuals that utilize public goods but do not contribute in their production are social cheaters , and they commonly appear in human infections in the form of *lasR* mutants that are unable to sense the main QS autoinducer signal, N-(3-oxo-dodecanoyl) homoserine lactone. Remarkably, the overexploitation of the cooperative individuals by social cheaters has the potential to create a “tragedy of the commons” therefore collapsing growth of the whole population; hence, it is interesting to determine how QS systems are maintained in bacterial populations. In this regard, we previously demonstrated that since QS defective mutants are more susceptible to abiotic stress (oxidative stress by H_2O_2) than wild-type individuals; such common stress provides a means of counter selection against the cheater mutants (3).**Methods:** Here, we explore the role of biotic stress in the form of reactive oxygen species promoting phenazines, competition against other bacterial species and temperate bacteriophages as means of selection for functional QS systems of *P. aeruginosa*. Using competition experiments between mixtures of the wild-type strain and a *lasR rhlR* mutant growing on protein as the sole carbon source as well as phenotypic determination (exoprotease, phenazine, autoinducer production, etc.), **Results:** we were able to show that pyocyanin production selects the wild-type strain and that some bacteriophages that preferentially infect the *lasR rhlR* mutant also select the wild-type, QS-proficient strain. **Conclusions:** Therefore, we speculate these factors maintain the presence of QS systems in bacterial populations in their natural environments, which could be relevant for bacterial ecology as well for the implementation of QS disrupting therapies that are proposed as an alternative to combat bacterial infections .

Author Disclosure Block:

R. García Contreras: None. **P. Castañeda Tamez:** None. **M. Saucedo-Mora:** None. **T. Maeda:** None. **T.K. Wood:** None.

Poster Board Number:

SATURDAY-026

Publishing Title:

The Selective Advantage of Synonymous Codon Usage Bias in *Salmonella*

Author Block:

G. Brandis, **D. Hughes**; Uppsala Univ., Uppsala, Sweden

Abstract Body:

Background: The genetic code in mRNA is redundant, with 61 sense codons translated into 20 different amino acids. Individual amino acids are encoded by up to six different codons but within codon families some are used more frequently than others. This phenomenon is referred to as synonymous codon usage bias. The genomes of free-living unicellular organisms such as bacteria have an extreme codon usage bias and the degree of bias differs between genes within the same genome. The strong positive correlation between codon usage bias and gene expression levels in many microorganisms is attributed to selection for translational efficiency. However, this putative selective advantage has never been measured and theoretical estimates vary widely.**Methods & Results:** By systematically exchanging optimal codons for synonymous codons in highly expressed genes we quantified the selective advantage of biased codon usage to range over an order of magnitude, from 0.2 - 4.2 x 10^{-4} per codon per generation.**Conclusions:** These data quantify for the first time the potential for selection on synonymous codon choice to drive genome-wide sequence evolution, and in particular to optimize the sequences of highly expressed genes. This quantification may have predictive applications in the design of synthetic genes and for heterologous gene expression in biotechnology.

Author Disclosure Block:

G. Brandis: None. **D. Hughes:** None.

Poster Board Number:

SATURDAY-027

Publishing Title:

Calculating Evolvability of Sialidase and Vlha from Avian Mycoplasmas in a Host Habitat Setting

Author Block:

M. A. MAY[1], N. Mullen[1], K. Pflaum[2], n. Ferguson-Noel[3], E. Tulman[2], D. Brown[4], S. Geary[2]; [1]Univ. of New England, Biddeford, ME, [2]Univ. of Connecticut, Storrs, CT, [3]Univ. of Georgia, Athens, GA, [4]Univ. of Florida, Gainesville, FL

Abstract Body:

The evolvability of a trait is its capacity to permanently change, most notably in response to diversifying natural selection. Naturally occurring evolvability as calculated by comparing selection values between homologous genes whose traits are exposed to different selective pressures. The avian pathogens *Mycoplasma synoviae* and *Mycoplasma gallisepticum* are often found in co-infections, and their genomes show extensive evidence of horizontal gene transfer. Two horizontally transferred virulence-associated traits, sialidase activity and members of the variable adhesin family VlhA, have previously been shown to be under diversifying selection in *M. synoviae* and stabilizing selection *M. gallisepticum* across diverse strains in *in vitro* culture. To test the hypothesis that these traits are significantly evolvable in *M. synoviae* and not *M. gallisepticum*, we examined the phenotypic, genotypic, and evolutionary adaptations in single strains of each species following passage through their host habitat. Isolates from a single point-source introduction of *M. synoviae* into a poultry flock were collected over a period of eighteen months, and isolates showed significant ($P<0.01$) variation in sialidase activity and the sialidase and expressed *vlhA* gene were under significant ($P<0.001$) diversifying selection. In contrast, the siaidase gene of *M. gallisepticum* retained 100% identity and its expression level remained unchanged during experimental infection of white leghorn chickens. Changes in the expressed *vlhA* allele occurred during infection; however, *post vivo* analysis demonstrated immediate reversion to an allele that was under strong stabilizing selection. Selection (ω) values from *M. synoviae* and *M. gallisepticum* were used to calculate Evolvability (E) values for each trait. Similar to *in vitro* analyses with diverse strains, sialidase and VlhA were demonstrated to be significantly ($P<0.05$) evolvable traits when expressed by *M. synoviae* following *in vivo* passage. To our knowledge, this is the first mathematical and functional demonstration of evolvability of bacteria occurring in a natural (non-experimental) habi

Author Disclosure Block:

M.A. May: None. **N. Mullen:** None. **K. Pflaum:** None. **N. Ferguson-Noel:** None. **E. Tulman:** None. **D. Brown:** None. **S. Geary:** None.

Poster Board Number:

SATURDAY-028

Publishing Title:

Rna Recombination and Mutation Enhance Adaptability During Acute Viral Infection

Author Block:

S. Bianco[1], Y. Xiao[2], I. Rouzine[2], R. Andino[2]; [1]IBM Almaden Res. Ctr., San Jose, CA, [2]Univ. of California San Francisco, San Francisco, CA

Abstract Body:

Background: While a high mutation rate fuels evolution, it also generates deleterious mutations. Recombination may resolve this paradox, alleviating the effect of clonal interference and purging deleterious mutations. **Methods:** We identify a poliovirus recombination determinant and generatea panel of variants with distinct mutation rates and recombination ability.**Results:** We find that recombination is essential to enrich the population in beneficial mutations and purge it from deleterious mutations. Strikingly, the concerted activities of mutation and recombination are key to virus spread and virulence in an infected animal. These experiments inform a mathematical model that shows that the optimal mutation rate in RNA viruses is determined by the trade-off between selection and the accumulation of detrimental mutations in the short evolutionary timeframe of acute infection.**Conclusions:** This work provides experimental proof and a conceptual frameworkto define how the interplay between mutation rates and recombination are tuned to promote evolution and adaptation.

Author Disclosure Block:

S. Bianco: None. **Y. Xiao:** None. **I. Rouzine:** None. **R. Andino:** None.

Poster Board Number:

SATURDAY-029

Publishing Title:

Symbiosis Through Warfare: Contact-Dependent Dynamics in the Human Gut Microbiome

Author Block:

A. G. Wexler[1], Y. Bao[1], J. Whitney[2], J. Xavier[3], W. Schofield[1], N. Barry[1], A. Russell[4], J. Mougous[5], A. Goodman[1]; [1]Yale Univ., New Haven, CT, [2]Univ. of Washington, Seattle, WA, [3]Mem. Sloan Kettering Cancer Ctr., New York, NY, [4]Fred Hutchinson Cancer Res. Ctr., Seattle, WA, [5]Howard Hughes Med. Inst., Univ. of Washington, Seattle, WA

Abstract Body:

The human gastrointestinal tract is home to a large, complex and diverse community of microorganisms that can confer numerous benefits to its host. Despite high microbial cell densities in the gut ($>10^{12}$ bacteria per gram), the factors known to shape our microbiomes—vitamins, dietary polysaccharides, metabolites, bacteriocins, host IgA—are diffusible and do not depend on cell-to-cell contact. Human-associated Bacteroidetes, one of two major phyla in the gut, encode functional type VI secretion systems (T6SSs). These macromolecular machines enable donor bacteria to inject antibacterial toxins called effectors into neighbouring recipient bacteria in a contact-dependent manner. However, the role of this weaponry in shaping gut microbial dynamics is unknown. Here we report that prominent human symbionts secure their membership within the microbiome through continuous attack on their immediate neighbours. We use bacterial genetics, gnotobiotic mouse studies and mathematical modeling to measure effector transmission rates exceeding one billion events per minute per gram of gut contents in live animals, and uncover strain-specific effector/immunity repertoires that predict inter-strain dynamics in vitro and in vivo. Moreover, we find that neighbouring species in the gut can partially protect sensitive strains from contact-dependent killing, thereby providing a mechanism for strain coexistence in the face of inter-bacterial warfare. Together, these results define a significant role for contact-mediated antagonism between human gut symbionts and may provide new strategies for precision microbiome manipulation.

Author Disclosure Block:

A.G. Wexler: None. **Y. Bao:** None. **J. Whitney:** None. **J. Xavier:** None. **W. Schofield:** None. **N. Barry:** None. **A. Russell:** None. **J. Mougous:** None. **A. Goodman:** None.

Poster Board Number:

SATURDAY-030

Publishing Title:

Relationships between Ammonia-Oxidizing and Nitrite-Oxidizing Bacteria at Low Substrate Concentrations in Freshwater Enrichments

Author Block:

M. Cai, P. K. H. Lee; City Univ. of Hong Kong, Hong Kong, Hong Kong

Abstract Body:

Nitrification is a vital oxidation process that links the reduced and oxidized inorganic nitrogen to sustain the global nitrogen cycle. Studies have shown that ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) are fastidious and slow growers, and their interactions are sensitive to sudden environmental changes. Previous research investigating the correlations between AOB and NOB have either conducted the experiments at a high ammonium concentration (2.8 to 60 mM) or using isolates of nitrifiers. In this study, we investigated the ecophysiological characteristics and the relationships between freshwater AOB and NOB by enriching AOB cultures with and without NOB at a relatively low ammonium concentration (0.05 to 1.5 mM).The cultures were enriched from freshwater aquarium biofilters over 1.5 years with ammonium as the sole energy source. Community compositions of the enrichments were determined by 16S rRNA gene amplicon sequencing. A positive correlation was found between nitrification kinetics and the ammonium/nitrite and bicarbonate concentrations. The maximum growth rates (μ_{max}) and half-saturation constant (K_m) indicated that the activity of *Nitrobacter*- and *Nitrospira*-like NOB depended strongly on the presence of the *Nitrosomonas*-like AOB and vice versus. Furthermore, the presence of NOB substantially shortened the lag phase of ammonium consumption, indicating that AOB can adapt to environmental changes more efficiently. Enrichments that had the highest growth rates had a lower abundance of targeted genes (*amoA, norA,* and *Nitrospira* 16S rRNA genes) in the quantitative PCR analysis. The lack of correlation between the abundance of nitrifying bacteria and the activity of nitrification indicates the importance of mutualism between the AOB and NOB guilds in determining the activity of the nitrification process. The metagenomes of the enrichments have been sequenced to further elucidate the metabolism of the nitrifying populations. Overall, this study has provided insights into the relationships between the ammonia-oxidizing and nitrite-oxidizing guilds at low substrate concentrations in the freshwater nitrification process.

Author Disclosure Block:

M. Cai: None. **P.K.H. Lee:** None.

Poster Board Number:

SATURDAY-031

Publishing Title:

Multiple Bacteriocin-Producing *Escherichia coli* Isolated from Coastal Marine Sediment in Hong Kong

Author Block:

L. Teh[1], S. C. K. Lau[2]; [1]The Hong Kong Univ. of Sci. and Technology, Kpwloon, Hong Kong, [2]The Hong Kong Univ. of Sci. and Technology, Kowloon, Hong Kong

Abstract Body:

Escherichia coli can produce bacteriocin, which is a kind of toxin protein that is only active against other closely related bacteria. Bacteriocin production is an anti-competitor strategy of *E. coli* for eliminating neighbouring cells that are competing for nutrients and space. *E. coli* may carry multiple bacteriocin genes to expand the killing range of target bacteria and enhance its competitiveness. The coastal marine sediment environment has been reported as a secondary habitat for *E. coli* and bacterial competition between *E. coli* and indigenous microorganisms is the major biotic factor affecting the survival of *E. coli* in the marine sediments. Bacteriocin has been suggested as a tool for *E. coli* to invade an indigenous bacterial community and establish their own niches. However, the occurrence of multiple bacteriocin-producing *E. coli* in this environment has not been studied. This study aimed to investigate the occurrence of multiple bacteriocin-producing *E. coli* in marine sediment and determine the dominant bacteriocin genes carried by these isolates. Both phenotypic and molecular approaches were utilized to investigate the occurrence of multiple bacteriocin-producing *E. coli* from coastal marine sediment. A collection of 48 isolates of *E. coli* originating from marine sediment in Hong Kong was initially screened for bacteriocin producing phenotype using agar overlay method. Twenty-six isolates were observed to be bacteriocin producers. PCR detection of bacteriocin producing genes followed by DNA sequencing of the PCR amplicons indicated that majority (n=22) of the 26 bacteriocin-producing isolates carry multiple bacteriocin genes. Colicin Ib (n=20) and colicin E7 (n=13) were found to be the most common bacteriocin genes. This is the first study to report that multiple bacteriocin-producing *E. coli* are ubiquitous in the marine sediment and that such environmental matrix may serve as a reservoir for these *E. coli*. Based on the results, we hypothesize that the ability to produce multiple bacteriocin might be beneficial for the survival of *E. coli* in marine sediment. Further studies will be conducted to investigate the relationship between the number of bacteriocin genes carried by *E. coli* and its survivability in the coastal marine sediments.

Author Disclosure Block:

L. Teh: None. **S.C.K. Lau:** None.

Poster Board Number:

SATURDAY-032

Publishing Title:

Evolutionary Enhancement of Social Motility: Insights into Interactions among Soil Bacteria

Author Block:

L. M. McCully, L. M. Smith, M. W. Silby; Univ. of Massachusetts Dartmouth, North Dartmouth, MA

Abstract Body:

The soil bacterium *Pseudomonas fluorescens* Pf0-1 is a model for soil survival, persistence, and biocontrol. When co-cultured with the soil bacterium *Pedobacter* sp. V48, a novel "social motility" phenotype emerges, allowing the co-culture to move on a hard agar surface, an environment where the mono-cultures of both species are immotile. Using experimental evolution, we sought to unveil mechanisms underlying social motility in this model community. Motile co-cultures on 2% agar frequently spawn faster-moving sectors, in which one species has evolved to facilitate faster migration. From over 100 sectors emerged from wild-type interactions, 87% of isolates conferring fast social motility were evolved *Pedobacter*. Evolved Pf0-1 have mostly emerged as a second evolutionary step during co-culture with *Pedobacter* strains which already confer faster social motility. While interaction of wild-type Pf0-1 and evolved *Pedobacter* resulted in social motility two times faster than the wild-type co-culture, interaction of evolved strains of both species resulted in a five-fold increase in social motility. Whole-genome sequencing has shown that most emerged *Pedobacter* strains have mutations in a region of genes related to polysaccharide biosynthesis; repeated selection of such mutants identifies this locus as important for enhancing social motility. Many of these strains have a mucoid phenotype, indicating probable overproduction of extracellular polysaccharides, and implicating EPS as a key driver of social motility. Transcriptomic analysis on mono- and co-cultures of evolved and wild-type strains show a broad range of changes in gene expression in both species. This analysis is beginning to reveal pathways that are altered as a consequence of evolutionary selection. In *Pedobacter*, many genes were upregulated in the polysaccharide biosynthesis locus, reiterating its importance in social motility. In Pf0-1, we observed changes in core aspects of carbon metabolism, as well as changes in flagellar assembly. These studies are revealing novel ways by which these two bacteria communicate and behave, providing new insight into how members of complex soil communities interact. Our research may also lead to development of bacterial communities as biocontrol agents, and approaches to enhance the colonization of biotic surfaces in the rhizosphere.

Author Disclosure Block:

L.M. McCully: None. **L.M. Smith:** None. **M.W. Silby:** None.

Poster Board Number:

SATURDAY-033

Publishing Title:

Redefining 'Core' Microbiome by Evaluating the Functional Role Microorganisms Play in the Assembly and Structure of Their Microbial Community

Author Block:

C. E. Brumlow[1], J. M. Carlson[1], E. Hollister[2], E. Hyde[3], J. Petrosino[2], T. P. Primm[1]; [1]Sam Houston State Univ., Huntsville, TX, [2]Baylor Coll. of Med., Houston, TX, [3]Univ. of California at San Diego, La Jolla, CA

Abstract Body:

Background: Our model vertebrate system for studying the recovery of a mucosal microbiome following a disruption event uses the Western mosquitofish, *Gambusia affinis*. The skin microbiome of *G. affinis*, unlike terrestrial animals, is mucosal and also directly accessible through the water column. This allows for non-invasive disruptions to the external microbiome and rapid sampling. **Methods:** In order to understand what drives microbiome community structure, recovery following two different disruptions was analyzed. A severe point-disruption involved rinsing fish until skin-derived bacterial culturable numbers were greatly reduced. At various time-points after the serial rinse, the skin microbiome of *G. affinis* was sampled and compared to an undisturbed, pre-treatment (PT) control sample. In a separate experiment, a group of fish were exposed to the antibiotic rifampicin in the water column for 3 days and then monitored during recovery. **Results:** After both disruptions culturable numbers drop >99.5% but rapidly recover to exceed the PT density before settling to a level comparable to PT. A profile of 20 community biochemical activities were altered by the disruptions, but returned to patterns similar to PT. In contrast, 16S profile revealed community composition did not return to PT taxa distributions as measured by principal components analysis. During succession of the microbial community after the disruptive events, a single taxa (genus *Myroides* after drug treatment, family *Aeromonadaceae* after physical rinse) dominates early (>80% abundance at 48 hrs), then consecutively declines. **Conclusion:** This data preliminarily supports the hypothesis that the functional roles of organisms within the microbial community may be more important than the taxonomic structure, which agrees with the major finding from the Human Microbiome Project that while community compositions from one body location vary widely between individuals, the biochemical pathways in those communities are remarkably similar.

Author Disclosure Block:

C.E. Brumlow: None. **J.M. Carlson:** None. **E. Hollister:** None. **E. Hyde:** None. **J. Petrosino:** None. **T.P. Primm:** None.

Poster Board Number:

SATURDAY-034

Publishing Title:

Impact of Road Salt Runoff on Soil Microbial Communities

Author Block:

K. Baker[1], J. Felker[2], A. Bowers[1], Z. Wummer[1]; [1]Penn State Harrisburg, Middletown, PA, [2]Penn State Berks, Reading, PA

Abstract Body:

Background: Roads are the dominant engineered feature in the US landscape. In most urban and suburban areas, it is unlikely that a individual at any location would be greater than 1 mile from a road surface. And yet, because of their ubiquity and utilitarian purpose, roads are inadequately addressed as sources of runoff impacting the surrounding environment. Many roads are heavily "salted" with deicing substances during the winter months and much of the salt is carried to surrounding areas via runoff and infiltration. Recently, transportation engineers have expressed concern regarding the impact of salt (NaCl) on soils and groundwater. We have investigated the impact of road salt on indigenous soil populations in roadside soils and on the persistence and transport of *E. coli* within these soils. **Methods:** Microcosms were constructed using an engineered sandy-loam soil. The microcosms were watered with artificial storm water (ASW) or with ASW supplemented (ASWS) with road salt. Several of the microcosms watered with ASWS were spiked initially with *E. coli* (10^4 CFU). The applied solutions drained through the microcosms and the resulting leachate was aseptically collected and analyzed for total heterotrophs (viable counts), *E. coli* (MPN), inorganic nutrients (N, P, salinity and trace metals) and hydraulic characteristics (flow).**Results and Conclusions:** Heterotrophic populations remained stable throughout the entire study with no significant differences between systems receiving ASW or ASWS. Although there was no change in the number of microorganisms in the leachate, there were significant changes in the hydraulic characteristics of the soil. Flow was significantly slower in microcosms watered with ASWS possibly reflecting either the development of microbial biofilm, changes in soil structure, or a combination of both of these factors.The presence of added *E. coli* had no effect on the numbers of total heterotrophs in the leachate. *E. coli* persisted in the spiked microcosms watered with ASW throughout the one month study, but declined to non-detectable levels within 2 – 3 weeks in microcosms receiving ASWS.

Author Disclosure Block:

K. Baker: None. **J. Felker:** None. **A. Bowers:** None. **Z. Wummer:** None.

Poster Board Number:

SATURDAY-035

Publishing Title:

Factors Affecting Growth of Difficult-to-Culture Oral Bacteria

Author Block:

S. R. Vartoukian[1], F. E. Dewhirst[2], W. G. Wade[1]; [1]Barts and The London Sch. of Med. and Dentistry, Queen Mary Univ. of London, London, United Kingdom, [2]The Forsyth Inst., Cambridge, MA

Abstract Body:

Background: Many oral bacterial species grow poorly on conventional bacteriological media. It has been suggested that growth may be inhibited by hydrogen peroxide in culture media when agar and phosphate are autoclaved together. In addition it is known that acid produced by some bacteria from glucose in culture media may inhibit the growth of other bacteria. The aim of this study was to assess the growth of difficult-to-culture oral bacteria on culture media with and without added glucose and to investigate the substitution of agar in media by gellan gum.**Methods:** *Tannerella forsythia* SP9_20 was cultured on Blood Agar Base No. 2 (BA) and Fastidious Anaerobe Agar (FAA), both supplemented with 5 % horse blood with and without cross-streaks of *Propionibacterium acnes*, and a paper disk soaked with glacial acetic acid for 7 d anaerobically. *T. forsythia* SP9_20, *Anaerolineae* bacterium HOT-439 SP9_5, and *Bacteroidetes* bacterium HOT-365 SP18_29_14 and SP18_29_15 were grown on a BHI-peptone based medium incorporating either 1.0% gellan gum (G) or 1.5% agar (A), and on BA, with *P. acnes* and *Fusobacterium nucleatum* cross-streaks. Growth was graded from - to +++ after 10 and 25 days incubation.**Results:** Independent growth of *T. forsythia* was stronger on FAA (++) than BA (+). In co-culture with *P. acnes* on FAA, a 9-mm zone of inhibition and area of haemolysis was seen around the *P. acnes* streak. A similar zone of inhibition was seen around the acetic acid disk.*T. forsythia* SP9_20, *Anaerolineae* HOT-439 SP9_5, and *Bacteroidetes* HOT-365 SP18_29_14 and SP18_29_15 showed weak growth (+) on BA and no growth (-) on G or A. When cultured with *P. acnes* or *F. nucleatum*, *T. forsythia* SP9_20, *Bacteroidetes* HOT-365 SP18_29_14 & *Anaerolineae* HOT-439 SP9_5 showed stronger growth on G (++, ++ & + respectively) than A (+, - & -), but weaker growth compared to BA (+++).**Conclusions:** Growth of *T. forsythia* is inhibited by acid produced by other bacteria in glucose-containing media. Replacing agar with gellan gum moderately enhances the growth of difficult-to-culture oral bacteria but dependence on helper strains remains a primary growth determinant.

Author Disclosure Block:

S.R. Vartoukian: None. **F.E. Dewhirst:** None. **W.G. Wade:** None.

Poster Board Number:

SATURDAY-036

Publishing Title:

Interspecific Interactions among Meat Spoilage-Related Lactic Acid Bacteria: Insights from the Transcriptome Profiling

Author Block:

M. Andreevskaya, P. Johansson, E. Jääskeläinen, R. Rahkila, T. Nieminen, L. Paulin, J. Björkroth, P. Auvinen; Univ. of Helsinki, Helsinki, Finland

Abstract Body:

Spoilage of modified-atmosphere packaged (MAP) meat is caused by the activity of several bacterial species, mainly lactic acid bacteria (LAB), which form a microbial community. Taking into account the differences in growth rates of spoilage LAB as pure cultures, it is not clear how the LAB with slower growth rates can maintain themselves in actively growing co-cultures in meat. We assume that these LAB, being a part of a common microbial community, might have different metabolism from that in pure culture, which would lead to enhanced growth in co-cultures. However, since most of the studies on bacteria have been done so far in pure culture, little is known about bacterial interspecific interactions in general. In the present study we explore the interactions among three LAB: *Leuconostoc gelidum* subsp. *gasicomitatum* (*Lg*), *Lactococcus piscium* (*Lp*) (both are predominant species in spoiled MAP meat) and *Lactobacillus oligofermentans* (spoilage-associated) using the comparison of RNA-seq based transcriptome profiles of their co-cultures (three pairs and the triplet) and pure cultures at three time points. Preliminary data analysis for the mixed culture of *Lg* and *Lp* revealed that for both species differentially expressed (DE) genes were distributed across multiple functional categories with "carbohydrate and amino acid metabolism" and "transcription" being most affected. Numbers of DE genes determined at the three time points indicate that in co-culture *Lg* adjusted its metabolism earlier than *Lp*. In *Lg*, the growth in mixed culture led to early downregulation of stress protection genes and dramatic shift in glucosamine metabolism from cell wall formation to energetic catabolism. As for *Lp*, the upregulation of several stress-related genes and downregulation of sugar transport were observed. Peculiarly, in both bacteria malolactic enzyme genes exhibited similar expression changes: at first they were upregulated in co-culture and later on downregulated. The metabolic role of malolactic fermentation is still unclear in these LAB genera. To conclude, the findings suggest that coexistence of only two bacterial species already requires global rearrangements in their gene expression. Currently, the transcriptome profiles of other mixed cultures are being analysed.

Author Disclosure Block:

M. Andreevskaya: None. **P. Johansson:** None. **E. Jääskeläinen:** None. **R. Rahkila:** None. **T. Nieminen:** None. **L. Paulin:** None. **J. Björkroth:** None. **P. Auvinen:** None.

Poster Board Number:

SATURDAY-037

Publishing Title:

Cheating Fosters Species Co-Existince in Well-Mixed Bacterial Communities

Author Block:

A. Leinweber[1], F. Inglis[2], R. Kümmerli[1]; [1]Univ. of Zurich, Zurich, Switzerland, [2]Washington Univ., St. Louis, MO

Abstract Body:

Most habitats on earth are populated by diverse bacterial communities. Explaining this high biodiversity is, however, challenging because competition between species occupying the same niche is predicted to lead to the exclusion of inferior community members. Spatial structure has been suggested to stabilize diversity by separating competitors from each other. Alternatively, it has been proposed that the strength of dominant competitors could be weakened through within-species competition, which in turn can relax between-species competition and foster species co-existence. Here, we test these two hypotheses using experimental communities of *Pseudomonas aeruginosa* and *Burkholderia cenocepacia*, two bacterial species naturally co-occurring in soil and human opportunistic infections. First, we manipulated the spatial structure of our communities by growing the bacteria in medium with varying viscosities. Using a mixture of competition assays and experimental evolution, we found that spatial structure did not promote species co-existence: *P. aeruginosa* always eradicated *B. cenocepacia*. Next, we introduced within-species competition by adding a P. *aeruginosa* cheating mutant to co-cultures, which can exploit the iron scavenging siderophores secreted by the wildtype PA under the iron-limited conditions. We found that this form of within-species competition indeed weakened the competitive strength of *P. aeruginosa* towards *B. cenocepacia*, and fostered species co-existence under well-mixed conditions.Taken together, our experiments identify within-species cheating as a novel mechanism, which can promote bacterial biodiversity in habitats with low spatial structuring as for example occurring in marine ecosystems.

Author Disclosure Block:

A. Leinweber: None. **F. Inglis:** None. **R. Kümmerli:** None.

Poster Board Number:

SATURDAY-038

Publishing Title:

Identifying Factors Involved in Interspecies Social Motility

Author Block:

L. M. Smith[1], S. C. Seaton[2], L. M. McCully[1], S. B. Levy[3], M. W. Silby[1]; [1]Univ. of Massachusetts Dartmouth, North Dartmouth, MA, [2]Univ. of North Carolina Asheville, Asheville, NC, [3]Tufts Univ. Sch. of Med., Boston, MA

Abstract Body:

Novel phenotypes emerge upon laboratory co-culturing of the soil bacteria *Pseudomonas fluorescens* Pf0-1 and *Pedobacter* sp. V48, namely 'social motility', the elicitation of movement when strains are in close contact. Identifying genes and pathways involved is key to unraveling mechanisms of this interspecies interaction. Pf0-1 mutants with altered social motility were identified through a transposon screen, highlighting genes related to a secretion system, flagellar assembly, and pili production. Hits in components of a Type 6 Secretion System (T6SS) led to impaired social motility, suggesting that this contact-dependent system, which relies on passing effectors from donor to recipient cell, plays a role in the interaction of these bacteria. Disruption of Pf0-1 flagellar and pilin genes suggests that these structures may not be required for social motility. We sought to decipher the role of T6S, flagella, and pilin in social motility. Strains were engineered with deletions of genes encoding putative VgrG proteins, secreted components of the T6SS. Some exhibit small changes in social motility, indicating that they may be exerting an effect on the interaction. Changes from different effector mutations and the presence of several predicted VgrG may indicate redundancy, possibly requiring multiple deletions to exhibit strong changes in phenotype, or the association of additional effectors to elicit a stronger response. To address the role of flagella in social motility, *fliC* was targeted, as it encodes the main structural component of flagella. Disruption of *fliC* abolishes social motility. Together with our transposon data, this suggests that while swimming motility is not required for social motility, the presence of flagella or flagellin in Pf0-1 is required. Likewise, deletion of *pilM* renders Pf0-1 defective for social motility, indicating that the movement is distinct from Type IV pili-mediated twitching motility. Together, these findings refine important factors involved in this interspecies interaction. Understanding social motility may facilitate the increased effectiveness of colonization of potential biological control agents on plant tissues, as well as providing insight into the mechanisms of interspecies interactions in microbial communities.

Author Disclosure Block:

L.M. Smith: None. **S.C. Seaton:** None. **L.M. McCully:** None. **S.B. Levy:** None. **M.W. Silby:** None.

Poster Board Number:

SATURDAY-039

Publishing Title:

Antibiotic Resistance Ecology in a Mutualistic Bacterial Community

Author Block:

B. Adamowicz, W. Harcombe; Univ. of Minnesota, Minneapolis, MN

Abstract Body:

Background: Although multispecies bacterial communities are common, our understanding of how species interactions influence microbes' survival and adaptation to stressful environments is limited[1]. Bacteria often obtain essential metabolites from the excretions of other species; in multispecies growth conditions, this dependency should modulate effective sensitivity of cooperating species to stressors such as antibiotics[2]. Specifically, we predict that, in obligate metabolic mutualisms, the effective minimum inhibitory concentration (MIC, the concentration at which no growth is observed) of each species will be lowered to that of the most sensitive species.**Methods**: We have genetically engineered an obligate metabolic mutualism between strains of *Escherichia coli*, *Salmonella enterica*, and *Methylobacterium extorquens* such that, when grown in minimal medium, each species requires a metabolite excreted by a partner to survive[3]. We then determined the MIC for monocultures by OD600 and species-specific fluorescent markers in shaken cultures, and compared this to the MIC observed for the community.**Results:** In tetracycline, community MIC was driven by the most sensitive community member as predicted. *S. enterica* was the most resistant in monoculture growth, with an MIC of >25 µg/mL; this decreased in community growth to 2µg/mL, which matched the monoculture MICs of *E. coli* and *M. extorquens*. In ampicillin, however, the MIC of the most sensitive community member, *S. enterica*, increased from 2µg/mL in monoculture to 5µg/mL in community. This suggests that, in contrast to our hypothesis, community growth protected the most sensitive member rather than being limited by it. However, as predicted, the MIC of *M. extorquens* was decreased in community growth (50µg/mL in monoculture to 5µg/mL in community) due to greater ampicillin sensitivity of its metabolic partners.**Conclusions:** Metabolic cross-feeding altered the effective MIC of bacteria to antibiotic stressors. Further work is needed to determine the mechanisms underlying these effects, particularly in growth in ampicillin, where community growth appears to be protective for sensitive species. Understanding how microbial species interactions influence response to stressors is critical for our understanding of eco-evolutionary dynamics in addition to having significant implications for the treatment of polymicrobial infections.

Author Disclosure Block:

B. Adamowicz: None. **W. Harcombe:** None.

Poster Board Number:

SATURDAY-040

Publishing Title:

The Effect of Increased Viscosity on Chemotaxis of *Bacillus* spp. Isolated from Songbird Plumage

Author Block:

M. Vroom, L. Tuhela; Ohio Wesleyan Univ., Delaware, OH

Abstract Body:

The plumage of birds is an ecosystem that harbors a diverse community of microbes including *Bacillus* spp., some of which are known to utilize feathers as the sole source of carbon, nitrogen, and sulfur by secreting keratinase enzymes that degrade β-keratin. *Bacillus* spp. are motile, chemotactic bacteria. Thus, amino acids from damaged parts of feathers could act as chemoattractants for plumage-dwelling *Bacillus* spp. Uropygial oil is used by birds as they preen and is thought to protect plumage from bacteria although not all uropygial oil has antibacterial properties. In this study, the inhibitory effect of increased viscosity on *Bacillus* chemotaxis was quantified to investigate the possibility that uropygial oil functions as a viscous physical barrier to inhibit the chemotaxis of feather-degrading *Bacillus* towards damaged areas on bird feathers, thereby preventing further deterioration. Motility assessments were conducted for over 60 *Bacillus* isolates obtained from songbird plumage via wet mount. Growth curves in coordination with hourly estimates of motility were subsequently performed. From these data, *Bacillus* 4201TV, which exhibited a maximum of 85% motility at 10 hours in the log phase, was chosen for chemotaxis assays. The chemotaxis of *Bacillus* 4201TV was quantified via capillary assays in modified Palleroni chambers. The amino acids of β-keratin tested as chemoattractants were proline, valine, and asparagine at concentrations of either 250 μM and 750 μM. *Bacillus* 4201TV exhibited a positive chemotactic response towards proline, valine, and asparagine at both concentrations with the greatest response ratios at 750 μM. To determine the effect of increased viscosity on chemotaxis, dual quantitative chemotaxis assays were performed using 750 μM proline or asparagine as chemoattractants, and an increase in viscosity was achieved by preparing the amino acids in 0.05% agar. Conditions of increased viscosity reduced the chemotactic response of *Bacillus* 4201TV toward 750 μM proline and asparagine by 41% ($p = 0.31$) and 97% ($p = 0.04$), respectively. The asparagine data suggest that the viscosity of uropygial oil may function as a physical barrier to inhibit the chemotactic movement of feather-degrading *Bacillus* spp. towards areas of feather damage, thus protecting bird plumage.

Author Disclosure Block:

M. Vroom: None. **L. Tuhela:** None.

Poster Board Number:

SATURDAY-041

Publishing Title:

Interactions between the Human Pathogen *Vibrio parahaemolyticus* and Marine Cyanobacteria

Author Block:

I. B. Gartmon, S. L. Klein, C. R. Lovell; Univ. of South Carolina, Columbia, SC

Abstract Body:

Vibrio parahaemolyticus is an opportunistic human pathogen that occurs naturally in brackish environments. This organism grows fastest in warm waters and with rising surface water temperatures, outbreaks of this organism are increasing in size and frequency. *V. parahaemolyticus* distributions are strongly correlated with blooms of marine phytoplankton, but specific interactions between abundant bloom forming marine cyanobacteria and *V. parahaemolyticus* have not been determined. The Type VI secretion system (T6SS), a *V. parahaemolyticus* virulence feature that permits injection of cytotoxic effector proteins into host cells, is thought to be activated in marine conditions. Studies to date have indicated that T6SS targets bacteria, potentially including cyanobacteria, and this study examined the frequency of the T6SS in environmental *V. parahaemolyticus* strains, using the T6SS marker gene *vipA1*. We also examined the nature of the relationship between *V. parahaemolyticus* and cyanobacteria. Thirty two environmental strains of *V. parahaemolyticus* were screened for *vipA1* and ten (31%) of these strains contained this gene. We infected cultures of two common species of cyanobacteria (*Procholorococcus marinus* and *Synechococcous bacillaris*) with different *V. parahaemolyticus* strains (some lacking T6SS, some with T6SS) and monitored cyanobacterial biomass via chlorophyll *a* fluorescence. We also tested the well known clinical *V. parahaemolyticus* strain por1 (T6SS +) as well as two deletion mutants, por1Δhcp1 (T6SS -) and por1Δhns (T6SS derepressed). *V. parahaemolyticus* stimulated the growth of both cyanobacteria species, regardless of its content of T6SS. *V. parahaemolyticus* is known to cause damage to eukaryotic microalgae, but we did not detect significant damage to the cyanobacteria despite the presence in *V. parahaemolyticus* of mechanisms that could produce damage. Stimulation of blooms of cyanobacteria could result in release of carbon and energy resources that might support elevated *V. parahaemolyticus* cell densities with a correspondingly greater threat to human health.

Author Disclosure Block:

I.B. Gartmon: None. **S.L. Klein:** None. **C.R. Lovell:** None.

Poster Board Number:

SATURDAY-042

Publishing Title:

Microbial Interactions Lead to Rapid Microscale Successions on Model Marine Particles

Author Block:

M. S. Datta[1], E. Sliwerska[2], J. Gore[1], M. F. Polz[1], O. X. Cordero[1]; [1]Massachusetts Inst. of Technology, Cambridge, MA, [2]ETH Zurich, Zurich, Switzerland

Abstract Body:

Background: Marine particles are nutrient rich microhabitats for dense microbial communities whose collective actions shape the fate of fixed carbon in the ocean. Although particular traits have been identified that allow marine bacteria to exploit particles (for instance, motility and exo-enzyme production), it remains unclear how community dynamics emerge from these traits, particularly in a complex microbial milieu like the ocean. We developed a model system that allowed us to track the dynamics of particle colonization with high spatiotemporal resolution and to explore the underlying drivers of these dynamics via culture-dependent and -independent approaches.**Materials:** We simulated POM with micro-particles made of chitin - a highly abundant biopolymer in the ocean - and incubated them in coastal seawater. Over nearly six days, bacteria from the surrounding seawater self-assembled into communities on the chitin particle microhabitats. At discrete time points, we harvested large pools of particles with which we performed 16S rRNA amplicon sequencing to reconstruct their average community assembly dynamics. Subsequently, we combined metagenomic sequencing with phenotypic assays to characterize the metabolic and behavioral traits underlying these dynamics.**Results:** Particle-associated bacterial communities underwent rapid, highly reproducible community turnover over six days. Although community turnover occurred continuously, we identified three discrete phases of colonization. Following initial stochastic colonization (Phase I), bacterial taxa enriched in chitin metabolism and rapid dispersal ability swept the communities (Phase II). In contrast, Phase III taxa could not metabolize chitin, but instead, consumed carbon sources produced in Phase II, thus marking a community-wide shift in metabolism away from chitin towards other nutrient sources.**Conclusion:** Our results demonstrate that marine bacterial communities assembled on model particles undergo rapid turnover, shifting from a community capable of degrading the particle substrate to one that cannot in a matter of hours. The timescale of this transition could influence the balance between organic matter consumption and biomass buildup, potentially a key factor shaping particle remineralization rates in the ocean.

Author Disclosure Block:

M.S. Datta: None. **E. Sliwerska:** None. **J. Gore:** None. **M.F. Polz:** None. **O.X. Cordero:** None.

Poster Board Number:

SATURDAY-043

Publishing Title:

Biotic Interactions Drive The Rapid Evolution of Penicillium On Cheese

Author Block:

E. A. Landis, I. Bodinaku, B. E. Wolfe; Tufts Univ., Medford, MA

Abstract Body:

Rapid microbial evolution is generally assessed in pure microbial cultures without the influence of neighboring microbes. But in naturally forming communities, microbes contend with competitors as well as fluctuations in resource availability, both of which could influence the rate and direction of evolution. In order to test the influence of biotic interactions, we serially transferred three wild strains of *Penicillium* onto nutrient-rich cheese curd agar both alone (- community) and with three neighboring microbes (+ community). We selected three mutant classes which differed from their ancestor strain in regards to color and spore production, and measured production of the mycotoxin cyclopiazonic acid. We then sought to identify genetic changes underlying differences in phenotype by using comparative genomic sequencing of relevant genes for one ancestral strain and three of its mutants. The effect of adding neighbors in the + community treatment was strain-dependent, with neighbors decreasing the frequency of mutants in two of the three strains we measured. In the three mutant classes we selected, spore production was significantly decreased in all mutants compared to the ancestor. In some mutants, production of cyclopiazonic acid was reduced to below detectable levels (<100 ppm). We were unable to identify any genetic changes underlying these differences, suggesting that phenotypic changes in *Penicillium* mutants may be due to changes in regulatory pathways. Understanding the nature of rapid microbial evolution in novel environments could illuminate the origins of microbial foods and inform how pathogenic microbes adapt to food systems.

Author Disclosure Block:

E.A. Landis: None. **I. Bodinaku:** None. **B.E. Wolfe:** None.

Poster Board Number:

SATURDAY-044

Publishing Title:

Diverse Germination Interactions Among Streptomyctetes

Author Block:

K. Vetsigian, **Y. Xu**; Univ. of Wisconsin-Madison, Madison, WI

Abstract Body:

Bacteria live in crowded and diverse communities and exhibit a wide range of social behaviors mediated by diffusing chemicals. While many types of interactions have been exhaustively studied, little is known about how the germination decisions of individual spores are affected by the activities of their microbial neighbors. In particular, it is currently unknown how widespread or diverse microbial germination interactions are, and there has been no systematic investigation of the spatial and temporal correlations of germinations they cause in communities of spores.Focusing on bacteria from the genus Streptomyces, we developed a high-throughput platform for automated quantification of spore germination on 2D surfaces through time-lapse microscopy. Our platform provides a full picture of germination events and subsequent mycelium growth for many thousands of individuals and allows monitoring of multiple communities in parallel. Fluorescent labeling allows us to investigate single- and two-species communities. By monitoring how the germination probability as a function of time is affected by the spore density and the germination of neighboring spores from same or different species, we quantified the germination interactions among a panel of Streptomyces strains.All species investigated had distinct germination interactions. While germinating spores from some species promoted the germination of their sister spores, other species inhibited their own germination and still others exhibited no interactions. We also discovered strong positive and negative inter-species interactions. These interactions could be reproduced with supernatants from newly germinated spores, which indicated that they are mediated by defusing chemicals. We also discovered that as spores of certain species age they enter a state of deep dormancy that can be fully reversed by chemicals released during germination.This work reveals that, similarly to small molecule interactions at the onset of sporulation, germination interactions among Streptomyces are common and diverse. Thus, bacterial germination is commonly affected by the community context, and different species have adapted different germination strategies. We expect our findings will trigger further activity aiming at determining the nature and diversity of the chemicals mediating the interactions and the ecological contexts favoring different germination strategies.

Author Disclosure Block:

K. Vetsigian: None. **Y. Xu:** None.

Poster Board Number:

SATURDAY-045

Publishing Title:

***pectobacterium Brasiliensis* gains A Competitive Advantage Within The Ecological Niche By The Production Of An Antimicrobial Molecule**

Author Block:

A. Durrant[1], A. Pitman[2], A. Mendoza[1], P. Fineran[3]; [1]Lincoln Univ., Lincoln, New Zealand, [2]Plant and Food Res., Lincoln, New Zealand, [3]Univ. of Otago, Dunedin, New Zealand

Abstract Body:

Background: *Pectobacterium* spp. are important pathogens that influence productivity of the potato industry, causing crop damage both in the field and post-harvest. The predominant pathogen was, until recently *Pectobacterium atrosepticum* (*Pba)*. However, *P. brasiliensis* (*Pbr*), has emerged as a more prevalent and aggressive pathogen [1]. The method by which *Pbr* has displaced *Pba* is unknown. Both bacterial species are routinely isolated together from fields[2], therefore, interactions are likely to occur between the two species. Few studies have focused on competition between two plant pathogens.**Methods:** Competition assays were conducted i*n vitro* and *in planta* between *Pbr* ICMP 19477 and *Pba* SCRI1043. A mutant library was produced for *Pbr* using random transposon mutagenesis and the mutants screened for the inability to out compete *Pba.* The disrupted genes were identified by next generation sequencing. Molecular and microbiology techniques were routinely used to further investigate the role of the identified genes on the competition phenotype of *Pbr* ICMP 19477. **Results:** *Pbr* ICMP 19477 produces a zone of inhibition when grown with *Pba* SCRI1043, indicative of the production of a diffusible antimicrobial molecule. Competition between the bacteria was established *in vitro* and *in planta*, with a greater affect seen in potato tubers. Four *Pbr* ICMP 19477 mutants were identified that did not produce a zone of inhibition. The disrupted genes were identified to be involved in quorum sensing regulation of carbapenem production. These mutants were significantly reduced in competition against *Pba* SCRI1043 in potato tubers. The wild type phenotype was restored by gene complementation.**Conclusions:**In mixed infections, *Pbr* ICMP 19477 out competes *Pba* SCRI1043 by actively inhibiting its growth by the production of a carbapenem molecule. It appears that carbapenem production is an important mechanism by which *Pbr* ICMP 19477 succeeds in its environmental niche. Therefore, this may contribute to the emergence of *Pbr* ICMP 19477 as a pathogen.

Author Disclosure Block:

A. Durrant: None. **A. Pitman:** None. **A. Mendoza:** None. **P. Fineran:** None.

Poster Board Number:

SATURDAY-046

Publishing Title:

Examining Bacterial Coexistence Among Members of the *Arabidopsis thaliana* Root Microbiome

Author Block:

M. J. Powers, J. L. Dangl, E. A. Shank; Univ. of North Carolina, Chapel Hill, Chapel Hill, NC

Abstract Body:

The plant root represents a topologically distinct environment that harbors significant bacterial diversity. These bacteria live in close proximity within the root's endophytic compartment or adhered to the root surface. We predict that bacterial interactions will therefore contribute to how root-associated bacteria colonize and persist in this complex community. To investigate this hypothesis, we explored co-culture interactions between 16 bacteria isolated from *Arabidopsis thaliana* roots, selecting a group of phylogenetically and functionally diverse bacilli, pseudomonads, and actinobacteria. Using combinations of four bacteria, we tested 210 different combinations in a liquid growth competition assay. Strain survival was determined by dilution plating, which was possible based on the distinct colony morphologies of these strains. Interactions were then classified as either coexistence (two or more strains survived) or dominance (one strain dominates). Based on a 16S rRNA gene relatedness metric, we used hierarchical clustering to categorize the 210 combinations into either high diversity combinations or low diversity combinations. In the high diversity group, the percentage of interactions that resulted in coexistence was 88%, whereas in the low diversity group, 58% of the interactions resulted in coexistence. These distributions differ significantly (Two Sample Proportions Test, $p < 0.001$). Thus, bacterial coexistence in liquid cultures appears strongly correlated with higher genetic diversity of the combined strains. In addition, approximately 43% of the combinations in the high diversity group were instances where three or four strains coexisted, compared to only 19% within the low diversity group. This observed trend between bacterial coexistence and increasing genetic diversity could potentially begin to explain the stability and functionality of root microbial communities. We are now determining whether our *in vitro* co-culture results can predict the ability of these bacteria to coexist in an *in planta A. thaliana* root colonization assay. Understanding these bacterial interactions in both an *in vitro* and *in planta* context will allow us to use chemical and genetic approaches to tease apart putative mechanisms for these interactions and their role in microbiome ecology.

Author Disclosure Block:

M.J. Powers: None. **J.L. Dangl:** None. **E.A. Shank:** None.

Poster Board Number:

SATURDAY-047

Publishing Title:

Mechanisms Of Microbial Community Assembly Across A Plant-Based Food System

Author Block:

E. R. Miller, B. E. Wolfe; Tufts Univ., Medford, MA

Abstract Body:

Use of molecular techniques in the phyllosphere has begun to shed light on patterns of microbial community composition, however the ecological processes shaping community structure are not well described. For vegetable fermentations, understanding the drivers of microbiome assembly is essential as successful fermentation relies on the presence of a key group of bacteria, the lactic acid bacteria (LAB). In this study, we developed a system to begin to identify ecological drivers of phyllosphere community composition in cabbages used for vegetable fermentation and how differences in the initial phyllosphere community results in changes in the final fermented vegetable product. Using three types of cabbage (Napa Savoy and red) at three sites in the Boston Area we determined the relative roles of host and geography in shaping phyllosphere community abundance and composition. Both site and cabbage variety influenced the overall bacterial abundance for the individual plants, although when looking solely at LAB there was no difference between site and variety. We then fermented the Napa cabbages from each of the three sites using an in vitro fermentation system to determine how phyllosphere community composition impacts fermentation dynamics. The pH of all ferments dropped rapidly suggesting a shift in microbial community composition to favor growth of the LAB. This shift was coupled with a change in the abundance of CFUs. Site differences in abundance were detected with the rural site taking longer for the pH to drop and having an overall lower CFU count at the end of the fermentation process. Ongoing work using amplicon metagenomic sequencing is determining the compositional shifts associated with the observed differences in abundance in the phyllosphere and fermentations. Lactic acid bacteria play the main role in vegetable ferments but are typically not added as a starter culture. Our work shows that reliance on naturally occurring lactic acid bacteria that are variable across space and plant varieties could have implications for the quality and safety of fermented vegetables.

Author Disclosure Block:

E.R. Miller: None. **B.E. Wolfe:** None.

Poster Board Number:

SATURDAY-048

Publishing Title:

Genome-Wide Identification Of *streptococcus Sanguinis* H_2o_2 Production And Regulation Genes Involved In Community Invasion Resistance

Author Block:

D. Ferrer, X. He, W. Shi; Univ. of California-Los Angeles, Los Angeles, CA

Abstract Body:

Foreign microbes are constantly entering and leaving the oral cavity, yet it remains stable with a relatively constant community of microbes. Using mouse oral flora as a model system, we recently identified a bacterial consortium comprised of three key species with distinct roles: the "sensor", *S. saprophyticus-O103,* the "mediator", *S. infantis-O102,* and the "killer" *S. sanguinis-O101*. They cooperate to detect foreign bacteria, conduct signal relays and regulate "killer's" H_2O_2 production to eliminate invaders. Normally, "mediator" inhibits "killer's" H_2O_2 production, however, "sensor" initiates a signal cascade resulting in the de-repression of H_2O_2 production. This study aimed to identify cellular pathways in "killer" whose activity can be modulated by the "mediator" to affect H_2O_2 production. The *S. sanguinis* SK36 mutant library was grown in 96-well plates. Individual mutants were mixed with *S. infantis*, which inhibits *S. sanguinis* H_2O_2 production, at a 1:1 ratio and spotted with horseradish peroxidase on BHI + leucocrystal violet agar plates. Plates were incubated at 37°C overnight; the H_2O_2 production was monitored as indicated by the development of blue colonies. The library was screened to identify mutants who escaped inhibition by *S. infantis* as well as mutants that had reduced production of H_2O_2 in the absence of *S. infantis.* The parent strain, *S. sanguinis* SK36 was spotted alone and with *S. infantis* on the plate as controls. Several *S. sanguinis* mutants were obtained that consistently escaped H_2O_2 production inhibition by *S. infantis.* These mutants carry deletions in genes that encode proteins with various functions, including a Zn-dependent hydrolase, acetyltransferases, tRNA synthetase, and many uncharacterized proteins. Additionally, a number of mutants were identified that consistently produced less H_2O_2 than the control. These mutants had deletions in ABC-type Mn/Zn transporters, a CMP binding factor, and many uncharacterized proteins. This large-scale mutant screen provided numerous gene candidates for further investigation into the signal relay between *S. infantis* and *S. sanguinis* in modulating H_2O_2 production by *S. sanguinis.* This will provide valuable insight into the communication that occurs between important members of the oral community and into the regulatory mechanism of invasion resistance.

Author Disclosure Block:

D. Ferrer: None. **X. He:** None. **W. Shi:** None.

Poster Board Number:

SATURDAY-049

Publishing Title:

Spatial Proximity Mediates Social Interactions in a Synthetic Two-species System

Author Block:

J. M. Chacón, W. R. Harcombe; Univ. of Minnesota, St. Paul, MN

Abstract Body:

Most microbial growth occurs on surfaces, but we still have little understanding of how spatial proximity influences social interactions. To investigate the influence of spatial structure, we developed a high-throughput method that uses scanners and custom image analysis software to track growth rates and yields of colonies on agar surfaces. As a first application we asked: how does competition vary as a function of colony distance, and is this relationship shaped by resource or competitor identity?We used *Escherichia coli* and *Salmonella enterica* as our model system and provided a variety of carbon sources. Colonies were either precisely placed at distances between 1.25 - 20mm apart or were randomly spread in large numbers on an agar plate. We also compared the strength of competition on an agar plate vs. in a well-mixed flask.We first found that, as expected, increased proximity to another colony significantly reduced colony yield, regardless of species. In general, competition between *E. coli* and *S. enterica* was asymmetric: *E. coli* colonies grew to similar size when near *E. coli* or *S. enterica* colonies, but *S. enterica* colonies grew larger when near *E. coli* colonies. However, the strength of the proximity effect, and the relative strength of intra- vs. interspecific competition, changed with carbon source: providing acetate caused a general decrease in productivity which reduced both the negative effect of close proximity and the difference in intra- vs. interspecific competition. Qualitatively, competition in acetate media was similar on agar and in a well-mixed flask, although *E. coli* grew better on agar. Interestingly, this qualitative similarity between flask and agar was absent when we provided non-overlapping carbon sources. In citrate and lactose media, *E. coli* and *S. enterica* have different carbon niches, and when together in a flask, grew to nearly additive amounts compared to monocultures. However, on a petri dish in close proximity, *S. enterica* and *E. coli* competed for space, and *S. enterica*'*s* faster growth rate on its private resource (citrate) gave it a competitive advantage.We conclude that spatial proximity both tunes competition of species and is an important resource in its own right for surface-living bacteria. In ongoing work, we are exploring whether the effect of spatial proximity scales differently when colonies engage in cooperative vs. competitive social modes.

Author Disclosure Block:

J.M. Chacón: None. **W.R. Harcombe:** None.

Poster Board Number:

SATURDAY-050

Publishing Title:

Green Fluorescent Protein Expression In *pseudogymnoascus Destructans* To Study Bat White Nose Syndrome

Author Block:

T. Zhang, P. Ren, M. DeJesus, V. Chaturvedi, **S. Chaturvedi**; Wadsworth Ctr., Albany, NY

Abstract Body:

Background: *Pseudogymnoascus destructans* (*Pd*) is the fungal etiologic agent of bat white-nose syndrome (WNS). WNS has led to mass die-offs of hibernating bats in many parts of the United States. Several bat species will face extinction if the pandemic spread is not stopped. *Pd* biology and pathogenesis studies are hampered by a lack of molecular toolbox. We describe a successful expression of codon optimized synthetic green fluorescent protein sGFP(S65T) in *Pd*.**Method:** The *sGFP(S65T)* gene was fused in frame with the *Aspergillus nidulans* promoter in the tumor inducing (Ti) plasmid (pRF-HUE) and the resulting plasmid pRFHUE-sGFP(S65T) was transformed into *Pd* by *Agrobacterium tumefaciens*-mediated transformation (ATMT) system with hygromycin B selection. The integration of sGFP(S65T) in *Pd* genome was analyzed by PCR and Southern hybridization. Transformatns were assessed for green fluorescence by confocal microscopy and by flow cytometry.**Results:** The single integration frequency of sGFP(S65T) was approximately 66%. The two randomly selected transformants with single integration event revealed ten-fold higher geometric mean fluorescence compared to the parent strain. Both spores and hyphal structures showed equally high fluorescence expression. Both transformants were mitotically stable after repeated sub-cultures on non-selective medium. The biology of sGFP(S65T) expressing transformants was not altered as judged by psychrophilic growth, sporulation, shape and size of vegetative structure, and urease production. Thus, constitutive expression of sGFP(S65T) is not toxic to *Pd*.**Conclusion:** The sGFP(S65T) transformants are currently under evaluation as reporters in animal experiments and environmental remediation.

Author Disclosure Block:

T. Zhang: None. **P. Ren:** None. **M. DeJesus:** None. **V. Chaturvedi:** None. **S. Chaturvedi:** None.

Poster Board Number:

SATURDAY-051

Publishing Title:

Metabolic Profiling of Bat White Nose Syndrome Pathogen *Pseudogymnoascus destructans* and Human Pathogen *Pseudogymnoascus pannorum* Reveals Clues to the Evolution of Pathogenic and Saprobic Lifestyles

Author Block:

H. DeFiglio[1], **S. Chaturvedi**[2], V. Chaturvedi[1]; [1]Wadsworth Ctr., Albany, NY, [2]NY State Dept. of Hlth., Albany, NY

Abstract Body:

Background: *Pseudogymnoascus destructans* (*Pd*) is a novel fungal pathogen of bat white-nose syndrome (WNS) and *Pseudogymnoascus pannorum* (*Pp*) causes humans and animal diseases rarely. Draft genome comparisons revealed *Pp* contained more deduced proteins with ascribed enzymatic functions than *Pd*. We performed metabolic profiling of two pathogens to validate *in silico* predictions.**Methods**: 96-well phenotype microarray plates (Biolog, Hayward CA), were inoculated with fungal spore suspensions OD_{600} = 0.2. The plates were incubated in dark at 15^0C and OD_{600} measured on day 7 (*Pp*) and day 10 (*Pd*). Negative control values were used to normalize recording of growth; each experiment was performed in duplicate on two separate occasions.**Results**: *Pp* exhibited high metabolic activity on a variety of carbohydrates (54% positive rates for analytes tested). Sucrose, sugar alcohols and cyclic oligosaccharides were utilized readily. In contrast, *Pd* grew poorly and on only 23% of carbon-source tested with preference for pyruvic acid, mannose and fucose. *Pd* exhibited robust metabolic activity on a majority of amino acids and peptides (61% positive rates for analytes tested).**Conclusions**: An abundance of carbohydrate degradation pathways provide an explanation for wide distribution of *Pp* in soil enriched with plant materials. A more limited metabolic profile of *Pd* validated *in silico* findings that it contained far fewer proteins and enzymes than Pp. Pd exhibited high preference for utilization of nitrogen sources. This observation provided a functional basis for the colonization and invasion of bat tissues.

Author Disclosure Block:

H. DeFiglio: None. **S. Chaturvedi:** None. **V. Chaturvedi:** None.

Poster Board Number:

SATURDAY-052

Publishing Title:

Prey Range Of Predatory *bdellovibrio* Strain Isolated From Bioswale Soil

Author Block:

N. Cullen, **S. O'Donnell**, L. E. Williams; Providence Coll., Providence, RI

Abstract Body:

Bdellovibrio are members of the delta-proteobacteria with an obligate predatory lifestyle. *B. bacteriovorus* invades the periplasm of Gram-negative prey bacteria, whereas *B. exovorus* attaches to the outside of prey cells. Both species digest prey bacteria in order to propagate. *Bdellovibrio* are found in a wide range of environments and may attack multiple different prey species, including animal and plant pathogens. This trait makes *Bdellovibrio* an attractive alternative to antibiotics, which are losing effectiveness with the rise in antibiotic resistance.To understand variation in prey range and predation efficiency within *Bdellovibrio*, we aimed to isolate naturally occurring strains of predatory bacteria from soil. Initially, we isolated potential prey bacteria from soil and classified them by 16S rRNA gene sequencing as strains of *Pseudomonas* and *Serratia*. We then used these strains as prey in enrichments of soil samples from a bioswale, which is an artificial landscape feature used to filter rainwater runoff. After purification by plaque formation on double agar overlay plates, we obtained an isolate showing predation on both *Pseudomonas* and *Serratia*. 16S rRNA gene sequencing classified this isolate as a member of *Bdellovibrio*, with ~96% identity to *B. bacteriovorus* 16S rRNA gene sequences and ~92% identity to *B. exovorus* 16S rRNA gene sequences.Based on 1000X phase-contrast microscopy, this soil *Bdellovibrio* isolate appears to invade prey cells, similar to the predatory strategy used by *B. bacteriovorus*. We will perform additional microscopy to determine whether this isolate invades the prey cell periplasm. In addition to *Pseudomonas* and *Serratia*, we will challenge this isolate with other Gram-negative bacteria, including isolates from freshwater environments, to assess prey range and investigate whether this isolate specializes on particular types of prey. This work will contribute to our understanding of predation in bacteria and explore the potential for therapeutic applications of predatory bacteria in the control of pathogens.

Author Disclosure Block:

N. Cullen: None. **S. O'Donnell:** None. **L.E. Williams:** None.

Poster Board Number:

SATURDAY-053

Publishing Title:

Isolation And Characterization Of Predatory Bacteria From An Estuary

Author Block:

B. Enos, L. E. Williams; Providence Coll., Providence, RI

Abstract Body:

Predatory bacteria are bacteria that attack and digest other bacteria in order to propagate. Predatory bacteria have been isolated from soil, marine and freshwater environments. The most well-studied predatory bacteria belong to four proposed families within the delta-proteobacteria. Certain species within these families, such as *Bdellovibrio bacteriovorus*, are found in a wide range of environments, whereas other species, such as *Halobacteriovorax marinus*, are restricted to marine environments. Both of these species are obligate predators that attack Gram-negative prey bacteria, including animal and plant pathogens. Because of this, predatory bacteria may serve as biocontrol agents and alternatives to antibiotics.To understand the diversity of predatory bacteria adapted to saltwater environments, we aimed to isolate predators from an estuary site in coastal Rhode Island. As a first step, we isolated seven strains of potential prey bacteria from the estuary site and classified these by 16S rRNA gene sequencing as *Vibrio* and *Enterovibrio*. We then used these strains as prey in enrichments of estuary water samples. An enrichment using a *Vibrio* strain as prey showed very small predatory bacteria by 1000X phase-contrast microscopy. We obtained a pure isolate of the predatory bacteria in this enrichment by plaque formation on double agar overlay plates.The predatory isolate appears to invade prey cells based on 1000X phase-contrast microscopy. Genus-specific PCR suggests that this isolate is not a member of *Bdellovibrio*. Moving forward, we will classify this isolate using 16S rRNA gene sequencing. We will also test prey range by challenging the isolate with the other potential prey bacteria isolated from the estuary. This work will broaden our understanding of diversity in predatory bacteria adapted to saltwater environments and contribute to efforts to develop predatory bacteria as biocontrol agents against bacterial pathogens.

Author Disclosure Block:

B. Enos: None. **L.E. Williams:** None.

Poster Board Number:

SATURDAY-054

Publishing Title:

A Highly Divergent Bioluminescent Isolate Suggests a Previously Undiscovered *Aliivibrio* Species

Author Block:

M. Whyte, C. F. Wimpee; Univ. of Wisconsin-Milwaukee, Milwaukee, WI

Abstract Body:

Bioluminescence is widely scattered across the tree of life with representatives in more than 700 genera, distributed among various microorganisms as well as fungi, plants, and animals. In bacteria, bioluminescence is confined to the Gammaproteobacteria, almost entirely in three genera of the family Vibrionaceae: *Vibrio*, *Photobacterium*, and *Aliivibrio*. This laboratory has been carrying out a long-term study of bacterial bioluminescence as a model system for bacterial molecular evolution. Methods used include PCR amplification of *lux* genes as well as other genes, followed by restriction enzyme fingerprinting, sequencing, and phylogenetic analysis. In a recent screening of seawater from St. Petersburg, Florida, we isolated 80 strains with an identical unusual *luxA* fingerprint pattern. One isolate, designated SP1, was chosen for further analysis. It was found that the *luxA* sequence of SP1 is significantly divergent from all other known bioluminescent bacterial species, sharing only 80% nucleotide sequence identity with the closest known relative, *Aliivibrio salmonicida*. Such a surprising evolutionary distance led us to hypothesize that SP1 represents an undescribed species. To test this hypothesis, we isolated and sequenced additional genes. Analysis of other *lux* genes shows that the SP1 *lux* operon shares the gene arrangement of *A. logei* and *A. salmonicida*. Besides *lux* genes, several housekeeping genes also place SP1 in the genus *Aliivibrio*. However, the very low sequence identity of several genes (*luxC*, 50%; *luxA*, 80%; *luxE*, 79%; *luxR*, 75%; *toxR*, 75%, *gyrB*, 88%; *mreB*, 89%) show that SP1, while sharing most similarity with the genus *Aliivibrio*, is significantly divergent from the other known species of that genus. These data suggest that SP1 represents a previously undiscovered species of *Aliivibrio*.

Author Disclosure Block:

M. Whyte: None. **C.F. Wimpee:** None.

Poster Board Number:

SATURDAY-055

Publishing Title:

Impact of Biochar Addition and Co-Inoculation with *Bradyrhizobium a*nd *Pseudomonas* Strains on Growth of Soybean

Author Block:

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Abstract Body:

Background: Plant growth promoting rhizobacteria (PGPR) provide an alternative to chemical fertilizers as they contribute to increased plant growth and development. Therefore, PGPR have been extensively documented for their positive impact on plants, but if added with biochar, enhancement of crop yield, also help in preventing fertilizer run-off and helping plants. We studied the effect of biochar application co-inoculated with *Bradyrhizobium japonicum* USDA 110 and *Pseudomonas putida* NUU8 strains on growth of soybean (*Glycine max* L. (Merr.).**Methods:** Plants were grown in loamy sandy soil taken from ZALF experimental field station, Müncheberg, Germany. We was used Pyrolysis char from wood (Pyreg-char) as a soil amendment at concentrations of 0.5 and 5%. A pot experiment was performed with seven different treatments, i.e., loamy sandy soil (control), soil + biochar 0.5 %, soil + biochar 0.5 % + *B. japonicum* USDA 110, soil + biochar 0.5 % + *B. japonicum* USDA 110+ *P. putida* strain NUU8, soil + biochar 5 %, soil + biochar 5 % + *B. japonicum* USDA 110, soil + biochar 5 % + *B. japonicum* USDA 110+ *P. putida* strain NUU8. The length and biomass of roots and shoots, as well nutrient uptake were measured soybean.**Results:**Generally, all treatments showed an increase in growth as compared to plants grown in untreated soil. The treatment - soil + biochar 0.5 % + *B. japonicum* USDA 110 + *P. putida* strain NUU8 provided the most benefits in nodulation, plant growth and nutrient uptake of soybean compared to all other treatments. It was observed that addition of biochar to soil influenced the overall growth of plants positively but co-inoculation of with B. *japonicum* USDA 110 and *Pseudomonas P. putida* NUU8 strains even enhanced this effect further. The treatment - "soil + biochar 0,5 % + *B. japonicum* USDA 110+ *P. putida* strain NUU8" also showed a significant increase in nutrient contents of soybean and soil.**Conclusions:**Hence, it can be concluded that both biochar and co-inoculation of with B. *japonicum* USDA 110 and *Pseudomonas P. putida* NUU8 strains are effective treatments for a sustainable soybean production on sandy soils.

Author Disclosure Block:

D. Jabborova: None.

Poster Board Number:

SATURDAY-056

Publishing Title:

Phosphate Solubilising Bacteria (PSB) with Multifarious Growth Promoting Traits from Tropical Forests of India Can Enhance Growth of Crop Plants

Author Block:

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Abstract Body:

Phosphorus (P), currently believed to be the most limiting nutrient in tropical regions. In India, about 46% of soil is classified as P-deficient. Utilization of mineral phosphate resources like rock phosphate for crop production is a big challenge as "P" becomes immobilized rapidly and become unavailable to the plants. Phosphate solubilising bacteria (PSB) with phosphate mineralizing activity and other growth promoting traits will be very useful for utilization of rock phosphates for growth enhancement of crop plants. Forest soils are rich source of organic "P" compounds and can harbour PSB with P mineralizing activity. Under this background, surveys were conducted in the tropical forests of West Bengal and Meghalaya, India. A total 83 candidate PSB were isolated using NBRIP agar. Upon further screening on agar plates containing URP, fifteen colonies showing halo zones were selected for quantification of P solubilisation. Among them 7 isolates could solubilise ≥200 mg URP/lt and were identified based on 16S rDNA homology as *Serratia nematodiphila* NGNB2, *Bacillus megaterium* NGNB17, *Burkholderia cepacia* PSKP1, *B. cepacia* JUNP1, *B. vietnamiensis* PSKP2, *Bacillus gingsensoli* JB1 and *Klyuvera cryocrescens* JBP1. Due to reported pathogenic nature of *Burkholderia*, only four isolates viz. NGNB2, NGNB17, JB1 and JBP1 were screened for phosphatase activities and other growth promoting traits like phytohormone production, HCN & siderophore production, K & Zn solubilization following standard methodologies.

Isolates	IAA production (mg/lt)	Acid phosphatase (U/lt)	Alkaline phosphatase (U/lt)	Solubilization of	Production of		
K	Zn	HCN	Siderophore				
NGNB17	0.7	94.5	13.0	-	-	-	-
NGNB2	ND	4.4	24.0	+	-	-	+
JB1	ND	3.3	18.0	-	-	-	+
JBP1	1.0	8.2	18.0	+	-	-	+

ND: Not detectedThese isolates were subjected to pot trial for growth promotion in maize, wheat and chickpea. It was observed that all the isolates could significantly enhance the biometric parameters like fresh and dry weight of root and shoot, length of root and shoot. The isolates despite their distinctly different ecological origin hold promise as bioinoculants for enhancing crop productivity due to their multifarious growth enhancing attributes.

Author Disclosure Block:

H. Chakdar: None. **P.L. Kashyap:** None. **P. Saxena:** None. **A.K. Srivastava:** None.

Poster Board Number:

SATURDAY-057

Publishing Title:

A "Trojan Horse" Strategy to Block Transmission of a Plant Pathogen - *Xylella fastidiosa*

Author Block:

A. K. Arora[1], T. A. Miller[2], R. Durvasula[3]; [1]Texas A&M Univ., College Station, TX, [2]Univ. of California, Riverside, CA, [3]Univ. of New Mexico, Albuquerque, NM

Abstract Body:

Background: Arthropod borne diseases are a big burden to global agriculture. The economic damage caused by these diseases runs into billions of dollars. Chemical insecticides are the main weapon against vector borne diseases, which control vector population. However, insecticide application is associated with environmental contamination and resistance development. Other methods including transgenic and paratransgenic insects are being developed to control these diseases. Paratransgenesis, which is a "Trojan Horse" strategy relies on a symbiont to deliver anti-pathogen molecules within the vector-gut. These molecules make the vector-gut hostile to pathogen survival, thereby decreasing vector competence. Here, we report a paratransgenic strategy to block transmission of *Xylella fastidiosa*, a global plant pathogen, by its vector, the glassy-winged sharpshooter (GWSS, *Homalodisca vitripennis*), using a commensal bacterium *Pantoea agglomerans*.**Methods:** Using the *E. coli* hemolysin secretion system we genetically engineered *P. agglomerans* to express two antimicrobial peptides (AMPs) - melittin and scorpine like molecule (SLM). These AMPs were selected based on their higher toxicity towards *Xylella* as compared to *Pantoea*.**Results:** Only 15.8% of the paratransgenic GWSS, which harbored melittin or SLM expressing *Pantoea*, acquired *Xylella.* This was significantly lower than the control group, in which 85.6% GWSS acquired *Xylella* ($p<0.0001$). These paratransgenic GWSS when allowed to feed on naive grape plants failed to transmit *Xylella*.**Conclusions:** We were able to block transmission of *Xylella*, which infects more than 150 plant species, by its most important vector, GWSS, using a transgenic bacterium. This is the first report of blocking transmission of a plant pathogen using a paratransgenic strategy. This will open the door of development paratransgenic strategies to control other vector borne plant diseases.

Author Disclosure Block:

A.K. Arora: None. **T.A. Miller:** None. **R. Durvasula:** None.

Poster Board Number:

SATURDAY-058

Publishing Title:

Lysinibacillus Sphaericus* Plant Growth Promoter and Phytoremediation Enhancer in *Canavalia ensiformis

Author Block:

S. A. Martínez, J. Dussán, Microbiological Research Center(CIMIC); Univ. de los Andes, Bogotá, Colombia

Abstract Body:

The recent genome sequencing of OT4b31, CBAM5 and III(3)7 strains revealed that *L.sphaericus* has nitrogen metabolism genes and multiple copies of metal resistance genes. Due to these insights, the aim of this study was to determine its role in the nitrogen cycle and lead phytoremediation process in contaminated soils with toxic metals.These strains of *L.sphaericus* and other four (OT4b26, OT4b32, OT4b49 and 2362) were tested for nitrogen metabolism (nitrogen fixation and nitrification) and indole acetic acid (IAA) production; the results were analyzed with a Kruskal-Wallis test.In the greenhouse study were used 100 seedlings per treatment: contaminated soil with lead (600ppm) with *L.sphaericus* consortium and respective control. The plant-growth parameters measured were: height, leaf number, dry weight, dry weight percentage, root length, shoot length, root:shoot, root mass, and foliar area; also were quantified nitrate and ammonia concentration in soil and lead concentration in root and soil as well. These measurements were performed every month for three months; the results were analyzed with a Student´s t-test.All *L.sphaericus* strains fixed molecular nitrogen (0,61-1,47mg/L) and oxidized ammonia to nitrate (0,26-0,7mg/L); III(3)7 and 2362 strains were the best nitrogen fixers, meanwhile OT4b31 and OT4b49 were the best ammonia oxidizer strains. All strains produced IAA in L-tryptophan supplemented media (3,3-5,0µg/mL); the best producers were the OT4b49, III(3)7, CBAM5 and 2362 strains.The green house study showed that in *L.sphaericus* consortium treatment height, foliar area, leaf number, root length and shoot length of *C.ensoformis* were statistically higher. According to nutrient content in soil, nitrate and ammonium decreased through time in both treatments. On the other hand, the bioaccumulation of lead in roots were higher in bacteria treatment; especially in the second sample where the lead concentration was almost two fold in contrast to the control.Finally we found a wide participation of *L.sphaericus* in the nitrogen cycle in soil and in lead phytoremediation. Then we consider that this microorganism can be a plant growth promoter bacteria (PGPB).

Author Disclosure Block:

S.A. Martínez: None. **J. Dussán:** None.

Poster Board Number:

SATURDAY-060

Publishing Title:

Preparation of Culture Media from Natural Ingredients as an Alternative to Produce *Bacillus sphaericus* Biomass for Use in Biological Pest Control Larvae in Commercially Important Crops

Author Block:

L. C. Sanchez, L. C. Corrales, L. L. Gonzalez, M. A. Calderon, N. G. Parra, D. P. Correa, M. A. Rodriguez; Univ. Colegio Mayor de Cundinamarca, Bogotá, Colombia

Abstract Body:

Background: One of the most important tools to verify the feasibility of a microorganism is the culture media. It´s use meant the way to visualize these microscopic particles and check their viability. Later, it was the way to get large amounts of biomass and find the most convenient substrate to activate any metabolic pathway. In this research, the objective went evaluate some culture media, to produce biomass and *Bacillus sphaericus* spore, which it gives an entomopathogen action. **Methods:** We selected seven substrates between grains, legumes and seeds. They were evaluated individually and in admixture. It was analized the production by direct and indirect viable cells recount. Solid media were tested with chickpeas, wheat and peas alone and in combination (GTA, by its initials in Spanish). Finally, *Bacillus sphaericus* entomopathogenic effect was evaluated on larvae of *Plutella xylostella* and *Tuta absoluta.* **Results:** The sporulation was 92% and the spores were terminals; just one, the treatment with chickpeas presented complete sporulation of 91% at 48 hours. About culture media with legumes and seeds, the culture media which contained sunflower presented the best performance and sporulation. The two best culture media, with more than 80% of sporulation were carried to scale of 1 L. After 7 hours of fermentation, GTA broth presented 3,20 x 10^9 cel/mL while G broth presented a recount of 3 x 10^{13}cel/ML after 10 hours. The native strain UCMCB3, presented the best entomopathogen effect in culture media, GTA with 90,91% mortality, at a concentration of 1, 6 x 10^8 spores/mL. This result is statistically significant when other doses ($p = 2.364^{e-07}$) **Conclusions:** It was concluded that the development of culture media with natural components meets the nutritional requirements for growth and are an alternative in terms of biomass production and obtaining primary or secondary bacterial metabolites

Author Disclosure Block:

L.C. Sanchez: None. **L.C. Corrales:** None. **L.L. Gonzalez:** None. **M.A. Calderon:** None. **N.G. Parra:** None. **D.P. Correa:** None. **M.A. Rodriguez:** None.

Poster Board Number:

SATURDAY-061

Publishing Title:

Characterization of the R-Pyocin Gene Cluster of *Pseudomonas chlororaphis* 30-84

Author Block:

R. Dorosky, L. S. Pierson, III, E. A. Pierson; Texas A&M Univ., college station, TX

Abstract Body:

Pseudomonas produces R-type pyocins, high molecular mass bacteriophage-derived bacteriocins that resemble phage tails. R-pyocin structural genes are encoded within the bacterial chromosome along with genes encoding a lysis cassette containing a holin and a lytic enzyme (1). Holins are small transmembrane proteins that facilitate cell lysis by accumulating and permeabilizing the cytoplasmic membrane thereby allowing the lytic enzyme into the periplasmic space and ultimately cell lysis (2). A previous study of the rhizosphere-colonizing bacterium *Pseudomonas chlororaphis* 30-84 revealed that production of the phenazine 2-OH-PCA was correlated with eDNA production as well as expression of an R2-type pyocin gene cluster (3). The present study demonstrates the functionality of *P. chlororaphis* pyocin lysis cassette, characterizes the pyocin particle, and shows the importance of cell lysis and pyocin release for competition among bacteria and the persistence of rhizosphere populations. To demonstrate the functionality of the lysis cassette, the holin and lytic genes were cloned separately or together into an expression vector behind an arabinose-inducible promoter. Co-expression of both genes resulted in cell lysis, whereas no cell lysis was observed when the genes were expressed separately. Moreover, cells of a *P. chlororaphis* holin-deletion mutant do not lyse when induced by UV radiation and do not accumulate as much eDNA as the wild type, consistent with an autolysis-deficient phenotype. Observations using transmission electron microscopy of the lysates of wild type following UV induction revealed rigid, rod-shaped, phage-like particles characteristic of R-type pyocins. This lysate inhibited the growth of some *Pseudomonas* species, but did not affect the growth of other bacterial genera tested. Mutation of the holin also affected the ability of *P. chlororaphis* to compete with pyocin-sensitive bacteria in biofilms and survive in the wheat rhizosphere. These results demonstrate that *P. chlororaphis* 30-84 possesses a fully functional lysis cassette and R-pyocin particle, and provides evidence for their ecological role in microbial interactions and rhizosphere dynamics.

Author Disclosure Block:

R. Dorosky: None. **L.S. Pierson:** None. **E.A. Pierson:** None.

Poster Board Number:

SATURDAY-062

Publishing Title:

Isolation and Identification of Causative Agent of Potato Common Scab and Potential Use of Antagonistic Bacteria as a Biological Control of Disease

Author Block:

A. Sarwar[1], C. R. Osorio, Male[2], C. Cabaleiro[2], Z. Latif[1]; [1]Univ. of the Punjab, Lahore, Pakistan, [2]Univ. of Santiago de Compostela, Santiago de Compostela, Spain

Abstract Body:

Background: Potato is considered as a cheaper nutritional source to eradicate hunger problem of the world. Common scab (CS) is a foremost bacterial disease of potato causing potato damage all around the world.**Methods:** The current study was designed to check the prevalence of CS in potato growing provinces like Punjab, Pakistan and Galicia, Spain. Samples of potato with tuber lesions collected from 25 different localities, were processed to find the causative agent of this disease. 124 bacterial strains were isolated and identified as *Streptomyces* spp. on the basis of morphological and physiological characteristics. Species specific 16S rRNA primers were used to confirm *Streptomyces* strains at species level. The antagonistic bacterial strain *Streptomyces hygroscopicus* AS-1 was isolated from soil and identified by 16S rRNA gene sequencing.**Results:** Out of 124 *Streptomyces* strains, 34 strains were confirmed as pathogenic due to the presence of *txtAB* gene. Among pathogenic *Streptomyces* strains, 56% strains were confirmed as *Streptomyces europaeiscabiei*, 25% *S. bottropensis*, 12% *S. turgidiscabiei* and 7% as *S. stelliscabiei* whereas 40% pathogenic *Streptomyces* strains were also positive for *nec1* and 23% for *tomA* genes. Isolated pathogenic *Streptomyces* strains were screened for CS disease symptoms while antagonistic *S. hygroscopicus* strain was evaluated to inhibit the CS disease production in pot assay. Disease severity index was established in tubers from 0-500. *S. turgidiscabiei* and *S. europaeiscabiei* showed highest disease severity index as 146 and 70 respectively. Antagonistic *S. hygroscopicus* reduced the disease severity index up to 18 and 7 for *S. turgidiscabiei* and *S. europaeiscabiei* strains respectively.**Conclusions:** This study provides an alternative strategy to control the spread of common scab in potato and other tuber related daily consumable foods for controlling hunger problem of the world.

Author Disclosure Block:

A. Sarwar: None. **C.R. Osorio:** None. **C. Cabaleiro:** None. **Z. Latif:** None.

Poster Board Number:

SATURDAY-063

Publishing Title:

Detection of Cccvd Variants in Palms by One Step Rt Late Pcr

Author Block:

R. K. Finol-Urdaneta[1], K. E. Pierce[1], R. Sambanthamurthi[2], M. Ong-Abdullah[2], L. Wangh[1]; [1]Brandeis Univ., Waltham, MA, [2]Malaysian Palm Oil Board, Selangor, Malaysia

Abstract Body:

Background: Palm tree products constitute major nutritional and economical resources in many tropical countries. Coconut *Cadang cadang* Viroid (CCCVd) infection results in slow but catastrophic productivity loss. A CCCVd variant causes orange spotting in oil palm. Disease symptoms are common to many other palm pathologies, making precise detection of the causative agent critical to field management and germplasm exchange procedures. But, association of viroid titers and viroid variants to disease progression remains unknown.**Method:** RT-LATE-PCR efficiently generates single-stranded amplicons improving assay sensitivity as compared to conventional RT-PCR. End-point detection with low temperature fluorescent probes is exquisitely specific and distinguishes sequence variations as shifts in melting temperature (T_M) of probe/target hybrids. Thus, these technologies allow for rapid, quantitative detection of palm viroids in a closed-tube One-Step reaction.**Results:** Several sets of LATE-PCR primers were designed and used to amplify cDNA from synthetic CCCVd RNA and infected palm isolates. CCCVd probes hybridized to synthetic DNA targets of reported CCCVd variants, at end point of PCR, and end point of RT-LATE-PCR of viroid RNA. Strong fluorescent signal showed T_M consistent with specific CCCVd variant sequences. An internal control was implemented which amplifies mitochondrial nad5 mRNA across "trans-splicing" junctions in a region encompassing exons 2 and 4, separated by >33,000 nucleotides. The lack of probe signal from samples without reverse transcriptase demonstrated the absence of DNA contamination in palm RNA samples, and/or the inability to generate the long product under our PCR conditions. This control allowed for sample quality control, and provided the means for relative quantification of CCCVd, by comparison of specific probe signals from control and pathogen LATE-PCR products.**Conclusions:** We have constructed a fast and reliable assay that detects, distinguishes and quantifies CCCVd variants in coconut and oil palms. Validation and implementation of the assay in the field will enable accurate diagnosis of infected palms and derived germplasm. Analyses of experimentally infected palms will contribute to the understanding of CCCVd patho-physiology in these economically important crops.

Author Disclosure Block:

R.K. Finol-Urdaneta: None. **K.E. Pierce:** None. **R. Sambanthamurthi:** None. **M. Ong-Abdullah:** None. **L. Wangh:** None.

Poster Board Number:

SATURDAY-065

Publishing Title:

Nematicidal Potential of Extracellular Metabolites of *Bacillus pumilus* and *Bacillus thuringiensis* Against *Meloidogyne javanica*

Author Block:

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Abstract Body:

Background: Nematodes are a group of pests detrimental to economically important plants. Most of the effective chemical nematicides are non-biodegradable, toxic and non-specific. Biological nematicides from microbes are generallyenvironmental friendly. In the present study a large pool (130) of *Bacillus* sp was screened for their nematicidal potential against root-knot nematode *Meloidogyne javanica.* **Method:**The eggs and larvae of *M. javanica* were isolated from infected roots of egg plant, after treatment with 1% bleach followed by sequential sieving 100, 250 and 400 mesh size. For hatching test 20-60eggs /ml were exposed to 1ml of cell free culture supernatant (CFCS) of each Bacillus species in sterile glass cavity block *(in triplicate).* The cavity-blocks were incubated in a moist chamber at 25°C and observations were made by counting number of hatched eggs after 24, 48 and 72h microscopically. The larvicidal activity was also observed similarly for three days by adding 25-40 larvae/ml instead of egg suspension. Partially purified and chemically characterized metabolites of *Bacillus pumilus* S108(a), *Bacillus pumilus* S113 and *Bacillus thuringiensis* S128 were selected for green house experiment on tomato seedlings by root dip and soil drench methods. Factorial ANOVA and Duncan's multiple-range test were performed using CoStat, 4. The mean %, standard deviation and probability were also determined by SPSS.**Result:** Only 7% of the test strains exhibited anti- hatching activity. *The* CFCS from few caused stimulation of hatching and the juveniles were immediately killed after hatching. Considerable larvicidal (> 50%) activity was exhibited by 115 *Bacillus* strains. ***The exposure-time and*** larvicidal potential was proportional to each other.The nematicidal metabolite of strains S108(a), S113 and S128 were heat-labile and protease-sensitive proteins. There was >50% reduction in knot/gall-formation in all the cases. The % reduction in nematode population/g root was 53%, 54% and 46% for strains S108(a), S113 and S144, respectively. **Conclusion:** The hatching stimulatory strains can be used to control nematode infection at an early stage of their life-cycle. The extracellular metabolites of strains S108(a), S113 and S128 can be used to control root-knot infection of *M. javanica.*

Author Disclosure Block:

S.N. Mukry: None. **S.A. Khan:** None.

Poster Board Number:

SATURDAY-066

Publishing Title:

Evaluation of Genotoxic Potential of Pesticides (Lufenuron and Emamectin) Using the *Allium cepa* Assay

Author Block:

M. Khawer, S. Sultan; Univ. of the Punjab, Lahore, Lahore, Pakistan

Abstract Body:

Background: Over the past few decades, the use of pesticides has been increased enormously in agriculture. These pesticides help in combating pests (insects, weeds etc), but at the same time, have potential threats for the plant itself and its consumers.**Methods:** In this, study two pesticides, lufenuron and emamectin were evaluated for genotoxicity and mutagenicity using *Allium cepa* chromosomal aberration test and Ames test, respectively. For genotoxicity evaluation, 7 concentrations (D1= 0.05%, D2= 0.1%, D3= 0.15%, D4= 0.2%, D5= 0.4%, D6= 0.6%and D7= 0.8%) of both pesticides were used.**Results:** Both pesticides were involved in decreased germination rates for onion seeds, inhibition of root length and decreased mitotic indices when compared to negative control (autoclaved water) in dose dependant manner. While the chromosomal aberrations (micro nuclei, vacuolated nuclei, chromosomal breakage, sticky chromosomes, vagrant chromosomes and chromosomal bridges) increased as the pesticide concentration increased. For mutagenicity detection, Ames test was performed using two *Salmonella typhimurium* strains i.e. TA98 and TA97a to detect frame shift mutations. For lufenuron doses used were LD1= 50 %, LD2= 75 % and LD3= 100 %. For emamectin doses used were ED1= 0.05 %, ED2= 0.1 % and ED3= 0.5 %. For both pesticides at least one concentration showed high mutagenicity.**Conclusions:** So, it was confirmed by that both pesticides have genotoxic and mutagenic effects. Additional mutagenicity tests should be conducted for further validation of these results which will confirm the predictions for pesticide's effect in an organism.

Author Disclosure Block:

M. Khawer: None. **S. Sultan:** None.

Poster Board Number:

SATURDAY-067

Publishing Title:

Microbiomes of Potato-Cyst-Nematodes Isolates Kept in Laboratory Conditions

Author Block:

D. N. Proenca[1], I. L. Conceicao[2], P. V. Morais[1]; [1]CEMUC, Univ. of Coimbra, Coimbra, Portugal, [2]CFE, Univ. of Coimbra, Coimbra, Portugal

Abstract Body:

Background: Potato is an important staple crop worldwide with an estimated production of 375 million tons every year. Portugal has a lower potato yield average comparatively to the average in European Union and may be attributed to the presence of plant-parasitic nematodes as, for example, potato-cyst nematodes (PCN), *Globodera* spp. Recently, in some European collections, PCN isolates kept in lab cultures started to die and were not able to multiply after the first generation obtained in potato cultures in pots. This is a problem shared by the Portuguese collections. This work is aimed to assess the microbiome of wild PCN and PCN maintained in laboratory collections.**Methods:** Cysts from *Globodera* spp. were extracted from soil infested with PCN. DNA was extracted from wild cysts (W-PCN), immediately after their isolation from the soil, and from cysts maintained in laboratory (L-PCN) in closed glass tubes, at room temperature, during 6 months. Molecular methods (DGGE and Illumina MiSeq) were used to assess the microbiomes (Bacteria, Archaea and Fungi) present in cysts. Sequence data were processed using MR DNA analysis pipeline (MR DNA, Shallowater, TX, USA).**Results:** DGGE profiles showed that the microbial community was different between wild and laboratory cysts. When comparing W-PCN with L-PCN, by the analysis of Illumina Miseq, it was possible to verify in L-PCN the increase of classes *Gammaproteobacteria* (30% to 60%, namely *Acinetobacter*) and *Bacilli* (8% to 20%, namely *Bacillus* and *Staphylococcus*) and a decrease in the percentage of microorganisms of the classes *Actinobacteria* (18% to 8%), *Sphingobacteriia* (7% to 1%), *Betaproteobacteria* (13% to 1%) and *Alphaproteobacteria* (20% to 7%). The number of bacteria and archaea involved in nitrification pathways in L-PCN was lower than in W-PCN. Moreover, we found the yeast *Saccharomyces* (30%) in L-PCN microbiome.**Conclusions:** The microbiomes of L-PCN and W-PCN are different and these differences might be involved in the loss of viability of the isolates in lab cultures. Saccharomyces was previously shown to kill nematodes and its presence in the L-PCN microbiome could also be related to the death of PCN maintained in lab conditions.

Author Disclosure Block:

D.N. Proenca: None. **I.L. Conceicao:** None. **P.V. Morais:** None.

Poster Board Number:

SATURDAY-069

Publishing Title:

The Cucumber Microbiome Shifts in Response to Rainfall

Author Block:

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Abstract Body:

Cucumber (*Cucumis sativus*) is a vining crop cultivated on the ground and commonly consumed raw. It has been implicated in multiple foodborne disease outbreaks in recent years. Research indicates that the levels of fecal indicator bacteria on crops spike immediately following a rainstorm, potentially representing an increased food safety risk. In this study, we profiled the surface bacterial communities of cucumber fruit in the days surrounding a rainstorm. Cucumber fruit samples (n=24) were collected from a Maryland organic farm 4 days before, and 1 and 4 days after a rain event (precipitation=12.45 mm). DNA was extracted from washes of fruit surfaces and used for microbial community profiling using Illumina sequencing. A 508 base pair fragment spanning the V1-V3 hypervariable regions of the 16S rRNA gene was amplified and sequenced using MiSeq v3. Resulting sequencing data was quality filtered and analyzed using QIIME v. 1.8. After quality filtering, 1.1 million reads were retained for alpha and beta diversity analysis. At a sampling depth of 29,287 sequences per sample, approximately 1,618 OTUs (97% similarity) were identified for each sample. Cucumber samples collected 4 days before, 1 day after, and 4 days after a rain event clustered separately, showing a significant influence of collection date on microbial community composition (R^2=0.225, p=0.002). The 4 day pre- and 1 day post-rain samples hosted significantly different communities (p=0.002), however the 4 day post-rain samples were not significantly different from either treatment. Rain resulted in a temporarily elevated relative abundance of Xanthamonadaceae (p=0.04), a group containing several plant pathogens; relative abundance returned to pre-rain levels by day 4 post-rain. Rain also resulted in an increase in Paenibacillaceae (p=0.01), a family comprising several beneficial and biocontrol species, which had only slightly decreased 4 days post-rain. This study demonstrated a shift in the structure of cucumber fruit surface-associated bacterial communities following a rain event on a Maryland organic farm that had only partially reversed within 4 days. The susceptibility of plant hosts to pathogen colonization could be enhanced by these weather-induced shifts in microbial species dominance.

Author Disclosure Block:

S.M. Allard: None. **A. Ottesen:** None. **S.A. Micallef:** None.

Poster Board Number:

SATURDAY-070

Publishing Title:

Bacterial Populations on the Surfaces of Organic and Conventionally Grown Almond Drupes

Author Block:

J. A. McGarvey; USDA ARS FTDP, Albany, CA

Abstract Body:

Aims: To compare the bacterial populations on organically and conventionally grown almond drupes before and after hull split. **Methods and Results**: We constructed 16S rRNA gene libraries containing approximately 3,000 sequences each from the bacteria from organically and conventionally grown drupes before and after hull split. We observed that before hull split both conventionally and organically grown drupes were colonized by relatively few types of bacteria that were mostly common phyllosphere-associated *Proteobacteria*. However, the organically grown drupes contained significantly more *Alphaproteobacteria* and the conventionally grown drupes contained significantly more *Gammaproteobacteria.* The conventionally grown drupes also contained significantly more sequences associated with the phylum *Actinobacteria.* After hull split, we observed a significant increase in bacterial diversity, with many newly appearing sequences that were not normally associated with the phyllosphere. **Conclusions:** Organic and conventional growing methodologies influence the types of bacteria on the pyllosphere and hull split results in a burst of microbial diversification. **Significance and Impact of the Study**: Production of organic produce is increasing due to consumer preferences, but it was unknown how this methodology affects the bacterial populations on the phyllosphere. This is the first study to compare the bacterial populations of organically and conventionally grown almond drupes.

Author Disclosure Block:

J.A. McGarvey: None.

Poster Board Number:

SATURDAY-071

Publishing Title:

Antagonistic Activity of Bacteria Against Root Rot Fungal Pathogens of Ginseng

Author Block:

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Abstract Body:

Korean *Panax ginseng* C.A. Meyer with thousand years of history, has been traditionally known as medicinal plant with mysterious powers in the orient. The efficacy of ginseng has been recognized based on oriental medical science theory and determined that ginseng roots and its extracts revitalize the body. Ginseng crop cultivation is highly vernacular to phytopathogenic fungi such as *Rhizoctonia solani, Cylindrocarpon destructans, Collectotrichium* and *Botrytis cinera.* The phytopathogen outbreak leads to replant disease in which the crop cannot be cultivated for consecutive years in the same soil. Our studies focuses on the biocontrol ability of bacterial species against fungal pathogens of ginseng. The bacterial strains were isolated from the rhizosphere of ginseng and in vitro tests were performed to check the efficiency of antagonism. Two effective strains were subjected to 16S rRNA partial gene sequencing and found belonging to the genus *Bacillus* and *Paenibacillus*. In vivo analysis was performed in pots under shade at green house conditions. The results of pot experiment showed the efficiency up to 67% reduction in root lesions. Bacterial inoculum was prepared in bulk and the culture filtrate was analyzed for the active metabolites responsible for the antagonistic properties. The culture filtrate analysis showed that the compounds belong to the surfactant group of lipopeptides such as Iturin and Plipastatin. The crude metabolites were purified and analyzed by LC-MS to confirm the mass of the metabolite and identify the molecular structure. Further work focuses on the identification of the gene corresponding to antifungal property.

Author Disclosure Block:

A. Sundararaman: None. **J. Young:** None. **C. Sung:** None. **S.S. Lee:** None.

Poster Board Number:

SATURDAY-073

Publishing Title:

Changes in the Rhizosphere Bacterial Community Composition in Cotton Plants

Author Block:

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Abstract Body:

The understanding of plant root-microbe associations has had an increasing interest during the last decades, as these interactions result in a dynamic microenvironment where bacteria play an important role in nutrient uptake and plant growth. The use of metagenomic methods has improved our understanding of the non-culturable component of the rhizosphere community. However, there are still gaps in the comprehension of this community, specially in field studies. Samples from bulk soil and soil up to 10 mm from the roots of *Gossypium hirsutum,* were taken from crops in experimental plots located in two sites under similar irrigation systems. Then, a 16S rRNA gene high-throughput sequencing method was used to explore the diversity and affiliation of the bacterial component (obtaining composite samples from 10 plants per treatment, amplified and analyzed by triplicate). Our aims were i) to observe the differences in the bacterial community structure between bulk soil samples and the rhizosphere of cotton plants in a long-term cultivated regosol, ii) to compare the rhizosphere communities in two soils with different characteristics, and iii) to observe if there are differences in the rhizosphere community under two different farming systems, i.e. organic and conventional. Our results showed that the structure of the bacterial community in bulk soil samples was different from that of the rhizosphere in *Gossypium hirsutum.* The bacterial phyla in the rhizosphere included Proteobacteria, Actinobacteria, Acidobacteria, Planctomycetes, among others. However, plants in the regosol, showed a higher proportion of bacteria belonging to the candidate phylum TM7. The conventional and organic farming system showed slightly differences in the composition of the bacterial community, although further studies most be done to clarify the modifications in the bacterial structure at higher taxonomic levels.

Author Disclosure Block:

R. Alcántara-Hernández: None. **A. Ponce-Mendoza:** None. **A. Wegier:** None.

Poster Board Number:

SATURDAY-074

Publishing Title:

***Bacteroidales* as Indicator of Fecal Contamination Source Tracking on Artificially Contaminated Tomato and Strawberry**

Author Block:

S. Garcia Alvarado, L. G. Hernandez, 66455, **J. A. Merino, 66455**, N. Heredia; Univ. Autonoma de Nuevo Leon, San Nicolas NL, Mexico

Abstract Body:

Background: Fecal contamination associated with the consumption of vegetables is a health issue worldwide. Current methods used to investigate the possible presence of pathogens, such as *Salmonella* spp. and *E. coli* O157: H7 in vegetables, are based in searching fecal indicator organisms; however, these are time-consuming and do not indicate the origin of contamination. It has been reported that the order *Bacteroidales* can be used as an alternative indicator and for tracking the source of contamination, due to host-specific sequences conserved in its genome. The objective of this work was to determine the association between fecal contamination, *Bacteroidales* and traditional bacteria indicators.**Methods:** Fecal sources from human and animal were used and applied on tomato and strawberry. Validation of oligonucleotides of host-specific (human, bovine and canine) markers of the 16S rRNA gene of *Bacteroidales* was performed using PCR. Tomato and strawberry samples were contaminated with diluted stool with an initial charge of *Bacteroidales* (between 6 and 8 log CFU/g) and 3 to 6 log CFU/g of traditional indicators of the various hosts. Traditional indicators (*E. coli*, total coliform and *Enterococcus)* were enumerated and the universal marker AllBac was quantified by qPCR. Host source was identified by PCR.**Results:** The limit of detection of PCR was 1.35 to 10.35 logarithmic gene copies, corresponding to an LOQ up to 2 cells. No correlation between levels of *Bacteroidales* and traditional bacterial indicators was observed. In all samples of contaminated vegetables, levels of *Bacteroidales* was 2 log CFU higher than traditional indicators; however, traditional indicators showed high variation and *E. coli* from bovine source was not detected in tomato samples. Identification of host specific markers in vegetables samples was 100% in almost all cases when the samples were contaminated with a 1:1000 dilution of feces. Contamination with dog feces was detected by PCR even at 1:100,000 dilution of feces, in 60% of cases.**Conclusions:** Based on these results, the use of Bacteroidales provide valuable information to identify the source of fecal contamination, and could help to reduce the risks of contamination of produce.

Author Disclosure Block:

S. Garcia Alvarado: None. **L.G. Hernandez:** None. **J.A. Merino:** None. **N. Heredia:** None.

Poster Board Number:

SATURDAY-075

Publishing Title:

Metagenomic Analysis of the Rhizobiomes of the Invasive Plant *Schinus terrebinthifolius* (Brazilian Pepper Tree) Suggest Active Alteration of Soil Microbiota Compared to Natives in Southeastern Florida

Author Block:

K. K. Dawkins, N. Esiobu; Florida Atlantic Univ., Boca Raton, FL

Abstract Body:

Background: The Brazilian peppertree (BP) is a category 1 invasive plant, displacing disturbed sites, mangroves and pinelands. BP is responsible for the loss of up to 3000 km^2 of terrestrial ecosystems in Central and Southern Florida. A 24.4 hectare plot previously invaded by dense BP vegetation could not be restored with natives until the top-soil itself was removed; which prompted our quest to determine the possible impact of BP and soil microorganisms. This report contrasts the rhizobiome (metagenomic) diversity and richness of BP and two adjacent non-invasive native plants across Southeastern Florida with the goal of identifying trends that could support allelopathy and or enemy recruitment strategies.**Methods:** Six sample sites spanning three counties across Southeastern Florida were sampled for rhizospheric soil of BP, Shepherd's needles (*Bidens alba)*, Firebush (*Hamelia* patens) and bare soil. Metagenomic DNA from rhizobacteria and fungi were extracted using a modified MoBio Powersoil DNA kit procedure. Bacterial 16S rDNA was sequenced and analyzed with the Illumina Mi-Seq system and QIIME software respectively. Arbuscular Mycorrhiza (AM) counts were done microscopically and the internal transcribed spacer (ITS) of extracted fungal DNA is being sequenced employing the Sanger sequencing method after cloning.**Results & Conclusion:**The average AM spore count of 221 +/- 38.4 spores/g of soil was significantly higher under BP compared to *Bidens alba* (151 +/- 28.4 spores/g), *Hamelia* patens (108 +/- 23.2 spores/g) and bare soil (83 +/- 14.4 spores/g) with a p value of <0.001. The presumptive Genus *Funneliformis* (32%) and *Septoglomus* (23%)were most abundant under BP. Over 5 million bacterial sequences were obtained producing 100,574 operational taxonomic units (OTU) where BP had the lowest average OTUs (9593). The most abundant phylum found throughout all samples were the *Proteobacteria* averaging 56.3%. Remarkably, BP soil had a 2 fold increase in prevalence of the phylum *Verrucomicrobia* (ANOVA, $F_{3,18} = 6.5$, $p = 0.004$). There was a drastic decrease in the order *Enterobacteriales* (16.4%) under BP with $p<0.001$ compared to bare soil (42.37%). These findings suggest BP may be recruiting specific microbes and eliminating others through allelopathy.

Author Disclosure Block:

K.K. Dawkins: None. **N. Esiobu:** None.

Poster Board Number:

SATURDAY-076

Publishing Title:

Investigation of an Antibiotic Biosurfactant Produced by a Plant-growth Promoting Strain of *Pseudomonas koreensis* Isolated from Connecticut

Author Block:

L. Iboki, E. Lewis Roberts, J. Thurber; Southern Connecticut State Univ., New Haven, CT

Abstract Body:

Fluorescent pseudomonads are of particular interest due to their biological control potential. The production of cyclic lipopeptide biosurfactants (CLPS) has been observed in some fluorescent pseudomonads and these CLPs have been linked to controlling growth of pathogenic microbes. While *Pseudomonas koreensis* has been isolated in both Korea and Mexico, only Mexican strains produce CLPs. The Mexican strains have also been shown to support plant health in tomato plants. In addition to a phylogenetic analysis, we examined biosurfactant production, antibiotic activity, and plant growth promotion activity of a new strain of *Pseudomonas koreensis* isolated in Connecticut, U.S.A.The 16s rRNA sequence of a fluorescent bacterium isolated from the rhizosphere of a Japanese Maple tree (*Acer japonica*) shared 97% identity with *Pseudomonas koreensis*. Evolutionary history of the Connecticut soil isolate was inferred by Maximum Parsimony analysis of the 16s sequence compared to other Pseudomonas strains including three *Pseudomonas koreensis* strains isolated in Mexico and two strains from Korea. The Connecticut isolate clustered with *P. koreensis* strains from Korea (95% bootstrap support). An atomized oil droplet assay detected a putative CLP biosurfactant produced by the new *P. koreensis* isolate grown on tryptic soy agar (TSA). A total of 0.3 µg (in 30µl methanol) of the biosurfactant spotted onto TSA plates showed marked antibiotic activity identified through clear zones of inhibition against *Staphylococcus aureus, Pseudomonas pudita,* and *Bacillus subtillis* overlaid on the plates. Addition of the bacterium to soils of twenty individual heirloom tomato (*Solanum lycopersicum*) plants resulted in significantly increased biomass (38% increase) over control plants. Furthermore, only 4 out of 48 individual tomato plants inoculated with the bacterium exhibited leaf rolling after being subjected to 3 days with no water at 40 °C, in contrast to 100% leaf rolling observed on un-inoculated controls.The ability to produce a biosurfactant differentiates our strain from the Korean isolates. In addition, our findings indicate that the Connecticut *P. koreensis* isolate could be used as a biological control agent, or to improve the health of tomato plants exposed to abiotic stresses.

Author Disclosure Block:

L. Iboki: None. **E. Lewis Roberts:** None. **J. Thurber:** None.

Poster Board Number:

SATURDAY-077

Publishing Title:

Bio-Augmentation Potentials of Indigenous *Azospirillum* Isolates in Drought Conditions

Author Block:

R. Anandan, D. Lakshmipriya, V. Nithyakalyani, L. Aswini, S. Rajkumar, P. Rajendran; Dr MGR Janaki Coll. of Arts and Sci. for, Chennai, India

Abstract Body:

Background: Indian sub-continent is facing a serious agricultural crisis in terms of fast shrinking cultivable lands. Genetically manipulated crops and farm chemicals have enhanced the farm production. But indiscriminate and unbalanced use of farm chemicals had led to a decline in the soil fertility. Urbanization has channelized water resources to domestic use inducing drought condition that has complicated the agro climate. South India, the 'cradle of paddy' is now largely embalmed with non-cultivable land mostly due to drought conditions. Soil microbes are actively investigated for their soil reclamation potential in drought conditions. In this background, this study was taken up to characterize *Azospirillum* spp. soil isolates and assess their *in situ* potentials to support plant growth and yield in drought conditions.**Methods:** Paddy field soil samples from 3 agronomic zones in Tamilnadu, India were screened for *Azopirillum* spp. using standard techniques. They were further confirmed with 16S rRNA PCR and evolutionary relationship established with MEGA6. *Azospirillum* soil isolates were screened for specific plant growth promoting traits that includes, IAA production, mineral solubilisation, extracellular enzymes, exopolysaccharide production (EPS), N2 fixation, siderophore and ammonia production. Isolates were tested for desiccation tolerance. *In situ* experiments were conducted with *Orzya sativa* (paddy) enforcing specific drought conditions with plant growth and yield as the end point.**Results:** A total of thirty two bacterial isolates were confirmed as *Azosipirillum* spp and their genotypic relationship established with 16S rRNA PCR. Their IAA production ranged from 9 - 25 μg/mL; Nase activity, 113-429 nmol C_2H_4/H/mg; and EPS, 32-214 mg/L. Two isolates exhibited remarkable desiccation tolerance and were proceeded to *in situ* studies. Both the isolates had a positive impact on the plant growth and yield with ASP9 having superior outcome especially when combined with an organic carrier, Panchakavya at drought conditions. Field trials in drought hit crop fields are underway to test their commercial application potentials.**Conclusions:** This study categorically demonstrates the bio-reclamation and plant growth promoting potentials of *Azospirillum* spp. especially under harsh field conditions

Author Disclosure Block:

R. Anandan: None. **D. Lakshmipriya:** None. **V. Nithyakalyani:** None. **L. Aswini:** None. **S. Rajkumar:** None. **P. Rajendran:** None.

Poster Board Number:

SATURDAY-078

Publishing Title:

Molecular Mechanisms for Virulence and Host Colonization of the Xylem Restricted Plant Pathogen Erwinia Tracheiphila

Author Block:

J. Rocha, L. Shapiro, R. Kolter; Harvard Med. Sch., Boston, MA

Abstract Body:

Erwinia tracheiphila (Enterobacteriaceae) is a Gram (-) plant pathogen that causes a fatal bacterial wilt in some cucurbit cultivars. *E. tracheiphila* is obligately insect-transmitted via the frass of striped or spotted cucumber beetles, which it is able to persistently colonize. Despite being one of the first bacterial phytopathogens described in the early 20th century, the molecular basis of plant pathogenicity and colonization capabilities for either plants or insect vectors are still unknown. The study of this model system is relevant both because of the economic losses due to bacterial wilt infection, and because whole genome sequencing data suggests *E. tracheiphila* may have recently emerged into its current niche, making it an important model for understanding the evolution and rapid emergence of plant pathogens.In this study, comparative genomic analyses were performed to identify candidate virulence genes and host colonization factors. Our *in silico* analyses revealed one *hrp* and one *inv/spa* Type III Secretion System (T3SS), and flagellar synthesis operons in the *E. tracheiphila* genome. The presence of these secretion systems is puzzling, as *E. tracheiphila* is xylem limited, which is an ecological niche where few microbes utilize T3SS or flagella. To investigate the functional importance of these operons, we developed the first molecular genetic tools for this bacterium. We generated *gfp*-transcriptional fusions for assessing gene expression *in vitro* and *in vivo* and constructed deletion mutants for determining factors essential for the colonization of both plant and insect hosts. Our results show that the T3SS *hrp* operon is up-regulated in the xylem during the early and middle stages of infection, relative to expression levels in pure culture. Inoculation experiments using clean deletion mutants showed that the *hrp* T3SS is essential for pathogenesis. We also showed that while flagellar motility increases the speed of movement through xylem and the rate of symptom appearance it is not absolutely essential for pathogenicity.These results provide the first insight into virulence mechanisms in this model. Continuing work will use the tools developed to examine additional virulence genes in order to understand this recently evolved plant pathogen.

Author Disclosure Block:

J. Rocha: None. **L. Shapiro:** None. **R. Kolter:** None.

Poster Board Number:

SATURDAY-079

Publishing Title:

Microbial Risk Assessment of Treated Wastewater Used for Urban Farming

Author Block:

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Abstract Body:

In Ethiopia, following urbanization especially in Addis Ababa, populations living around the suburbs have been using treated wastewater by stabilization pond for urban agriculture and produce different vegetables for their daily consumption and also outlet to the urban population. However, the suitability of the treated wastewater for irrigation purpose has never been studied before, so that this suitability assessment has crucial concern of the health of communities that live around the irrigation and also to the whole population of the city who consume the products. 500 ml of wastewater samples were taken from the inlet and outlet points of stabilization pond of Addis Ababa Water & Sewerage Authority and another 500 ml of water sample from the irrigation area of the urban farming with sterile bottles and transported to Ethiopian Public Health Institute (EPHI) water quality laboratory with icebox and analyzed for microbial tests such as: Total coliform count, Fecal coliform count and E.coli counts.The following table presents the Microbial level of the three water samples taken from different sites which indicates there is a significant decrement in the Microbial load before and after treatment of the wastewater:

Microbial tests	**Sample source**		
	Inlet of sewages	**Outlet of sewages**	**Irrigation site**
Total coliform counts	1.5 x10^6CFU/ml	3x10^4CFU/ml	3x10^4CFU/ml
Fecal coliform counts	5x10^5CFU/ml	1x10^3CFU/ml	1.1x10^3CFU/ml
E-coli counts	4x10^5CFU/ml	6x10^2CFU/ml	6.2x10^2CFU/ml

Generally, although the results show a significant change of Microbial load by the treatment pond (about 2 logs), the result revealed that the treated water still insufficient to use for irrigation purpose as it is recommended by WHO; the level of fecal coliform for irrigation should be less than 1000/100ml (WHO, 2006).

Author Disclosure Block:

T.A. Bayleyegn: None.

Poster Board Number:

SATURDAY-080

Publishing Title:

Impact of Different Temperatures on Biology and Time Concentration Mortality of *Plutella xylostella* L. (Lepidoptera; Plutellidae) Larvae against *Bacillus thuringiensis* (Bt) and Deltamethrin

Author Block:

W. Jaleel[1], S. Saeed[2], H. Yurong[1], Q. Saeed[2], M. N. Naqqash[3]; [1]South China Agricultural Univ., Guangdong, Guangzhou, China, Guangzhou, China, [2]Bahauddin Zakariya Univ., Multan, Multan, Pakistan, [3]Dept. of plant production and technologies, Faculty of Agricultural Sci. and Technology, Niğde Univ.,, Nidge, Turkey

Abstract Body:

Background: *Plutella xylostella* L.is a most damaging pest of brassicae crops in most countries of world i.e. Pakistan, China e. t. c. with preferred hosts cabbage (*Brassica oleracea* var. capitata) and cauliflower (*B.oleracea* var. botrytis). So cost of control of *P. xylostella* is around 4.5 billion dollars annually on world-wide basis primarily with chemical control.Effective management of this pest is essential due to the fact that these vegetables are economically very important crops with high cosmetic value.**Methods:** Evaluation of suitable temperature for its development and second testing was performed by bioassay with leaf dip method on unselected population of *P. xylostella* 3rd instar larvae against deltamethrin and *Bt Cry1Ac* at different temperatures i.e. 15°C, 20°C, 25°C and 30°C.**Results:** Total larval duration was significantly longer at 15°C temperature. Fecundity was 288.09±4.65 eggs per female in its life at 20°C that was significantly higher than other temperatures. The *P. xylostella* mortality was higher (87.50%) against deltamethrin at 30°C after 24 hours. So, same trend was depicted against *Bt Cry1Ac.* Results concluded that with the increase in temperature, mortality of *P. xylostella* was increasedagainst deltamethrin and *Bt Cry1Ac*.**Conclusions:** According to result that was life duration of *P. xylostella* was inversely proportional to temperature but mortality was directly proportional to temperature. Base on the ecological assumption temperaturestrategies is the best tool for Integrated Pest Management against *P. xylostella* at farmer level.

Author Disclosure Block:

W. Jaleel: N. Other; Self; Ph.D Scholar. N. Other; Spouse/Life Partner; Research Assistant. **S. Saeed:** N. Other; Self; Associate Professofer. **H. Yurong:** N. Other; Self; Professor. **Q. Saeed:** N. Other; Self; Lecturer. **M.N. Naqqash:** N. Other; Self; Ph.D Scholar.

Poster Board Number:

SATURDAY-081

Publishing Title:

Elucidating Molecular Underpinnings of Extracellular Electron Transfer in Metal Breathing Microbes Using Evolutionary Genomics Approaches

Author Block:

S. Phadke, K. Carpenter, O. Bretschger; J. Craig Venter Inst., La Jolla, CA

Abstract Body:

Extracellular electron transfer (EET) is a process through which microbes shuttle electrons from donor substrates to solid phase electron acceptors such as various metal oxides. Microbial EET is ubiquitous in natural ecosystems and directly determines microbial fitness in some environments; however, we largely lack the knowledge of how selection on rates of EET shapes microbial genome composition as well as genetic networks involved in EET. We combined the power of experimental evolution and next generation sequencing to identify the rates as well as patterns of single nucleotide polymorphisms (SNPs) and Indels in pure cultures of EET-active bacteria grown under controlled laboratory conditions in the presence of electrochemical stimuli. We compared SNP and Indel patterns in biofilms vs. suspension communities to understand if cellular phenotype affects the genome-wide mutational landscape. Our analysis reveals that the genetic makeup of cells in biofilm vs. suspension communities diverges during evolution. Our approach also allows us to reveal previously hidden players in the genetic networks underlying the EET activity. Because EET-active microbes have been held as promising biocatalysts in technologies such as wastewater treatment and biosensing that integrate bioelectrochemical stimuli, our results emphasize the need to characterize evolutionary trajectories of these important biocatalysts for developing robust biotechnological applications as well as understanding the evolutionary context of dissimilatory metal reducing bacteria.

Author Disclosure Block:

S. Phadke: None. **K. Carpenter:** None. **O. Bretschger:** None.

Poster Board Number:

SATURDAY-082

Publishing Title:

Control of Nanofiltration Membranes Biofouling by Using a New Anti-Fouling Coating

Author Block:

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Abstract Body:

Background: Membrane biofouling is a major impediment limiting a more widespread use of membrane technologies. The formation of biofilm on the membrane surface can cause considerable technical problems and economical losses, especially in reverse osmosis (RO) and nanofiltration processes. **Methods and Results:** This work describes a new multi-functional formulation that permits either reversible or irreversible coating of membrane during normal operation. The formulation consists of antimicrobial micelles which was formed by natural and synthetic materials, these include functionalized chitosan, phosphatidylcholine, primary, secondary and tertiary amine as well as zwitterionic compounds. Tests show that the coating exhibit both contact-killing and anti-adhesion properties that are effective in retarding biofilm formation on the membrane surface. The treated membranes can maintain better than 2 log reduction (99 %) of *E. coli* and effectively prevent surface colonization even in the presence of soiling agents. Comparisons made with uncoated membrane showed no reduction in water permeation and dye rejection rate, indicating minimal impact of the treatment on the original membrane filtration property. Moreover, stability tests showed that the membrane retained better than 85% of the filtered anti-biofoulant after normal cross-flow operation.**Conclusions:** This new coating material can be applied in filtration membrane systems in order to prevent fouling problems, and improve the industrial efficiency.

Author Disclosure Block:

Y. Li: None. **H.R. Zhang:** None. **K.L. Yeung:** None. **N. Zhan:** None.

Poster Board Number:

SATURDAY-083

Publishing Title:

Role of Nutritional Richness, Hypoxia, Bioactive Cell-Wall Glycopeptidolipid on Biofilm Formation in *Mycobacterium avium* subsp. *hominissuis*

Author Block:

Y. Tateishi[1], S. Matsumoto[1], Y. Nishiuchi[2]; [1]Niigata Univ., Niigata, Japan, [2]Osaka City Univ., Osaka, Japan

Abstract Body:

Background: *Mycobacterium avium* subsp. *hominissuis* (MAH) is the major causative agent of non-tuberculous mycobacteriosis, the representative case of zoonotic and environment-related infectious disease in the industrialized countries. Although glycopeptidolipid (GPL), a MAH-specific bioactive surface molecule, has been suggested to confer colony morphology and swarming in previously, the precise mechanism of biofilm formation in MAH is yet to be know. In this study, we investigated the atmospheric and nutritional condition and the role of glycopeptidolipid (GPL) on biofilm formation in MAH. **Methods:** Seventeen environmental isolates were obtained from bathrooms of MAH lung disease patients' residence. Biofilm formation assay was performed in glass tubes and in polystyrene plates, in normal eutrophic media (7H9/ADC) or oligotrophic media (only containing less than one-tenth amount of albumin and glycerol), under hypoxic (5% O_2), hypercapnic (5% CO_2), or ambient condition, respectively at 37 ^oC. The ultrastructure of biofilm was analyzed by surface electron microcopy. Experiment of exogenous supplementation of GPL was performed to obtain a final concentration of GPL as 100, 500, and 1,000 μg ml^{-1} when mixed with growing rough mutant cells. **Results:** MAH reference strain 104 formed biofilm as surface pellicle in glass tubes only in eutrophy under hypoxic condition, neither in eutrophy under atmospheric condition nor in oligotrophy. Thirteen strains (76 %) formed biofilm in polystyrene plates under hypoxic condition, and 16 strains (94 %) formed thick biofilm under hypoxia as equal to or greater than under hypercapnia in glass tubes. GPL-producing wild type strains developed much thicker biofilm than GPL-deficient rough mutants. The biofilm of wild type strains had robust membranous structure and possessed a plenty of bacteria compared with that of rough mutants. Exogenous supplementation of GPL in rough mutants developed the same phenotype of biofilm as wild type strains. **Conclusions:** Both hypoxia and nutritional richness are necessary factors for biofilm formation in MAH. Furthermore, GPL plays a major role on biofilm development in MAH. This study provides a new insight into the molecular pathogenesis and efficacious prevention of biofilm infectious disease.

Author Disclosure Block:

Y. Tateishi: None. **S. Matsumoto:** None. **Y. Nishiuchi:** None.

Poster Board Number:

SATURDAY-084

Publishing Title:

Synergistic Activity in Biofilm Formation and Biocontrol Between *Bacillus subtitlis* and *Bacillus cereus*

Author Block:

Y-y. Yu[1], F. Yan[1], J-h. Guo[2], Y. Chai[1]; [1]Northeastern Univ., Boston, MA, [2]Nanjing Agricultural Univ., Nanjihn, China

Abstract Body:

Rhizosphere bacteria *Bacillus subtilis* and *Bacillus cereus* are widely used biological control agents (BCAs). Our previous studies show that *B. subtilis* SM21 and *B. cereus* AR156, when applied together, were more efficient in controlling tomato wilt disease caused by *Ralstonia solanacearum*. This suggests a synergistic activity in biocontrol between these two species. *B. subtilis* is able to form structurally complex multicellular communities, known as biofilms. Biofilm plays a key role in process of biological control of tomato wilt disease by *B. subtilis*. We thus hypothesize that the promoted biocontrol efficacy upon co-application of *B. subtilis* and *B. cereus* was due to enhanced biofilm formation by *B. subtilis* during bacterial interaction. In this study, we found that *B. cereus* AR156 was able to enhance biofilm formation by *B. subtilis* SM21 upon co-culture. In addition, matrix gene expression was upregulated and pellicle biomass increased upon co-culture. We separated the biofilm-inducing active compound from AR156 pellicle by sonication and filtration of the supernatant. This active compound is able to stimulate *B. subtilis* biofilm formation in a non-biofilm-inducing medium (LB medium) in a dose-dependent manner. In search of potential sensor(s) for the active compound, we noticed that biofilm of a SM21 derivative bearing Δ *kinC* mutation was not induced by the compound, suggesting the histidine kinase KinC might be involved in sensing the active compound. In conclusion, our preliminary data showed that *B. cereus* AR156 enhanced *B. subtilis* SM21 biofilm and the stimulation is possibly mediated by activating *B. subtilis* KinC. This study provided molecular evidence for the synergistic biocontrol activity.

Author Disclosure Block:

Y. Yu: None. **F. Yan:** None. **J. Guo:** None. **Y. Chai:** None.

Poster Board Number:

SATURDAY-086

Publishing Title:

Prevalence of Antibiotic Resistance among Biofilm Forming Bacteria Isolated from Domestic Environments in Dubai

Author Block:

S. Balaji, K. A. Vasudev, P. Srinivasan, S. Singh, **N. Sood**, T. S. Gokhale, R. Subramanian; Birla Inst. of Technology & Sci., Dubai, United Arab Emirates

Abstract Body:

Background: Biofilm forming bacteria in domestic environments are potential source of pathogenic microbes and food spoilage. Comprehensive studies on biofilm bacterial communities in household environments and antibiotic resistance remain unexplored in UAE. The present study was to isolate, characterize and to screen for antibiotic resistance in bacteria isolated from biofilms.**Methods:** Samples from households comprising of kitchen sinks, household drain systems and shower curtains were obtained and grown on nutrient, selective and differential media. A total of 27 different colonies were isolated from 12 samples. Bacterial isolates were identified based on colony morphology, Gram's staining and biochemical characterization.**Results:** Several genera were found that include *Pseudomonas, Shigella, Bacillus, Salmonella, Vibrio, Enterobacter*, *Campylobacter* and *Proteus* species. Nearly two third of the isolates were *Enterobacter* and *Proteus* species. The selected 27 isolates were subjected to antibiotic resistance test on nutrient medium using the standard antibiotics disks. Most of the bacterial isolates were resistant to tested antibiotics. Further studies on 16s rRNA based identification and phylogenetic profiling in progress.**Conclusions:** The preliminary study on biofilm in household environments is significant to catalogue in this region due to sparse work conducted so far. The data could be useful to correlate certain infectious diseases and the role of biofilm forming bacteria in domestic environments.

Author Disclosure Block:

S. Balaji: None. **K.A. Vasudev:** None. **P. Srinivasan:** None. **S. Singh:** None. **N. Sood:** None. **T.S. Gokhale:** None. **R. Subramanian:** None.

Poster Board Number:

SATURDAY-087

Publishing Title:

Synergistic Virulence Generation by *Pseudomonas aeruginosa* Strains, Implications for Application in Bioelectrochemical Systems

Author Block:

E. M. Bosire, L. M. Blank, M. Rosenbaum; RWTH Aachen Univ., Aachen, Germany

Abstract Body:

Pseudomonas aeruginosa virulence factors, especially phenazines, have gained attention for application as redox mediators in bioelectrochemical systems (BES). Other pathogenicity factors like rhamnolipids and siderophores might play an important role in enabling biofilm formation on the electrodes. In bacterial-based BES, bacteria metabolise substrates and liberated electrons are shuttled to an external electron acceptor (anode) *via* soluble redox mediators among other modes of electron transfer. One of the promising applications of these technologies is electricity generation from wastewater. Like in natural communities, microorganisms often build synergistically interacting microbial consortia in wastewater BES. In these interactions, consumption of fermentation products by *P. aeruginosa* leads to increased phenazine production and consequently increased electroactivity of the whole community. This natural phenomenon can be a basis to design synergistically interacting co-cultures, where *P. aeruginosa* is the redox mediator producer. However, the physiological roles of these secondary metabolites, how they are employed under varying ecological conditions across the *P. aeruginosa* strains, and the capacity of the phenazines as redox mediators in BES is yet to be fully understood. To investigate the performance of *P. aeruginosa* strains (PA14, PA01 and a BES isolate KRP1), we cultured them in media containing different substrates including fermentation products, assessed their virulence factor production and electroactivity. Our study reveals remarkable differences among the three strains in the production of phenazines, rhamnolipids and the tendency to form biofilms under the BES conditions and different substrates. Further, the strains exhibit different electroactivity capacities due to differences in phenazine spectra. The virulence factor generation and the electrochemical behaviour of the phenazines observed will not only be useful in improving BES performance but also in understanding the usage of phenazines in the different redox-controlled ecological niches of *P. aeruginosa*.

Author Disclosure Block:

E.M. Bosire: None. **L.M. Blank:** None. **M. Rosenbaum:** None.

Poster Board Number:

SATURDAY-088

Publishing Title:

Optimization of ALI Assay for Model Archaeon and Isolation of Novel Adhesion Mutants

Author Block:

G. Legerme, M. Pohlschroder, R. Esquivel; Univ. of Pennsylvania, Philadelphia, PA

Abstract Body:

Biofilm formation is a fundamental adaptation to environmental stress that is found throughout the prokaryotic domains. The Air-liquid interface (Ali) assay is a method used to quantify cell surface adhesion, the first step in biofilm formation. Although this assay has been successfully used to screen transposon insertion libraries for several bacterial species, an analogous assay had not been optimized for any archaeal species. In the model archaeon *H. volcanii*, we have now adapted the Ali assay for use in 96-well plates, allowing us to rapidly screen a transposon insertion library for adhesion mutants. Using this protocol, we identified 19 genetically diverse adhesion mutants. One of these mutants contains an insertion between genes encoding CheB and CheW, chemotaxis machinery components primarily known for their effect on motility in bacteria. In a second mutant, we identified an insertion in *pilB1*, a gene that encodes a paralog of the *H. volcanii* ATPase PilB3, which plays an essential role in surface adhesion by aiding in the biosynthesis of adhesion pili, suggesting the efficacy of this approach for identifying proteins that play roles in pilus biosynthesis and function, as well as in adhesion. Moreover, considering that PilB1 is not essential for *H. volcanii* surface adhesion like PilB3 is, as seen in using the 12-well Ali assays, this suggests the possibility of differential supplemental expression of specific adhesion pili in different conditions. In fact, in using the 12-well assay, of the five *H. volcanii* PilB paralogs and of its 42 predicted pilins, only PilB3 and six pilins appeared to be required for adhesion. Since *pilB1* is co-regulated with genes encoding additional predicted pilins, these pilins may be subunits of pili that mediate surface adhesion under certain conditions. We are currently determining the specific differences between conditions used for the 12-well assay compared to the 96-well assay that affect the surface adhesion of the *pilB1* mutant. Additional adhesion mutants contained insertions in genes that encode transcriptional regulators as well as in genes encoding componentsof metabolic pathways. Further characterization of these mutants should advance our understanding of the mechanisms underlying archaeal biofilm formation..

Author Disclosure Block:

G. Legerme: None. **M. Pohlschroder:** None. **R. Esquivel:** None.

Poster Board Number:

SATURDAY-089

Publishing Title:

Adhesion of Bacteria to Microplastics in Freshwater

Author Block:

M. Hossain, M. Jiang, Q-H. Wei, L. Leff; Kent State Univ., Kent, OH

Abstract Body:

Microplastics (plastic debris less than 5mm in diameter) are of particular concern to the environment. Previous research has shown that microplastics provide a substratum for bacterial adhesion in the oceans. However, there is a paucity of information on the adhesion of freshwater bacteria to plastics, especially microplastics. We examined the following microplastics: polypropylene (PP), polystyrene (PS), high-density polyethylene (HDPE), and low-density polyethylene (LDPE). Each type of plastic possesses specific surface morphologies and characteristics which influence adhesion of the bacterial species: *E.coli*, *A.calcoaceticus*, *B.cepacia*, *S.paucimobilis*, *P.aeruginosa*, *Synecococchus* spp.Scanning Electron Microscopy (SEM) was used to visualize and quantify the colonization of recently discarded "new" microplastics at weeks 0, 4, and 8 composed of PP, PS and PE (representing both HDPE and LDPE) and "eroded" microplastics sampled from the beaches adjacent to Cleveland, OH. The bacteria were allowed to colonize microplastics in water from Lake Erie. Fluorescent microscopy was also used to quantify bacterial colonization. Quantification of EPS was performed to determine how plastic surface characteristics affect bacterial adhesion properties.Imaging showed a gradual increase in attachment of bacteria on all forms of microplastics. PS exhibited high microbial attachment through the entirety of the study, while HDPE, LDPE and PP had relatively minimal microbial attachment at week 4 and had biofilm matrices by week 8. Eroded microplastics had higher bacterial attachment than new microplastics.Imaging indicated that presence of bacterial flagella affects bacterial attachment and EPS production, with peritrichous *E.coli* displaying highest bacterial abundance on microplastics while producing the least amount of EPS. Meanwhile, non-flagellated *A.calcoaceticus* had lowest abundance and highest amount of EPS production.Our findings indicate that bacterial attachment to microplastics in freshwater is influenced by the unique properties of different classes of microplastics. As the plastic ages in the environment, its surface becomes eroded causing higher bacterial attachment. In addition, bacteria adopt varying strategies to attach to the plastic surfaces based on their physiological properties.

Author Disclosure Block:

M. Hossain: None. **M. Jiang:** None. **Q. Wei:** None. **L. Leff:** None.

Poster Board Number:

SATURDAY-090

Publishing Title:

Biofilm Formation on 304 Stainless Steel in Marine Water Studied by Optical and Scanning Electron Microscopy

Author Block:

B. S. Neves, A. M. N. Korres; Inst. Federal de Educação, Ciência e Tecnologia do Espírito Santo, Vitória, Brazil

Abstract Body:

Biofilms are complex communities of microorganisms attached to surfaces or associated with interfaces. Adherence of bacteria to metal surfaces can initiate the biofilm formation within which the metabolic processes can significantly affect of the corrosion surface. This study reports microbial biofilm formation on 304 stainless steel surface in marine water by the counting (CFU/cm^2) and image by optical and scanning electronic microscopy. Experiments were performed in laboratory. Steel coupons were immersed in marine water collected from Ilha do Boi Beach (Vitória, ES, Brazil) during four weeks. CFU/cm^2 and images were determined for each time using optical stereomicroscope (Leica EZ4 HD) and scanning electron microscope (Zeiss 900, Germany). Samples were sputtered (Super Cool Sputtering System, Bal-Tec®, SCD 050, Germany) and images were obtained in SEM. Results showed biofilm was formed in all situations, except on the first week. CFU/cm^2 indicated biofilm formation on second, third and fourth week, respectively $5{,}15 \times 10^4$, $9{,}15 \times 10^5$ e $8{,}74 \times 10^5$. First week showed number of CFU/cm^2 of $2{,}50 \times 10^2$, indicating biofilm was not formed considering a value of 10^3 and 10^5 cells per cm^2 to biofilm formation. Images from stereomicroscope in fresh samples showed the steel surface with microbial adhesion offering a real dimension of the preview image, with the zoom facilitated look the details of the agglomeration, texture and color microbial growth. The results of the test EDS associated with scanning electron microscope images validated the data indicating the existence of iron oxides because the surface of the coupon, sodium chloride because to sea water, and also organic substances due to the formation of biofilms. The high-resolution images show the biofilm growth during the period of the study. Morphological characterization by optical microscope showed the presence of elliptical yeast like organisms. The study of biofilms becomes important in marine environment because its adhesion to surfaces may cause biofouling and deterioration of structures, leading losses for various sectors.

Author Disclosure Block:

B.S. Neves: None. **A.M.N. Korres:** None.

Poster Board Number:

SATURDAY-091

Publishing Title:

***Deinococcus aquaticus,* Life or Death in a Biofilm Driven by Desiccation**

Author Block:

A. Zarling, F. Mayer, K. Laux, S. Mitchell, N. Weiss, R. Krueger, A. Limbach, A. Dorner, J. Draven, G. Dungar, C. Schmitz, R. Koll, Z. Stuebs, S. Mueller-Spitz, A. Horkman; Univ. of Wisconsin Oshkosh, Oshkosh, WI

Abstract Body:

Desiccation is a state where cells are exposed to extremely dry conditions, which results in numerous physiological changes and may lead to cell death upon rehydration. The extremophile *Deinococcus radiodurans* is resistant to long-term desiccation related to its ability to repair DNA damage. Desiccation tolerance is assumed to be a common trait for all members of this genera. However, whether this trait provides an ecological advantage for deinococci inhabiting biofilm environments that are prone to extreme variation in water potential is a question of interest. *D. aquaticus* are common members of shallow freshwater biofilms, yet it is unknown what controls their dominance and survival under these harsh conditions. To address this question, the survival and morphological changes of four *D. aquaticus* strains was explored following desiccation and rehydration over a six week period. All cultures were grown to mid-exponential phase prior to desiccation to ensure the cells possessed the machinery essential for DNA repair. Regardless of the biofilm habitat, the four isolates survived six weeks of desiccation; however, each strain showed different patterns of regrowth after rehydration. Strain P17 had doubled cell counts after week one and two showing the potential for reductive division post-rehydration. However, between 60-88% of the starting culture for the other three strains (P34, P43, and P71) died following one week of desiccation. Two strains (P34 and P71) were particular sensitive to long-term desiccation with less than 5% of the starting culture remaining viable. Even though two strains (P34 and P43) were isolated from the same biofilm habitat and share genome-wide similarity, survival was significantly different. The variation in the long-term desiccation survival was attributed to an extracellular matrix excreted by the individual strains. Based upon scanning electron micrographs of desiccated cells, both P17 and P34 were heavily encased in a protective matrix. This thick matrix was not seen for strains P71 or P43. We hypothesize that resistance to desiccation stress in *D. aquaticus* relates to strain viability in expression of an extracellular matrix when grown in a pure culture and predict these strains may protect other ecotypes present in a mixed community.

Author Disclosure Block:

A. Zarling: None. **F. Mayer:** None. **K. Laux:** None. **S. Mitchell:** None. **N. Weiss:** None. **R. Krueger:** None. **A. Limbach:** None. **A. Dorner:** None. **J. Draven:** None. **G. Dungar:** None. **C. Schmitz:** None. **R. Koll:** None. **Z. Stuebs:** None. **S. Mueller-Spitz:** None. **A. Horkman:** None.

Poster Board Number:

SATURDAY-092

Publishing Title:

Growth-Phase Dependent Attachment of *Acidithiobacillus ferrooxidans* to Metal Sulfide Ores

Author Block:

J. Iordanou, S. Popko, **S. E. Blumer-Schuette**; Oakland Univ., Rochester, MI

Abstract Body:

Initial contact between *Acidithiobacillus ferrooxidans* and surfaces is important for the development of a monolayer biofilm that facilitates direct dissolution of metal sulfides. Typical extracellular structures used to mediate cell-surface attachment include flagella and pilus systems. While the genome sequence for *A. ferrooxidans* ATCC 23270 does not encode for flagella, both type IV and tight adherence pilus systems have been identified from the available genome sequence. Using the Integrated Microbial Genomes database to query for additional pilus systems, we also identified genes encoding for chaperone/ usher pili in the genome of *A. ferrooxidans* that may also be used for attachment to surfaces. Here, we propose that the pilus systems used in surface attachment are expressed in a growth phase dependent manner, and are crucial for attachment to hydrophobic surfaces. Initially to determine if there are growth phase-dependent effects on attachment, we measured biofilm deposition by *A. ferrooxidans* grown on soluble iron(II). Crystal violet staining of the biofilm formed on hydrophilic (borosilicate glass) and hydrophobic (polystyrene) surfaces were used to quantify biofilm formation over time. Polystyrene supported cell attachment, with over twice as much biofilm being deposited. Increases in biofilm also correlated with active growth of the planktonic cells, as determined by enumeration using microscopy. Aside from inert surfaces, we also monitored the ability of cells grown on soluble iron to attach to pyrite, an insoluble iron sulfide. Comparing the number of planktonic cells left after incubation with pyrite at various growth time points, we observed a 58% reduction in *A. ferrooxidan*s attachment during the transition to stationary phase. This result was reproduced in three independent cultures. In order to determine which pilus systems are being expressed prior to entry into stationary phase, we designed primer sets for quantitative RT-PCR to monitor expression of key genes in the three distinct pilus gene clusters. Our results so far indicate a role for attachment mechanisms that are hydrophobic in nature and are expressed during active growth of the planktonic culture. Data on the expression of the three pilus systems will add to our mechanistic understanding of how A. ferrooxidans initiates attachment to metal sulfides.

Author Disclosure Block:

J. Iordanou: None. **S. Popko:** None. **S.E. Blumer-Schuette:** None.

Poster Board Number:

SATURDAY-093

Publishing Title:

Quorum Sensing in *Paracoccus denitrificans* PD1222 Inhibit Cell Aggregation

Author Block:

K. Morinaga, M. Toyofuku, N. Nomura; Univ. of Tsukuba, Tsukuba, Japan

Abstract Body:

Various bacteria communicate with each other via signal molecules to control their behavior. Especially, cell density dependent cell to cell communication is called quorum sensing. While most quorum sensing studies are based on pathogenic bacteria, numerous bacteria in the environment possess' quorum sensing systems, which functions are not fully understood (Churchill MEA, *et al.*, 2011). To investigate the role of quorum sensing in a non-pathogenic bacteria, we used *Paracoccus denitrificans*, which is frequently isolated from activated sludge or soils. *P. denitrificans* synthesizes N-hexadecanoyl-AHL (C16-HSL) (Schaefer AL, *et al.*, 2002). To examine the phenotypes regulated by C16-HSL, we constructed a non-AHL-producing mutant (*luxI* mutant) of *P. denitrificans* Pd1222. The *luxI* mutant strongly aggregated while the wild-type did not. The aggregation of the *luxI* mutant was not observed when C16-HSL was added to the culture. This result suggests that quorum sensing in *P. denitrificans* inhibits cell aggregation. SEM analysis showed that the *luxI* mutant produced fiber-like extracellular matrix that was not observed in the wild-type. To identify the genes involved in this quorum sensing-inhibited aggregation, we screened transposon mutants of the *luxI* mutant. Several transposon mutants of the *luxI* mutant that did not aggregate were obtained and the positions where the transposon was inserted were identified. Most transposons were inserted in putative polysaccharide synthesis genes, suggesting that quorum sensing in *P. denitrificans* Pd1222 regulates aggregation through the production of extracellular polysaccharides. Our results imply that *P. denitrificans* Pd1222 avoids forming cell aggregates when the cell density is high.

Author Disclosure Block:

K. Morinaga: None. **M. Toyofuku:** None. **N. Nomura:** None.

Poster Board Number:

SATURDAY-094

Publishing Title:

***In Vitro* Response to Guanidine Desinfectant Cleaner (GDC) on Multidrug Resistant Gram Negative Bacteria**

Author Block:

M. G. Quinteros[1], P. Rigou[1], R. D. Marino[2], A. Farinati[1]; [1]SALVADOR Univ., MARTINEZ, BUENOS AIRES, Argentina, [2]Hosp. FJ Muñiz, BUENOS AIRES, Argentina

Abstract Body:

Background: The multidrug resistant bacteria are a concern in the hospital-acquired infections. The bacterial cells are in planktonic or within biofilm (BF). This have intrinsic resistance to conventional antimicrobial substances, including disinfectants. This event has motivated new approaches to eliminate or disrupt BF after it has already been formed. **Aim:** to study the response of a chemical derivative of guanidine (poli hexametilen guanidine clorhidrate-PHG) (GDC) on planktonic (PL) and biofilm (BF) multidrug-resistant bacteria (MDR).**Methods:** bacteria species used were: IMP13; KPC and VIM producer *Pseudomonas aeruginosa* (Pae) n=3; CMY-2 and CTX-M-2 *Escherichia coli* (Eco) n=2; CTX-M-15 and KPC-2 *Klebsiella pneumoniae* (Kpn) n=2; *Acinetobacter baumannii* (Aba) n=1. The minimal inhibition dilution (MID) for all those planktonic bacteria was 1/100 of GDC. **Biofilm:** A dilution of 100 microliters overnight from each bacteria was inoculated in 6 TS Broth (TSB) recipient and a glass coupon (GC) is put in as a biofilm support. All isolates were incubated at 37^0C with an without GDC in a recommended dose (1/100) and were observed at 24 hours (previously we determined the BF formation at different hours). Everything was read by optical microscopy previous crystal violet tinction.**Results:** we observed that the MID was 1/500 and 1/400 for planktonic MDR. Over MDR BF the 1/100 dose was not effective; except for Eco: bacterial growth was zero for the dose and time recommended by the manufacturer. Some BF exposed to disinfectant at recommended dose should be interacting longer; *Acinetobacter baumannii* biofilms should be tested at higher doses of disinfectant in order to evaluate the dose at which it is eradicated.**Conclusions:** GDC is useful to clean contaminated surfaces with MDR planktonic bacteria but not for BF at recomended doses.

Author Disclosure Block:

M.G. Quinteros: None. **P. Rigou:** None. **R.D. Marino:** None. **A. Farinati:** None.

Poster Board Number:

SATURDAY-095

Publishing Title:

Anti-Bacterial and Anti-Biofouling Effects of Self-Assembly Cationic Polymers Coated on Multiple Substrates

Author Block:

H. ZHANG[1], K. L. YEUNG[2]; [1]The Hong Kong Univ. of Sci. and Technology, Hong Kong, Hong Kong, [2]Hong Kong Univ. of Sci. and Technology, Hong Kong, Hong Kong

Abstract Body:

Background: Water related Industrial applications including cooling tower systems, water reservoir tanks and membrane filtration processes are susceptible to microbial contamination. Various kinds of chemical biocides are used to remove the bacteria. However, many microorganisms in aquatic environment tend to colonize on surface of substrates by encapsulating themselves into slimy matrix, which is known as biofilm. Once biofilms are formed, their resistance to biocides is greatly improved, and result in huge loss in industrial processes. **Method and Result**: This work studied the effect of cationic self-assembly polymers on biofouling reduction. Single polymer and combination of different polymers were formulated into micelles and applied onto different substrates including PVC, stainless steel and glass. Contact killing tests were carried out on *E.coli*, *P. aeruginosa*, *S. aureus and B. subtilis*. 4 log (99.99%) reduction was achieved on all tested bacteria strains. The formulation was coated onto different substrates using dopamine as adhesive by layer-by-layer method. The stability of coating was measured by immersing samples for over 2 weeks. X-ray photoelectron spectroscopy was applied for the quantification of functional moieties on the coated substrates surface. The anti-biofouling test was analyzed by scanning electron microscope. The quantification of bacteria attached onto the coated surface was measured by Direct Enumeration technique. In the anti-adhesion test, the coating can achieve 2 log (99%) reduction of *E.coli* attachment onto PVC and 85% reduction of E.coli attachment onto stainless steel. **Conclusion:** The study showed both high bactericidal and high anti-adhesion efficacy of formulated cationic polymer coating. The coating can be stably attached onto multiple substrates using layer by layer methods, which can be used in academic research and industrial applications.

Author Disclosure Block:

H. Zhang: None. **K.L. Yeung:** None.

Poster Board Number:

SATURDAY-096

Publishing Title:

Fungal Isolates Form Biofilms in Hemodialysis Fluids

Author Block:

R. H. Pires[1], L. Teodoro[1], L. G. Lopes[1], C. H. G. Martins[1], M. C. Jamur[2]; [1]Univ.e de Franca, Franca, Brazil, [2]Univ.e de São Paulo, Ribeirao Preto, Brazil

Abstract Body:

Background: In hemodialysis procedure, the dialysate is constituted by hemodialysis machines mixing treated water (by reverse osmosis), acid and bicarbonate concentrates. Appropriate water purity has been standardized by a variety of organizations, professional societies and pharmacopoeias. The absence of any type of antimicrobial in the water makes it susceptible to microbial contamination downstream of the water treatment, which contributes to the development of biofilms in the dialysate pathway of the proportionating system of hemodialysis machines. This study aimed to evaluate the ability of biofim formation by fungal isolates in the dialysate and its constituent solutions, comparing to the RPMI culture medium.**Methods:** *Aspergillus, Fusarium* and *Penicillium* isolates were recovered from the water circuit of a hemodialysis facility. The biomass of the biofilms were determined by the crystal violet method and its ultrastructures were visualized by scanning electron microscopy (SEM).**Results:** *Fusarium* strains (10 isolates) were the most homogeneous group in biofilm-forming ability on both dialyate and other solutions. *Aspergillus* (18 isolates) showed high variability in biofilm formation. *Aspergillus* isolate 186 formed biomass in the dialysate equal to the biomass formed in RPMI, while 33.3% and 44.4% of the isolates formed more biomass in base or acid concentrates than in the dialysate. The biomass formed in the dialysate, for all strains of *Penicillium* (7 isolates), was greater than that formed in the concentrated acidic or basic. SEM analysis of *Aspergillus* biofilms formed on RPMI medium showed dense aggregation of hyphae, presence of conidial structures and matrix. **Conclusions:** The demonstration that fungal biofilm can be formed in dialysis fluids strengthens the need for the inclusion of fungi and, in particular, biofilms, in quality of dialysis fluid for haemodialysis therapy.

Author Disclosure Block:

R.H. Pires: None. **L. Teodoro:** None. **L.G. Lopes:** None. **C.H.G. Martins:** None. **M.C. Jamur:** None.

Poster Board Number:

SATURDAY-097

Publishing Title:

***Agrobacterium*-Mediated Transient Expression Using Modified Geminiviral Replicons Provides Rapid High-Yield Production of Vaccine Antigens and Monoclonal Antibodies in Plants**

Author Block:

A. Diamos, T. Anderson, J. M. Crawford, H. S. Mason; Arizona State Univ., Tempe, AZ

Abstract Body:

Agrobacterium-mediated expression systems have been investigated as alternatives to traditional fermentation-based systems for the production of recombinant proteins. We have developed a system that uses *Agrobacterium* to deliver modified bean yellow dwarf virus replicons to plant cells, which, unlike many other plant expression systems, is capable of producing heteromultimeric proteins with more than 2 heterosubunits (Huang et al., 2010). However, the system had not been optimized to maximize yield and reduce plant cell death.To address these concerns, we evaluated *Agrobacterium* strains LBA4301, LBA4404, GV3101, and EHA105 harboring geminiviral replicons by syringe infiltration to leaves of *Nicotiana benthamiana*. We found that strain EHA105 reduced tissue cell death, enhanced reporter GFP expression, and provided a >2-fold increase in monoclonal antibody production measured by ELISA. To determine whether viral replication components also played a role in cell death, we created modified vectors that varied the wildtype expression of Rep and RepA. We found that a modest reduction of both Rep and RepA reduced cell death, and despite also reducing the accumulation of replicon DNA as measured by semi-quantitative PCR, resulted in a ~50% increase in production of Norwalk virus capsid protein measured by ELISA. We evaluated diverse genetic elements for their capacity to improve mRNA stability and efficient utilization by the plant cell. Using protein gel quantification and ELISA, we found that the 5' untranslated from *Nicotiana benthamiana* homologs of the photosystem K subunit gene enhanced GFP production by as much as 40% more than the widely used omega leader from tobacco mosaic virus. We also found that the tobacco Rb7 matrix attachment region positioned 3' of the gene terminator enhanced monoclonal antibody production by up to 3-fold using ELISA. Together, we used these modifications to produce Norwalk virus capsid protein at 1.8mg per gram leaf fresh weight, more than twice the highest level ever reported in a plant-based system, and the monoclonal antibody rituximab at 1 mg per gram leaf fresh weight.

Author Disclosure Block:

A. Diamos: None. **T. Anderson:** None. **J.M. Crawford:** None. **H.S. Mason:** None.

Poster Board Number:

SATURDAY-098

Publishing Title:

Analysis of UV Protective Compounds from Cyanobacterial Isolates

Author Block:

s. nosheen; Univ. of the Punjab, Lahore, Pakistan

Abstract Body:

Ozone layer is continuously decreasing due to increasing atmospheric pollutants as a result UV radiations reaching on the surface of Earth are increasing day by day. Living organisms have different mechanisms to cope up with hazardous UV radiations, even some organisms produce UV protective compounds. In cyanobacteria, production of UV protective compounds is a very competent defense mechanism. In this study cyanobacterial isolates were analyzed for the production of UV protective compounds by using quite efficient extraction method to analyze how common and diverse these substances are present among them. UV absorption spectrum of extracted compounds was analyzed by UV spectrophotometer. Hyper UV protective compounds producing isolates (three filamentous and two unicellular isolates) were selected and their compounds were analyzed for UV and Nitrate stress. Various peaks of absorbance were observed, in all three regions of UV. Strains showed variations in the distribution of UV protective compounds. Overall trend that was observed according to number of peaks is UV-C > UV-B > UV-A. UV stress induces the production of UV protective compounds and nitrate stress also affects its production. There was not only increase in the number of peaks but absorbance values were also increased and trend shifts toward the production of more UV-B and UV-A compounds as compare to control. These multipurpose UV protective compounds can find applications in various fields and industries including pharmaceutical, paint, sunglasses and most important skin care and cosmetics industry.

Author Disclosure Block:

S. nosheen: None.

Poster Board Number:

SATURDAY-099

Publishing Title:

Genetically Inducible Recovery of Cyanobacterial Biomass by Autoaggregation

Author Block:

M. Fisher[1], **C. Keal**[1], V. Patil[2], D. Nielsen[2], R. Curtiss, III[3]; [1]Southern Connecticut State Univ., New Haven, CT, [2]Arizona State Univ., Tempe, AZ, [3]Arizona State Unversity, Tempe, AZ

Abstract Body:

One attractive method to produce renewable energy is the development of biofuels made by cyanobacteria, which can be engineered to produce biofuels, biofuel precursors or bioproducts from CO_2, sunlight and water. A major obstacle to economical production of such bioproducts is harvesting cyanobacteria from their liquid media. Centrifugation, for instance, is energy intensive and increases production cost. Therefore, low energy mechanisms for biomass harvest are necessary. Herein we describe a novel process for biomass harvest in which cyanobacteria were engineered to express genes encoding type V secreted adhesins. In their native organisms, type V secreted proteins are necessary for adhesion of the bacterium to their target host cells. Additionally, when over produced, they elicit autoaggregation and subsequent precipitation from the culture medium. We wished to determine whether expression of such adhesins would have a similar effect in cyanobacteria. Therefore, *Synechocystis* PCC 6803 was engineered to express either aipA, *taaP*, *tibA* or *yadA* under control of the promoter $P_{nrsB,}$ which can be induced by the presence of Nickel. These strains were named SD1011, SD1012, SD1013 and SD1014, respectively. In addition, the *tibA* gene was placed under control of the promoter P_{cmpA}, which can be induced by CO_2 limitation. This strain was named SD1022. Following construction of these strains, we tested them for their ability to precipitate out of solution. SD1011, SD1012, SD1013 and SD1014 were grown in 125 ml of BG-11 to an OD_{730} of 1.0. After a 24-hour induction with 6 μM Ni^{++}, 25 ml of the induced culture was aliquoted into a sterile, 125 ml Erlenmeyer flask. The adhesion producing strains SD1011, SD1012, SD1013 and SD1014 precipitated out of solution within 8 hours. We observed similar results with SD1022 by CO_2 limitation. Importantly, we noted that volume and the shape of the culture flask influenced the outcomes of agglutination. SD1022 grown in 800 ml of BG-11 in a septation flask failed to show dramatically different autoagglutination from the wild-type strain. These data indicate: *Synechocystsis* is capable of producing functional type V secreted proteins, that these proteins properly translocate to the outer membrane and they are able to function as autoagglutinating adhesins.

Author Disclosure Block:

M. Fisher: None. **C. Keal:** None. **V. Patil:** None. **D. Nielsen:** None. **R. Curtiss:** None.

Poster Board Number:

SATURDAY-100

Publishing Title:

Synthetic Ecology for Quantitative Prediction of Anti-Contamination Strategies in Biofuel-Producing Cultures of *Synechocystis* PCC 6803

Author Block:

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Abstract Body:

We previously engineered *Synechocystis* to secrete fatty acids such as laurate, a jet fuel precursor. An outdoor 4,000-liter photobioreactor (PBR) of *Synechocystis* culture that became contaminated by heterotrophic bacteria had reduced yield of laurate. We hypothesized that chemical additives such as salt, alkalinity and/or antibiotic will inhibit growth of these heterotrophs while allowing *Synechocystis* to flourish, improving laurate yields.To test this hypothesis, we used factorial analysis to model heterotroph growth response to three factors: NaCl concentration, pH, and kanamycin concentration. Growth was tested in rich media (LB broth), and also in laurate-producing *Synechocystis* cultures. Forty contaminant isolates were combined to create a single Defined Consortium, which was then used as inoculum for each of 19 growth conditions generated by central composite design. ANOVA of heterotroph growth measured a statistically significant response surface regression ($p < 0.05$). We tested the model using two different Defined Consortia (a total of 80 isolates), and also two different Undefined Consortia derived from lake water. Growth of both Defined and Undefined Consortia matched model predictions, supporting our hypothesis that these additives inhibit heterotroph growth. Furthermore, these data support that levels of these additives can quantitatively predict heterotroph growth independent of the species present.Attempts to generate a surface response regression in *Synechocystis* cultures were unsuccessful, indicating the presence of additional factors contributing to growth. However, a time-course assay showed that laurate is protected for over 72 hours in the presence of additives (as measured by FID GC), compared to less than 24 hrs without additives.We conclude that this study is a proof-of-principle for further investigation of using a Defined Consortium to quantitatively model "generalized" PBR contamination under controlled conditions. We propose this is possible due to the widely conserved mechanisms of resistance to salt, alkalinity, and antibiotics across diverse bacterial phyla, which we will investigate using deep sequencing of 16s rDNA followed by metabolic prediction with PICRUSt.

Author Disclosure Block:

R. Allen: None. **A. Zevin:** None. **R. Curtiss:** None. **R. Krajmalnik-Brown:** None.

Poster Board Number:

SATURDAY-101

Publishing Title:

Characterization of Plant Cell Wall-Acting Esterases: The Effect of Ionic Liquids on Ferulic Acid Release from Corn Stover

Author Block:

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Abstract Body:

Plant cell wall-acting esterases play an important role in facilitating the microbial degradation of lignocellulosic biomass through the cleavage of ester bonds on the substituted polymers. Recombinant feruloyl and acetyl esterases from *Pycnoporus cinnabarinus*, *Volvariella volvacea*, *Penicillium funiculosum*, and *Penicillium purpurogenum* were heterologously produced in *Pichia pastoris*, biochemical characterized and their activity tested in ionic liquids (ILs) as solvent on model and natural substrates. This is the first detailed investigation concerning the effect of ionic liquids on acetyl and feruloyl esterases. Heterologous production of the acetyl esterases from *V. volvacea* and *P. purpogenum* and a type-B feruloyl esterase from *P. funiculosum* was successfully achieved. In addition, the gene coding for CE1 from *Pycnoporus cinnabarinus* was cloned, expressed and the corresponding protein biochemically characterized for the first time and shown to be an acetyl esterase. The use of 1-Ethyl-3-methylimidazolium acetate ionic liquid resulted in a 2 to 5.5-fold increase in the activity of all the esterases on model substrates such as *p*-nitrophenyl acetate and methyl caffeate, and approximately 3-fold on the release of ferulic acid from corn stover by PfFaeB. Two other previously characterized feruloyl esterases (AnFaeA and TsFaeC), an L-arabinofuranosidase (TxAbf) and three xylanases from *Trichoderma viride*, *Neocallimastix patriciarum* and *Podospora anserina*) were also tested with ionic liquids. The ILs induced positive and negative effects depending on the enzyme and the substrate used. The tolerance of the enzymes to ILs was also dependent on the temperature of incubation. The obtained results suggested that the effect of ILs on enzymes activities is thermo-substrate dependent and that the stability and kinetic constants are modified by the presence of ILs. Depending on the enzyme, the hydrolytic activity on lignocellulosic biomass can be improved or reduced in the presence of these solvents. Further work is needed to discover and evaluate the factors that affect the interactions between ILs, enzymes and substrates in order to contribute to the optimization of enzymatic lignocellulose degradation process.

Author Disclosure Block:

P. Palacios: B. Collaborator; Self; Suzy Bulot, Pablo Alvira. D. Employee; Self; Mireille Haon. F. Investigator; Self; Sana Raouche, Eric Record. I. Research Relationship; Self; Claire Dumon. J. Scientific Advisor (Review Panel or Advisory Committee); Self; Craig B.Faulds.

Poster Board Number:

SATURDAY-102

Publishing Title:

Phosphorus Limitation Enhances Biodiesel Production in Dark-Grown Algae Fed Grain or Lignocellulosic Hydrolysates

Author Block:

N. Shrestha, **M. A. Schneegurt**; Wichita State Univ., Wichita, KS

Abstract Body:

Algae grown in the dark on pure sugars or plant biomass hydrolysates accumulate lipids suitable for biodiesel. We have previously reported on the growth of *Chlorella kessleri* on grain and lignocellulosic hydrolysates. Nitrogen limitation has been shown previously to increase the accumulation of lipids by algae. While this scheme works well with media supplemented with pure sugars, biomass hydrolysates are complex mixtures that contain a variety of nitrogen sources. It is difficult to reduce their bioavailable nitrogen content. We have investigated the potential of phosphorus limitation to enhance the accumulation of algal lipids, since it may be straightforward to remove phosphorus compounds using physicochemical means such as functionalized beads. *Chlorella* was grown in a series of media creating a range of nitrogen or phosphorus limitations, either autotrophically in the light or heterotrophically in the dark on sucrose or a lignocellulosic hydrolysate of big bluestem (*Andropogon gerardii*). Algal growth was measured by direct microscopic cell count and by chlorophyll content, while lipid accumulation was estimated using a Nile red assay. Nutrient limitation led to lower growth rates and stationary-phase culture densities, as expected. Nitrogen starvation led to increased lipid content when *Chlorella* was grown on sucrose, as previously reported. Similarly, phosphorus starvation led to increases in lipid accumulation of 5- to 7-fold on a dry weight basis. Iron starvation did not elicit an increase in lipid content. When big bluestem hydrolysates were used to support heterotrophic growth, lipid accumulation also was enhanced 3- to 7-fold by nitrogen or phosphorus limitation. There were differences in the composition of the lipids by FAME analysis. While the lipid content of cells increased, the biomass yields from starved cultures were concomitantly lower, such that the total lipid content by volume of culture was not substantially increased. A two-stage reactor system may work, where algae are grown in nutrient-replete medium to high density and then moved to nutrient-limited conditions to increase lipid yields. The sharing of technologies (fermenters) and feedstocks (grain and grass hydrolysates) between bioethanol and biodiesel production may provide new opportunities for flexible production of renewable liquid fuels using existing infrastructure.

Author Disclosure Block:

N. Shrestha: None. **M.A. Schneegurt:** None.

Poster Board Number:

SATURDAY-103

Publishing Title:

Isolation and Characterization of Microorganisms Capable of Growing on Corn Fibre

Author Block:

V. N. Anakwenze[1], C. A. Anwadike[1], A. B. Ilodinso[1], C. C. Ezemba[2]; [1]Nnamdi Azikiwe Univ., Awka, Anambra, Nigeria, Awka, Nigeria, [2]Renaissance Univ., Ugbawka, Enugu, Nigeria, Awka, Nigeria

Abstract Body:

Corn fibre is the fibrous matter remaining after the wet-milled corn has been sieved. The potential of using these corn fibres for the production of cellulolytic enzyme was assessed. Microorganisms capable of utilizing corn fibre as the sole carbon source were evaluated. Corn fibres were pre-treated with 25% ammonia and then added to the compounded medium (MM1). Appropriate dilutions of soil sample obtained from Science village, Nnamdi Azikiwe University, Awka, Anambra State, were used to inoculate the medium. The isolates obtained were assessed for the production of cellulase enzyme by flooding with congo red. The isolates include *Aspergillus flavus*, *Aspergillus niger*, *Geotrichum* sp.and *Penicillium* sp. *A. flavus* showed the highest zone of hydrolysis while none was observed on *A. niger.* This study has shown that *A. flavus* producing the highest zone of inhibition could be used for cellulase production using pretreated corn fibre as substrate.

Author Disclosure Block:

V.N. Anakwenze: None. **C.A. Anwadike:** None. **A.B. Ilodinso:** None. **C.C. Ezemba:** None.

Poster Board Number:

SATURDAY-104

Publishing Title:

One-Pot Enzymatic Pretreatment of Lignocellulosic Biomass for Bioenergy Production

Author Block:

J. L. Tran, T. Nadelson, B. Wang, K-H. Chu; Texas A&M Univ., College Station, TX

Abstract Body:

Background: Lignocellulosic biomass is abundant, available, and rich in organic material, making it a potential source for sustainable bioenergy production. Available lignocellulosic biomass includes wood, agricultural and forest residues, and some urban waste like yard trimming, paper waste, and saw dust. As the biomass are complex biopolymers composed of mainly cellulose, hemicelluloses, and lignin, it is necessary to pretreat the lignocellulosic biomass to produce fermenting sugars. Current practice of pretreating lignocellulosic biomass, commonly using chemical methods followed by enzymatic hydrolysis for depolymerization, is costly and not sustainable.**Methods, Results and Conclusions:**In this study, we explore the possibility to pretreat lignocellulosic biomass with direct application of enzymes for effective depolymerization. Specific objective of this study is to investigate potential for using engineered-enzymatic producing *E.coli* to pretreat lignocellulosic biomass to release sugars and monomers for producing triacylglycerol (TAG) by TAG-accumulating bacteria. TAG, a major component in fats, oils and lipids derived from plants, animals, fungi, and bacteria, is the material for the production of biodiesel, a clean and renewable liquid form of bioenergy. Cellulose-degrading gene, *Egls*, and Xylose-(most common hemicellulose) degrading gene, *XynA*, from Bacillus subtilis strain 168, and lignin-degrading genes, *DypB*, from *Rhodococcus jostii* RHA1 have been cloned into plasmid vectors and separately transformed into *E.coli* cells. The *E.coli* cells are currently lignocellulosic degrading gene expressions at low levels.On-going research efforts improve gene expression and test for activity of enzyme producted to optimize the pretreatment of lignocellulosic biomass for higher TAG production. The results of this study are expected to minimize environmental impacts of lignocellulosic biomass disposal while generating value TAG feedstock for biodiesel production.

Author Disclosure Block:

J.L. Tran: None. **T. Nadelson:** None. **B. Wang:** None. **K. Chu:** None.

Poster Board Number:

SATURDAY-105

Publishing Title:

Lignocellulose-Derived Byproducts Inhibition on the Activity of Halotolerant Xylose Reductase from *Debaryomyces nepalensis*

Author Block:

B. Paidimuddala[1], S. Gummadi[1], R. Ashish[2]; [1]Indian Inst. of Technology Madras, Chennai, India, [2]Indian Inst. of Technology, Madras, Chennai, India

Abstract Body:

Background: Xylitol is a multi-beneficial natural sugar substitute that can be produced from lignocellulose derived xylose. Xylose reductase (XR) that converts xylose to xylitol could be exploited for the development of enzyme based bioprocess to surpass the shortcomings associated with current chemical and microbial processes. But the lack of knowledge on the effect of major lignocellulose derived byproducts (LDBs) that generated while preparation of hydrolysates, on enzyme, leading to end up with failures. To study this, we used previously cloned novel halotolerant XR from *Debaryomyces nepalensis* (*Dn*XR).**Methods:** In this study, for the first time, we investigated the effect of phenol, furfural and acetate on the activity of *Dn*XR. In the presence of LDBs, enzyme activity was determined by spectrophotometric monitoring of the change in A_{340} with NADPH as a cofactor and stability of *Dn*XR was assessed at 30°C. The kinetic parameters were determined by non- linear regression and half-life of the enzyme was calculated by considering first order deactivation kinetics. The given values are mean of triplicate measurements.**Results:** The IC_{50} (half maximal inhibitory concentration) of phenol, furfural and acetate were found to be 20, 50 and 55 mM respectively. At their IC_{50}, the enzyme showed catalytic efficiency of 0.55 ± 0.03 mM^{-1} s^{-1} when compared to control. All LDBs showed competitive inhibition on enzyme activity as evident from increased K_M and decreased catalytic efficiency (k_{cat}/K_M) in the presence of inhibitor. Phenol which originates from lignin of lignocellulose, was exhibited maximum inhibition with IC_{50} 20 mM on the activity of enzyme among three. Nevertheless, half- life of enzyme at 10 mM phenol was reduced to 1 h from 14 h (control). Whereas, at 50 mM acetate and 30 mM furfural the half-life of enzyme was found to be 5 h and 2 h respectively.**Conclusions:** We found that phenol acting as a potent inhibitor of *Dn*XR. The hydrolysates with low concentration of LDBs should be used in enzyme based approach. The results of this study would give more insights to develoep an enzymatic bioprocess for the production of xylitol at industrial grounds.

Author Disclosure Block:

B. Paidimuddala: None. **S. Gummadi:** None. **R. Ashish:** None.

Poster Board Number:

SATURDAY-106

Publishing Title:

Interactions And Metabolic Output Of Acetoclasitc Hydrogenotrophic Archaea Grown In Co-Culture With Cellulose Degrading Bacteria

Author Block:

F. S. Mayer; Univ. of Wisconsin Oshkosh, Oshkosh, WI

Abstract Body:

The biodigester on the University of Wisconsin Oshkosh campus is the first of its kind in North America; the energy produced by this dry digester is utilized by the university. The feedstock material consists of organic waste including food waste from the University of Wisconsin Oshkosh, as well as food and yard waste collected from the city of Oshkosh and agricultural waste from nearby farms. The feedstock is degraded by microbes via a dissimilatory food web resulting in methane and CO_2 production. This gas mixture is then combusted to harness electrical and thermal energy. Two main types of microbes that make this process possible are cellulose degrading bacteria and methanogenic archaea. These microbes work as part of a nutritional symbiosis; cellulose degrading bacteria digest the feedstock and produce acetate, hydrogen and CO_2 while methanogenic archaea are able to utilize these products to produce methane. Strains of acetoclastic, methanogenic archaea were isolated from the biodigester and cultured using media designed to mimic the digester environment. In addition, a strategy to isolate a cellulose degrading bacterium was undertaken using cellulose filter disks inoculated with digetate from the biodigester. The digester bay itself was used as the incubator for the duration of the 28-day cycle. This approach allowed for cultivation and subsequent isolation of cellulose degrading bacteria. Finally, co-culture studies were conducted involving two microbial species already growing in pure culture: *Methobacterium formicicum* (an hydrogenotrophic, methanogenic archaea) and *Clostridium thermocellum* (a cellulose degrading bacterium). The cultures were maintained under anaerobic conditions and headspace pressures were monitored to detect gas production and consumption. The results of these preliminary studies suggest that cellulose degrading bacteria are capable of supporting the growth of methanogenic archaea.

Author Disclosure Block:

F.S. Mayer: None.

Poster Board Number:

SATURDAY-107

Publishing Title:

Characterization of Keratin Hydrolysate for Use as a Feedstock for Biofuel Production

Author Block:

V. A. Pawar[1], A. S. Prajapati[1], A. Kumar[1], D. H. Patel[2], R. B. Subramanian[1]; [1]Sardar Patel Univ., Anand, India, [2]P D Patel Inst. of Applied Sci., Anand, India

Abstract Body:

Protein is one of the most abundant biomolecules found on the Earth. Keratin is an example of such a non-food protein found in nature. Keratin hydrolysate are used in feedstock as supplements. However, recent developments in metabolic engineering direct the possibility of using such feedstock for biofuel production. The amino acids could be converted into 2-keto acids or tricarboxylic acid intermediates by deamination. The present work aims at characterization of keratin hydrolysate for use as a feedstock for biofuel production**.** In the present study, the gene encoding keratinase from *Bacillus altitudinis RBDV1* was cloned in *Escherichia coli*. The amino acid sequence inferred from the 1130-bp nucleotide sequence revealed that the protein is 376 amino acids long & has an estimated molecular weight of ~41,433 Da. Sequence analysis of Keratinase gene revealed sequence similarities with members of the Subtilisin family of proteases. The recombinant keratinase was purified by affinity chromatography. Its molecular weight was found out to be ~44,000 Da. For further characterization, effect of temperature & IPTG concentrations was studied on the expression of recombinant proteases & solubilization of the expressed enzymes. The recombinant keratinase was characterized for various catalytic properties. The property of the hydrolysate was evaluated by HP-TLC. The amino acid profile of this keratin hydrolysate will then be used for biofuel production using the genetically modified *E.coli.*

Author Disclosure Block:

V.A. Pawar: None. **A.S. Prajapati:** None. **A. Kumar:** None. **D.H. Patel:** None. **R.B. Subramanian:** None.

Poster Board Number:

SATURDAY-109

Publishing Title:

Mixed Cultures of Freshwater Microalgae and Bacteria for High Lipid Productivity

Author Block:

D. E. Berthold, 33199, **K. G. Shetty**, K. Jayachandran, 33199, M. Gantar, 33199; Florida Intl. Univ., Miami, FL

Abstract Body:

Incorporating biofuels derived from microalgae biomass into energy production is becoming a realistic choice for achieving goals of reducing the fossil fuel dependency. Microalgae biofuel is a feasible technology since microalgae have high growth rates and lipid levels and can be processed using infertile land and wastewater. The microalgae biofuel industry is however facing productivity issues that hinder the development of large-scale projects. Microalgae success is largely dependent on lipid accumulation that usually occurs under depleted nutrient conditions. Nutrient-deficient conditions that stimulate lipid storage, in turn, inhibit biomass production.Manipulating the conditions that allow for both high lipid and cell growth rates during cultivation is desired. In order to overcome cell and lipid productivity issues, we propose the use of mixed cultures of oleaginous freshwater microalgae and bacteria. Ten samples from the South Florida region were used for isolation of microalgae and bacteria. Three species of freshwater microalgae and forty strains of bacteria were isolated and cultured on BG-11 and nutrient agar respectively. For mix culturing, bacteria cultures were incubated separately in 10ml of nutrient broth medium for 1 week, harvested, and then combinations of individual strains of algae and bacteria were inoculated and incubated in 24-well plates at 25 °C under continuous fluorescent lighting (50 μmol photons $m^{-2}s^{-1}$). Growth and lipid content were assessed for 15 days by recording the *in vivo* chlorophyll-a fluorescence and Nile Red fluorescence, respectively. Results indicate that one strain of microalgae, *Coelastrum sp.* 46-4, when grown in mixed culture with one particular strain of bacteria *sp.* 121-1-1 resulted in 63% and 31% increase of biomass and lipid content respectively. Analysis of microalgae growth on bacteria-free supernatant revealed biomass increase from 0.089 to 0.26 g L^{-1} day^{-1} and lipid productivity increase from 21 to 68 mg l^{-1} day^{-1}. Additional analysis of the mix culturing of *Coelastrum sp.*46-4 and bacteria *sp.* 121-1-1 is required in for identifying the factor responsible for observed growth and lipid increase. Our goal is to identify prospective bacterial growth-promoters that improve the productivity of microalgae biofuel technology.

Author Disclosure Block:

D.E. Berthold: None. **K.G. Shetty:** None. **K. Jayachandran:** None. **M. Gantar:** None.

Poster Board Number:

SATURDAY-110

Publishing Title:

Boosting Power Output of Microbial Fuel Cells by Chemically Activating the Graphite Cathode

Author Block:

P. Song[1], L. Zhang[2], Z. Lu[2], D. Li[2], Y. Liu[2], G. Huang[2], J. Ma[3], L. Cai[2]; [1]Thomas Nelson Community Coll., Hampton, VA, [2]East China Univ. of Sci. and Technology, Shanghai, China, [3]Univ. Libre de Bruxelles, Brussels, Belgium

Abstract Body:

The broad application of microbial fuel cells (MFCs) has been hindered by its low power output and high cost of the cathode material, which is often made of precious metals. In this study we chemically treated graphite with H_3PO_4 and used it as the catalyst-free MFC cathode. We observed a dramatic improvement of the oxygen reduction reactions (ORR) near the cathode when the graphite was treated with 14.62 M H_3PO_4 for 12h between 30 and 50 ºC. The resulting power density was 7.9 W/m^2, 2.4 times higher than that of the untreated control. Fourier Transformation Infrared (FTIR) spectra and Methylene Blue Analysis revealed that H_3PO_4 treatment increased specific areas of the graphite and allowed more oxygen-containing functional groups to bind to the surface, which facilitated ORRs. Since the chemical activation process involves mainly one simple immersion step, with no requirement for heating, electrochemical processing or the use of expensive chemicals, it is a highly cost-effective approach to improve the performance of MFCs. We recommend *in situ* chemical treatment of graphite cathodes in scale-up MFCs to optimize their various industrial operations.

Author Disclosure Block:

P. Song: None. **L. Zhang:** None. **Z. Lu:** None. **D. Li:** None. **Y. Liu:** None. **G. Huang:** None. **J. Ma:** None. **L. Cai:** None.

Poster Board Number:

SATURDAY-111

Publishing Title:

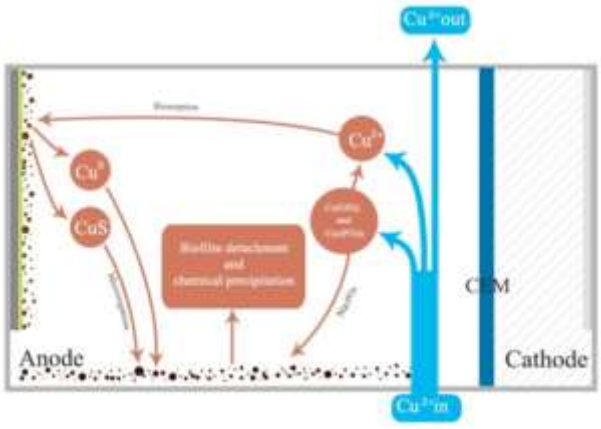
Behavior of Cu2+ in Anode of Microbial Fuel Cells

Author Block:

P. Song[1], D. Chang[2], L. Zhang[2]; [1]Thomas Nelson Community Coll., Hampton, VA, [2]East China Univ. of Sci. and Technology, Shanghai, China

Abstract Body:

Abstract:We investigated the behavior of copper ions in the anodic chamber of microbial fuel cells (MFCs) by gradually increasing the concentration of Cu^{2+} from 2.78 to 111.11 mg/L. The transformation, distribution, and removal of Cu^{2+}, as well as its effect on voltage output were analyzed. Results showed that at low levels ($[Cu^{2+}]$ < 19.44 mg/L initially and ended at 0.36 mg/L after batch operation), Cu^{2+} could improve the voltage output of MFCs; while when $[Cu^{2+}]$ exceeded 22.22 mg/L, it would inhibit MFC performance. At low $[Cu^{2+}]$, the average Cu^{2+} removal efficiency was over 95% with the maximal efficiency reaching 99.99%. XPS analysis revealed that 21% of Cu^{2+} was reduced to Cu^{0}, which may have acted as nanoparticles to enhance the MFC performance of MFC. The columbic efficiency was decreased, mainly due to the chemical oxygen demand (COD) from the Sulfate Reducing Bacteria. To our knowledge, this is the first report to show that Cu^{2+} gets reduced to its metallic form on the MFC anode, and its addition improves the voltage output of MFCs. We also show that reduction and precipitation were the major mechanisms for the removal of Cu^{2+} in MFCs.



Author Disclosure Block:

P. Song: None. **D. Chang:** None. **L. Zhang:** None.

Poster Board Number:

SATURDAY-112

Publishing Title:

Synthesis of Fluorescent Cus Nanoparticles by a Highly Metal-Resistant Antarctic Bacterium. Potential Application as Photosensitizer in Solar Cells

Author Block:

L. Saona[1], N. Órdenes-Aenishanslins[2], N. Bruna[3], C. Norris[2], **J. Pérez-Donoso**[3]; [1]UCHILE, Santiago, Chile, [2]UChile, Santiago, Chile, [3]UNAB, Santiago, Chile

Abstract Body:

Background: Fluorescent nanoparticles or quantum dots (QDs) are semiconductor nanoestructures used in biotechnological and industrial applications (biomedicine, renewable energies and optoelectronics). Their interesting properties are consequence of composition, structure and nanometric size. Most QDs are chemically produced and constituted by elements such as Cd, Te, Se, In and Zn. In this context, very few has been reported regarding the production of Cu-based fluorescent nanoparticles, constituting an opportunity to give added value to the principal exportation product of Chile.During the last years, biological methods for QDs biosynthesis have gained interest because they are cheap, eco-friendly, non-toxic and in most cases generate QDs with new properties. The molecular bases of bacterial biosynthesis of NPs are still unknown, however the participation of cellular peptides, proteins, polymers and thiols has been reported.**Methods and Results:** Based on this, our laboratory has recently developed a method for the biosynthesis of fluorescent Cu-nanoparticles using a highly Cu-resistant strain isolated from Antarctica (MIC 20 mg/mL). Due to its Cu resistance and uptake, probably consequence of improved catalase activity, this bacterium represents a unique candidate to evaluate biosynthesis of NPs at high Cu concentrations.In this work we report the intracellular aerobic biosynthesis of fluorescent CuS nanoparticles during exponential growth phase at 28ºC. Biosynthesized NPs are aqueous-soluble (indicative of an organic capping), with a monodisperse size distribution below 10 nm (HR-TEM), constituted by CuS (EDX) and display a unique emission peak between 500 and 700 nm when excited with UV light (Synergy Fluorescence reader H1M).**Conclusion:** Because of their semiconductor and spectroscopic properties these NPs represent a potential candidate for developing biological solar cells. We are testing purified CuS NPs produced by this novel Antarctic strain as sensitizers on “quantum dots sensitized solar cells”.

Author Disclosure Block:

L. Saona: None. **N. Órdenes-Aenishanslins:** None. **N. Bruna:** None. **C. Norris:** None. **J. Pérez-Donoso:** None.

Poster Board Number:

SATURDAY-113

Publishing Title:

Biosolubilization of Coal in Pakistan

Author Block:

M. Ali; Univ. of the Punjab, Lahore, Pakistan

Abstract Body:

Background: Pakistan with about 185 billion tons is known to contain sixth largest reservoir in the world. Coal fired power plant, Thermo catalysis and Pyrolysis are common practices across the world, but as they are not economical and ecofriendly, compelling the modern world towards a better approach of coal solubilization. Therefore, recently coal biosolubilization is considered as promising technology for coal conversion into value added products i.e. humic acid, fulvic acid, methane and other aromatic compounds. The present study is specifically undertaken to investigate the solubilization of coal by aerobic microorganisms.**Methods:** Efficient coal solubilizing microorganisms were isolated from samples of water, soil and coal taken from Salt range, Chakwal. After screening by agar plate assay, bacterial strains AY2, AY3 and fungal strains AY5, AY6 were shown to have effective coal solubilization activity. UV-Vis spectroscopy, infrared spectroscopy and SEM were performed to characterize the solubilization residues. Intensity of biosolubilization was determined by measuring the net weight loss of the coal pieces.**Results:** Coal solubilization was observed to be about 25.93% by AY2, 36.36% by AY3 and 50% by AY6 while AY5 showed maximum coal solubilization (66.67%). UV spectrum showed an increase pattern of absorbance while IR spectrum indicated alterations in comparison to the original coal. Aromatic acids and other organic functional groups of hydroxyl, cyclane, carbonyl, ether linkage and aromatic rings were conceived in graph. Evidence for the presence of microorganisms and surface erosion of coal residues in contrast to control coal samples were obtained through SEM.**Conclusions:** the microorganisms isolated from coal mines have enough potential of coal solubilization and conversion into valuable products suggested various applications in different fields.

Author Disclosure Block:

M. Ali: None.

Poster Board Number:

SATURDAY-114

Publishing Title:

High Culturability of the Microbial Community from Hydrogen Producing Reactors

Author Block:

L. Fuentes[1], L. Braga[1], E. Castelló[2], **C. Etchebehere**[1]; [1]Biological Res. Inst. Clemente Estable, Montevideo, Uruguay, [2]BioProA Lab, Montevideo, Uruguay

Abstract Body:

Background: Production of hydrogen by dark fermentation is an alternative to produce a clean fuel using agro-industrial wastes. To improve the process it is necessary to understand the microbiology of the system. Several studies have been conducted analyzing the microbial communities from hydrogen producing bioreactors using molecular tools, but only in few cases a partial culturing strategy were included. The aim of this work was design strategies to isolate the microorganisms predominant in hydrogen producing reactors.**Methods:** Based on previous work and revision of the literature, it was possible to conclude that the microbial communities from hydrogen producing reactors are mainly composed by four different physiological guilds: 1-spore forming strict anaerobic fermenters, 2-non-spore forming strict anaerobic fermenters, 3-facultative fermenters 4-lactic acid bacteria. According to the growth characteristics of these microorganisms we designed strategies to isolate them from samples taken from hydrogen producing reactors. The isolation strategies were tested in two samples taken from different hydrogen producing reactors fed with raw cheese whey. The isolates were characterized according to the 16S rRNA gene sequence analysis and the production of hydrogen and other metabolites were determined in the pure cultures. To determine the coverage of our isolation strategies from the total community we analyze the samples used for the isolation by 16S rRNA gene pyrosequencing.**Results:** The results showed that the four strategies were successful for the isolation of the predominant organisms reaching coverage of more than 90% of the OTUs present in the community. 43 strains were isolated and characterized as belonging from the genera *Acetobacter, Micrococcus, Chryseobacterium, Rahnella, Lactobacillus, Pseudoclavibacter, Propionibacterium, Megasphaera, Bifidobacterium* and *Clostridium.* From those, the strains characterized as *Clostridium, Rahnella* and *Megasphaera* produced hydrogen by fermentation. The phylogenetic analysis showed a close relationship between the isolates 16S rRNA gene sequences and the sequences retrieved in the pyrosequencing analysis.**Conclusions:** It was demonstrated for first time the high culturability of the hydrogen producing bioreactors biomass.

Author Disclosure Block:

L. Fuentes: None. **L. Braga:** None. **E. Castelló:** None. **C. Etchebehere:** None.

Poster Board Number:

SATURDAY-115

Publishing Title:

Anaerobic Digestion of Algae: Microbes, Gas and Temporal Changes

Author Block:

A. Doloman[1], Y. Soboh[2], C. Miller[1], R. Sims[1]; [1]Utah State Univ., Logan, UT, [2]Palestine Technical Coll.s - Arroub, Arroub, Palestinian Territory

Abstract Body:

Background: Anaerobic digestion (AD) is a microbiologically coordinated process with dynamic relationships between bacterial players. Although key bacterial players carrying out AD of various substrates have been identified in the literature, a comprehensive understanding of dynamic changes in the bacterial composition during the AD process is currently incomplete. The objective of this research was to assess changes in bacterial community composition that coordinates with anaerobic co-digestion of algal biomass cultivated on municipal wastewater treated in facultative lagoons. **Methods:** Duplicates of a high-rate continuous-flow reactor with up-flow anaerobic sludge blanket (UASB) were used to achieve high rates of algae decomposition and biogas production. Sodium hydroxide was added to the algal feed to balance the carbon-to-nitrogen ratio of the feedstock, with final C/N ratio of 21/1. Samples of the sludge blanket were collected throughout AD and extracted DNA was subjected to next-generation sequencing (MiSeq platform) using methanogen *mcrA* gene specific and universal bacterial primers (338F and 785R). Resulting sequences were quality filtered and analyzed in the MOTHUR software. Statistical analysis of significant differences between samples at various time points was performed within the MOTHUR pipeline. **Results:** Analysis of the data revealed that samples taken at different stages of AD had varying bacterial composition, with initially low abundance of key phyla in the seeding inoculum from the wastewater lagoons sludge. A group consisting of *Bacteroidales, Pseudomonadales* and *Enterobacteriales* was proposed to be responsible for the hydrolysis of algal biomass. The methanogenesis phase was dominated by *Methanosarcina mazei,* which contributed to the production of biogas with up to 90% of methane composition. **Conclusion:**. Results of observed changes in the composition of microbial communities during AD can be used as a roadmap to stimulate identified key bacterial species of each phase of AD in order to increase yield of biogas and rate of biomass decomposition. Waste grown algae have a high biogas potential and this research demonstrates a successful exploitation of methane production from algae without any biomass pre-treatment.

Author Disclosure Block:

A. Doloman: None. **Y. Soboh:** None. **C. Miller:** None. **R. Sims:** None.

Poster Board Number:

SATURDAY-116

Publishing Title:

Assessing the Potential of Different Tropical Biomasses as Feedstock for Biogas Production Through Anaerobic Digestion

Author Block:

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Abstract Body:

Second generation biofuels are considered a potential alternative to face the peak oil crisis. The challenge in the development of these non contaminant fuels is the finding of a viable biomass. Puerto Rico, a sub-tropical island, is rich in flora and other potential biomasses. In this island, climbing vines compose the 20% of the flora population, meanwhile marine algae and seagrasses are highly abundant in the littoral zone. This fact lead us to study the potential of these biomasses as feedstock for biogas production through anaerobic digestion. We used 10 species of biomasses and submitted them to a aqueous extraction pretreatment through blending, in which we separated the water-soluble and non-soluble fractions. Both fractions were anaerobically digested and compared with untreated biomass. Reactors were prepared using 160 mL serum bottles and inoculated with effluent from anaerobic reactors previously enriched with similar biomasses. Methane production and biodegradation were measure by GC and HPLC. Results showed that climbing vines yielded more methane than seagrasses and most algae. The effect of the pretreatment varies with every biomass, but liquid fraction yielded less methane than untreated biomass in all cases. Up to 78.16 L/Kg of methane was obtained from the solid fraction of *Epipremnum spp.*, potentially producing up to 720 W/hr per Kg of biomass, meanwhile untreated *Dioscorea spp.* and *Acanthophora spp.* yielded almost 60 L/Kg (~560 W/hr per Kg). These three feedstocks showed a net methane production greater than the woody biomass production average of 50 L/kg, what makes them excellent candidates for industrialization.

Author Disclosure Block:

W.R. Morales-Medina: None. **L.A. Ríos-Hernández:** None.

Poster Board Number:

SATURDAY-117

Publishing Title:

Microbiome Dynamics in Sea Water Anaerobic Digestors from Different Geographical Locations Across Puerto Rico

Author Block:

K. Navarro[1], D. Derilus[2], F. Godoy-Vitorino[1], C. Louime[2]; [1]Inter American Univ. of Puerto Rico, San Juan, PR, [2]Univ. of Puerto Rico, San Juan, PR

Abstract Body:

Background: We have evaluated the feasibility of the conversion of marine algae into biogas through bench-scale anaerobic digestors inoculated with different sea water samples and designed to overcome sodium and sulfur inhibition through manipulation of the salinity and pH levels. The present study aimed at characterizing the microbiome of salt water anaerobic digester samples collected along the coast of Puerto Rico in different salinity and pH regimes. We hypothesize that the populations that evolve in each chamber will have different composition according to the salinity levels of the system the geographical origin of the samples. **Methods:** Higher salinity was experimentally induced through daily introduction of dried ground sargassum to the anaerobic digestors with microalgae. Genomic DNA extraction was done from the 30ml filtered seawater and sequenced with the miseq Illumina platform. Sequence analyses was performed with QIIME using the RDP database as reference. **Results:** A total of 1,751,790 sequences were binned into 122,668 OTUs.Alpha rarefaction plots indicate that richness is higher in samples acquired from the Caribbean sea compared to those obtained from Atlantic coastal areas, as is significantly richer in high salinity and high pH. A total of 74 phyla were found with a dominance of Proteobacteria across all samples followed by Bacteroidetes and Planctomycetes. A total 1,224 genus were found across all samples with a dominance of 1,176 bacterial genus (with dominance of *Vibrio* and Pirellulacea) and 48 from Archaea with dominance of Desulfobacteraceae. Phyla distribution of Archaea shows that Parvarchaeota (dominate in most samples followed by Crenarchaeota (*Nitrospumilus*) and Euyarchaeota. We found that *Desulfococcus* was significantly associated with moderate pH while *Desulfoccus* dominated at higher pH values and higher salinity. Methanogenic taxa were distributed across salinity and pH. **Conclusion:** We found that microbial communities can be partitioned according to the salinity and pH regimes with an increase in sulfate reduction at high salinity and moderate pH.

Author Disclosure Block:

K. Navarro: None. **D. Derilus:** None. **F. Godoy-Vitorino:** None. **C. Louime:** None.

Poster Board Number:

SATURDAY-118

Publishing Title:

Bacterial Degradation of Reactive Textile Dyes

Author Block:

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Abstract Body:

Most of the textile industries in Bangladesh dispose reactive dyes in the environment without any treatment and pollute the environment severely. To obtain bacteria having remarkable ability to decolorize and degrade reactive textile dyes, 30 bacterial isolates were isolated from the effluents collected from two textile industries and two leather industries. Screening of these isolates for dye decolorization and degradation capability was performed in nutrient broth medium by using eight structurally different reactive textile dyes. Among these bacterial isolates, 12 isolates showing one or more dye decolorizing ability within 48 h of incubation were identified based on morphological, cultural and biochemical characteristics. Decolorization and degradation capabilities of *Aeromonas, Pseudomonas* and *Bacillus* was optimized under different physicochemical conditions by using Novacron Super Black G (NSB-G), one of the eight reactive dyes. These bacteria decolorized and grew well up to 500 mgl^{-1} of NSB-G. *Aeromonas sp., Bacillus sp.* and *Pseudomonas sp*. showed significant decolorization by 93, 92 and 91%, respectively at 200 mg/l of NSB-G after 96 h of incubation under optimum conditions. Biodegradation and decolorization of reactive dye was confirmed using UV-VIS Spectrophotometry and Fourier Transform Infrared Spectroscopy (FTIR). Peaks at 600 nm during the course of decolorization were observed and the peak of the parent dye compound was completely disappeared after 96 h of incubation. Analysis by thin layer chromatography indicated that the dyes were degraded into many parts. These results clearly indicated that the dye had been catabolized and utilized by these bacterial isolates. Finally azo-reductase activities have been characterized in the biodecolorized supernatant. High decolorization extent supports the notion that these bacterial isolates might be potential in the biological treatment of dyeing mill effluents.

Author Disclosure Block:

A. Azad: None.

Poster Board Number:

SATURDAY-119

Publishing Title:

Marine Microbial Consortium Applied to Rbbr Textile Dye Detoxification and Discoloration

Author Block:

G. A. L. Vieira[1], V. M. Oliveira[2], L. D. Sette[1]; [1]São Paulo State Univ., Rio Claro - SP, Brazil, [2]Campinas State Univ., Paulínia - SP, Brazil

Abstract Body:

Background: Marine environments are susceptible to contamination by industrial wastes, and also may represent a target niche for microbial prospecting for bioremediation. Marine microorganisms are adapted to saline conditions and have potential for being used in many biotechnological processes, as bioremediation, a promising approach for the degradation of environmental pollutants using metabolic potential. The aim of this study was to evaluate the potential of marine microbial consortium in detoxify and discolor RBBR textile dye applying experimental design.**Methods:** Microbial consortium were structured using two ligninolytic fungi isolated from marine invertebrates of Brazilian coast, and two bacteria from oil reservoir (off-shore), previously selected based on their capacity to produce enzymes and to degrade hydrocarbons. Experimental design (Plackett & Burman - matrix 20, with four central points) was used to evaluate 13 independent factors in the RBBR textile dye detoxification and discoloration. Erlenmeyer flasks containing 50 ml of medium, the microbial consortium and RBBR were kept in incubators for 7 days at 140 rpm and 28 °C. Toxicity assay was applied using *Artemia* sp. as bioindicator (n=30) in triplicate and discoloration percentage was calculated by the ratio between initial and final spectrophotometric absorbance ($\lambda_{594\ nm}$).**Results:** This first experimental design (P&B 20) was successfully applied. Four assays showed high rates of detoxification (89 - 98% *Artemia* sp. survivors) and 59 - 82% of discoloration. Assay 11 showed high levels of detoxification and discoloration, 94% and 82%, respectively. It is important to highlight that this assay contained the maximum dye concentration (750 ppm RBBR) and 100% of salinity (artificial sea water), revealing the power of marine microbial consortium for environmental pollutants bioremediation. Moreover, the absent of glucose and other independent variables in assay 11 characterize low cost to the process.**Conclusions:** Experimental design was a useful tool in this work, since statistical analyses of independent factors allow the evaluation of its influence and effects in the process. Some variables were eliminated and other fixed for future experiment in order to get the optimization of bioremediation process.

Author Disclosure Block:

G.A.L. Vieira: None. **V.M. Oliveira:** None. **L.D. Sette:** None.

Poster Board Number:

SATURDAY-120

Publishing Title:

Decolorization of Synthetic Textile Dyes Using Yeasts Isolated from Fruit Peels and Leaves

Author Block:

A. NGO, G. Dedeles, M. Devanadera; Univ. OF SANTO TOMAS, Manila, Philippines

Abstract Body:

Background: Discharge of synthetic dyes poses a big threat as they are poorly biodegradable and toxic due to their complex nature. In search for alternatives to physical and chemical treatments, decolorization of dyes by different microbes is now being considered as a promising approach **Objectives:** Delorization of synthetic dyes by yeast co-cultures and consortia from leaves and fruit peels was assessed with 50 ppm (parts per million) dye concentration. **Methods:** Yeasts from leaves and fruit peels were screened for potential decolorization at 25-50 ppm. Decolorization parameters were optimized for the synergistic property and development of yeasts co-cultures and consortium. Possible decolorization reaction was initially assessed by cell immobilization, SDS-PAGE, and FTIR analysis. **Results:** A total of 16 organisms were isolated from rose, mango, and pineapple leaves and pineapple fruit peels. Only 4 organisms showed highest decolorization of four synthetic dyes namely- Direct Pink B (DPB), Disperse Yellow 5G (DY5G), Direct Fast Orange S (DFOS), and Reactive Turquoise Blue G (RTBG). The optimum condition for best decolorizers of selected dyes at 50 µg/mL were *Candida guilliermondii* Y011 for DPB at pH 9, 37^{o}C; *C. dubliniensis* Y014 for DY5G at pH 4, 25^{o}C; *C. guilliermondii* Y004 for DFOS at pH 7, 25^{o}C, and *C. famata* Y003 for RTBG at pH 4, 35^{o}C. All of the 4 yeast isolates did not show any antagonistic activity when subjected to lawn-spotting method for the formation of co-cultures and consortium. The best co-cultures obtained 61% decolorization of DPB, 65% decolorization of DY5G, 41% decolorization of DFOS and 50-51% decolorization of RTBG. Immobilized yeast cells were active in decolorizing the dyes and SDS-PAGE analysis confirmed the presence of an extracellular protein. FTIR results also showed that there are changes in the functional group of Direct Pink B while minimal changes in the functional groups of the Reactive Turquoise Blue G implying that there was a different pathway of decolorization. **Conclusion:** Yeasts in co-cultures and consortia can decolorize toxic synthetic dyes with different possible decolorization pathway like enzyme degradation and bioaccumulation. This could be used for possible treatment in wastewater systems.

Author Disclosure Block:

A. Ngo: None. **G. Dedeles:** None. **M. Devanadera:** None.

Poster Board Number:

SATURDAY-121

Publishing Title:

Stirred Tank Bioreactor with Immobilized *Aspergillus terreus* Qms-1: Textile Effluent Treatment Approach

Author Block:

Q. Laraib[1], M. Shafique[2], M. Sohail[1]; [1]Univ. of karachi, karachi, Pakistan, [2]federal urdu Univ., karachi, karachi, Pakistan

Abstract Body:

Background: Textile sector is one of the greatest generator of liquid effluent pollutants all over the world, contributing to serious health hazards. Biodegradation and biodecolorization of Congo red (a direct di-azo dye) by a fungal strain *Aspergillus terreus* QMS-1 was studied in a self-designed lab scale stirred tank bioreactor (STR).**Method:** The strain QMS-1 was isolated from effluent of a local textile industry and was immobilized on pieces of natural loofah sponge for biodegradation of dye in a stirred tank bioreactor (STR). The reactor operation was carried out under aerobic condition with working volume 1L at room temperature and pH 5.0 in continuous flow mode with dye concentration of 100 ppm in simulated textile effluent. The reactor was run on fill with hydraulic retention time (HRT) of 24 hr.**Result:** Overall, the color was removed by 97% with 100 ppm and HRT of 12 hr.**Conclusions:** The STR system was found a cost-effective, stable and easily maintained system for the in-situ treatment of textile waste water by *Aspergillus terreus* QMS-1. It also represents an inexpensive and readily available process for the development of biomass that has a significant potential of dye decolorization.

Author Disclosure Block:

Q. Laraib: None. **M. Shafique:** None. **M. Sohail:** None.

Poster Board Number:

SATURDAY-122

Publishing Title:

Microbial Ecology and Pharmaceutical Removal in an Ecological Wastewater Treatment Plant

Author Block:

I. N. Balcom; Lyndon State Coll., Lyndonville, VT

Abstract Body:

Background: The environmental effects of pharmaceutical and personal care products (PPCP) from wastewater treatment plants (WWTP) is an area of emerging concern due to widespread contamination and PPCPs' potential to cause adverse effects at relatively low concentrations. However, very little is known about the treatment of PPCP by ecological WWTP. **Methods:** To assess role of plants in the treatment PPCP by an ecological WWTP at a highway rest stop facility, we characterized the microbial communities residing on plant roots immersed in wastewater. Microbial genomic DNA from aqueous wastewater, plant root biofilm samples and single pharmaceutical compound enrichment cultures was sequenced by massively parallel sequencing. The phylogenetic structure of the microbial communities was analyzed with the lowest common ancestor algorithm. The system's microbial xenobiotic metabolism was examined by comparing the location, identity, and relative abundance of known xenobiotic gene sequences. **Results:** The concentrations of PPCP in the system were in many cases much greater than typical values reported for conventional WWTP. Whole metagenome shotgun sequencing of twelve WWTP and eight enrichment culture samples generated more than 388 million and 177 million paired-end reads, respectively. **Conclusions:** Recalcitrant PPCP (such as carbamazepine) detected at relatively high concentrations may accumulate as the wastewater is reused in the toilets and urinals. Two distinct communities were found in the aqueous and plant root biofilm samples. The initial treatment tanks were dominated by microbial taxa of fecal origin (Bacilli), while the latter tanks had higher microbial species richness. These patterns highlight the importance of providing sufficient physical heterogeneity to allow the development of diverse microbial biofilm communities. We propose that the presence of plants in the WWTP protect the system from disturbances as PPCP inputs change with the changing population of Interstate rest stop visitors. This is achieved by promoting microbial functional richness, thereby increasing metabolic resilience as plant root exudates impart structurally diverse carbon sources.

Author Disclosure Block:

I.N. Balcom: None.

Poster Board Number:

SATURDAY-123

Publishing Title:

Anaerobic Toxicity of Eight Aromatic Pharmaceutical and Personal Care Products

Author Block:

A. W. Porter, S. Wolfson, J. Campbell, L. Y. Young; Rutgers Univ., New Brunswick, NJ

Abstract Body:

Pharmaceutical and personal care products are emerging contaminants that are found in the environment as the result of incomplete removal over the course of the wastewater treatment process. We hypothesize that biodegradation of these chemicals during the anaerobic digestion step could be limited due to toxic effects on the microbial community. Eight aromatic compounds (atenolol, bisphenol-A, diphenhydramine, ibuprofen, naproxen, nonylphenol, octylphenol, and triclosan) commonly found in municipal waste treatment were tested to determine if the presence of each individually would have a negative effect on the microorganisms in anaerobic digestate by measuring methane production as a proxy for anaerobic microbial activity. Methane production was quantified by GC and was slightly reduced in cultures amended with pharmaceutical compounds, however, it was almost completely repressed in the presence of the antimicrobial product triclosan. Surfactant by-products octylphenol and nonylphenol also showed moderately decreased methane production. HPLC analysis of naproxen amended cultures showed evidence of biotransformation within the 34 day sampling period, although there was little change in concentration of the other substrates tested. The microbial community within the anaerobic digester is very complex, but there were noticeable differences in the denaturing gradient gel electrophoresis fingerprints that corresponded to the cultures with decreased methane production. These data indicate that some substrates had little effect on anaerobic microbial activity, but were not biodegraded within the timeframe of this study. Further investigations into bioavailability of these substrates may be necessary to understand the anaerobic biodegradation potential of pharmaceutical and personal care products, as any that are not transformed during wastewater treatment will be released with treated effluents and may ultimately accumulate in anoxic sediments.

Author Disclosure Block:

A.W. Porter: None. **S. Wolfson:** None. **J. Campbell:** None. **L.Y. Young:** None.

Poster Board Number:

SATURDAY-124

Publishing Title:

Anaerobic Transformation of Naproxen by Methanogenic and Sulfidogenic Consortia

Author Block:

S. J. Wolfson, A. W. Porter, L. Y. Young; Rutgers Univ., New Brunswick, NJ

Abstract Body:

Pharmaceutical compounds, now recognized as environmental contaminants, are potential carbon sources for microbes. Naproxen, a common nonsteroidal anti-inflammatory drug, has been detected in wastewater treatment influent, treated effluent, and environmental waters. The biodegradation potential of naproxen in environmental sediments and the mechanism of naproxen transformation under two anaerobic conditions was investigated. To determine microbial degradation of naproxen in anaerobic wastewater digestion, methanogenic enrichment cultures were established with wastewater digestate as inoculum. The microbial degradation potential of naproxen in the environment was determined using anoxic estuarine sediment inoculum under sulfate reducing conditions. Enrichment cultures were fed 1mM naproxen as the sole carbon source. Substrate loss was monitored with HPLC, and GC/MS was used to identify intermediate metabolites. Both methanogenic and sulfate reducing primary enrichment cultures transformed 1mM naproxen to 6-o-desmethylnaproxen (DMN) in 23 and 59 days, respectively. Continual enrichment, naproxen addition, and culture transfer resulted in consistent stoichiometric transformation of naproxen into DMN with no loss of the transformation product observed over one year. Methane production and sulfate loss confirm the loss of one carbon and not complete mineralization of the 14-carbon naproxen compound, indicating demethoxylation. Community 16S rRNA fingerprinting revealed differences in organisms present in naproxen transforming cultures and unamended cultures. The enrichment of two robust consortia specialized in demethoxylating naproxen and provides insight into the transformation of methoxyaromatic contaminants in anaerobic systems.

Author Disclosure Block:

S.J. Wolfson: None. **A.W. Porter:** None. **L.Y. Young:** None.

Poster Board Number:

SATURDAY-125

Publishing Title:

Biotransformation of Sulfadiazine by a Mixed Microbial Culture

Author Block:

X. Li[1], R. Levine[1], Y. Zhang[1], D. Snow[1], L. Durso[2], Y. Leng[1]; [1]Univ. of Nebraska-Lincoln, Lincoln, NE, [2]USDA, Lincoln, NE

Abstract Body:

Background: Certain microbes can transform antibiotics in the environment. However, little is known about the identity of these microbes and their antibiotic biotransformation processes. The objectives of this study were to (1) isolate bacterial strains capable of transforming antibiotics, (2) determine the biotransformation kinetics of antibiotics, (3) characterize the effects of background carbons on the biotransformation kinetics, and (4) identify biotransformation products under various environmental conditions.**Methods:** Sulfadiazine (SDZ) was used as the model antibiotic in this study due to its frequent occurrence in livestock wastes. Surface soil from a cattle feedlot was collected to enrich potential SDZ degrading bacteria. A mixed culture was obtained after several cycles of enrichment in a mineral solution containing 10 mg/L SDZ as the sole carbon and energy source.**Results:** Despite repeated efforts, no single SDZ degrading strain could be isolated from the mixed culture. 16S rRNA gene sequence analysis showed that the culture consisted primarily of two major bacterial species, *Brevibacterium epidermidis* and *Castellaniella denitrificans*. The degradation kinetics of SDZ by the mixed culture could be described using a mirrored logistic function, with a biotransformation rate measured to be at 4.86 $mg \cdot L^{-1} \cdot d^{-1}$. Three types of background carbons were tested: diluted R2A medium, glucose, and humic acid. The mixed culture had the fastest and slowest SDZ biotransformation rates when diluted R2A and humic aicd were used as the background carbon, respectively, at concentrations equivalent to SDZ on a carbon basis. The mixed culture could also degrade other sulfonamide compounds such as sulfamethazine and sulfamerazine, at transformation rates slower than that of SDZ, but could not degrade sulfathiazole. Using liquid chromatography tandem mass spectrometry, we identified 2-aminopyrimidine (2-AP) as a major biotransformation product of SDZ in the absence and presence of the background carbons tested. Another biotransformation product detected was confirmed to not be 4-aminobenzenesulfonate, the remaining structure after the cleavage of 2-AP from SDZ.**Conclusions:** This work presents a comprehensive study of microbial biotransformation of SDZ under various environmental conditions.

Author Disclosure Block:

X. Li: None. **R. Levine:** None. **Y. Zhang:** None. **D. Snow:** None. **L. Durso:** None. **Y. Leng:** None.

Poster Board Number:

SATURDAY-127

Publishing Title:

Isolation and Characterization of Alachlor-degrading Bacterium *Acinetobacter* sp. Gc-A6

Author Block:

H. Lee, H. Kim, J. Yun, J-O. Ka; Seoul Natl. Univ., Seoul, Korea, Republic of

Abstract Body:

Background: Alachlor is a widely used herbicide for preemergence control of annual grass and broadleaf weeds. Owing to its frequent detection in environments, toxicity and mutagenicity, it is important to elucidate degradation mechanism of alachlor and eliminate alachlor and its metabolites from the environments. However, there is limited information on degradation of alachlor and the degradation rate of alachlor is relatively low. In this study, we isolated an alachlor-degrading bacterium, and investigated its ability to degrade alachlor. Also, metabolites produced during degradation of alachlor were identified and novel alachlor degradation pathway was proposed.**Methods:** An alachlor-degrading bacterium was isolated through enrichment processes from agricultural soils. The degradation pathway of alachlor was studied by GC-MS analysis.**Results:** An alachlor-degrading bacterial strain, GC-A6, was isolated and identified as *Acinetobacter* sp. by 16S rRNA gene sequence analysis. The isolate was able to utilize alachlor as a sole source of carbon and energy for its growth in mineral medium. The strain GC-A6 completely degraded 200 mg L^{-1} of alachlor within 72 hours, showing rapid alachlor degradation. The isolate degraded alachlor through two pathways. Alachlor was initially degraded to 2-chloro-*N*-(2,6-diethylphenyl)acetamide which was subsequently degraded through two pathways, 2,6-diethylaniline pathway and 7-ethylindoline pathway. 2,6-diethylaniline was transformed to *N*-(2,6-diethylphenyl)formamide, which was then completely mineralized. 7-ethylindoline also was completely mineralized.**Conclusions:** *Acinetobacter* sp. GC-A6 could utilize and completely mineralize alachlor with high degradation rates. Moreover, the strain could degrade alachlor simultaneously through two pathways, without accumulating any intermediates. Therefore, the *Acinetobacter* sp. GC-A6 may be useful for removal of alachlor and its metabolites from the contaminated environments.

Author Disclosure Block:

H. Lee: None. **H. Kim:** None. **J. Yun:** None. **J. Ka:** None.

Poster Board Number:

SATURDAY-128

Publishing Title:

Molecular and Ecological Characteristics of Carbamate Pesticide-Degrading Bacteria

Author Block:

D-U. Kim, H. Lee, J-O. Ka; Seoul Natl. Univ., Seoul, Korea, Republic of

Abstract Body:

Background: Several studies have focused on the isolation and characterization of carbamate insecticide-degrading microorganisms. In spite of these studies, little information on degradative pathways and genes of carbamate insecticides is available. In this study, novel carbamate insecticide-degradative pathways of *Sphingobium* sp. was characterized, nucleotide sequence of carbofuran degradative plasmid pJE1 was analyzed, and a carbofuran degradation-related gene of pJE1 was identified.**Methods:** Intermediate metabolites was determined by GC-MS analysis. Library construction for plasmid sequencing was performed using the shotgun method. After complete nucleotide sequencing, open reading frames (ORFs) were predicted by the RAST server. For the comparison of plasmid nucleotide sequences, the Artemis Comparison Tool was used. Determined the level of expression of the examined genes using the standard curves. Ct values and normalized them based on 16S rRNA expression and relative gene expression was quantified using the $2^{-\Delta\Delta Ct}$ method. **Results:** *Sphingobium* sp. JE1 was observed to degrade carbofuran through a new pathway, via carbofuran-phenol and 5-hydroxycarbofuran. Another significant character of JE1 was that it had carbofuran hydrolase gene dissimilar to the previously reported *mcd* gene. *Sphingobium* sp. JE1 has a carbofuran-degradative 220kb-sized plasmid pJE1 and pJE1 observed to have novel catabolic genes. First of all, according to the phylogenetic analysis, ParA from pJE1 was classified as a novel clade. In particular, the type IV secretion-like system was present in the same order as in other aromatic compound degrading bacteria even they had low sequence similarity. Relative mRNA expression analysis showed that VirB4, one of the type IV secretion-like system-related gene, is involved in carbofuran degradation. The result shows that, in association with carbofuran degradation, pJE1 has an unique macromolecular translocation system similar to the type IV secretion system.**Conclusions:** *Sphingomonas* sp. JE1 was shown to be capable of degrading carbofuran, using it as a sole source of carbon and energy, through a novel pathway producing an unknown red compound. pJE1 had type IV secretion system-like clade, and relative mRNA expression analysis showed that VirB4 was related to carbofuran degradation.

Author Disclosure Block:

D. Kim: None. **H. Lee:** None. **J. Ka:** None.

Poster Board Number:

SATURDAY-129

Publishing Title:

Nanoremediation of Microbial Contaminants Screened from Algal-Wastewater Systems

Author Block:

A. Limayem[1], A. Micciche[1], E. Haller[1], B. Nayak[2], F. Gonzalez[1], S. S. Mohapatra[1]; [1]Univ. of South Florida, Tampa, FL, [2]Pinellas County Utilities, Largo, FL

Abstract Body:

Background: Algal biofuels have regained interest as environmentally attractive sources of renewable energy. The life cycle assessment (LCA) indicates that algal biofuel production is not sustainable if wastewaters are not used for algae cultivation. However, microbial contaminants inhabiting wastewaters give yield loss of up to 95%. Our preliminary molecular screening from local wastewater systems evidenced the prevalence of lytic bacteria detrimental to algae growth. The objective of this study was to test the efficacy of a scalable nanoremediation on lytic bacteria and prove its mechanistic effect through Transmission Electron Microscopy (TEM).**Methods:** Previously screened strains of *Pseudomonas, Micrococcus,* and *Bacillus* through 16S rRNA DNA sequencing from anaerobically digested wastewaters received from local algae bioreactors were tested against polymeric chitosan-based nanoparticles, 3 kDA, combined with Zinc oxide (CZNPs). The minimal inhibitory concentration (MIC) test conforming to NCCLS Standards was performed using 96 well plates loaded with CZNPs at a 2 fold dilution prior to the addition of 50 µL of ~5×10^5 CFU/mL of bacteria in tryptic soy broth media. Plates were mixed, incubated for 24h at 37°C, and observed visually for the last clear well containing the minimum concentration for inhibition. The mechanistic effects of CZNPs on the lytic bacterial contaminants were visualized through TEM involving osmium tetroxide fixation. Contaminants were exposed for 24h before fixation to a concentration 65.5mg/ml of CZNPs.**Results:** The MICs of CZNPs on *Pseudomonas, Micrococcus,* and *Bacillus* averaged over trials were 0.42, 3.33, and 1.46 mg/mL respectively. TEM performed on lytic bacteria revealed protrusions of microbial cell membranes. Observations of cellular structure, electron density, and cell wall appearance of primarily *Pseudomonas, Micrococcus,* and *Bacillus* were markedly impacted by CZNPs compared to microbial control cells.**Conclusions:** Synergism of chitosan and ZnO proved to be inhibitive of *Pseudomonas, Micrococcus,* and *Bacillus*. This broad spectrum of activity has not been noted before. TEM analyses demonstrated attachment and lysis of microbes at 24h past treatment. Conclusively, CZNPs inhibit the lytic bacterial group and is a promising *in situ* intervention agent.

Author Disclosure Block:

A. Limayem: None. **A. Micciche:** None. **E. Haller:** None. **B. Nayak:** None. **F. Gonzalez:** None. **S.S. Mohapatra:** None.

Poster Board Number:

SATURDAY-130

Publishing Title:

Biodegradation of Aromatic Contaminants at Estuarine Frontal Boundaries

Author Block:

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Abstract Body:

Background: Though previously considered to be persistent in the environment, there is growing evidence that aromatic contaminants such as PAHs and nitrogenous explosives are metabolized relatively rapidly. Estuarine frontal boundaries are transitional zones within coastal waters that may harbor conditions promoting rapid aromatic organic matter biodegradation within a watershed. **Methods:** Bacterial production (leucine incorporation method) and mineralization rates of phenanthrene and energetics (*e.g.* TNT, RDX, HMX) were measured in coastal waters around the Lower Outer Banks, NC, Key West, FL, Charleston, SC, and Corpus Christi, TX. In addition to comparing metabolic rates at frontal transition zones between adjacent water mases, mixing experiments were performed to mimic conditions that may occur at fronts where nearshore waters (*e.g.* mangrove lagoons, Cypress bogs) mix with offshore water. **Results:** Bacterial production was often enhanced at discreet frontal boundary interfaces relative to the adjacent water masses on each side of the front (e.g. ca. 20-100%, Bogue Sound, NC) as was phenanthrene mineralization (ca. 5-fold increase, Bogue Sound, NC). Mixing experiments between DOC-rich end members (mangrove lagoon, FL; Cypress bog, NC) and estuarine or marine end members stimulated bacterial production above that predicted from conservative mixing and often disproportionately stimulated mineralization of phenanthrene and energetics (e.g. five-fold increase in ratio of mineralization to production). **Conclusions:** Transient but common estuarine features such as frontal boundaries appear to stimulate heterotrophic bacterial metabolism and may serve to attenuate anthropogenic contaminants released from terrestrial runoff. Boundaries between water masses may create important transition zones where organic matter is more rapidly metabolized and transformed than in adjacent coastal waters.

Author Disclosure Block:

M.T. Montgomery: None. **J.N. Atar:** None. **T.J. Boyd:** None. **R.B. Coffin:** None. **C.L. Osburn:** None.

Poster Board Number:

SATURDAY-131

Publishing Title:

Detection of Fungal Capabilities to Metabolize 2,4,6-Trinitrotoluene Using Cyclic Voltammetry

Author Block:

J. E. Liquet y González, J. Castellanos, I. Cortez, V. Miranda, R. Padilla, F. Morales, C. Vega, S. Hernandez, C. Ríos-Velazquez; Univ. of Puerto Rico-Mayagüez, Mayagüez, Puerto Rico

Abstract Body:

2,4,6-Trinitrotoluene (TNT) is a man-made explosive used in military shells, bombs, and grenades, in industrial uses, and in underwater blasting. TNT is present in the environment throughout diverse locations, and also known to accumulate in animals and plants. Although its effects on human health are not completely understood, the Environmental Protection Agency, has classified it as a possible human carcinogen. Biodegradation is regarded as an option to clean up many environmental pollutants. However, there is a need for TNT biodegradation experiments using fungi where the metabolic activity can be easily detected. Cyclic voltammetry (CV) is an electrochemical technique that uses an alternating current, oxidizes and reduces molecules, producing a detectable signal. The main focus of this research is the use of CV as an effective technique to detect the TNT degradation capabilities of a fungal bioprospect isolated in Puerto Rico. Spores suspensions of the prospective TNT-degrader, *Aspergillus sp.*, were spreaded on Potato Dextrose Agar, and after 7 days of incubation, 5mm fungal disks were placed in tubes with Bushnell-Haas broth with 68ppm of TNT for 58 days. After applying CV for TNT, three oxidized peaks were found with the increasing current (called Peak 1, 2 and 3) and one that was reduced (Peak 4). In the first five days of incubation, the TNT concentration decreased almost 10ppm in average for Peak 1 and Peak 4. After 58 days of incubation, the TNT concentration had decreased almost 20 ppm on average for the mentioned peaks. For Peak 2 and 3 we did not found a drastic decrease in concentration, only a reduction of 8ppm on average after 58 days of incubation. There was no noticeable change in the fungus morphology after 58 days of incubation with exposure to TNT, determined by using a scanning electron microscope. Further studies to determine the biodegradation product are underway. These findings demonstrate not only that the fungal bioprospect is metabolizing TNT, but also confirm the use of a feasible method to detect metabolic activity in biological systems. The results will allow the discovery of enzymes with potential industrial application and could propose a cost-effective mechanism to biodegrade environmental TNT into a less-hazardous compounds.

Author Disclosure Block:

J.E. Liquet y González: None. **J. Castellanos:** None. **I. Cortez:** None. **V. Miranda:** None. **R. Padilla:** None. **F. Morales:** None. **C. Vega:** None. **S. Hernandez:** None. **C. Ríos-Velazquez:** None.

Poster Board Number:

SATURDAY-132

Publishing Title:

Arsenic Detoxification by Geobacter Species

Author Block:

Y. Dang[1], D. Walker[1], K. E. Vautour[1], S. Dixon[2], D. E. Holmes[2]; [1]Univ. of Massachusetts Amherst, Amherst, MA, [2]Western New England Univ., Springfield, MA

Abstract Body:

Insight into the mechanisms for arsenic detoxification by *Geobacter* species is expected to improve the understanding of global cycling of arsenic in iron rich subsurface sedimentary environments. Analysis of thirteen different *Geobacter* genomes showed that all of these species have genes coding for an arsenic detoxification system (*ars* operon), 3 species (*G. uraniireducens*, strain OR-1, and *G. lovleyi*) have genes required for arsenic respiration (*arr* operon), and 3 (*G. uraniireducens*, *G. lovleyi*, and *G. metallireducens*) have a gene coding for L-methionine-dependent methyltransferase (ArsM) which is required for arsenic methylation. The *ars* operon from *G. sulfurreducens* consists of 10 genes, all of which show increased transcription in the presence of arsenite and arsenate. Four copies of an arsenic repressor-like protein were detected in the genome of *G. sulfurreducens*, however, only one of these genes (*arsR1*) encodes a protein that regulates transcription of the *ars* operon. Elimination of *arsR1* from the *G. sulfurreducens* chromosome resulted in enhanced transcription of genes coding for the arsenic efflux pump (Acr3) and arsenate reductase (ArsC). When the gene coding for Acr3 was deleted, cells were not able to grow in the presence of either the oxidized or reduced form of arsenic, while *arsC* deletion mutants could grow in the presence of arsenite but not arsenate. A number of other heavy metal efflux proteins besides Acr3 were identified in the *G. sulfurreducens* genome, however, transcriptomic studies showed that none of these proteins are likely to be involved in arsenic efflux, even when the gene coding for Acr3 has been deleted. These studies shed light on how *Geobacter* influences arsenic mobility in anoxic sediments and may help us develop methods to remediate arsenic contamination in the subsurface.

Author Disclosure Block:

Y. Dang: None. **D. Walker:** None. **K.E. Vautour:** None. **S. Dixon:** None. **D.E. Holmes:** None.

Poster Board Number:

SATURDAY-134

Publishing Title:

Mechanism of Iodate Reduction by Members of the Genus Shewanella

Author Block:

H-D. Shin[1], S-K. Wee[1], B. Lee[2], M. Lee[2], T. J. DiChristina[1]; [1]Georgia Inst. of Technology, Atlanta, GA, [2]Pacific Northwest Natl. Lab., Richland, WA

Abstract Body:

Background: An attractive remediation of iodine-contaminated environments is iodine immobilization via microbial iodine transformations, including iodate (IO_3^-) reduction, which is a major component of the iodine biogeochemical network. It has been proposed that IO_3^- is reduced by microbial NRs, although this hypothesis has not been extensively investigated. In this study, NO_3^-- and metal-reducing *Shewanella* species were examined for IO_3^- reduction activity and genetic mutagenesis were performed to identify the components shared by the metal, NO_3^-, and IO_3^- reduction systems of *Shewanella* species.**Methods:** 13 *Shewanella* species were anaerobically cultivated in modified marine broth with lactate as electron(e^-) donor and IO_3^- as e^- acceptor to determine IO_3^- reduction activities. The *crp* (cAMP-responsive regulator of catabolite repression)-deficient mutant of *S. oneidensis* (M3013) was also used to examine the involvement of *crp*-regulated anaerobic respiration. To study involvement of NRs on IO_3^- reduction, the mutant Δ*napA* (lacking the periplasmic NR of *S. oneidensis* MR-1) was constructed by in-frame gene deletion mutagenesis and the IO_3^- and NO_3^- reduction activities of Δ*napA* were compared to the wild-type.**Results:** 8 of the 13 species reduced IO_3^-, with extents of reaction varying from 23-97% of the initial IO_3^- amount. *S. putrefaciens* 200 displayed the highest IO_3^- reduction activity of the 8 species. However, the 5 marine species, *S. hanedai, S. frigidimarina, S. dinitrificans, S. baltica and S. liohica,* were unable to reduce IO_3^-. *S. oneidensis* Δ*napA*, on the other hand, was unable to reduce NO_3^-, yet displayed wild-type IO_3^--reduction activity. In addition, the *crp*-deficient mutant M3013 was unable to reduce IO_3^- or NO_3^- under anaerobic condition. Interestingly, *S. oneidensis* Δ*napA* and M3013 reduced NO_2^- faster and to larger extents of reaction than the wild-type. Δ*napAα* and Δ*napAβ* mutants of *S. putrefaciens* 200 were also constructed and both mutants displayed wild-type IO_3^- reduction activity.**Conclusions:** A variety of *Shewanella* species showed IO_3^- reduction activity, NR-deficient mutants of *S. oneidensis* MR-1 and *S. putrefaciens* 200 retained wild-type IO_3^- reduction activity, indicating that NR is not the primary enzymatic system for IO_3^- reduction. The *crp*-deficient mutant was unable to reduce NO_3^- or IO_3^-, which indicates that IO_3^- reduction may be regulated by the *crp* protein.

Author Disclosure Block:

H. Shin: None. **S. Wee:** None. **B. Lee:** None. **M. Lee:** None. **T.J. DiChristina:** None.

Poster Board Number:

SATURDAY-135

Publishing Title:

Comparative Tolerance Assessment of Fresh and Marine Water Phototrophic Microorganisms to Nano And Bulk Zinc Oxides

Author Block:

L. Gibson, D. Berthold, K. Jayachandran, **K. G. SHETTY**; Florida Intl. Univ., MIAMI, FL

Abstract Body:

Zinc oxide (ZnO) nanoparticles (NPs) have vast range of applications, release of these NPs into waste streams and further into the environment may adversely impact non-target organisms. Natural variations in tolerance to nano ZnO may exist among various aquatic phototrophic microbial species, and the availability of tolerant species may lead to better understanding of tolerance mechanism and potential applications in bioremediation. In order to elucidate the effects of zinc oxides on phototrophic microorganism growth, two fresh and marine water microalgae and cyanobacterial species, were used in a replicated study the cultures were tested using 3 growth media treatments, media containing no added zinc, and nano and bulk ZnO at 10 ppm. Fresh water species were grown in 75ml of BG-11 media and the marine species in BG-11 marine media. The culture flasks were incubated on a shaker at 25°C under continuous fluorescent lighting. Changes in terms of growth and morphology of the species to the treatments were assessed by measuring optical density and microscopic observations for a total of 20 days. Results showed marked difference among species tested to varying ZnO concentrations. Most of the microalgae showed morphological changes as a result of exposure to ZnO, which included decreased cell size and discoloration. Among species tested the cyanobacterial species *Limnothrix* and *Tolypothrix* were the most sensitive to both types of ZnO. Although slightly lower compared to the growth in bulk ZnO media the marine species *Neochloris* (OD 0.64) and fresh water species *Scenedesmus* (OD 0.52) showed the highest growth in nano ZnO media. The marine species *Neochloris* (OD 0.64) showed significantly higher growth than *Sphearocystis* (OD 0.09) when grown in nano ZnO media. Morphological changes as a result of exposure to ZnO included decreased cell size and discoloration. These findings provide strong impetus for screening larger collection of diverse indigenous microalgae for tolerance to NPs and which may yield potential candidates for bioremediation of NPs.

Author Disclosure Block:

L. Gibson: None. **D. Berthold:** None. **K. Jayachandran:** None. **K.G. Shetty:** None.

Poster Board Number:

SATURDAY-136

Publishing Title:

Identification and Genetic Analysis of Zinc Resistance in *Bacillus toyonensis*

Author Block:

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Abstract Body:

Heavy metal pollution in soil and water-systems neighboring mines and recycling yards are a major concern. Zinc (Zn) is of particular interest because it is not only essential to life but is also an anthropologically deposited and concentrated pollutant. Current methods utilize electrolytic plants for remediation; however this gestation period is long and may be unable to combat rising amounts of Zn deposition and subsequent pollution. Bioremediation utilizing microbes could supplement current phytoremediation methods by mobilizing and expediting Zn recovery and recycling. To assess the feasibility of Zn reclamation through bioremediation, we investigated the genetic basis of heavy metal tolerance in bacteria isolated from a recycling yard. Bacteria SR-13 was isolated from soil and water samples taken near a recycling yard by the Mississippi river in Minneapolis, Minnesota. Initial characterization of SR-13 was done using gram stain, biochemical testing and was found to be gram positive and catalase positive. Maximum tolerable concentration (MTC) analysis using Zinc (II) Chloride was determined in LB + Agar media. The MTC of SR-13 was found high and growth was observed up to a concentration of 4 millimolar (mM). SR-13 was then sequenced using 16s rRNA 1492R and 27F primers that amplified the host genome and identified the strain as *Bacillus toyonensis*. To further elucidate the genetic mechanism of Zn resistance, *B. toyonensis* was subjected to transposon mutagenesis using a conjugation method with F+ *Escherichia coli* (*E.coli*) that contained a pRL27 plasmid with a Tn5 transposable element, and kanamycin resistance. Transposon containing mutants were selected by plating on combination 25ug/mL kanamycin + 50ug/mL ampicillin + LB + Agar plates. Zn intolerable mutants were then observed using replica plating from the antibiotic plate onto a 2mM Zn (II) Chloride + LB + agar plate. Successful disruption of the host genome of *B. toyonensis* created a Zn intolerable mutant (MV-1) and was also sequenced. The mutant was also identified as the same as the non-mutagenized strain. Transposon mutagenesis concluded that *B. toyonensis* has a genetic element that gives resistance to Zn in its environment. Future experiments are being conducted to identify the disrupted gene as well as other resistance genes in *B. toyonensis* that could be used for bioremediation applications.

Author Disclosure Block:

V.S. Krawiec: None. **R.B. Kumar:** None.

Poster Board Number:

SATURDAY-137

Publishing Title:

A Null Mutation in Sulfate Adenylyltransferase Supresses Lead Precipitation in Caulobacter Crescentus

Author Block:

R. E. McCorkle, K. A. Moore, M. J. Hickman, G. B. Hecht; Rowan Univ., Glassboro, NJ

Abstract Body:

Background: Microbial mineralization of lead is not well understood. Previous work by our group has demonstrated that microbes are able to precipitate lead in the form of $Pb_9(PO_4)_6$, which can otherwise only be produced synthetically at around 200°C. In contrast, bacterial mediation of this process occurs at room temperature, though the mechanism by which bacteria precipitates this lead phosphate is not known. We have isolated spontaneous lead hyper-precipitating (*hyp*) strains of *Caulobacter crescentus*, a freshwater oligotrophic bacterium that is normally not an efficient lead precipitator, and created several non-precipitating (*nop*) suppressor strains. Whole-genome sequencing revealed that the *hyp* mutants have changes in the CC3625 cysteine synthase gene. Some of the *nop* suppressor strains have additional changes in CC1117, a gene encoding a LysR transcriptional regulator. We report here the analysis of RCCR60, a suppressor strain where the *nop* mutation is unlinked to CC1117. **Methods:** Whole genome sequence analysis was performed to locate mutations unique to strain RCCR60. Cr30-mediated transductions were carried out to demonstrate which mutations are important for the precipitation phenotype. The growth of RCCR60 on minimal medium was compared to our CC1117 mutant strains. **Results:** Transductions using a selectable marker linked to a nonsense mutation in CC1482 demonstrated that locus is both necessary and sufficient for the *nop* phenotype of RCCR60. CC1482 encodes a sulfate adenylyltransferase that is an early step in converting sulfate into H_2S, an important substrate for CC3625. Unlike mutations in LysR, the CC1482 null allele does not confer cysteine auxotrophy and only confers a partial suppression of the lead precipitation phenotype. **Conclusions:** We propose that the CC1482 gene is important for cysteine synthesis but also that *Caulobacter* contains a second sulfur acquisition pathway independent of CC1482. Current experiments are aimed at quantifying the rate and efficiency of lead precipitation and cysteine synthesis in our strains, including the CC1117 and CC1482 mutant strains.

Author Disclosure Block:

R.E. McCorkle: None. **K.A. Moore:** None. **M.J. Hickman:** None. **G.B. Hecht:** None.

Poster Board Number:

SATURDAY-138

Publishing Title:

Cooper Reduction by *Shewanella* Isolated from the Chilean Antarctica

Author Block:

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Abstract Body:

Background: Several environmental microorganisms have adapted to the harsh conditions of the Antarctica, including temperature, pH, nutrient limitation, radiation and metals/metalloids, among others. A common bacterial genus that is found in these environments is *Shewanella*, which is characterized by the use of a wide range of electron acceptors during anaerobic respiration such as fumarate, nitrate, sulfur, Fe(III), Mn(III), Cr(VI), Cr(VI) and others. During an expedition to the Chilean Antartica, a marine bacterium belonging to this genus was isolated and compared with other *Shewanella* species regarding metal reduction.**Methods:** Metal quantification was accomplished through Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES).**Results:** The results indicate that *S. antartica* CH displays high tolerance to copper salts, whose concentration was determined in supernatants of treated cells. Growth curves for *S. antartica* CH, *S. oneidensis* MR1, *S. baltica* OS155 and *S. putrefaciens* growing under different concentrations of NaCl (1%-5%), pH (5,5-9,0) and copper (2-4 mM) were constructed.**Conclusions:** *S. antarctica* CH reduced copper (when added as Cu^{+2}) to elemental copper (Cu^{0}) in anaerobic conditions. This antarctic bacterium showed better growth regarding other *Shewanella* species exposed to different copper salts and/or high NaCl concentrations. The pH value of the culture medium was critical for the growth and metal reduction by the Antarctic strain.

Author Disclosure Block:

M. Valdivia: None. **D. Loyola:** None. **M. Jara:** None. **C. Vasquez:** None.

Poster Board Number:

SATURDAY-139

Publishing Title:

Detection of Copper and Mercury Resistance Genes in Purple Non-Sulfur Bacteria from Different Aquatic Environments in Puerto Rico

Author Block:

K. M. Rodriguez-Nuñez, J. Rullan-Cardec, C. Rios-Velazquez; Univ. of Puerto Rico Mayaguez Campus, Mayaguez, Puerto Rico

Abstract Body:

Environmental Protection Agency revealed in 2014 that 79.7% of rivers and streams used as drinking supply in Puerto Rico (PR) were impaired with metals such as copper and mercury. Copper is the first metal and fifth cause of impairment along with mercury as the fourth metal contaminating the studied waters. Mercury is bio-accumulative and its consumption leads to kidney damage while copper intake can cause liver disease.There have been several reports of human intoxication by these metals. For example, Agency for Toxic Substances and Disease Registry recorded mercury poisoning of 290 civilians in PR by eating fish grown in contaminated waters. Techniques such as metal precipitation and ion exchange resins are employed internationally to remove such metals but their high cost and low effectiveness limit their use. Other approach include the use of microbes such as PNSB for the successfully removal of heavy metals from water. In PR, our Laboratory of Microbial Biotechnology and Bioprospecting has a collection of PNSB isolated from different aquatic environment such as Bromeliad phytotelmata and benthic hypersaline Microbial Mats (MM). However the biotechnological potential of these bacteria and its application in heavy metal bioremediation is unknown. In this study the presence of genes related to copper and mercury resistance was addressed molecularly by PCR, using specific primers for mercuric reductase (*merA*) and *cop*-like genes. From a total of 39 isolates (14 from MM and 25 from Bromeliad phytothelmata) evaluated in this study, positive amplification products for mercuric reductase genes (1,238 bp) and for *cop*-like genes (750bp) were found in 7% and 14% of the isolates respectively from MM, and 16% *cop*-like genes from Bromeliads isolates. Other amplifications of non-expected size were obtained and will be confirmed *in silico* to validate their genetic identity. The *in silico* analysis of the 16S rDNA of the isolates where amplified genes were detected suggest PNSB from *Rhodopseudomonas, Rhodospirillaceae and Rhodobacter* family. The results not only confirm the presence of genes related to metal resistance in isolates from extreme and unexplored microenvironments; but also its biotechnological potential applications in metal bioremediation.

Author Disclosure Block:

K.M. Rodriguez-Nuñez: None. **J. Rullan-Cardec:** None. **C. Rios-Velazquez:** None.

Poster Board Number:

SATURDAY-140

Publishing Title:

Expression and NMR Studies of Membrane Proteins from Mercury-Resistant *Bacillus* Sp. and Its Use in Mercury Bioremediation

Author Block:

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Abstract Body:

Background: Mercury exists naturally in small amounts in the environment. Its level is rising due to industrialization and other anthropogenic activities. Mercury resistant bacteria harboring *m*er operon encode for certain functional soluble and transmembrane proteins which are involved in bacterial mercury detoxification system.**Methods and Results:** Mercury (Hg) resistant bacterial isolate was isolated from wastewater polluted soil collected close to tanneries of district Kasur, Pakistan. Selected bacterial strain was screened out on the basis of Hg-resistance (40-50µg/ml). Biochemical characterization, 16S ribotyping and phylogenetic analysis revealed that the isolate characterized as *Bacillus* sp. AZ-1 (KT270477). Two genes (*mer*F and *mer*E) were amplified and expressed into small membrane proteins MerF (81-residues) and MerE (89-residues). In order to perform structural studies of MerF and MerE by NMR spectroscopy, it is necessary to produce milligram quantities of isotopically labeled protein; as is the case for most membrane-associated proteins, this is challenging. We described the successful expression of full-length of both proteins using a fusion partner in pHLV and pET31b(+) vectors that direct the over-expressed protein to the inclusion bodies. Following isolation of the fusion proteins by affinity chromatography, MerF was chemically cleaved with cyanogen bromide and MerE was cleaved by thrombin respectively. Both polypeptides were purified by size exclusion chromatography. Solution NMR two-dimensional HSQC spectra of uniformly ^{15}N-labeled MerF-polypeptides in SDS containing NMR buffer were fully resolved, with a single resonance for each amide site. Finally, *Bacillus* sp. AZ-1 was immobilized in sodium alginate beads and checked the Hg-detoxification potential. It showed an extensive capability to detoxify 88% (15 out of 17µg/ml) of mercury from industrial effluent. **Conclusions:** Mercury resistant Bacillus sp. possessing the mer operon including merF & E and other functional genes coding for resistance should be given prime consideration for use in bioremediation.

Author Disclosure Block:

A. Amin: None. **S.J. Opella:** None. **Z. Latif:** None.

Poster Board Number:

SATURDAY-141

Publishing Title:

Biosorption of Lead by Microbes and Agro-Waste Materials: An Ecofriendly and Inexpensive Approach for Remediation of Heavy Metals from Environment

Author Block:

S. JANAKIRAMAN; Bangalore Univ., Bangalore, India

Abstract Body:

Accumulation of heavy metals in the environment is of major concern as they are not biodegradable and therefore causes serious health problem to living organisms. In recent years, biosorption of heavy metals by microbes and agro-waste materials have gained increasing attention over the conventional methods. A total of one hundred and twenty fungi were isolated from soil samples collected form heavy metal contaminated sites. Two species of *Aspergillus* exhibited resistance to 1500mM of lead (Pb) on agar medium. A biosorbent was designed by growing these fungal organisms on luffa sponge (an agro-waste material) under shaken condition for 72 hours at 30^0 C. The percentage of lead adsorption by the designed biosorbent was determined initially by gravimetry method and further confirmed by Atomic Adsorption Spectrophotometry (AAS) and Inductively Coupled Plasma- Optical Emission Spectrometry (ICP-OES) showing 35%, 56%, 63% respectively with *A niger*, whereas for *A terreus* it showed 32%, 44%, 59%. The Fourier Transform Infrared Spectroscopy (FTIR) analysis revealed that the functional groups OH^-, $=CH^+$ present on our designed biosorbents are facilitating the biosorption. The textural properties of the designed biosorbent was morphologically analyzed by Scanning Electron Microscopy (SEM).The practical applicability of the designed biosorbent for heavy metal removal from heavy metal contaminated environment is discussed.

Author Disclosure Block:

S. Janakiraman: None.

Poster Board Number:

SATURDAY-142

Publishing Title:

Biological Treatment of Cyanide by Isolated Bacteria from Gold Leaching Plants in Colombia

Author Block:

V. López, **C. X. Moreno-Herrea**, M. A. Márquez, Grupo de Microbiodiversidad y Bioprospección (MICROBIOP), Grupo de MIneralogía Aplicada y Bioprocesos (GMAB); Univ. Natl. de Colombia, Medellín, Colombia

Abstract Body:

Background: The technologies used into cyanide contaminated water treatment depend of a combination of physic and chemistry factors. Due to cyanide is highly toxic, it must be treated before it to be discharged into effluents. It is unknown the bacterial communities diversity which are associated with places contaminated with cyanide who could have an *in situ* remediation process potential. The biological degradation is the process of breaking and transforming hazardous materials into simple nontoxic substances by a biological treatment. Despite the technological interest of cyanide-oxidizing bacteria, the ecology physiological role and diversity of bacteria in gold leaching plants from Colombian is still unknown. **Methodology:** In this research the microbiological treatment for the removal of cyanide compounds by 4 species isolated from gold leaching plants in two Colombian mines were studied. Theses were inoculated in LB medium with 200 ppm of cyanide as preliminary test of degradation and later were evaluated in M9 liquid medium with 200, 500 and 1000 ppm and monitored during 168 hours. **Results:** The pH was constant and the cell counting increased to 10^8 after 8 days of incubation under aerobic conditions. All the isolates revealed degradation in function of time. The isolated that showed higher degradation percentages using LB medium was identified as *Rhodococcus* sp., which degraded until 53% and the isolated which showed better performance in M9 medium had the affiliations to *Microbacterium* sp. which degraded until 40% of the cyanide in the medium. **Conclusions:** To the best of our knowledge, this is the first report of these bacteria as degraders and habitants of places contaminated with cyanide in Colombia. Our results indicate that there is a wide diversity of cyanide-oxidizing bacteria that could be applied for conventional gold leaching plants in aerobic conditions.

Author Disclosure Block:

V. López: None. **C.X. Moreno-Herrea:** None. **M.A. Márquez:** None.

Poster Board Number:

SATURDAY-143

Publishing Title:

Characterization of *Pseudomonas veronii* 2E Extracellular Polymeric Substances as Cd(II) Ligand for Metal Removal

Author Block:

M. L. Ferreira[1], A. Casabuono[2], A. Couto[3], S. A. Ramirez[1], **D. L. Vullo**[1]; [1]Univ. Natl. de Gen. Sarmiento, Los Polvorines, Buenos Aires, Argentina, [2]FCEN-Univ. de Buenos Aires, Buenos Aires, Argentina, [3]FCEN-Univ. Buenos Aires, Buenos Aires, Argentina

Abstract Body:

Biological treatments are innovative technologies available for industrial wastewaters containing metals. Some bacteria are adapted to metal polluted environments as consequence of the development of survival strategies or just by secretion of extracellular polymeric substances (EPS). EPS are microbial products excreted (soluble) or associated to the cell surface and are composed by a complex mixture of proteins, polysaccharides, nucleic acids, lipids and other polymeric compounds. They can protect cells from metal toxicity due to the presence of different functional groups available as ligands. The aim of this study was to evaluate the complexing capacity of the major component in EPS produced by the indigenous strain *P.veronii* 2E for potential application in wastewater treatment. EPS were produced by growing cells at 32 ºC in a minimal medium M9-2% (v/v) glycerol, precipitated from culture supernatants with ethanol and purified by both dialysis (MW>12,400Da) and enzymatic treatments (DNase I and Proteinase K). After treating this last fraction (EPSenz) with sodium deoxycholate, two major fractions were obtained: lipopolysaccharide LPS and extracellular polysaccharide ExP, both in similar proportions. FT-IR spectroscopy was performed to study the functional groups present in LPS and ExP that may interact with Cd(II). The ability of EPS, EPSenz, LPS and ExP to complex Cd(II) was determined by an electrochemically monitored titration (ASV) at pH 7.5 (25±1°C). One family of moderate strength binding sites was observed in all fractions with a log K´ (conditional formation constant) of 5.5, while the ligand concentration (µmoles of binding sites/g dry weight) was 65 µmol/g EPS, 500 µmol/g EPSenz, 278 µmol/g LPS and 16 µmol/g ExP. LPS is the purified fraction with great affinity for the complexation of the cation, consistent with the high biosorptive capacity of the whole cells observed in previous studies. The simple production and purification of the soluble EPS is a promissory alternative as a metal retaining agent to minimize hazardous discharges of industrial wastes and to open the possibility of metal recovery.

Author Disclosure Block:

M.L. Ferreira: None. **A. Casabuono:** None. **A. Couto:** None. **S.A. Ramirez:** None. **D.L. Vullo:** None.

Poster Board Number:

SATURDAY-144

Publishing Title:

Metal Accumulation by Sporulated Bacteria on Feathers

Author Block:

E. Góngora, L. N. Osorio, J. Dussán; Univ. de los Andes, Bogotá, Colombia

Abstract Body:

Background: Human activities release toxic metals to the environment polluting the air, water and soil. Sporulated bacteria (SB), ubiquitous in most habitats, have the ability to absorb/adsorb those metals. As birds can bioaccumulate metals and their feathers possess a microbiota that includes various SB, these may interact with the present metals.Here, we evaluate the ability of SB to accumulate Pb, both, *in vitro* and when present on feathers so that it may later be used for bioremediation processes. **Methods:** We sampled hummingbirds and swabbed their plumages to sample the SB present on feathers, grew them on single-feather liquid and solid media after performing heat shock (to isolate spores), and identified them by sequencing the 16S rRNA gene.We used two strains of *Lysinibacillus sphaericus* (obtained from feathers and beetle larvae) to evaluate the Pb adsorption on the surface of the spores after culture in a Pb-enriched liquid sporulation medium. We confirmed Pb adsorption with an energy dispersive X-ray analysis (EDX). Pb uptake was measured with a spectrophotometric analysis (SA) and analyzed with an ANOVA.The ability of 10 SB isolates to degrade keratin was determined by the presence of halos and degradation of feather particles in solid and liquid feather media, respectively. Then, we evaluated the capacity of SB to accumulate Pb using a single-feather medium by confirming the presence of Pb in cells and spores by EDX. **Results:** We confirmed the presence of metals on the feathers by EDX and SA. Over 50 morphotypes were obtained and 37 were sequenced. 65.7 % of them were SB (Bacillaceae), 8.6 % belonged to Pseudomonadaceae and the remaining 25.8 % were not identified.We observed that spores from both strains could adsorb Pb on their surface. The accumulation capacity varied between strains and with the initial Pb concentration and the adsorption time.30 and 100 % of the SB degraded keratin on the solid and liquid feather media, respectively. The SB could also accumulate Pb using feathers as their C and N substrate. **Conclusions:** We conclude that the evidence here presented suggests that there is a possible accumulation of Pb present on bird feathers as a naturally occurring process. We also demonstrated that the process may be potentially implemented for bioremediation in water sources, for example, using the spores of these SB.

Author Disclosure Block:

E. Góngora: None. **L.N. Osorio:** None. **J. Dussán:** None.

Poster Board Number:

SATURDAY-145

Publishing Title:

Quantification of Biomass Production and Organic Acid Production for Three Fungal Candidates

Author Block:

A. Lobos; Univ. of South Florida, Tampa, FL

Abstract Body:

An environmentally friendly fungal bioleaching process with organic acids produced by *Aspergillus niger, Penicillium chrysogenum* and *Penicillium simplicissimum* will be investigated as an alternative leaching method for the recovery of lithium/cobalt from spent rechargeable Li-ion batteries. The first approach for this plan is to quantify fungal growth by recording the biomass production and measuring pH changes on two different mediums; high performance liquid chromatography will be used to quantify organic acid production for the three fungal species over 10 days to determine the best candidate for fungal bioleaching of lithium and cobalt from spent lithium-ion batteries. The three fungal candidates were cultured in sabouraud dextrose broth and czapek dox broth for 10 days at 30°C while shaking at 125rpm; samples were collected in triplicates every 24 hours and processed to determine dry biomass production, pH of medium and organic acid production. Preliminary results with fungi in sabouraud dextrose broth after 10 days of growth: pH of medium was measured before inoculation of fungi, mean pH (5.37 $\pm$ 0.042); *Aspergillus niger* dry weight biomass mean (3.35 g/L $\pm$ 2.254 g/L), mean pH (3.57 $\pm$ 0.899), mean concentration of oxalic acid (51.104 mM $\pm$ 2.187 mM); *Penicillium chrysogenum* dry weight biomass mean (1.862 g/L $\pm$ 0.905 g/L), mean pH (4.21$\pm$ 0.057), mean concentration of oxalic acid (11.16 mM $\pm$ 3.016 mM); *Penicillium simplicissimum* dry weight biomass mean (3.106 g/L $\pm$ 1.505 g/L), mean pH (4.89 $\pm$ 0.053), mean concentration of oxalic acid (5.26 mM $\pm$ 2.568 mM). Preliminary results indicate that *Aspergillus niger* will be the top candidate due to high production of oxalic acid. The data collected in this study will expand knowledge on the process for organic acid production in three fungal species and help select the top candidate for fungal bioleaching. After quantification of organic acid production and fungal growth is complete, metal tolerance tests will then be done to determine the toxicity of lithium and cobalt to each fungal candidate; bioleaching experiments will then be conducted to recover lithium and cobalt from spent rechargeable lithium-ion batteries.

Author Disclosure Block:

A. Lobos: None.

Poster Board Number:

SATURDAY-147

Publishing Title:

Microbial Functional Diversity Predicts Groundwater Contamination and Ecosystem Functioning

Author Block:

Z. He[1], P. Zhang[1], L. Wu[1], A. Rocha[2], Q. Tu[1], Z. Shi[1], Y. Qin[1], J. Wang[1], D. Curtis[1], J. Van Nostrand[1], L. Wu[1], D. Elias[2], D. Watson[2], M. Adams[3], M. Fields[4], E. Alm[5], T. Hazen[6], P. Adams[7], A. Arkin[7], J. Zhou[1]; [1]The Univ. of Oklahoma, Norman, OK, [2]Oak Ridge Natl. Lab., Oak Ridge, TN, [3]Univ. of Georgia, Athens, GA, [4]Montana State Univ., Bozeman, MT, [5]Massachusetts Inst. of Technology, Cambridge, MA, [6]Univ. of Tennessee, Knoxville, TN, [7]Lawrence Berkeley Natl. Lab., Berkeley, CA

Abstract Body:

Background: Anthropogenic activities have significantly impacted the biosphere of Earth due to contamination of air, water and soil environments, thus decreasing biodiversity and destabilizing ecosystem functions. However, little is known about how environmental contamination affects the biodiversity of groundwater microbial communities and its feedbacks to ecosystem functioning.**Methods:** We used a comprehensive functional gene array (GeoChip 5.0) to analyze the functional diversity of groundwater microbial communities from 69 wells at the Oak Ridge Field Research Center (Oak Ridge, TN), representing a wide range of uranium, nitrate and other contaminant concentrations, as well as pH and dissolved gases (e.g., CO2, CH4, N2O).**Results:** It is hypothesized that the functional diversity would decrease as environmental contamination (e.g., uranium, nitrate) increased, or at low or high pH; however, specific populations capable of utilizing or resistant to those contaminants would increase. Thus, it would be possible to predict groundwater contamination and ecosystem functioning using those key microbial functional genes. Our results indicate that the functional richness/diversity significantly ($p < 0.05$) decreased as uranium (but not nitrate) increased in groundwater, and about 5.9% of specific key functional populations (e.g., dsrA, cytochrome genes, nirK, norB, nosZ, napA) significantly ($p < 0.05$) increased as uranium or nitrate increased. Also, we showed that microbial functional diversity could successfully predict uranium and nitrate contamination and ecosystem functioning.**Conclusions:** This study provides new insights into our understanding of the effects of environmental contamination on groundwater microbial communities and the potential for predicting environmental contamination and ecosystem functioning.

Author Disclosure Block:

Z. He: None. **P. Zhang:** None. **L. Wu:** None. **A. Rocha:** None. **Q. Tu:** None. **Z. Shi:** None. **Y. Qin:** None. **J. Wang:** None. **D. Curtis:** None. **J. Van Nostrand:** None. **L. Wu:** None. **D. Elias:**

None. **D. Watson:** None. **M. Adams:** None. **M. Fields:** None. **E. Alm:** None. **T. Hazen:** None. **P. Adams:** None. **A. Arkin:** None. **J. Zhou:** None.

Poster Board Number:

SATURDAY-148

Publishing Title:

Mediation of Coal Mine-derived Acid Mine Drainage with the Introduction of Pristine Soil

Author Block:

S. Sharma; The Univ. of Akron, Akron, OH

Abstract Body:

Active or abandoned coal mines generate acid mine drainage (AMD), which are fluids systems with low pH and High Fe(II) Concentrations, produced by oxygenation of coal mine associated pyrite (FeS) exposed during mining activities. AMD remains one of the greatest threats to the quality of surface water in Appalachian coal mining regions of the U.S. Many remediation processes that focus on Fe removal are costly and require extensive maintenance. Therefore, there is a need to develop less costly and more sustainable AMD treatment approaches. The activities of Fe (II) oxidizing bacteria (FeOB) that are associated with AMD may be exploited to catalyze the oxidative precipitation of Fe from fluids. In this study we introduced AMD fluids collected from abandoned mine works to pristine soil and evaluated the rates of Fe (II) oxidation and abundances of FeOB over time. We are also evaluating the change in the microbial communities that occur when AMD infiltrates initially pristine soil using 454- pyrosequencing-derived 16S rRNA gene sequences. Our results indicate that upon mixing of AMD with pristine soil, microbial communities mediate rapid oxidative precipitation of Fe from AMD. Finally, this study presents an effective system to analyze the mediation of Fe oxidation of AMD by microbial community present in pristine soil, which further can be used to develop an effective and economical alternative to already present remediation methods.

Author Disclosure Block:

S. Sharma: None.

Poster Board Number:

SATURDAY-149

Publishing Title:

Metagenomics Study of Endophytic Fungi of *Avicennia marina* Using Next Generation Technology

Author Block:

A. Bahkali[1], M. Abdel-Wahab[2], A. El-Gorban[1], M. Hodhod[1]; [1]King Saud Univ., Riyadh, Saudi Arabia, [2]Sohag Univ., Sohag, Egypt

Abstract Body:

Endophytic fungi are an excellent source of new pharmaceutical chemicals and may be potential sources of bioactive medicinal compounds. Metogenomics study of environmental samples enables rapid analysis of the composition and diversity of microbial communities at various habitats. We have assessed the endophytic fungi of *Avicennia marina* using next generation generation technology (NGS) by assessing its PCR amplicon of the partial LSU rDNA sequences using LROR and LR3 primers with Illumina metagenomics technique used to generate a total of 35,886 reads from four healthy looking samples of leaves of *A. marina*. Seventy-two OTUs were recorded from the four samples, of which 30 OTUs were single read. Yeast species dominating the fungal of the samples. The Basidiomycetous yeast genus *Malassezia* dominated the fungal community of the samples representing 98.6 % of the reads. Other common yeast genera were: *Hortaea, Candida* and *Schwanniomyces*. Common filamentous fungal genera were: *Trichoderma, Neocallimastix, Geranomyces, Ramaria, Laetiporus, Mucor* and *Gonapodya*.

Author Disclosure Block:

A. Bahkali: None. **M. Abdel-Wahab:** None. **A. El-Gorban:** None. **M. Hodhod:** None.

Poster Board Number:

SATURDAY-150

Publishing Title:

The Genetic Diversity and Composition of the Rhizosphere Microbiome in Healthy and *Phellinus noxius* Infected Trees

Author Block:

K. S. W. Tsang, C. K. Wong, H. S. Kwan; The Chinese Univ. of Hong Kong, Hong Kong, Hong Kong

Abstract Body:

Brown root rot is a fatal disease affecting over 200 plant host species globally. The pathogen is a soil-dwelling white rot basidiomycetes fungus *(Phellinus noxius)* and if left untreated, leads to host death within a year. The rhizosphere microbiome plays an important role in plant health. Currently there are limited studies that observe the changes occurring within the rhizosphere when the plant is faced with a pathogen. The purpose of this study is to investigate the changes a *P. noxius* infection has on the diversity and composition of bacteria and fungi present in the rhizosphere microbiome of two different plant species. Rhizosphere soil samples were taken in triplicates from 4 diseased and 2 healthy *Ficus microcarpa* and *Mallotus paniculatus* trees around Hong Kong. Each set of healthy and diseased samples were taken in the same immediate area to avoid bias. Bacterial 16S and fungal ITS amplification was and sequenced on the Ion Torrent PGM system. The resulting data was demultiplexed, quality filtered and analyzed using the QIIME pipeline. Representative OTUs were clustered at 97% similarity within each microbial group and aligned to reference sequences using Greengenes for bacteria and QIIME/UNITE for fungi. In general, the bacterial and fungal rhizosphere compositions between the two species of tree are vastly dissimilar. However, within each species, the composition trends remain relatively similar. There is also a significant shift in the rhizosphere composition between the diseased and healthy trees. Interestingly, there is an increased presence of pathogenic *Fusarium* species in the diseased samples of both species, which suggests an opportunistic colonization. The majority of fungal species found in diseased samples are saprotrophic, with the genus *Humicola* being particularly dominant in *M. paniculatus* diseased samples. The bacterial chao1 index shows the diversity of *P. noxius* infected trees was significantly reduced in comparison to healthy trees; however there is no such difference within the fungal samples. In conclusion, the rhizosphere composition is different between species, however retains similarities within a species. There is also a significant difference in the microbial composition between healthy and *P. noxius* infected tree samples.

Author Disclosure Block:

K.S.W. Tsang: None. **C.K. Wong:** None. **H.S. Kwan:** None.

Poster Board Number:

SATURDAY-151

Publishing Title:

Survey of *Methylobacteria* Species Found in Association with Several Genera of Fungi in Massachusetts

Author Block:

S. Drinan, M. James-Pederson, R. Hirst; Stonehill Coll., Easton, MA

Abstract Body:

Methylobacteria *(Methylobacterium spp.*) are pink pigmented facultative methylotrophs (PPFM) capable of utilizing one-carbon compounds, such as methanol, as a carbon source. Methylobacteria have been isolated from a variety of substrates including but not limited to soil, air, freshwater, dust, lake sediments, and plants. They have been found in association with over 70 different types of plants and the association can be symbiotic, epiphytic or endophytic; utilization of methanol released from plant stomata by *Methylobacterium* has been well documented. Although ubiquitous in nature and predominant in the phyllosphere, to date there have been no reports of *Methylobacterium* species isolated from white-rot fungi, which are also capable of releasing methanol as a byproduct of lignin degradation. Our laboratory recently identified a species of *Methylobacterium* living in association with the white-rot fungi, *Armillaria gallica*. To determine if this association extended beyond a single genus of fungi, we analyzed the *Methylobacterium* species colonizing the surface of a wide range of fungal genera collected from the Stonehill College campus in Easton, MA. 18S rRNA gene sequence analysis was used to identify the genus of each fungus collected from the wooded areas over a 3 month period of time running from July to October, 2015. Potential *Methylobacterium* species were isolated from fungal stamps using selective media containing methanol as the only carbon source. 16S rRNA gene sequence and comparative analysis using EZtaxon was used to identify the genus and species of the associated bacteria. The results showed that a diverse pool of *Methylobacterium* (*M. komagate, M. goesingense, M. mesophilicum, M. longum, M. pseudosasicola, and M. phyllostachyos*) are common colonizers of a wide range of fungi from the phylum basidiomycota including *Amanita, Megacollybia, Russula, Boletus and Gymnopus*. These results suggest that Methylobacteria may be widely associated with fungus providing candidate associations that could be investigated to better understand the role Methylobacteria may play in the ecology of fungi.

Author Disclosure Block:

S. Drinan: None. **M. James-Pederson:** None. **R. Hirst:** None.

Poster Board Number:

SATURDAY-152

Publishing Title:

Study of the Ecology of Anthrax Foci on the Azerbaijan Border Areas with Georgia

Author Block:

M. Baghirova[1], S. Mammadova[2], N. Imamov[3], M. Hasanova[3], G. Ahmadov[3], G. Ahmadova[3]; [1]Azerbaijan Vet. Scientific Res. Inst., Baku, Azerbaijan, [2]Republican Vet. Lab., Baku, Azerbaijan, [3]Gakh Zonal Vet. Lab., Gakh, Azerbaijan

Abstract Body:

Background: Anthrax is a livestock-borne zoonotic disease that is endemic in the South Caucasus region. There is a lack of understanding of the regional epidemiology of this pathogen and the transboundary factors related to its persistence. This project is a regional collaboration between research institutes in Azerbaijan and Georgia aimed at increasing the local and regional understanding of anthrax ecology, ecological risk factors, and the genetic relationships and distribution among Azerbaijani and Georgian *B. anthracis* strains. This abstract will present data from the Azerbaijan activities of this joint project. **Methods:** Thirty villages in seven rayons along the Azerbaijan border with Georgia (Gazakh, Agstafa, Tovuz, Samukh, Gakh, Zagatala, and Balakan) which have had historic cases of both human and animal anthrax were included. At least 1,500 and up to 3,000 soil samples are being collected from four sites within each village. Sample collection sites were targeted to known burial sites and areas of previous contamination, as evidenced by livestock outbreaks or human case records. GIS (Geographic Information System) data is being collected and will be analyzed using ecological niche modeling. Soil samples will be tested by bacteriology and confirmed with PCR. **Preliminary Results:** Two sample collection sessions have been conducted with a total of 327 samples collected from Zagatala (96) and Gakh (231) rayons of Azerbaijan. Bacteriological tests indicated 37 suspected positive samples. All suspect samples were extracted for confirmation by PCR. All extracts of confirmed positive samples will be tested by real-time PCR in Azerbaijan and sent to the Lugar Center in Georgia for genotyping. **Conclusion:** The activities of this project are ongoing. This regional collaboration will provide the potential for the cooperative development of strategies for the control and trans-boundary management of anthrax in the South Caucasus.

Author Disclosure Block:

M. Baghirova: None. **S. Mammadova:** None. **N. Imamov:** None. **M. Hasanova:** None. **G. Ahmadov:** None. **G. Ahmadova:** None.

Poster Board Number:

SATURDAY-153

Publishing Title:

Diverse Groups of *Escherichia coli* Persisting in Cattle Pasture

Author Block:

G. NandaKafle[1], T. Seale[2], T. Flint[1], M. Nepal[1], S. Venter[2], V. S. Brozel[1]; [1]South Dakota State Univ., Brookings, SD, [2]Univ. of Pretoria, Pretoria, South Africa

Abstract Body:

It is widely believed that *Escherichia coli* does not thrive for extended periods outside the intestines of warm-blooded animals, but recent studies show that *E. coli* strains can persist in soils without any known fecal contamination. The objective of this study was to determine whether any *E. coli* strains were able to persist in a cattle pasture, and whether these are different from *E. coli* occurring in bovine and human feces. Soil samples were collected from four pastures free from grazing animals since the previous summer. Cattle were introduced in July and soil, run-off and bovine feces samples were collected every week for four weeks. *E. coli* were isolated using Membrane Lactose Glucuronide agar. A Maximum- Likelihood tree of concatenated *uidA* and *mutS* sequences was constructed using MEGA6.06. Isolates were allocated to phylogroups using the Clermont protocol and analyzed by multi-linear logistic regression model. Survival was determined by incubating 45 isolates in sterile pasture soil from Fall to Spring under field conditions. Isolates were evaluated for propensity to form biofilm and curli, and for RpoS activity. Three types of clusters emerged from the 390 isolates, *viz.* bovine associated, clusters devoid of cattle isolates representing isolates endemic to the pasture environment, and clusters with both. All isolates clustered with *E. coli sensu stricto*, distinct from Clades I, III, IV and V. The soil endemic population was comprised largely of phylogroup B1. The phylogroup regression tree supported significant difference ($p < 0.001$) between bovine and soil-persisting clusters. Soil persisting cluster isolates displayed lower decline than bovine and human sewage cluster isolates ($p < 0.05$). Soil endemic isolates had a higher average RDAR score than other isolates but this did not correlate with degree of biofilm forming in either LB or liquid soil extract. All isolates but one displayed RpoS activity. The results indicate the existence of soil persistent *E. coli sensu stricto* that are distinct from the Clades and appear to maintain populations that persist in the soil environment.

Author Disclosure Block:

G. NandaKafle: None. **T. Seale:** None. **T. Flint:** None. **M. Nepal:** None. **S. Venter:** None. **V.S. Brozel:** None.

Poster Board Number:

SATURDAY-155

Publishing Title:

Antimicrobial Activities of Actinomycetes Isolated from the Rhizosphere of *Genista umbellata* Collected from South-Eastern Spain (AlmeríA)

Author Block:

I. Gonzalez, F. J. Reche, M. M. Estevez, M. de la Cruz, C. Díaz, V. González, R. Serrano, N. de Pedro, J. Tormo, O. Genilloud; Fundación MEDINA, Centro de Excelencia en Investigación de Medicamentos Innovadores en Andalucía, Granada, Spain

Abstract Body:

It is well known that actinomycetes are one of the most productive microbial communities in soil and they are especially rich in plant rhizospheres. In an attempt to isolate novel actinomycetes to discover new potential producers of antimicrobial compounds with interest to be developed as novel antibiotics to fight human pathogens as well as phytopathogens, we studied the rhizosphere microbial community of *Genista umbellata,* an endemic plant in south-eastern Spain, from the area of Almerimar (Almería). To assess the diversity of the microbial community obtained following the different isolation approaches, all the isolates were initially identified to the genus level on the basis of their micromorphology, to be later characterized chemotaxonomically on their fatty acid composition. The microbial population was highly represented by a wide diversity of members of the genus *Streptomyces*, as well as isolates of the families *Micromonosporaceae, Nocardiaceae, Thermomonosporaceae, Streptosporangiaceae* and *Pseudonocardiaceae*. A selection of strains including the most diverse microorganisms from each cluster were selected to be cultivated and fermentation extracts were screened for the production of new antimicrobial agents active against a panel human pathogens (*Pseudomonas aeruginosa, Acinetobacter baumannii*, *Staphylococcus aureus* MRSA, *Candida albicans, Aspergillus fumigatus)* as well as phytopathogens (*Collecotrichum acutatum, Botrytis cinerea*). Our results confirm that rhizospheres are an extremely rich reservoir for the isolation of a wide diversity of actinomycetes, many of them still representing a rich untapped source of secondary metabolites with antimicrobial activities with potential application in different therapeutic areas and biotechnological fields.

Author Disclosure Block:

I. Gonzalez: None. **F.J. Reche:** None. **M.M. Estevez:** None. **M. de la Cruz:** None. **C. Díaz:** None. **V. González:** None. **R. Serrano:** None. **N. de Pedro:** None. **J. Tormo:** None. **O. Genilloud:** None.

Poster Board Number:

SATURDAY-156

Publishing Title:

Purification and Characterization of Potent Antimicrobial Agent with Antitumor Activitiy Recovered from Actinomycetes in Beni-Suef, Egypt

Author Block:

A. El-Gendy[1], A. Gaber[1], R. Rashad[2], M. Sayed[1], H. Hassan[1]; [1]Microbiol., Beni-Suef, Egypt, [2]Histology, Beni-Suef, Egypt

Abstract Body:

Background: The emergence of extensive antibiotics resistant bacteria increased the demands for digging out new sources of antimicrobial agents. Actinomycetes, especially *Streptomyces spp.,* have recently grasped considerable attention worldwide due to production of many useful bioactive metabolites, however few studies regarding Actinomycetes isolated from soil niche in Beni-Suef had been conducted. **Methods:** Actinomycetes were isolated from 24 soil samples using ISP4 agar and screened for production of antimicrobial activities against 7 clinical bacterial pathogens and 1 yeast using modified spot on lawn technique from which ethyl acetate total extracts of the most potent isolates were screened for their antitumor activities against two cell lines; MCF-7 and HEPG-2 cells. Genotypic characterization by 16s rRNA gene sequencing and presence of genes encoding for PKS and NRPS were conducted for one most potent isolate followed by optimization of its production using different growth parameters; carbon and nitrogen sources, pH, incubation time and starting inoculum size from which a successive purification steps using RP-HPLC followed by Ms and H NMR analysis were achieved.**Results:** A number of 52 Actinomycetes were recovered, from which 20 showed antimicrobial activities to at least one tested organism. The most potent isolate AGM12-1, which was active against all tested organisms and with IC50; 3.3 and 1.1 µg/ml against MCF-7 and HEPG-2 respectively, was characterized as *Streptomyces vinaceusdrappus* with presence of PKS and NRPS genes. Mannitol, ammonium sulfate, pH 7, 5% starting inoculums and incubation for 11 days at 30 °C were the optimum conditions used to maximize the production and hence allowed purification of one active compound to homogeneity using RP-HPLC, with m/z 488.05 and the NMR structural elucidation showed that this compound was a derivative of diketopiperazine.**Conclusions:** We isolated several Actinomycetes from soil samples in Beni-Suef from which a compound with potent antimicrobial and antitumor activities had been purified and characterized. These results have to shed light for the importance of Actinomycetes, especially those from soil reservoir, as vital source for the discovery of many novel and useful biological active compounds.

Author Disclosure Block:

A. El-Gendy: None. **A. Gaber:** None. **R. Rashad:** None. **M. Sayed:** None. **H. Hassan:** None.

Poster Board Number:

SATURDAY-157

Publishing Title:

Isolation, Identification and Characterization of Nitrogen-Fixing Bacteria from the Rhizosphere of Plants Inhabiting the Mine "El Bote" from Zacatecas, Zac. Mexico

Author Block:

A. Castanon; Univ. Autonomous of Zacatecas, Zacatecas, Mexico

Abstract Body:

Mining site's soils represent a hostile environment for vegetal life, however, nitrogen-fixing bacteria might be key for the health of these ecosystems through the nitrogen input. A search for nitrogen-fixing bacteria of the rhizosphere of plants inhabiting mine tailings was carried out in order to understand the nitrogen cycle's flux from this environment. Methods employed to seek these microorganisms are focused on the identification of genes that participate in the nitrogen fixation like the gene *nifH* through PCR. However, due to the enormous phylogenetic diversity among the nitrogen fixing bacteria and the complexity of such genes it's hard to get accurate results.Enrichment cultures in nitrogen-free medium inoculated with soil rhizosphere coming from a mine tailing were made in order to isolate possible diazotrophic bacteria. Once we obtained the strains, a molecular analysis was carried out on the same by first isolating genomic DNA and then amplifying through PCR the ribosomal gene 16S (rDNA 16S) and thus identify such strains. We concluded with the amplification through PCR of the gene *nifH* using 4 sets of primers specific for diazotrophs species previously reported and 3 pairs of primers degenareted to cover more genetic diversity.We obtained a total of 11 strains that were capable of growing in nitrogen-free medium and the the gene sequence analysis showed that most of them corresponded to nitrogen fixing bacteria genera highly studied like *Rhizobium* and other diazotroph genera also reported like *Serratia, Acinetobacter, Xanthomonas* and *Streptomyces*. As for the identification of the gene *nifH*, from the 7 pairs of primers tested, only one of the degenarated primers amplified an expected DNA fragment size in all of the strains tested, nevertheless, the sequenciation of one those amplicons showed that it does not match the *nifH* gene.The results obtained in this work suggest that a considerable diversity of nitrogen fixing rhizobacteria might exist in the rhizosphere of plants that inhabit the mine "El Bote". Molecular methods for detecting functional genes like *nifH* represent a good tool for the identification of nitrogen-fixing bacteria despite of the results; however, an analysis of the great variety of primers that target this gene should be done first in order to get more accurate results.

Author Disclosure Block:

A. Castanon: None.

Poster Board Number:

SATURDAY-158

Publishing Title:

Microbial Diversity in Armenian Mining Areas Revealed by Molecular Approach

Author Block:

A. Margaryan[1], N-K. Birkeland[2], H. Panosyan[1], A. Trchounian[1]; [1]Yerevan State Univ., Yerevan, Armenia, [2]Univ. of Bergen, Bergen, Norway

Abstract Body:

Background: Metal-rich natural and factitious habitats are extreme environments for the development and evolution of unique microbial communities, which could have important applications in bioremediation of heavy-metal contaminated environment. The main objective of this study was to determine the bacterial and archaeal diversity in Alaverdi Copper mine waste (sludge), Akhtala tailing, and Shamlugh copper stone rock using molecular approaches. **Methods:** PCR-DGGE fingerprinting and 454 pyrosequencing have been used to reveal the microbial community in the collected samples. **Results:** The bacterial community structure analysis by PCR-DGGE fingerprinting method revealed an abundance of the representatives of *Modestobacter, Microbacterium, Rhodococcus* and *Saccharomonospora* genera in Alaverdi sludge. In Shamlugh copper stone rock the members of the genera *Stenotrophomonas, Massilia, Dechloromonas, Limnobacter, Thiobacillus, Thalassospira, Actinobacterium* and uncultured phylawere found. Akhtala tailing was rich in representatives of the genera *Acidobacteria*, as well as uncultured groups of Acidobacteriaceae and other uncultured bacteria. Tagged 16S rRNA gene pyrosequencing data confirmed a domination of *Alphaproteobacteria, Gammaproteobacteria* and *Bacteroidetes* in Akhtala tailings, and also revealed the presence of *Verrucomicrobia, Acidobacteria, Actinobacteria,* etc. and some uncultured bacterial groups.Archaeal PCR-DGGE analyses indicated the presence of uncultured lineages in Alaverdi sludge, *Ferroplasma acidiphilum* in Akhtala tailing, Candidatus *Nitrososphaera gargensis, Methanosphaera* sp. and uncultured groups of Methanobacteriaceae in Shamlugh copper stone rock samples. **Conclusions:** The majority of the bacteria detected by DGGE fingerprinting were most closely related to uncultivated organisms. Most of the bacterial sequences shared less than 95% identity with their closest match in GenBank, indicating the presence of a unique bacterial community in Armenian Mines..

Author Disclosure Block:

A. Margaryan: None. **N. Birkeland:** None. **H. Panosyan:** None. **A. Trchounian:** None.

Poster Board Number:

SATURDAY-159

Publishing Title:

Chemoheterotrophic Bacteria Contribute to Biogenic Evolution of Speleothems in Cave Formations

Author Block:

S. JOSHI; NORTH EASTERN HILL Univ., SHILLONG, MEGHALAYA, India

Abstract Body:

Background: Caves offer a stable and protected environment devoid of all light sources. The microbial community living in such aphotic conditions in caves are dependent on sources of energy other than light and derive energy from the surrounding atmosphere, minerals and rocks. The present work explored the role of bacterial biofilms of various texture and colourations in caves in North-east India which represent some of the longest caves in the subcontinent and have not received much attention of scientists.**Methods:** Chemohererotrophs isolation was done from the speleothems of the caves in the region. Bacterial morphology, precipitated microstructural crystal morphologies, mineral compositions and association of microbial colonies with crystals were examined by SEM-EDX. Quantitative analysis of the mineral precipitated was done in Sequential X-ray Fluorescence Spectrometer (XRF). The ultrastructural morphology of calcium carbonate (CaCO3) crystals precipitated by the bacteria associated were studied using electron microscopy.**Results:** Thirty-two bacterial species belonging to sixteen genera majority being *Bacillus* and *Pseudomonas* isolated from these caves indicated the capability to precipitate calcite. SEM studies revealed crystal polymorphs generated *in vitro* by the isolates resembling the microscopical observations of natural speleothems. The EDX spectrum showed the *in vitro* precipitated crystals predominately composed of calcium carbonate hypothesizing the relevance of bacterial biofilm in cave geomicrobiology and biogenic evolution of cave which was supported by XRF analysis and Raman spectroscopy. The isolated bacteria under *in vitro* conditions precipitated CaCO3 in the media when supplemented with proper calcium source which was not the case in dead cells suggesting that calcification required metabolic activity in the speleothemic environment.**Conclusions:** The findings indicate the geomicrobiological contribution in the precipitation of calcium carbonates in caves and subterranean environments. Thus, microbiological investigations along with SEM studies, XRF and Raman spectroscopic observations add evidences to the hypothesis about the biogenic influence to the genesis of speleothem.

Author Disclosure Block:

S. Joshi: None.

Poster Board Number:

SATURDAY-161

Publishing Title:

Molecular Characterization of a Novel Partitivirus Infecting the Bats White Nose Syndrome Etiological Fungus *Pseudogymnoascus destructans*

Author Block:

P. Ren, S. Rajkumar, H. Sui, P. Masters, S. Chaturvedi, V. Chaturvedi; Wadsworth Ctr., NYSDOH, Albany, NY

Abstract Body:

From the White Nose Syndrome (WNS) etiological fungus *Pseudogymnoascus destructans*, we isolated a mycovirus (named PdPV-1). The virus is a bipartite virus since it contains two double-stranded RNA (dsRNA) segments designated as LS and SS. The cDNAs from PdPV-1 genomic dsRNAs were constructed and sequenced. The LS dsRNA was 1,683 bp in length with an open reading frame (ORF) that encodes 539 amino acids (molecular mass of 62.7 kDa). The SS dsRNA was 1,524 bp in length with an ORF that encodes 434 amino acids (molecular mass of 46.9 kDa). Both LS and SS ORFs were embedded only on the positive strand of each dsRNA segment. The predicted amino acid sequence of LS contained motifs representative of RNA-dependent RNA polymerase (RdRp); whereas the amino acid sequence of SS showed homology with the putative capsid proteins (CPs) from various mycoviruses. Phylogenetic analysis using the RdRp and CP sequences of mycoviruses suggests that both segments constitute the genome of a new virus that belongs to the family *Partitiviridae*. In agreement with the phylogenetic result, virus particles were successfully purified from this WNS causative fungus *P. destructans*. Electron microscopy analysis showed that the purified virions were isometric with an estimated diameter of 33 nm. Reverse transcription PCR (RT-PCR) and its sequences revealed that all isolates of *P. destructans* collected from various states of US were infected with this novel partitivirus; however, its closely related fungal species, such as *P. roseus* and *P. appendiculatus*, did not contain the virus.

Author Disclosure Block:

P. Ren: None. **S. Rajkumar:** None. **H. Sui:** None. **P. Masters:** None. **S. Chaturvedi:** None. **V. Chaturvedi:** None.

Poster Board Number:

SATURDAY-162

Publishing Title:

Survey of Bacterial Infectious Agents in Bats from the Country of Georgia

Author Block:

L. Urushadze[1], Y. Bai[2], L. Osikowicz[2], C. McKee[3], I. Kuzmin[4], A. Kandaurov[5], P. Imnadze[1], M. Kosoy[4]; [1]Natl. Ctr. for Disease Control and Publ. Hlth.., Tbilisi, Georgia, [2]Ctr.s For Disease Control and Prevention, Fort Collins, CO, [3]Ctr.s For Disease Control and Prevention, Fort Collines, CO, [4]CDC, Atlanta, GA, [5]Ilia State Univ., Tbilisi, Georgia

Abstract Body:

Background: Bats (Order: Chiroptera) are hosts of a wide range of zoonotic pathogens**.** The importance of chiropterans as reservoirs of viruses potentially transmissible to humans and other animals has become more evident with the passage of time. Little is known though about presence of bacterial infectious agents in bats, and no studies on this subject have been conducted in Georgia.**Objectives:** Bats of 10 species were sampled in 7 localities of Georgia in June 2012. Tissue specimens from 245 bats were available for testing for the presence of bacterial pathogens. **Methods:** A real-time PCR assay was performed in a triplex for detection of *Brucella* (IS711), *Bartonella* (tmRNA), and *Yersinia* (pal) from bat spleen tissues. An individual real-time PCR assay was used for detection of *Leptospira* (LipL32) in bat kidney tissues**.** Conventional PCR targeting the 16SrRNA gene was performed for detection of multiple strains of other bacteria in spleen tissues. Culturing was used for detection and identification of *Bartonella* species in blood samples and a phylogeny of *Bartonella* isolates was evaluated by sequence analysis of the *gltA* gene.**Results**: 113 of 221 bats (51%) were positive for at least one bacterial agent. *Bartonella* and *Leptospira* species were the most frequently detected agents with 20% *Leptospira*-positive bats and 51% *Bartonella*-positive. The *gltA* sequence analysis differentiated nine unique *Bartonella* phylogenetic clusters with a divergence corresponding to species levels. Four spleen samples were found positive for *Brucella* DNA by IS711 with two of them further confirmed by *Brucella*-specific csp31 and one by 16S rRNA. Sequence analysis of the three targets demonstrated 99.0% identity to known *Brucella* species.**Conclusion:** The proposed study was the first investigation of multiple bacterial agents conducted in bats from Georgia. Further analysis and interpretation of the bacteriological and genetic results will benefit surveillance systemsfor emerging pathogens in bats and outline potential risks to human and animal health in Georgia.

Author Disclosure Block:

L. Urushadze: None. **Y. Bai:** None. **L. Osikowicz:** None. **C. McKee:** None. **I. Kuzmin:** None. **A. Kandaurov:** None. **P. Imnadze:** None. **M. Kosoy:** None.

Poster Board Number:

SATURDAY-164

Publishing Title:

Detection of *Borrelia, Ehrlichia,* and *Rickettsia* spp. in Ticks in Adair County of Northeast Missouri

Author Block:

D. A. Hudman, N. J. Sargentini; ATSU/Kirksville Coll. of Osteopathic Med., Kirksville, MO

Abstract Body:

Background: The most common ticks in Missouri, all of which are associated with human pathogen transmission, are the lone star tick (*Amblyomma americanum*), American dog tick (*Dermacentor variabilis*), and the black-legged tick (*Ixodes scapularis*). Ticks are especially common in Northeast Missouri, but poorly studied despite the fact that Missouri is one of five states that accounts for over 60% of all Rocky Mountain spotted fever cases and one of three states that accounts for 30% of all reported *E. chaffeensis* infections. We conducted this research to improve our understanding of tick populations and tick-borne pathogen presence. **Methods:** Actively questing ticks were collected using a tick drag-flag method and by carbon dioxide traps. Ticks were identified and pooled by species and life stage (adults = 5; nymphs = 25, larvae = 100). Tick pools were tested for the presence/absence of *Borrelia* spp., *Ehrlichia* spp., and *Rickettsia* spp. using polymerase chain reaction (PCR). All positive reactions were confirmed by a second round of PCR and representative positive PCR products were purified and sent off-site for sequence verification. **Results:** A total of 15,162 ticks were collected, of which 13,980 were grouped in 308 pools. Infection rates were calculated as the maximum likelihood estimation (MLE) with 95% confidence intervals (CI). Of the 308 pools tested, 229 (74.4%) were infected with bacteria and the overall MLE of the infection rate per 100 ticks was calculated as 2.9% (CI 2.6-3.2). Infection rates varied among life stages, 28.6% (CI 24.0-34.0) in adults, 7.0% (CI 5.1-9.9) in nymphs, and 0.95% (CI 0.8-1.2) in larvae. In the 116 adult lone star pools, infection rates for *Borrelia*, *Ehrlichia*, and *Rickettsia* spp. were 4.3%, 13.2% and 19.1%, respectively. In the 52 nymph lone star pools, infection rates for *Borrelia*, *Ehrlichia*, and *Rickettsia* spp. were 1.6%, 2.4% and 2.7%, respectively. In the 20 adult American dog tick pools, the infection rate for *Ehrlichia* spp. was 18.3%. **Conclusions:** We have performed the first in-depth survey of ticks and tick-borne human disease in Northeast Missouri. We not only determined the presence of *Borrelia, Ehrlichia* and *Rickettsia* species, but have also implicated high prevalence. One novel finding was the presence of *E. chaffeensis* and *E. ewingii* in five of the 120 lone star larvae pools, which is suggestive of transovarial transmission.

Author Disclosure Block:

D.A. Hudman: None. **N.J. Sargentini:** None.

Poster Board Number:

SATURDAY-165

Publishing Title:

Reduced Pathogenic Potential of Environmental *Campylobacter jejuni* Carried by the American Crow

Author Block:

K. Sen[1], K. Francisco[1], J. Ton[1], J. Lu[2], D. Lye[2]; [1]Univ of Washington, Bothell, WA, [2]EPA, Cincinnati, OH

Abstract Body:

Campylobacter spp. are major causes of gastroenteritis worldwide. Recent studies have implicated wild birds in the zoonotic transmission of these bacteria. The virulence potential of *Campylobacter* shed in crow feces obtained from a wetland area in Bothell, Washington (where over 10,000 crows roost nightly) was studied. *Campylobacter* was isolated from 64 % of the fecal (n=58), and 20% (n=20) of the water samples collected during 2014 - 2015 by a unique filter method. PCR analysis confirmed the isolates to be *C. jejuni*. Similar analysis for virulence gene markers flagellin A (*fla*A) and CadF revealed 95% and 90% of the fecal, and 100% of the water isolates to have *flaA* and *cad*F genes, respectively. PCR analysis for the major toxin, cytolethal distending toxin (CDT), encoded by the *cdt*ABC gene cluster, revealed 89% of the fecal and 100% of the water isolates to have the cluster. A functional CDT toxin is produced by the expression of three tandem genes, *cdt*A, *cdt*B, and *cdt*C. Sequence analysis of *cdt*ABC in the isolates revealed a truncated cluster. Two kinds of mutation were observed. Some strains had a 668-bp deletion across *cdt*A and *cdt*B and a further 51 bp deletion within *cdt*B. Other strains had an additional 20 bp deletions in *cdt*B. In either case, a functional toxin is not expected. Source tracking studies using restriction fragment length polymorphism and sequencing of the *fla*A gene correlated the water isolates to crows. To investigate the possibility of a niche specific selection of these strains (with truncated *cdt*ABC) by the crows, additional fecal samples (n=11) were obtained from two other non-wetland locations within Washington. *C. jejuni* was isolated from 5 samples and all isolates demonstrated a truncated *cdt*ABC. Although the former deletion pattern has been reported in one study (<3% of isolates), the source was not correlated to crows (1). Examination by tissue culture assay did not demonstrate a CDT toxin activity in these isolates. Patients suffering from *Campylobacteriosis* have been clearly shown to elicit circulating antibodies to the entire CDT toxin (1). Our study suggests that North American crows (*Corvus brachyrhyncous*) carry the bacteria *C. jejuni,* but with a dis-functional toxin protein that is expected to drastically reduce its potential to cause diarrhea. Whether this phenomenon is host (crow) specific, needs to be explored further.

Author Disclosure Block:

K. Sen: None. **K. Francisco:** None. **J. Ton:** None. **J. Lu:** None. **D. Lye:** None.

Poster Board Number:

SATURDAY-166

Publishing Title:

Prevalence of *Brucella Suis, Campylobacter jejuni, Escherichia coli* O157:H7, and *Yersinia enterocolitica* in Georgia's Feral Pig Communities

Author Block:

J. K. Lama, D. Bachoon; Georgia Coll. & State Univ., Milledgeville, GA

Abstract Body:

Feral pigs carry zoonotic pathogens and can disseminate these pathogens to domestic pigs, other animals, and humans. Fecal samples were collected from 64 feral pigs from ten counties in Georgia. DNA was extracted from the fecal samples and assayed using qPCR for *Brucella suis*, *Campylobacter jejuni*, and *E. coli* O157:H7, and *Yersinia enterocolitica*. Out of 64 samples analyzed, *B. sui*s was detected in 21% of the pigs and *E. coli* O157:H7 were detected in 14% of the feral pigs. However, *Campylocater jejuni* was not detected in any of the pig samples. These pathogenic bacteria carried by feral pigs represent a growing threat to public health and agriculture in the State of Georgia.

Author Disclosure Block:

J.K. Lama: None. **D. Bachoon:** None.

Poster Board Number:

SATURDAY-167

Publishing Title:

Prevalence and Enumeration of Campylobacter at Different Steps of Broiler Slaughterhouses in South Korea

Author Block:

J-S. Moon; Animal, Plant, and Fisheries Quarantine and Inspection Agency, Anyang-si Gyeonggi-go, Korea, Republic of

Abstract Body:

Background: In this study, prevalence and enumeration of Campylobacter in carcasses at different steps of the broiler slaughterhouses were investigated between April and November 2012 in South Korea. **Methods:** The two differently sized typical chicken processing plants accredited HACCP system was managed the critical control points for fecal contamination reduction and chlorinated water concentration (20ppm) at air chiller step and body core temperature (4.4°C) of carcasses at final storage step. Sampling was conducted at four points (anus swap of live chicken, post-evisceration/pre-washing, post washing/pre-chilling, and post chilling) in the slaughter process of whole chicken carcasses. **Results:** All experimental procedures for isolation, identification and enumeration of C. jejuni, C. coli, and C. lari were conducted according to the culture method by Food Safety and Inspection Service. In both plants, the prevalence of Campylobacter increased considerably after evisceration (from 5.8% to 20.5% in plant A; from 5.8% to 15.4% in plant B) and decreased after the chilling step (from 20.5% to 10.9% in plant A; from 15.4% to 2.9% in plant B). However, distribution of quantification of Campylobacter contamination against carcasses reduced from evisceration (41.7%) to final carcasses (20.0%) in the more than 103 CFU/ml. Among isolates from 119 samples, C. coli (52.9%) were the most prevalent species in plants. **Conclusions:** Our finding suggested that low level of Campylobacter contamination imply during broiler processing from two plants accredited HACCP system.

Author Disclosure Block:

J. Moon: B. Collaborator; Self; H. J. Park, J. H. Lee, J. S. Lim, J. W. Chon, K. H. Seo, Y. J. Kim, E. J. Heo, H. J. Yoon, Y. H. Kim, S. H. Wee.

Poster Board Number:

SATURDAY-168

Publishing Title:

Molecular Subtyping and Source Attribution of *Campylobacter* Isolated from Food Animals

Author Block:

G. H. Tyson[1], H. Tate[1], J. Abbott[1], T-T. Tran[1], C. Kabera[1], E. Crarey[1], S. Young[1], P. McDermott[1], M. Campbell[2], S. Thitaram[2], S. Zhao[1]; [1]U.S. Food and Drug Admin., Laurel, MD, [2]USDA, Athens, GA

Abstract Body:

Campylobacter spp. commonly cause gastrointestinal illness in humans, and poultry meats have long been considered the predominant source of these infections. Nevertheless, few in-depth source attribution studies have attempted to compare *Campylobacter* isolated from food animals to isolates causing human illness. As a result, we obtained over 1,300 *Campylobacter* isolates from a number of sources, including dairy and beef cattle, pigs, poultry, and retail poultry meat. Each isolate was subtyped using pulsed-field gel electrophoresis (PFGE), and queried against the CDC PulseNet database to identify human isolates with indistinguishable patterns. As expected, many of the patterns from poultry (49.5%) had indistinguishable patterns from those of human isolates, suggesting retail poultry are a likely source of some human illnesses. Isolates from beef and dairy cows had an even higher proportion of PFGE patterns indistinguishable from those from human patients, with 56.6% and 65.0%, respectively. In contrast, only a small portion of *Campylobacter* PFGE patterns from pig isolates (9.5%) were found to have indistinguishable patterns from human isolates. These data suggest that food sources or environmental contamination associated with cattle may be larger contributors to *Campylobacter* infections than previously recognized, and help further our understanding of potential sources of human campylobacteriosis.

Author Disclosure Block:

G.H. Tyson: None. **H. Tate:** None. **J. Abbott:** None. **T. Tran:** None. **C. Kabera:** None. **E. Crarey:** None. **S. Young:** None. **P. McDermott:** None. **M. Campbell:** None. **S. Thitaram:** None. **S. Zhao:** None.

Poster Board Number:

SATURDAY-169

Publishing Title:

WGS of S*almonella* Subspecies *enterica* Serotype Tennessee Obtained from Peanut Butter Outbreaks

Author Block:

M. W. Allard; U.S. FDA, College Park, MD

Abstract Body:

Establishing an association between possible food sources and clinical isolates requires discriminating the suspected pathogen from an environmental background, and distinguishing it from other closely-related foodborne pathogens. We used whole genome sequencing (WGS) to *Salmonella* subspecies *enterica* serotype Tennessee (*S.* Tennessee) to describe genomic diversity across the serovar as well as among and within outbreak clades of strains associated with contaminated peanut butter. We analyzed 71 isolates of *S.* Tennessee from disparate food, environmental, and clinical sources and 2 other closely-related *Salmonella* serovars as outgroups (*S.* Kentucky and *S.* Cubana), which were also shot-gun sequenced. A whole genome single nucleotide polymorphism (SNP) analysis was performed using a maximum likelihood approach to infer phylogenetic relationships. Several monophyletic lineages of *S.* Tennessee with limited SNP variability were identified that recapitulated several food contamination events. *S.* Tennessee clades were separated from outgroup salmonellae by more than sixteen thousand SNPs. Intra-serovar diversity of *S.* Tennessee was small compared to the chosen outgroups (1,153 SNPs), suggesting recent divergence of some *S.* Tennessee clades. Analysis of all 1,153 SNPs structuring an S. Tennessee peanut butter outbreak cluster revealed that isolates from several food, plant, and clinical isolates were very closely related, as they had only a few SNP differences between. SNP-based cluster analyses linked specific food sources to several clinical S. Tennessee strains isolated in separate contamination events. Environmental and clinical isolates had very similar whole genome sequences; no markers were found that could be used to discriminate between these sources. Finally, we identified SNPs within variable *S.* Tennessee genes that may be useful markers for the development of rapid surveillance and typing methods, potentially aiding in traceback efforts during future outbreaks. Using WGS can delimit contamination sources for foodborne illnesses across multiple outbreaks and reveal otherwise undetected DNA sequence differences essential to the tracing of bacterial pathogens as they emerge. More on FDA Whole Genome Sequencing can be found at http://www.fda.gov/Food/FoodScienceResearch/WholeGenomeSequencingProgramWGS/ and http://www.ncbi.nlm.nih.gov/pathogens/

Author Disclosure Block:

M.W. Allard: None.

Poster Board Number:

SATURDAY-170

Publishing Title:

Ribotype Diversity of *Clostridium difficile* Strains from Diverse Environmental Sources and Geographical Areas

Author Block:

M. ALAM[1], J. Miranda[1], J. McPherson[1], A. Anu[1], A. Apu[1], E. Z. Kao[1], G. T. Chao[1], K. R. Wasko[1], W. Chowdhury[2], M. Ali[3], S. Parveen[4], J. Masuoka[5], K. W. Garey[1]; [1]Univ. of Houston Coll. of Pharmacy, Houston, TX, [2]Georgia Southern Univ., Statesboro, GA, [3]Metropolitan State Univ. of Denver, Denver, CO, [4]Univ. of Maryland Eastern Shore, Princess Anne, MD, [5]Midwestern State Univ., Wichita Falls, TX

Abstract Body:

Background: Based on U.S. population-based data, community-acquired *C. difficile* infection (CA-CDI) is associated with 32% of all CDI cases. Environmental spores of *C. difficile* from human and animal fecal contamination can persist for months. The objectives of this study were to investigate the ribotype diversity of *C. difficile* strains, and their molecular characteristics from a wide-range of environmental sources.**Methods:** Non-clinical environmental surface swab samples were collected from from 4 countries (6 States of USA (n=1938), Germany (n=100), France (n=100), and Taiwan (n=200) and analyzed using anaerobic culture and molecular methods. Samples were enriched in 25 ml of brain heart infusion (BHI) broth with 0.1% Na-taurocholate and incubated at 37C in an anaerobic chamber for 48h. One ml of culture broth was centrifuged, and treated with 70% ethanol. Ethanol treated culture were plated on to CCFA and incubated 48h anaerobically. Suspected colonies were then identified and characterized by PCR (tcdA, tcdB, tpi, ctdA and ctdB genes) and ribotyped using fluorescent PCR ribotyping. **Results:** Of the 2,338 total samples 8.8% (205) were positive for *C. difficile*. Toxigenic *C. difficile* were present in 61.4% (126/205) among isolates. Twenty different ribotypes (001, 002, 012, 017, 014-020, 027, 078-126, UM9, UM10, UM11, UM12, UN13, UM14, UM16, UM18, UM24, UM26, UM41, UM78, and UM87) of *C. difficile* were found among 144 strains screened. Most common ribotyes were 014-020, UM11, UM12, 002, and 078-126.**Conclusions:** Non-clinical environmental samples were frequently positive for potentially pathogenic *C. difficile* with diverse ribotypes in all geographical locations. Further studies are needed to prevent and control environmental sources of toxigenic *C. difficile* in and around our community environs.

Author Disclosure Block:

M. Alam: None. **J. Miranda:** None. **J. McPherson:** None. **A. Anu:** None. **A. Apu:** None. **E.Z. Kao:** None. **G.T. Chao:** None. **K.R. Wasko:** None. **W. Chowdhury:** None. **M. Ali:** None. **S. Parveen:** None. **J. Masuoka:** None. **K.W. Garey:** None.

Poster Board Number:

SATURDAY-171

Publishing Title:

Isolation and Molecular Analysis of Waterbloom-Forming Cyanobacterial Genus *Microcystis* Occurred in Agricultural Water Reservoir with Biotope

Author Block:

T. Nishizawa[1], M. Sutou[1], A. Satou[1], K-I. Harada[2], M. Shirai[3]; [1]Ibaraki Univ., Ibaraki, Japan, [2]Meijo Univ., Nagoya, Japan, [3]Aikoku Gakuen Univ., Chiba, Japan

Abstract Body:

There has been increased concern over the effects on wildlife of toxins, particularly those associated with harmful algal blooms in water reservoirs and recreational areas. Cyanobacteria, which include the genera *Anabaena, Microcystis, Nostoc*, and *Planktothrix*, produce structurally diverse peptides as secondary metabolites. The best known are the heptatoxic cyclic peptides, microcystins which are synthesized on large enzyme complexes comprising non-ribosomal peptide synthetase and polyketide synthase. This type of toxin has been responsible for the deaths of wild birds that have drunk water contaminated with cyanobacterial waterblooms in Japan. In 2008, a mass death of egrets and herons with steatitis were found at the agricultural reservoir with biotope, Kanagawa Prefecture, Japan, occurring cyanobacterial waterblooms. The objective of this study was to elucidate cyanobacterial community structure in the agricultural reservoir according to molecular analysis. To assess the cyanobacterial community, 16S rRNA-based terminal restriction fragment length polymorphism and clone library analysis have been utilized, as well as for the typing and monitoring of the cyanobacterial strains. Our results indicated that the *Microcystis* strain was observed to be the dominant species of cyanobacteria in the reservoir. In this study, a total of 59 axenic *Microcystis* strains were obtained from the reservoir. Phylogenetic analysis of the 16S-23S internal transcribed spacer region indicated that these strains classified into two groups of the genus *Microcystis*. Two microcystin biosynthetic gene (*mcy*)-possessing strains were identified according to the *mcy*-based PCR amplification. These results suggested that the *Microcystis* strains occurred in the reservoir have a taxonomic status different from toxic *Microcystis* strains which are obtained from other eutrophic freshwater in Japan to date.

Author Disclosure Block:

T. Nishizawa: None. **M. Sutou:** None. **A. Satou:** None. **K. Harada:** None. **M. Shirai:** None.

Poster Board Number:

SATURDAY-172

Publishing Title:

Phenotypes And Virulence Among *staphylococcus Aureus* Usa100, Usa200, Usa300, Usa400, And Usa600 Clonal Types

Author Block:

J. M. King, K. Kulhankova, C. S. Stach, B. G. Vu, W. Salgado-Pabon; The Univ. of Iowa, Iowa City, IA

Abstract Body:

Background: *S. aureus* lineages circulating worldwide and frequently associated with serious illness include clonal complex (CC) 5/USA100, CC30/USA200, CC8/USA300, CC1/USA400, and CC45/USA600. The USA300 (the current community epidemic lineage in the U.S) causes predominantly severe skin and soft tissue infections. The USA100/CC5, 200/CC30, and 600/CC45 lineages cause the majority of bloodstream infections and infective endocarditis (IE) worldwide yet remain largely uncharacterized. To elucidate key characteristics common to invasive *S. aureus* strains, we characterized a subset of isolates from five USA types associated with human disease. **Methods:** To test strains in vitro we used PCR and immunoblotting to determine superantigen gene profile and expression levels, respectively, rabbit erythrocyte assays to determine cytolytic potential, microtiter plate assays to determine biofilm formation, and blood survival assays. We also tested these strains in vivo in our rabbit model of IE and sepsis. **Results:** Intraclonal variation in hemolysis, biofilm formation, and blood survival is seen among strains from different lineages while expression of a high-level superantigen (SAg) (e.g. TSST-1, SEB, or SEC) or the egc is consistently seen in USA100, USA200, USA400, and USA600 strains. Isolates from each clonal group induce similar lethality in rabbits but USA300 isolates are deficient at causing IE. **Conclusions:** Intraclonal variability is common in the parameters frequently used to determine virulence of S. aureus strains however, strains most frequently associated with serious illness encode a high level SAg or the egc SAgs. Therefore, studies relating to S. aureus virulence should include USA100, 200, 400, and 600 isolates and consider SAg profiles.

Author Disclosure Block:

J.M. King: None. **K. Kulhankova:** None. **C.S. Stach:** None. **B.G. Vu:** None. **W. Salgado-Pabon:** None.

Poster Board Number:

SATURDAY-173

Publishing Title:

Leveraging a Real-Time PCR Assay for the Determination of the Germination-State of *Bacillus* Spores in Context of Wide-Area Spore Decontamination Efforts

Author Block:

T. M. Mott, **A. M. Woodson**, S. L. Welkos, C. K. Cote; USAMRIID, Frederick, MD

Abstract Body:

The events following the intentional release of *Bacillus anthracis* spores in 2001 strongly reinforced the need for reliable methods of decontamination and remediation of affected sites. Furthermore, identifying methods that are cost-effective, safe and minimize environmental damage is top priority. Given the inherent resistance of the spores to inactivation and the greater sensitivity of germinated bacilli, the main focus of this work is to evaluate germination as an enhancement to *B. anthracis* decontamination methods. The evaluation and validation of such a method relies on the accurate quantification of germinated and ungerminated spore populations in a given test-sample. Current means of quantification, such as culture-based methods, are labor and time intensive. To overcome these caveats, our lab has leveraged a real-time qPCR assay that uses changes in threshold cycle to detect viable germinated spores recovered from different coupon surfaces. A magnetic bead-based DNA extraction and purification step is employed prior to evaluation with our real-time PCR assay that targets a 159 bp fragment of the 30s ribosomal protein S21gene *rpsU*. Evaluated for both *B. anthracis* and its surrogate *Bacillus thuringiensis*, this method can accurately detect 50 femtograms of chromosomal DNA harvested from germinated spores. Using the resulting cycle threshold values, the percentage of viable germinated spores can be extrapolated from a DNA standard curve generated from a known amount of bacteria. Such methods of quantification are essential for testing the efficacies of secondary decontaminants in a laboratory setting or field trial and potentially confirming the remediation of sites affected by the intentional or accidental release of *B. anthracis* spores.

Author Disclosure Block:

T.M. Mott: None. **A.M. Woodson:** None. **S.L. Welkos:** None. **C.K. Cote:** None.

Poster Board Number:

SATURDAY-175

Publishing Title:

Prevalence of Lysogens and Virulence Genes Show That Tropical Soil Environment as a Gene Reservoir

Author Block:

A. A. Gonzalez[1], J. Marrero[1], A. Baerga[2], G. A. Toranzos[1]; [1]Univ. of Puerto Rico,Rio Piedras Campus, San Juan, PR, [2]Univ. of Puerto Rico,Med. Sci. Campus, San Juan, PR

Abstract Body:

Pangenomic studies indicate that *E. coli* are adaptable to survive in the environment. However, few studies are available of the impact these have on public health. Bacteria harboring virulence genes to colonization and control host defense represent a concern to public health. Previous work in our laboratory has demonstrated the presence of virulence genes as part of the environmental microbiota. Mobile genetic elements contribute to bacterial genome evolution by increasing virulence capabilities. As a follow-up to previous studies we demonstrate the presence of batteries of virulence genes present in mobile elements and they diversity within environmental populations. Our aim is to determine the prevalence of lysogenic virus and virulence genes in 83 environmental *E. coli* isolates from pristine areas of tropical rainforest in Puerto Rico. Primers were designed to amplify integrases, fimbriae and pro-inflammatory genes. Chemical lysogenic phage inductions indicated a total of (23.8%) isolates to be positive for lysogens. Integrases were present as follows: P2 (53.8%), P4 (26.3%), Hk97 (22.5%), Lambda (18.8%), Wphi (17.5%), Hk022 (7.5%) and phiV10 (1.3%). Conserved domain of colonization factor (*eae*) was detected in (7.5%) in previous studies. Interestingly, some of the isolates positive for (*eae*) previously failed to amplify the variable portion, decreasing the prevalence to (5%). Sequencing demonstrated diversity and presence of two different (*eae*) alleles: Zeta (ζ) and Kappa (κ). To our surprise, its co-receptor (*tir*) was also absent in some (*eae*) positive. Another unexpected finding was the amplification of unknown product, sequencing revealed a Type II secretion system (TIISS). One strain isolate harbored pro-inflammatory TcpC (0.01%). Our findings clearly indicate that the environmental microbiota indeed a gene reservoir. This is one of the first reports that aimed to identify virulence genes in pristine environments, indicating the presence of novel genes critical in colonization and control host response.

Author Disclosure Block:

A.A. Gonzalez: None. **J. Marrero:** None. **A. Baerga:** None. **G.A. Toranzos:** None.

Poster Board Number:

SATURDAY-176

Publishing Title:

Study of the Role of Avin03910 Depolymerase in the Metabolism of Polyhydroxybutyrate Polyester (Phb) in *Azotobacter vinelandii*

Author Block:

L. A. Adaya[1], J. Guzman[1], D. Pfeiffer[2], D. Jendrossek[2], G. Espin[1], D. Segura[1]; [1]Univ. Natl. Autonoma de México, Cuernavaca, Mexico, [2]Univ. of Stuttgart, Stuttgart, Germany

Abstract Body:

Background: Polyhydroxybutyrate (PHB) is polyester that is synthesized and intracellular accumulated as a carbon and energy reserve in several bacteria. In *A. vinelandii* the genes coding for the PHB biosynthetic enzymes, as well as several regulatory elements involved in the control of its synthesis, have been characterized. However, little is known about the mobilization process allowing the utilization of accumulated PHB when it is needed. The sequence of *A. vinelandii* DJ revealed seven genes that are thought to encode intracellular PHB depolymerase (the enzymes responsible for the mobilization of PHB). However, direct evidence for the *in vivo* roles of their gene products is missing.**Methods:**In this study, we selected a gen (*Avin03910*), representing the most probable candidate to be involved in the catabolism of PHB and investigated the physiological function of their gene product: (i) with *Escherichia coli* recombinant strains expressing AvinPhbZ$_1$ (*Avin03910*), protein; (ii) with a mutant *A. vinelandii*.**Results**: Evidence for significant PHB depolymerase activity *in vitro* was obtained for the product of *Avin03910* expressed in *E. coli*. The protein was active on amorphous and artificial granules only, but not on crystalline PHB, like other intracellular depolymerase.On the other hand, we found that the protein Avin03910 is associated with the granules of PHB. The mutant AvinPhbZ$_1^-$ had a phenotype of increased PHB accumulation and its purified native granules showed a diminished PHB mobilization and degraded more slowly than the wild type.**Conclusions**: This phenotype, together with its inability to degrade intracellular PHB under conditions stimulating mobilization in the wild type strain, suggest that the depolymerase AvinPhbZ$_1$ is the main responsible for intracellular PHB degradation. The presence of other PHB depolymerase in this bacterium cannot be discarded and will also be discussed.

Author Disclosure Block:

L.A. Adaya: None. **J. Guzman:** None. **D. Pfeiffer:** None. **D. Jendrossek:** None. **G. Espin:** None. **D. Segura:** None.

Poster Board Number:

SATURDAY-177

Publishing Title:

Genetic Regulation of Omega-3 Polyunsaturated Fatty Acid Synthesis in the Deep Sea Bacterium *Photobacterium profundum* Strain Ss9

Author Block:

M. N. Allemann, E. E. Allen; Univ. of California San Diego, La Jolla, CA

Abstract Body:

One of the better-characterized bacterial adaptations to the deep sea environment includes the biosynthesis and incorporation of polyunsaturated fatty acids, such as eicosapentaenoeic acid (20:5n-3; EPA) into membrane phospholipids. There is considerable biomedical interest in these molecules due to their beneficial role in cardiovascular health and neural development. In *Photobacterium profundum* strain SS9 the biosynthetic pathway for EPA has been previously characterized and consists of five genes *pfaA-E,* which encode a polyketide synthase. The *pfaA-D* genes form an operon that consists of two transcriptional units. While it is known that a variety of culture parameters, such as temperature and hydrostatic pressure, can influence the levels of EPA in SS9, there is no information about regulatory mechanisms behind these changes. Additionally, this secondary lipid pathway and the dissociated Type II fatty acid synthase appear to coordinate with one another by an unknown mechanism. Genome sequencing of an SS9 chemical mutant, designated EA2, that overproduces EPA with reduced monounsaturated fatty acid content revealed no changes in the *pfa* operon or its promoter regions. Previous transcript analyses of the *pfa* operon in EA2 had indicated elevated transcript abundance. Given this information we hypothesize that an uncharacterized *trans* regulatory mechanism controls *pfa* expression. To begin to address what genes regulate the *pfa* operon, we have prepared a gene fusion between the *pfaA* gene and the E.coli *lacZ* gene and crossed this into the SS9 genome using allelic exchange. We report the use of a variety of transposons in this reporter strain and the isolation of mutants with altered *pfaA::lacZ* activity. An enhanced understanding of the genetic determinants controlling microbial omega-3 polyunsaturated fatty acid synthesis will expand opportunities to direct high-level production of these molecules in native or recombinant microbial strains.

Author Disclosure Block:

M.N. Allemann: None.

Poster Board Number:

SATURDAY-178

Publishing Title:

Growth of *Sphingomonas wittichii* Rw1 on Dibenzo-*p*-Dioxin Requires Plasmid and Chromosomally Encoded Genes

Author Block:

T. Mutter, H-K. Chang, G. Zylstra; Rutgers Univ., New Brunswick, NJ

Abstract Body:

Sphingomonas wittichii RW1 is one of a few strains known for the ability to grow on the related compounds dibenzofuran (DBF) and dibenzo-*p*-dioxin (DD) as the sole source of carbon. The genes for the initial steps in the DBF catabolic pathway (ring hydroxylating dioxygenase, ring cleavage dioxygenase, and a hydrolase) which result in the formation of salicylate and a five carbon fragment have been localized to a megaplasmid designated pSWIT02 in RW1. Plasmids highly similar to pSWIT02 have been found in other DBF degrading *Sphingomonas* strains. However, despite having the pSWIT02-encoded DBF degradation pathway these other bacteria are not capable of growth on DD. We hypothesized that the unique ability of *S. wittichii* RW1 to grow on DD is due to a combination of the DBF degradation genes on pSWIT02 and chromosomally encoded genes specific to DD degradation. We initially targeted the ring cleavage enzyme encoded by the *dbfB* gene on pSWIT02 as previous studies indicated that this enzyme has very low activity against the DD pathway intermediate trihydroxybiphenylether and high activity against the DBF pathway intermediate trihydroxybiphenyl. We knocked out the pSWIT02 *dbfB* gene through disruption with a kanamycin resistance cassette. Growth curves indicate that RW1delta*dbfB* grows extremely slowly on DBF and accumulates the ring cleavage substrate trihydroxybiphenyl. Growth curves also indicate that RW1delta*dbfB* grows normally on DD as the sole source of carbon indicating that *dbfB* is not necessary for the DD catabolic pathway. Previously published proteomic and transcriptomic data indicated that the ring cleavage enzyme encoded by the chromosomal gene SWIT3046 is upregulated during growth on DD but not on DBF. Knockout of gene SWIT3046 resulted in a strain RW1delta3046 that grows normally on DBF but that does not grow on DD. The double knockout RW1delta*dbfB*delta3046 does not grow on either DBF or DD. These results prove that separate ring cleavage enzymes are absolutely necessary for DBF and DD degradation. These results also show that a combination of plasmid (pSWIT02) and chromosome genes are necessary for growth of RW1 on DD.

Author Disclosure Block:

T. Mutter: None. **H. Chang:** None. **G. Zylstra:** None.

Poster Board Number:

SATURDAY-179

Publishing Title:

Atypical Iron Sulfur Cluster Biosynthesis in Sulfate Reducing Bacteria

Author Block:

V. Trotter[1], A. Saini[1], M. N. Price[1], J. He[1], J. V. Kuehl[1], K. M. Wetmore[1], N. L. Liu[1], G. M. Zane[2], S. R. Fels[3], T. R. Juba[2], M. Shatsky[1], A. P. Arkin[1], P. D. Adams[1], J-M. Chandonia[1], J. D. Wall[2], A. M. Deutschbauer[1], **G. P. Butland**[1]; [1]Lawrence Berkeley Natl. Lab, Berkeley, CA, [2]Univ. of Missouri, Columbia, MO, [3]Yale Univ., New Haven, CT

Abstract Body:

Iron sulfur (FeS) cluster containing proteins make essential contributions to many key cellular processes but can readily be damaged by environmentally encountered oxidative stress, exposure to toxic metals or reactive N-oxyanions. Damage of key FeS enzymes such as pyruvate-ferredoxin oxidoreductase (PFOR) has been proposed as the source of inviability of sulfate reducing bacteria (SRBs) and other anaerobes when encountering oxygen. Using *Desulfovibrio vulgaris* Hildenborough (DvH) as a model SRB, we have characterized DvH strains harboring mutations in known FeS cluster biosynthesis factors and assayed the relative contributions of these factors to FeS cluster biosynthesis by monitoring activity of FeS dependent enzymes including PFOR. To date, enzymatic assays suggests that mutations in DVU1021(*sufB*), DVU1382 (*sufA*) and DVU0664 (*nifS*) have a detrimental effect on multiple FeS enzymes, although functional redundancy between biosynthesis factors appears to be significant. Only DvH strains lacking DVU1021 or DVU1382 displayed severe growth defects in rich growth media, consistent with the hypothesis that the SufBC predicted FeS scaffold complex is the likely primary site of *de novo* FeS cluster assembly in DvH and may work with DVU1382 to mature multiple FeS enzymes, deficiencies in which can impact the growth rate of the cell. In order to fully uncover the relationships between DvH FeS cluster biosynthesis factors, we have utilized a TnSeq-based procedure (RB-TnSeq) to conduct high throughput genetic interaction screening. We have observed synthetic lethality between SufBC and NifSU systems (no *sufB*/*C* transposon insertions in *nifS*/*U* background or *v.v.*) which is consistent with partial functional redundancy. Strikingly, the observed viability of a *nifS* mutant in DvH where it is predicted to be the sole cysteine desulfurase, contradicts current FeS cluster biosynthesis dogma regarding the essentiality of cysteine desulfurases as a sulfur source for *de novo* FeS cluster formation. Further work is ongoing to confirm *nifS* genetic interactions.

Author Disclosure Block:

V. Trotter: None. **A. Saini:** None. **M.N. Price:** None. **J. He:** None. **J.V. Kuehl:** None. **K.M. Wetmore:** None. **N.L. Liu:** None. **G.M. Zane:** None. **S.R. Fels:** None. **T.R. Juba:** None. **M.**

Shatsky: None. **A.P. Arkin:** None. **P.D. Adams:** None. **J. Chandonia:** None. **J.D. Wall:** None. **A.M. Deutschbauer:** None. **G.P. Butland:** None.

Poster Board Number:

SATURDAY-180

Publishing Title:

Characterization of Three Different Diphenylmethane Degrading *Pseudomonas* Species from the New Jersey Passaic River

Author Block:

A. J. Roberts, G. J. Zylstra; Rutgers Univ., New Brunswick, NJ

Abstract Body:

Diphenylmethane (DPM) is a hazardous compound produced by a number of industries. It is known that some (but not all) biphenyl degrading organisms are also capable of metabolizing DPM using the same enzymes as those that are involved in the biphenyl catabolic pathway. This is not surprising given the structural similarities between the two compounds. We hypothesized the reverse situation that DPM degrading organisms would also be capable of degrading biphenyl with the secondary hypothesis that the same catabolic enzymes would be involved for both DPM and biphenyl degradation. Starting with Passaic River (NJ) sediment we isolated three different *Pseudomonas* species capable of growth on DPM as the sole source of carbon. Subsequent testing showed that all three isolates could grow on biphenyl. Using PCR primers previously shown to amplify a wide variety of genes encoding the large subunit of biphenyl dioxygenase we identified a putative *bphA1* gene in all three *Pseudomonas* species. Sequence analysis of the PCR product revealed that even though each Passaic River strain was a different species they all had the exact same nucleotide sequence. The sequence is 91% identical to the canonical *bphA1* gene of *Burkholderia xenovorans* LB400 with the majority of the differences in the wobble position of the codons. Comparison of the three Passaic River isolates *bphA1* to other sequences in the GenBank database show that the Passaic River sequences represent a new subgroup within the LB400 family. Using reverse transcriptase PCR of mRNA extracted from succinate, DPM, and biphenyl grown cells we showed that the same *bphA1* gene sequence was induced during growth on DPM and biphenyl demonstrating that the same initial dioxygenase is involved in both DPM and biphenyl degradation. Interestingly, we isolated a spontaneous variant of *Pseudomonas* sp. strain AJR09 that lost the ability to grow on DPM, biphenyl, and salicylate (strain AJR10). PCR analysis showed that strain AJR10 lost the *bphA1* gene confirming that DPM and biphenyl are metabolized by the same initial dioxygenase and that the genes for salicylate degradation may be linked by an excisable element to the genes for DPM and biphenyl degradation. Because we found three different species containing the exact same gene for DPM degradation we conclude that the DPM degradation genes are horizontally transferred among bacteria in the Passaic River.

Author Disclosure Block:

A.J. Roberts: None. **G.J. Zylstra:** None.

Poster Board Number:

SATURDAY-181

Publishing Title:

***Sphingomonas wittichii* Rw1 Dibenzofuran 4,4a Angular Dioxygenase Attacks Biphenyl at a Lateral Position**

Author Block:

R. Faisal[1], H-K. Chang[2], G. Zylstra[2]; [1]Rutgers Univ., New brunswick, NJ, [2]Rutgers Univ., new brunswick, NJ

Abstract Body:

Sphingomonas wittichii RW1 is one of only a few microorganisms known to degrade dibenzo-*p*-dioxin (DD) and dibenzofuran (DBF). Due to the toxic, carcinogenic, and endocrine disruption characteristics of dioxin-like compounds the molecular and biochemical study of the enzymes involved in the DD and DBF degradative pathways is of great interest. Dibenzofuran 4,4a-dioxygenase (DBFDO) is the first enzyme involved in the DD and DBF degradation pathway. This enzyme is a heterodimer of two polypeptides DxnA1 (45KDa) and DxnA2 (23KDa) and catalytically adds two atoms of molecular oxygen at two adjacent carbon atoms where one of the carbons is a bridge atom between the two benzene rings. This enzyme is thus often called an angular dioxygenase. Based on studies with the purified enzyme DBFDO is known to hydroxylate other aromatic compounds. We hypothesized that DBFDO would oxidize biphenyl at the 2,3 (nonangular lateral) position and that the enzymatic activity would be sufficient to allow growth on biphenyl as the sole carbon source in an organism with the remainder of the biphenyl catabolic pathway. We initially cloned the four genes necessary for DBFDO into *E. coli* in the pET30a expression vector. This included the genes for the two oxygenase subunits *dxnA1* and *dxnA2*, the reductase *redA2*, and the ferredoxin *fdx3*. *E. coli* BL21 strains harboring this clone when induced had the ability to attack DD and DBF at the angular position and biphenyl at the 2,3 lateral position as determined by HPLC and GC-MS. In order to prove that this observed activity is sufficient to allow growth we cloned the *S. wittichii* RW1 *dxnA1-dxnA2- fdx3-redA2* genes into the biphenyl degrading organism *S. yanoikuyae* B1 in place of the *bphA1-bphA2* genes encoding the oxygenase component of biphenyl dioxygenase thus placing the RW1 DBFDO under control of the B1 biphenyl pathway promoter. The engineered B1 strain with the RW1 DBFDO grew on biphenyl with a growth rate similar to that of the wild type B1 strain. Our work shows that RW1 DBFDO has both angular and lateral dioxygenase activity depending on the substrate and that this activity can be sufficient for growth on alternate substrates.

Author Disclosure Block:

R. Faisal: None. **H. Chang:** None. **G. Zylstra:** None.

Poster Board Number:

SATURDAY-182

Publishing Title:

Screening and Identification of Motility Defective *H. volcanii* Mutants

Author Block:

E. Yang, R. Esquivel, M. Pohlschroder; Univ. of Pennsylvania, Philadelphia, PA

Abstract Body:

Archaeal flagella are structurally related to bacterial type IV pili and thus present a novel, yet still poorly understood mechanism for swimming motility in prokaryotes. Previous studies in the model archaeon *Haloferax volcanii* have utilized a random genomic insertion mutant library generated by transposon mutagenesis with the Mu transposon for efficient gene discovery of components involved in colony pigmentation and metabolite biosynthesis. Recently, we have conducted motility assays with mutants from this insertion library in order to screen for mutants of *H. volcanii* lacking swimming motility.Over 7000 transposon mutants were screened for non-motile phenotypes using low percentage agar motility assays. Subsequently, genomes of non-motile mutants without a significant growth defect were submitted for sequencing to identify the Mu-Transposon insertion sites.The screen resulted in the identification of 28 non-motile *H. volcanii* mutants of which 19 have been sequenced thus far. A subset of the transposon insertions was identified to be located adjacent to or in genes encoding known flagella biosynthesis or regulatory components, thus confirming that the screen could successfully identify motility mutants. These included the prepilin peptidase (PibD) that processes the flagellins as well as components of the chemotaxis machinery. Additional insertions were identified in genes encoding: an ABC transporter permease, an adjacent ABC transporter ATP-binding protein, an arylsulfatase, a cell division inhibitor, and a diaminopimelate decarboxylase. These genes have not previously been identified to play a role in flagella biosynthesis or regulation. Insertions in several hypothetical genes were identified; HVO_2876, which is adjacent to a methyltransferase is most intriguing because recent mass spectrometry has revealed that the major flagellin is methylated. The machinery required for this post-translational modification, which seems essential for H. volcanii flagellin stability, remains elusive.Our findings have demonstrated that the screen successfully identifies critical components for either the biosynthesis or regulation of archaeal flagella-dependent motility. The characterization of the several diverse motility mutants obtained from our motility screen will provide a foundation for a better understanding of this crucial archaeal cellular process.

Author Disclosure Block:

E. Yang: None. **R. Esquivel:** None. **M. Pohlschroder:** None.

Poster Board Number:

SATURDAY-183

Publishing Title:

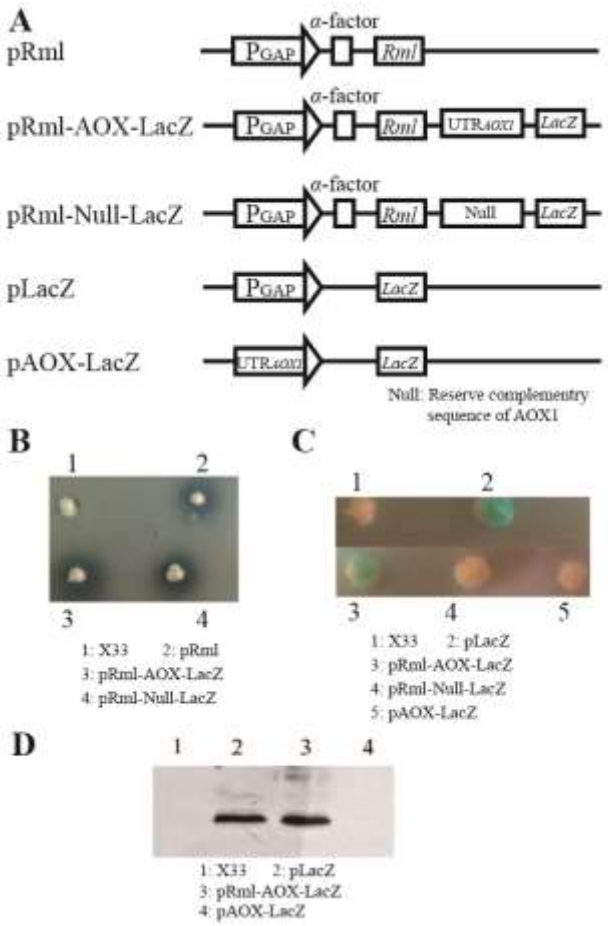
Endogenous Internal Ribosome Entry Site Mediates Protein Synthesis in *Pichia pastori*s

Author Block:

C. Li, Male, Y. Lin, S. Liang; South China Univ. of Technology, Guangzhou, China

Abstract Body:

Background: Most eukaryotic mRNAs are translated in a cap-dependent manner. But under conditions it is compromised , an alternative initiation pathway, internal ribosome entry site (IRES), is used for protein synthesis. *Pichia pastoris* is widely used for high-level production of recombinant industrial proteins. However, an endogenous IRES elements have not been reported in *P. pastoris*. Co-expression of two or more genes, which is achieved using functional IRES element, is an important and useful method for improving recombinant protein production and metabolic engineering. **Methods:** The endogenous 5'-UTR of AOX1 was investigated using bicitronic assays containing an upstream *Rhizomucor miehei* lipase (Rml) cistron and a downstream LacZ ORF in *P. pastoris* (Figure 1A). Real-Time PCR and Western blot are used to detect the expression. **Results:** As shown in Figure 1C and D, *lacZ* was expressed in the pRml-AOX-LacZ but not in pRml-Null-LacZ, indicating that a requirement for 5'-UTR of AOX1 in the translation of the downstream ORF. However, the pAOX-LacZ clones remained white on MDX plates (Figure 1C). And detected by Real-Time PCR and Western blot (Figure 1D), the *lacZ* was not expressed in pAOX-LacZ. These suggested that 5'-UTR of AOX1 did not contain a cryptic promoter that subsequent cap-dependent translation. These combined data demonstrated that insertion of the 5'-UTR of AOX1 upstream of the LacZ resulted in translation of the second ORF. The expression of Rml resulted from a cap-dependent ribosome scanning mechanism when the production of LacZ reflected the IRES activity of 5'-UTR of AOX1. **Conclusions:** The 5'-UTR of AOX1 is an endogenous IRES elements and the IRES-dependent translation initiation mechanism existed in *P. pastoris*.



Author Disclosure Block:

C. Li: None. **Y. Lin:** None. **S. Liang:** None.

Poster Board Number:

SATURDAY-184

Publishing Title:

Evidence-Based Validation of Quorum Quenching from *Lysinibacillus sphaericus* in the Bioremediation of Oil Sludge

Author Block:

C. Gómez, J. Dussán; CIMIC, Univ. de los Andes, Bogota, Colombia

Abstract Body:

Quorum quenching (QQ) is the disruption of quorum sensing (QS), mainly by enzymatic degradation of autoinducers. A well-known case of QQ are AHL-lactonases. Since pathogenicity is usually a QS-regulated process, QQ is a promissory base to develop novel strategies against pathogenic bacteria. Its effects are also remarkable in the performance of bacterial consortia in applications as bioremediation. Herein, we describe a potential novel AHL-lactonase gene from *L. sphaericus* and the interaction of this bacterium with a commercial consortium in the bioremediation of oil sludge in soil.We evaluated the presence of the locus in the studied strains by PCR; sequenced and characterized them by the following strategies: searching for homology (BLAST), for conserved domains and motifs (multiple alignments), and a phylogenetic analysis (ML). Then, we assessed the expression of the locus by RT-qPCR. The locus was expressed in *E. coli* in order to determine its effects over the pathogenicity of *Pectobacterium carotovorum* in an *in vivo* assay. The experiment was performed by triplicate and the results were analyzed with a Kruskal-Wallis test. These results were contrasted with data collected from a bioremediation assay carried out using the evaluated strains together with a commercial consortium made up by AHL-producing bacteria.The locus was present in five *L. sphaericus* strains. All the sequences exhibited the characteristic motifs and domains involved in Zn-binding from AHL-lactonases. Sequences were grouped in one clade within the phylogeny of the lactonases from firmicutes, showing 70 % aa identity with the lactonase AhlS. Bootstrap values were between 60 - 100. The locus is transcribed in the evaluated strains and its presence is correlated with an antagonist effect over *P. carotovorum* when bacilli or transfected *E. coli* titer was three magnitude orders higher than *P. carotovorum* titer. In field experiments, *L. sphaericus* inhibited the bacterial consortium on an oil sludge-contaminated soil: *L. sphaericus* titer increased gradually, whereas the consortium titer decreased to <10 CFU/mL after 60 days from inoculation.The presented evidence supports the existence of QQ activity from *L. sphaericus*, possibly due to a lactonase. This enzyme would be able to suppress the pathogenicity in *P. carotovorum* and to inhibit the function of a bacterial consortium for bioremediation.

Author Disclosure Block:

C. Gómez: None. **J. Dussán:** None.

Poster Board Number:

SATURDAY-185

Publishing Title:

LasM, a Novel Membrane Protein Essential for *Legionella pneumophila* to Survive in Water

Author Block:

L. Li, S. P. Faucher; McGill Univ., Ste-Anne-de-Bellevue, QC, Canada

Abstract Body:

Background: *Legionella pneumophila* is an opportunistic pathogen that can infect human and result in a severe form of pneumonia called Legionnaires' disease. This bacterium is found ubiquitously in freshwater, both natural and man-made water systems, and is typically transmitted through inhalation of contaminated aerosols. Therefore, the ability of *L. pneumophila* to survive in water is essential for the propagation to human. It is known that a gene, *lpg1659*, is highly up-regulated in *L. pneumophila* exposed to water. This gene encodes a hypothetical protein with no putative functions, but a similar protein is found in many *Legionella* species and other aquatic bacteria, suggesting an important role of this protein for the survival in aquatic environment. **Objective:** The primary goal of this study is to characterize the *lpg1659* gene, particularly on its importance for the survival of *L. pneumophila* in water and its regulation. **Methods:** Bioinformatic analysis was done to predict the potential functions of Lpg1659. Deletion mutant and over-expression strains were constructed and their survival in water was monitored by CFU counts. RT-qPCR was used to test if *lpg1659* is regulated by RpoS, a known stress response regulator in *L. pneumophila*. Wet mount analysis, live/dead staining and heat shock assay were performed to study the effect of *lpg1659* deletion on cell morphology, membrane integrity and heat tolerance. Infection assay was also done to test the effect on intracellular growth. **Results:** Lpg1659 was predicted to be a membrane protein involved in transport and binding. Deletion of *lpg1659* resulted in a survival defect in water at 42°C. Interestingly, over-expression was found to promote the survival of *L. pneumophila* in water. Result of RT-qPCR shows that the expression of *lpg1659* is positively regulated by RpoS in water. In addition, deletion of *lpg1659* did not affect the cell shape and length, as well as membrane integrity. Both the deletion mutant and the wild-type had similar CFU reduction after short-term heat shock. Lastly, *lpg1659* was found to be dispensable for intracellular growth in the amoebae *Acanthamoeba castellanii* and human macrophage-like THP-1 cells. **Conclusion:** The novel membrane protein encoded by *lpg1659*, which was found to be regulated by RpoS, is important for the survival of *L. pneumophila* in water and is therefore named LasM (for *Legionella* aquatic survival membrane protein).

Author Disclosure Block:

L. Li: None. **S.P. Faucher:** None.

Poster Board Number:

SATURDAY-186

Publishing Title:

Enhanced Expression of a Luxi Homolog and Utilization of Ahl Binding Resin Xad-16 Improve the Detection of Accumulated Ahl Signals from the Grapevine Crown Gall Bacterium *Novosphingobium sp.* Rr 2-17

Author Block:

L. Dailey[1], H. M. Gan[2], N. Halliday[3], P. Williams[3], A. Hudson[1], M. Savka[1]; [1]Rochester Inst. of Technology, Rochester, NY, [2]Monash Univ. Malaysia Genomics Facility, Selangor, Malaysia, [3]Univ. of Nottingham, Nottingham, United Kingdom

Abstract Body:

This research is focused on investigating the acyl-homoserine lactone (AHLs) signaling compounds produced by the grape crown gall tumor colonizing bacterium, *Novosphigobium* sp. Rr2-17, whose genome sequence was recently determined by our laboratory. The luxI homolog (novI) responsible for AHL synthesis was identified from its genome sequence based on a similarity search and cloned into the IPTG inducible expression vector, pSRKKm. This recombinant plasmid was then transferred into the parent wild type Rr2-17 strain to enable the inducible expression of novI. Inducible expression of novI is predicted to enhance the identification of additional AHLs signals not previously identified from the wild type bacterium. The AHLs produced by strain Rr2-17 (pSRKKm::novI) were analyzed by LC-ESI MS/MS after ethyl acetate extractions from varying concentrations of the AHL binding resin XAD-16. This enabled the major and minor AHL of *Novosphingobium* to be identified and quantified, and demonstrate the use of XAD-16 resin as a more effective method for optimal AHL signal recovery. The major AHL produced was identified as OH-C8-homoserine lactone and IPTG induction of novI expression in Rr2-17 (pSRKKm::novI) increased its concentration 6.8-fold when compared to the wild type Rr2-17 (pSRKKm), vector only. Similar increases were identified with two minor AHLs, OH-C10-homoserine lactone and C8-homoserine lactone. The presence of 20% w/v of XAD-16 resin in the extraction procedure bound 99.3 percent of the major AHL produced by IPTG-induced Rr2-17 (pSRKKm::novI) strain. Through the identification and quantification of the AHLs produced by *N. sp.* Rr2-17, we expand our knowledge of its quorum sensing system and are the first in the genus *Novosphingobium*.

Author Disclosure Block:

L. Dailey: None. **H.M. Gan:** None. **N. Halliday:** None. **P. Williams:** None. **A. Hudson:** None. **M. Savka:** None.

Poster Board Number:

SATURDAY-187

Publishing Title:

Methanotrophically Mediated Bioaggregation to Control Sand Dust

Author Block:

A-M. Harik, T. Hazen; Univ. of Tennessee, Knoxville, Knoxville, TN

Abstract Body:

Sandstorms are commonplace in many parts of the world including Qatar, these storms have negative impacts on the economy, environment, infrastructure, and public health. Traditional prevention techniques use chemical stabilizers and cementing agents; however, their application is expensive and may have negative impacts on public health. We proposed using native extracellular polysaccharide (EPS) producing methanotrophic bacteria for bioaggregation. The proposed technique would involve application of an EPS producing methanotrophic bacterial culture to the sand's surface followed by application of purified EPS. The preference is for native methanotrophic bacteria to be used, to minimize the disruption to the native ecosystem. We have collected native methanotrophic bacteria from Qatar sand. Until they can be identified and researched, two well studied strains of Methanotrophs are being used for preliminary EPS testing. Application of purified EPS to sand has shown signs of significant aggregation, along with increases in shear strength. Once a strain of bacteria is chosen by the research team, and the EPS production optimized, a bioreactor will be built to produce EPS for lab scale sand stabilization studies. Lab scale studies will monitor time dependent changes in shear strength during application, microbial community changes, and ability to withstand critical threshold wind velocities. Future plans include field scale studies and the construction of a fluidized bed bioreactor for production of the liquid medium and EPS. Bioaggregation with EPS producing bacteria utilizing methane - an indigenous resource to Qatar - has cost saving benefits specific to Qatar.

Author Disclosure Block:

A. Harik: None. **T. Hazen:** None.

Poster Board Number:

SATURDAY-188

Publishing Title:

Divergent Taxonomic and Functional Responses of Microbial Communities to Field Simulation of Aeolian Soil Erosion and Deposition

Author Block:

X. Ma[1], C. Zhao[2], Y. Gao[1], B. Liu[2], T. Wang[1], T. Yuan[3], L. Hale[3], J. D. Van Nostrand[3], S. Wan[2], J. Zhou[3], Y. Yang[1]; [1]Tsinghua Univ., Beijing, China, [2]Henan Univ., Kaifeng, China, [3]Univ. of Oklahoma, NORMAN, OK

Abstract Body:

Aeolian soil erosion and deposition have worldwide impacts on agriculture, air quality and public health. Aeolian soil erosion, exacerbated by anthropogenic perturbations, has become one of the most alarming processes of land degradation and desertification. In contrast, dust deposition imposes a potential fertilization effect. Therefore, determining microbial responses to soil erosion and deposition is crucial for elucidating the mechanistic underpinnings in driving soil nutrient cycling, which remains little understood. Here we report the first in-depth, holistic study via 16S rRNA gene amplicon and GeoChip techniques to analyze microbial communities subjected to field simulation of soil erosion and deposition. In a semiarid grassland of Inner Mongolia, China, we found that microbial communities were altered (P<0.039) by simulated soil erosion and deposition, with substantial increase in abundance of biocrust-forming *Cyanobacteria*. *amyA* genes encoding α-amylases were increased (P=0.01) in relative abundance by soil deposition, which was positively correlated (P=0.02) to increased dissolved organic carbon (DOC) content in dust. However, most of the microbial functional genes associated with carbon, nitrogen, phosphorus and potassium cycling were decreased or unaltered by both erosion and deposition, probably arising from acceleration of organic matter mineralization by aeolian breakdown of soil aggregates. These divergent responses suggest that microbial communities are sensitive to soil erosion and deposition, supporting the necessity to include microbial components in evaluating ecological consequences. In addition, Mantel tests showed strong, significant correlations between soil nutrients and functional gene composition but not taxonomic composition, demonstrating close relevance of microbial function traits to nutrient cycling.

Author Disclosure Block:

X. Ma: None. **C. Zhao:** None. **Y. Gao:** None. **B. Liu:** None. **T. Wang:** None. **T. Yuan:** None. **L. Hale:** None. **J.D. Van Nostrand:** None. **S. Wan:** None. **J. Zhou:** None. **Y. Yang:** None.

Poster Board Number:

SATURDAY-189

Publishing Title:

Phylogenetic Diversity of Bacterial Community on Asian Dust Assessed by Pyrosequencing Analysis

Author Block:

S. Srinivasan, E. Joo, E. Kim, S. Jeon, M. Kim; Seoul Women's Univ., Seoul, Korea, Republic of

Abstract Body:

Background: Asian dust or yellow sand events in the East Asia are the major issue of environmental contamination and human health, with increasing concern. The high amount of dust particles especially the coarse particles (PM_{10}) is transported by the wind from the arid and semi-arid tracks to Korean Peninsula bring the bacterial population that alters the terrestrial and atmospheric microbial communities. **Methods:**In this study, we aimed to explore the bacterial population of Asian dust samples collected during November-December 2014. The dust samples were collected using high volume samplers, and the genomic DNA was isolated using commercial kit. The variable regions (V3-V6) of 16S rRNA gene were amplified using PCR and pyrosequencing were performed using Roche-454 (454 GS FLX, Roche). The data was analysed using Mothur and V programs. **Results:** The pyrosequence data showed the genus *Sphingomonas* (~50%) belonged to the phylum *Proteobacteria* (~86%) are the predominant population in the atmosphere. The genus *Bacillus* (7%) belonged to the phylum *Firmicutes* (~15%) are increased during Asian dust compared to non-Asian dust samples. **Conclusions:**This study showed that the significant relationship of bacterial populations between Asin dust samples and non-Asian dust samples in Korea, which could significantly affect the microbial population in the environment.

Author Disclosure Block:

S. Srinivasan: None. **E. Joo:** None. **E. Kim:** None. **S. Jeon:** None. **M. Kim:** None.

Poster Board Number:

SATURDAY-190

Publishing Title:

Indoor Microbial Air Quality of a 42-Year-Old Elementary School

Author Block:

L. Sturmer; Skyline Coll., San Bruno, CA

Abstract Body:

Background: The average public school building in the U.S. is over 40 years old and 29% have never been renovated (1). Almost any building surface can nourish microbial growth. The high occupant density in schools increases the concentration of airborne particulate matter from human microbiota (2). Schools are required to meet ventilation standards, but despite this, there are still no concentration standards or personal exposure limits for airborne microbes (3). Additionally, children may more susceptible to indoor pollutants than the general population.**Aim:** The objective of this study was to perform a survey of indoor microbial air quality of a water-damaged, 42-year-old school operating as a child development center in the San Francisco Bay Area.**Methods:** A total of 496 air samples were collected within the old building and 180 air samples were collected from nearby newer buildings and outdoor locations. Air samples were taken with an impact air sampler onto nutrient media selected for bacteria and fungi. Data were examined using a three-dimensional approach and multivariable analysis.**Results:** Indoor airborne bacteria were 139% higher in the old building compared to neighboring renovated buildings and 76% higher than outdoor air. Airborne fungi were 128% more numerous compared to the renovated buildings and 112% lower than outdoors. The variety and number of microorganisms inside the older building were different than outdoors. No direct source of contamination was found, however, open windows correlate with a 105% increase in indoor fungal concentration ($P<0.001$).**Conclusions:** Data confirm that ventilation and filter maintenance help lower microbial aerosols and should be maintained in accordance with California regulations.

Author Disclosure Block:

L. Sturmer: None.

Poster Board Number:

SATURDAY-191

Publishing Title:

Metagenomic Profiling of Antimicrobial Resistance Genes from Airborne Microbial Communities

Author Block:

T. L. Dickerson, D. Swales, M. Krause, A. Ferris, J. L. Jacobs; MRIGlobal, Rockville, MD

Abstract Body:

Since the adoption of antibiotics in the early 20th century, a plethora of clinical pathogens have acquired resistance to one or more modern-day antibiotics. Consequently, antimicrobial resistance (AMR) is recognized as a severe threat to human and animal health worldwide. Recent work has demonstrated that AMR bacteria are widely prevalent in the environment, perhaps exacerbated by the widespread use of antibiotics for clinical or agricultural purposes. Along these lines, the principal objective for this study was to assess the temporal dynamics of airborne bacterial communities and the dispersion of AMR genes present within them. Dry air filter units were used to collect air samples daily at four locations in- and outside a transit center in the National Capital Region. Microbial biomass was eluted from each filter, concentrated by ultrafiltration, and DNA was extracted for downstream shotgun metagenomic sequencing on a ThermoFisher Ion Proton. In addition, DNA libraries were prepared from pooled samples and subjected to a functional metagenomics screen for susceptibility testing against seven antibiotics. Antibiotics tested include chloramphenicol, ciprofloxin, trimethoprim, colistin, tetracycline, penicillin and meropenem and resistance was found against many of these antibiotics at the Clinical and Laboratory Standards Institute (CLSI) minimum inhibitory concentration (MIC) level. The prevalence of AMR genes was also assessed within the shotgun metagenomics data. Bacterial profiles from the four sites surveyed showed a diverse population of bacteria, with the genus Pseudomonas representing the largest proportion of sequences. These results suggest that airborne microbial communities may serve as a dynamic reservoir for the dispersion of antimicrobial resistance factors in the environment, potentially complicating the existing world-wide public health crisis to combat AMR pathogens.

Author Disclosure Block:

T.L. Dickerson: None. **D. Swales:** None. **M. Krause:** None. **A. Ferris:** None. **J.L. Jacobs:** None.

Poster Board Number:

SATURDAY-192

Publishing Title:

Proteins Expressed by *Sphingomonas aerolata* in Response to Aerosolization

Author Block:

V. Krumins[1], M. Abadjev[1], S. Boeren[2], T. Kruse[2], P. Schaap[2], H. Smidt[2], L. J. Kerkhof[1], G. Mainelis[1], D. E. Fennell[1]; [1]Rutgers Univ., New Brunswick, NJ, [2]Wageningen Univ., Wageningen, Netherlands

Abstract Body:

Background: The objective of this study was to analyze the proteomic response of an atmospheric microorganism, *Sphingomonas aerolata*, to aerosolization and the presence of a volatile substrate while airborne. **Methods:** The genome of *Sphingomonas aerolata* strain NW12 (AJ429240.1), a psychrotolerant bacterium isolated from ambient air, was sequenced using the Illumina HiSeq2000 platform, resulting in 3344 predicted protein sequences. *S. aerolata* was grown in liquid minimal media containing acetic acid as the sole substrate, aerosolized into rotating gas-phase bioreactors, and incubated in the airborne state for two days. Cells were collected onto membrane filters, and proteins extracted and run on SDS PAGE gels. Peptides were fragmented by in-gel trypsin digestion and analyzed by LCMS-MS. Putative proteins were identified by referencing the sequenced genome. The proteomes of stationary phase liquid-grown, acetate-fed cells; airborne cells in the presence of 20 ppmv ethanol; and airborne cells without ethanol, were compared.**Results:** After aerosolization and 2 days of airborne incubation, 20 to 80 x 10^6 cells were recovered. The protein digest yield was 25-30%, and 1370 putative *S. aerolata* proteins were identified from the aerosolized cells. Compared with the *S. aerolata* proteome in liquid culture, 109 proteins were downregulated and 37 increased. These included several related to the TCA cycle, cell membrane and cell wall transporters, and ribosomes, suggesting an active response to the stresses of aerosolization.There were relatively few differences between aerosolized cells presented with a gaseous substrate and starved aerosols. Six proteins were significantly and positively correlated with culturability, all of which are related to cell membranes, protein synthesis or DNA repair.**Conclusions:** *S. aerolota* respond to aerosolization stress by activating the TCA cycle, membrane and cell wall proteins involved in transport and secretion, and ribosomal proteins. Six proteins were identified that correlate with culturability, indicating that protein, membrane and DNA repair are critical to maintenance of viability.

Author Disclosure Block:

V. Krumins: None. **M. Abadjev:** None. **S. Boeren:** None. **T. Kruse:** None. **P. Schaap:** None. **H. Smidt:** None. **L.J. Kerkhof:** None. **G. Mainelis:** None. **D.E. Fennell:** None.

Poster Board Number:

SATURDAY-193

Publishing Title:

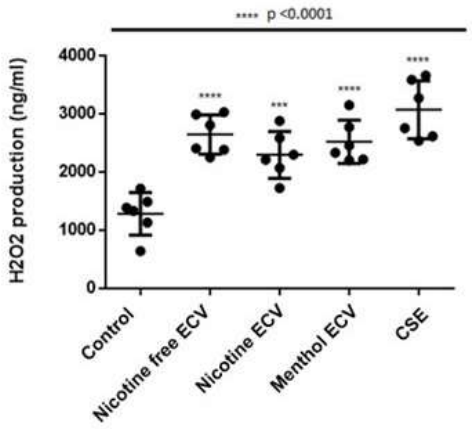
Effect of E-Cigarette Vapour and Cigarette Smoke Extract on S. *pneumoniae* Virulence

Author Block:

L. Miyashita, J. Grigg; Queen Mary Univ. of London, London, United Kingdom

Abstract Body:

Background: The potential for Electronic cigarettes (EC) to cause adverse health effects remains unclear, yet their popularity have significantly increased over the past few years. Vulnerability to invasive pneumococcal disease is associated with exposure to tobacco smoke (Grigg *et al.* 2012). Cigarette smoke extract increases oxidative stress which in turn mediates the adhesive response of airway epithelial cells, allowing increased invasion of *S. pneumoniae* via the host expressed platelet-activating factor receptor (PAFR). Hydrogen peroxide, a reactive oxygen species, is a major pneumococcal virulence factor produced in quantities known to be cytotoxic to lung cells. At present the direct effect of CSE and EC vapour (ECV) on pneumococcal virulence remains unclear. In this initial study we sought to determine whether oxidative stress is increased in *S. pneumoniae* during CSE and ECV exposure.**Methods:** Hydrogen peroxide concentration was measured in *S. pneumoniae* D39 cultures exposed to either 2% or 0% ECV/CSE for one hour. Cultures were filter sterilised and the supernatant was quantified for H_2O_2 levels against a standard curve using a colorimetric assay. Catalase was included as a control.**Results:** H_2O_2 production significantly increased in pneumococcal cultures exposed to ECV and CSE ($p<0.0001$) compared to unexposed controls. Catalase control reduced H_2O_2 concentration.**Conclusions:** We have for the first time assessed the effect of ECV and CSE on pneumococcal virulence. H_2O_2 production significantly increased in cultures exposed to ECV/CSE, thus suggesting a direct effect on pneumococcal virulence. This research can now be expanded by assessing the effect of supernatant H_2O_2 on pneumococcoal adhesion to lower airway cells, as preliminary data suggests adhesion is increased in ECV/CSE exposed bugs.



Author Disclosure Block:

L. Miyashita: None. **J. Grigg:** None.

Poster Board Number:

SATURDAY-194

Publishing Title:

Concentration and Particle Size Distribution of Endotoxin and Beta-glucan Present in Ambient Particulate Matter

Author Block:

A. L. Pabon, E. Gonzalez-Figueroa, W. Torres-Rivera, L. B. Mendez; Univ. del Este, Carolina, PR

Abstract Body:

Asthma is the most common chronic childhood disease in the US. Among the environmental factors associated with increased asthma morbidity are exposures to indoor allergens, bioaerosols (e.g. endotoxins and beta-glucans) and air pollutants, such as particulate matter (PM). The main objective of this study was to determine the concentration and size distribution of endotoxin and beta-glucans present in ambient PM. Air samples were obtained in the vicinity of an elementary school in Puerto Rico. Samples were collected for 2 consecutive weeks with an 8-stage cascade impactor and particles were extracted from the filters with pyrogen-free water by sonication for 2 hours. Endotoxin and beta-glucans present in each stage were measured using a kinetic chromogenic limulus amebocyte lysate and glucatell assay, respectively. The mass median aerodynamic diameter (MMAD) and the geometric standard deviation (GSD) were calculated using the log-probability method. Endotoxin concentrations varied between 0.78 and 18.80 pg/m^3. In contrast, the beta-glucans levels ranged between 87.52 and 522.77 pg/m^3. Approximately 50% of the detected endotoxins and beta-glucans were associated with particles of 2.5 μm or less in diameter. This data will be used as complimentary information for exposure assessments to evaluate if endotoxin and beta-glucans present in ambient PM are associated with respiratory effects in asthmatic children.

Author Disclosure Block:

A.L. Pabon: None. **E. Gonzalez-Figueroa:** None. **W. Torres-Rivera:** None. **L.B. Mendez:** None.

Poster Board Number:

SATURDAY-195

Publishing Title:

Effect of Soil Type, Composting, and Antibiotic Use on Fate of Antibiotic Resistant Bacteria and Resistance Genes in Dairy and Beef Manure Applied Soil

Author Block:

C. Pankow, C. Chen, R. Williams, G. Guron, K. Xia, M. Ponder, A. Pruden; Virginia Tech, Blacksburg, VA

Abstract Body:

Application of livestock manure to land is known to increase antibiotic resistant bacteria (ARBs) and antibiotic resistance genes (ARGs) in soil. The objective of this study was to determine the effect of soil type (sandy loam, clay loam or silty clay) on the fate of ARBs and ARGs in soil microcosms, which may be used to predict fate in land applied cattle manure. Four manure types (beef or dairy, with or without chlortetracycline, sulfamethazine, and tylosin or pirlimycin and cephapirin antibiotics, respectively) with or without composting were amended to three locally-available soil types at a rate achieving 10% bioavailable nitrogen content for a total of 39 experimental conditions. Three sacrificial replicate samples were collected over time to culture bacteria resistant to cefotaxime, ceftazidime, clindamycin, tetracycline, erythromycin, and vancomycin on R2A (heterotrophs) and MacConkey (enteric bacteria) agar. There were significant reductions ($p < 0.0001$) in heterotrophs resistant to clindamycin, erythromycin, tetracycline, and vancomycin from day 1 to day 57. Significant reductions ($p < 0.0001$) to erythromycin and tetracycline resistant enteric bacteria also occurred. Soil type only had a significant effect on enteric bacteria resistant to erythromycin ($p < 0.0001$), with the highest log CFU/g in the silty clay condition. Heterotrophic and enteric bacteria showed no significant difference in log CFU/g with respect to the type of compost amended or whether the cattle were administered antibiotics. Ongoing analysis with metagenomics and quantitative polymerase chain reaction will serve to further illustrate overall microbial community changes and quantify ARGs. Results thus far indicate that prior antibiotic use or composting did not have a measureable effect on culturable ARBs once applied to soil, while soil type may have limited influence on certain culturable bacteria.

Author Disclosure Block:

C. Pankow: None. **C. Chen:** None. **R. Williams:** None. **G. Guron:** None. **K. Xia:** None. **M. Ponder:** None. **A. Pruden:** None.

Poster Board Number:

SATURDAY-196

Publishing Title:

Impact of Different Arable Farming Systems on the Abundance and Diversity of Bacterial Community and Antimicrobial Resistance Determinants

Author Block:

E. Gómez-Sanz[1], S. Jaenicke[2], R. Wittwer[3], A. Goesmann[2], M. M. van der Heijden[3], B. Duffy[1], T. H. M. Smits[1]; [1]Zurich Univ. for Applied Sci., Waedenswil, Switzerland, [2]Justus-Liebig-Univ., Giessen, Germany, [3]Agroscope, Zürich, Switzerland

Abstract Body:

This study examines soil management systems for abundance, diversity and dynamics of microbial communities and antimicrobial resistance (AMR) genes in a Swiss experimental farming trial with four setups: Organic production with plough (OCP) and reduced tillage (ORT) and Conventional production with plough (CCP) and no tillage (CNT). The organic treatments were fertilized with slurry and the conventional systems with mineral fertilizer (N, P, K and Mg). A combination of amplicon sequencing and total DNA metagenomics was used to generate an overview of the complete diversity, while qPCR assays were employed to examine the AMR gene abundance and dynamics. Actinobacteria and Alphaproteobacteria were the Bergey's classes most abundant in all systems. Beta- and Gamma-proteobacteria, and Sphingobacteria were also predominant in diverse order relative to the system. Within the 5 most abundant Bergey's genera, Gp6, Gaiella and Sphingomonas were present in all systems, with Gp6 as predominant in all but C-NT, for which Gaiella was dominant. Gp4 was abundant in the same 3 systems, while Gemmatimonas was only present in C-NT system. Lysobacter was abundant in O-RT and C-T whilst Spartobacteria genera incertae sedis was rich in O-T and C-NT. Based on ARG-ANNOT database, resistance genes to most clinically relevant families were detected in low counts. The fluoroquinolone resistance gene *oqxB* was the most abundant in all systems (yet plasmid -confering resistance- or chromosomal -normally do not confer resistance- location need to be role out), followed by the fluoroquinolone *qepA* (O-RT, N-T) or tetracycline resistance *otrA* (O-T, C-NT) genes. Currently, we are examining the temporal dynamics of the four soil management systems. From preliminary data, it appears that higher bacterial counts were observed in both organic systems (mean 4.1 versus 2.9 billion g/soil). Temporal increase in bacterial counts was observed in all systems a few days after fertilization with highest counts on day 21. Ongoing qPCRs on all soils and slurry samples are quantifying a set of AMR genes of interest, including those most abundant by the sequencing methods.

Author Disclosure Block:

E. Gómez-Sanz: None. **S. Jaenicke:** None. **R. Wittwer:** None. **A. Goesmann:** None. **M. M. van der Heijden:** None. **B. Duffy:** None. **T.H.M. Smits:** None.

Poster Board Number:

SATURDAY-197

Publishing Title:

Effect of Antibiotic Exposure on the Presence and Abundance of Resistance Genes in Cattle Manure and Ranch Soil

Author Block:

B. Weeder[1], D. Nguyen[1], E. Layton[2], G. Nyerges[1]; [1]Pacific Univ., Forest Grove, OR, [2]North Powder Charter Sch., North Powder, OR

Abstract Body:

Antibiotic resistance is considered a major threat to human health. Half of the antibiotics produced are used for agricultural purposes in the United States. The use of antibiotics in animal husbandry was showed to contribute to the dissemination of resistance genes in environmental microbial communities, and potentially plays a crucial role in creating a major reservoir of resistance genes.Our study investigated the presence and abundance of 14 different tetracycline resistance genes and 2 sulfonamide resistance genes in samples collected from areas of varying animal exposure on a beef cattle ranch in North Powder, Oregon, using qualitative polymerase chain reaction (PCR) and quantitative polymerase chain reaction (qPCR) methods. In addition, PCR amplification targeting conserved regions that flank a variable middle region of class 1 integrons and subsequent DNA sequencing were used to examine the presence and structure of these genetic elements in the collected samples.Of the 14 tetracycline genes tested for, 13 were present in samples from the farm. 6 genes (*tetO, tetM, tetA, tetQ, tetS,* and *tetW*) were further tested for copy number in the samples. After adjusting for variation in bacterial presence between samples (using 16S rDNA copy number), levels of both *tetM* and *tetS* were significantly higher (one way nested ANOVA with a Tukey post-hoc test) in the feedlot area (high exposure) when compared to the rangeland (low exposure). *tetM* presence was also significantly higher in the quarantine pen samples (highest exposure). Furthermore, sequence data of the integrons revealed resistance to aminoglycosides and trimethoprim.Our study indicates that the use of antibiotics creates a selective pressure favoring the survival of bacteria with various mechanisms to tolerate exposure to these drugs and results in elevated level or resistance genes in manure and in soil.

Author Disclosure Block:

B. Weeder: None. **D. Nguyen:** None. **E. Layton:** None. **G. Nyerges:** None.

Poster Board Number:

SATURDAY-198

Publishing Title:

Effect of Composting Method and Antibiotic Residue in Manure on the Fate of *E. coli* and Antibiotic Resistance Genes in Cattle Manures

Author Block:

R. K. Williams, P. Ray, K. Knowlton, L-A. Krometis, M. Ponder, A. Pruden; Virginia Tech, Blacksburg, VA

Abstract Body:

Cattle manure can be an abundant source of antibiotic resistant bacteria (ARBs) and resistance genes (ARGs) and thus its use as a soil amendment poses an emerging concern with respect to its potential to contribute to the spread of antibiotic resistance along the farm to fork continuum. The objective of this study is to determine the effect of composting on the abundance and antibiotic resistance profiles of *Escherichia coli* and ARGs relative to raw manures. Four manure types (beef with or without chlortetracycline, sulfamethazine, and tylosin or dairy with or without pirlimycin and cephapirin antibiotic administration) were mixed with alfalfa hay, pine bark mulch, and sawdust (C:N = 23-26%) and composted at small-scale (wet mass: 20-22 kg) using compost tumblers [71 cm (L) × 64 cm (dia.)] under static versus turned conditions, for a total of 8 treatments, which were each performed in triplicate. Compost samples were collected on day 0, 4, 7, 14, 21, 28, 35 and 42 to enumerate *E. coli* and total heterotrophs using MacConkey and R2A agars, respectively. At the conclusion of composting, *E. coli* colonies were isolated and screened for antibiotic resistance using the Kirby-Bauer disk diffusion test. Results indicate that composting was associated with an increase in resistance to ampicillin, chloramphenicol, and cefotaxime for *E. coli* that survived the composting procedure. A second round of small-scale static composting was performed using only dairy manures (with or without pirlimycin and cephapirin residues) where the thermophilic phase (>55°C) was maintained for fifteen days using an external heat source to evaluate the effect of prolonged thermophilic phase on *E. coli*. In the first round, *E. coli* counts persisted and actually increased by 2 logs over the course of the experiment for all manure types and composting methods. In the second round, non-detectable levels of *E. coli* were achieved by the final day of composting (day 42). Metagenomic analysis is currently underway to explore more deeply how the antibiotic and composting treatment affected profiles of ARGs and gene transfer elements. This study demonstrates that composting may actually increase antibiotic resistance of *E. coli* if it is not effectively eliminated during treatment.

Author Disclosure Block:

R.K. Williams: None. **P. Ray:** None. **K. Knowlton:** None. **L. Krometis:** None. **M. Ponder:** None. **A. Pruden:** None.

Poster Board Number:

SATURDAY-199

Publishing Title:

Prevalence and Diversity of Antibiotic Resistant of *Escherichia coli* in Food Animals in Ibadan, Nigeria

Author Block:

E. Amosun, O. Olatoye; Univ. of Ibadan, Ibadan, Nigeria

Abstract Body:

Background: Beef and chickens are the generally acceptable protein sources across meat consumers in Nigeria. Foodborne diseases and superbug transmission along the food chain result from contamination by gut microbiota of which Escherichia coli is an indicator organism **Methods:** The prevalence and diversity of antibiotic resistant of Escherichia coli in slaughtered cattle, dairy cows and free-range local chickens in Ibadan were investigated by phenotypic and genotypic methods. Escherichia coli was isolated using EMB agar; isolates were confirmed by PCR method following DNA chelex extraction from 70 faecal samples of cattle in abattoir, another 70 bovine raw milk from dairy farms and 50 free range local chicken cloacae swab samples. The antibiotic resistance profile of the isolates was determined using breakpoint assays on LB Agar plates **Results:** A total of 96 (50.53%) E. coli isolates were obtained from 190 samples. The isolates included 60 (85.71%) of 70 faecal samples of cattle in abattoir, 26 (37.14%) of 70 bovine raw milk and 10 (20%) of 50 free range local chicken cloacae swab samples. This primer produced positive amplified DNA bands of identical molecular weight all isolates of Escherichia coli. Overall, Escherichia coli showed resistance to streptomycin (39.58%), sulfamethoxazole/trimethoprim (28.5%), ampicillin (28.1%), amoxillin / clavulanic acid (17.7%), kanamycin (13.5%), chloramphenicol (12.5%), cefoxitin (9.4%), florfenicol (6.3%) and nalidixic acid (3.13.%). All the ten Escherichia coli isolated from free range chicken showed sensitivity to all the nine antibiotics. Multi drug resistance in three or more antibiotics was observed in (75.0%). Forty-one (42.7%) were sensitive to all the antimicrobial agents while fifty-five (57.3%) were resistant to at least one of the tested antibiotic agents. Twenty-five unique resistant patterns were detected.**Conclusions**: This study showed that antibiotics resistance genes are circulating among the cattle. This could either results from misuse of antibiotics in these animals or acquired resistance through horizontal gene transfer (HGT) and they also serve as reservoirs for pools of resistance genes.

Author Disclosure Block:

E. Amosun: None. **O. Olatoye:** None.

Poster Board Number:

SATURDAY-200

Publishing Title:

***Robinsoniella peoriensis*: A Model Anaerobic Commensal Bacterium for Acquisition of Antibiotic Resistance?**

Author Block:

T. R. Whitehead; USDA/ARS/NCAUR, Peoria, IL

Abstract Body:

Background: *R. peoriensis* was characterized in our laboratories from swine manure and feces as a Gram-positive, anaerobic bacterium. Since then strains of this species have been identified from a variety of mammalian and other GI tracts, suggesting it is a member of the commensal microflora. *R. peoriensis* has also been isolated from human infections. Recently our laboratory isolated new antibiotic resistant (AR) *R. peoriensis* strains. Therefore it is of interest to determine the AR profiles of *R. peoriensis* strains from different ecosystems and locations worldwide. **Methods:** *R. peoriensis* strains were collected from the US, France, Sweden and Germany. Sources included human wounds, turtle feces, and mouse GI tract. AR profiles were determined using agar plate disc-diffusion technique. Strains were plated onto BHI agar and antibiotics (10 µg) were added to blank discs. The plates were then transferred to an anaerobic glovebox for growth at 37°C and observed for resistance. Strains resistant to antibiotics were subjected to PCR analyses to determine the presence of resistance genes. **Results:** All strains of *R. peoriensis* shared resistance to ampicillin, gentamycin, kanamycin, and nalidixic acid, and were sensitive to vancomycin and fusidic acid. Variation in resistance was observed with tetracycline, erythromycin, and tylosin. The type strain, PPC31, another swine strain and a strain from turtle feces were found to be sensitive to all three antibiotics. One Swedish strain from a wound infection was also sensitive to the three antibiotics, whereas a second strain from human blood was resistant to tetracycline only. A strain from a French neonate was resistant to erythromycin/tylosin but not tetracycline. Initial studies indicate at least several of the strains are resistant to ciprofloxacin. PCR analyses indicated the presence of the *tet*(L) gene in at least one strain. **Conclusions:** All strains of *R. peoriensis* shared similar profiles to certain antibiotics, but the presence and absence of resistance to tetracycline and erythromycin suggests that strains may have acquired resistance genes, as indicated by the presence of *tet*(L). Strains may serve as models for testing for movement of resistance genes from other commensal and pathogenic bacteria. Further analyses of resistance genes in strains may provide insight into movement of resistance genes in the GI tract of various animals.

Author Disclosure Block:

T.R. Whitehead: None.

Poster Board Number:

SATURDAY-201

Publishing Title:

Low Carriage Rate of Esbl-Producing *Enterobacteriaceae* in Companion Animals on the Island of Barbados

Author Block:

T. A. R. Alleyne, N-A. McMillan, **S. N. Workman**; Univ. of the West Indies, Cave Hill Campus, Bridgetown, Barbados

Abstract Body:

Companion animals have been identified as reservoirs of extended-spectrum beta-lactamase-(ESBL-) producing enteric bacteria and there is evidence of their role in zoonotic transmission. This study sought to determine the carriage rate of ESBL-producing *Enterobacteriaceae* in local companion animals and to assess the susceptibility of isolates to commonly used antibiotics. Rectal swabs from 305 dogs and 63 cats from 6 veterinary clinics and one rescue pound were screened for ESBL-producing Enterobacteriaceae by plating on eosin methylene blue agar supplemented with 2 mg/L cefotaxime. ESBL production was confirmed by the combined disc test and ESBL type was determined by multiplex PCR with primers specific for conserved regions of TEM-, SHV- and CTX-M genes. The susceptibility of isolates to a panel of 10 antibiotics was determined. The carriage rate of ESBL-positive enterics was 5.3% and 1.6% in dogs and cats, respectively. There was no significant difference in carriage rate between cats and dogs (χ^2= 1.586; p = 0.326). Higher carriage rates were reported for sick animals (χ^2= 7.976; p = 0.005) and animals treated with antibiotics within the six months prior to sampling (χ^2= 7.185; p = 0.014). Of the 19 canine isolates, most were *Klebsiella pneumoniae*. The feline isolate was *E.coli*. High levels of resistance to tetracycline (95%), sulphamethoxazole-trimethoprim (90%) and ciprofloxacin (65%) were observed. Isolates were susceptible to carbapenem antibiotics. Most isolates (85%) exhibited co-resistance and carried CTX-M-type ESBLs. The bla_{TEM} and bla_{SHV} genes were detected in 75% and 40% of isolates, respectively.This is the first investigation into carriage of ESBL-producing enteric bacteria in companion animals on the island and, to our knowledge, the first report of its kind for the region. It revealed that local companion animals can harbour multi-drug resistant ESBL-producing *Enterobacteriaceae.* Further investigation into the role of pet dogs and cats as reservoirs of human infection on the island is warranted.

Author Disclosure Block:

T.A.R. Alleyne: None. **N. McMillan:** None. **S.N. Workman:** None.

Poster Board Number:

SATURDAY-202

Publishing Title:

Microbial Diversity and Antibiotic Resistance in the Chesapeake Bay and Upper Watershed (Winter of 2013)

Author Block:

V. Lahanda Wadu, J. Rivers, N. Boire, S. Riedel, N. Parrish; The Johns Hopkins Med. Inst.s, Baltimore, MD

Abstract Body:

Background: Antimicrobial resistance (AR) is an emerging threat. The Chesapeake Bay (CB) and upper watershed (UW) were previously found to harbor AR in multiple bacteria during the summer months. During the winter of 2013, we conducted a pilot study to survey bacterial diversity and resistance in isolates from the CB and UW.**Methods:** Water samples were collected from 9 sites on the CB and UW. Samples (500 ml) were collected and passed through a 0.2 µm filter. Filters were inoculated into Mueller Hinton broth and incubated at 35°C. All bacterial isolates were identified to the species level using MALDI-ToF MS. Antimicrobial susceptibility testing (AST) was performed for all enteric Gram-negative (G-) isolates using Etest and 14 antibiotics.**Results:** A total of 36 bacterial isolates were recovered from all sites including 6 Gram-positive (G+) and 30 G- organisms. Of the G+ isolates, nearly all (5/6, 83.3%) were *Bacillus* spp; a single *Enterococcus faecium* was found at the Baltimore Inner Harbor. Of the G- bacteria, 36.7% (11/30) belonged to the *Enterobacteriaceae*. The remainder of the G- organisms included predominantly *Pseudomonas* spp (2/30, 0.07%) and *Aeromonas* spp (13/30, 43.3%). A single strain of *Plesiomonas* was recovered from the Potomac River. No enteric bacteria were isolated from Gunpowder Falls State Park or the Monocacy River. Most enteric isolates were susceptible to the fluoroquinolones, cephalosporins and aminoglycosides. However, all isolates were resistant to ampicillin with MIC's ranging from 48 to >256 µg/ml. Eight isolates (72.7%) were resistant to chloramphenicol and tetracycline with MIC's ranging from 8 to 32 and 4 to >256 µg/ml, respectively. Two isolates recovered from the UW, an *Enterobacter cloacae* and a *Citrobacter freundii* were resistant to imipenem (MIC's, 1.5 and 16 µg/ml, respectively).**Conclusions:**Resistance to chloramphenicol and tetracycline exist in the CB and UW, a finding consistent with those of a prior study conducted in the summer of 2012. In that study, both the number of enteric bacteria and the amount of AR was increased versus the winter study. Taken together, these data suggest that resistance to these 2 antibiotics persists, regardless of season. Further studies are needed to better understand potential sources of antibiotic exposure in the CB and UW and the patterns of resistance seen in this study.

Author Disclosure Block:

V. Lahanda Wadu: None. **J. Rivers:** None. **N. Boire:** None. **S. Riedel:** None. **N. Parrish:** None.

Poster Board Number:

SATURDAY-203

Publishing Title:

Are Carbapenemases Produced by *Vibrio* Species Isolated from Bottlenose Dolphins (*Tursiops truncates*) in the Gulf of Mexico?

Author Block:

G. A. Pankey[1], D. S. Ashcraft[1], S. Shen[2], D. J. Grimes[2]; [1]Ochsner Clinic Fndn., New Orleans, LA, [2]Univ. of Southern Mississippi/Gulf Coast Res. Lab., Ocean Springs, MS

Abstract Body:

Background: Antimicrobial resistance is a global problem. *Vibrio* species from bottlenose dolphins have not been studied for carbapenemase-production. The Gulf of Mexico shoreline water allows contact between dolphins and humans, which might allow transference of carbapenemases. **Methods:** Fifteen bottlenose dolphins from Sarasota Bay, Florida and 16 from Barataria Bay, Louisiana were cultured during the 2011 National Oceanic and Atmospheric Administration (NOAA) Health Assessment. A total of 899 bacterial isolates were originally collected from the 31 dolphins, and 350 isolates were viable for testing. Identification and susceptibility testing was performed on 71 *Vibrio* spp. Forty-nine were collected from dolphins in the Sarasota Bay; 22 were from dolphins in the Barataria Bay. Isolates were collected from the following areas: blowhole (29), genital (12), fecal (11), and water (19) from immediate area where dolphins were captured. Identification was determined by 16s rRNA PCR and Sanger sequencing. Susceptibility testing was performed using the Vitek® 2 System. Any isolate found to be non-susceptible to carbapenems was screened for carbapenemase by a Neo-Rapid CARB kit. *Vibrio* spp. that were tested included: *V. alginolyticus* (n = 35), *V. harveyii/owensii/campbellii* (n = 17), *V. parahaemolyticus* (n = 13), and *V. fluvialis* (n = 6). **Results:** MICs (µg/mL) determined by the Vitek were: imipenem < 4 (99% susceptible), 8 (1% intermediate); meropenem < 0.5 (100% susceptible); and doripenem < 1. There are no Clinical and Laboratory Standards Institute breakpoints for testing doripenem and *Vibrio*. The one *V. alginolyticus* isolate with an imipenem MIC of 8µg/mL was negative when screened for carbapenemase. **Conclusions:** No carbapenemases were detected in any of the *Vibrio* species collected from dolphins in the Barataria Bay or Sarasota Bay area in 2011. Current studies are needed to determine if carbapenemase-production by *Vibrio* has occurred.

Author Disclosure Block:

G.A. Pankey: None. **D.S. Ashcraft:** None. **S. Shen:** None. **D.J. Grimes:** None.

Poster Board Number:

SATURDAY-204

Publishing Title:

Antibiogram Characterization of *Vibrio* Isolates Recovered from Aquaculture Environment

Author Block:

E. O. Igbinosa; Univ. of Benin, Benin City, Nigeria

Abstract Body:

Background: The use of antimicrobial agent to control livestock infection, for prophylaxis and growth promotion has development resistant strains of pathogenic bacteria. This issue is of major concern to human and animal health, as it leads to a greater risk of therapeutic failure of standard infection management. The aim of this study was to evaluate the presence of *Vibrio* isolates recovered from some fish pond facilities in Benin City, Nigeria, and determining their antibiogram properties and evaluating the public health implications of the findings. **Methods:** Samplings were conducted during the months of March and September 2014. Samples were screened for the isolation of vibrios using standard culture-based methods. Polymerase chain reaction (PCR) was used to confirm the identities of *Vibrio* species using the genus specific and species-specific. *Vibrio* species were detected at all studied sites between the order of 10^3 and 10^6 CFU/100 ml. A total of 550 presumptive *Vibrio* isolates were subjected to PCR confirmation for *Vibrio* organisms, 334 isolates tested positive, giving an overall *Vibrio* prevalence rate of 60.7 %.**Results:** Overall, 334 isolates from fish pond were obtained and identified of which 32.63% were *V. fluvialis*, 20.65% *V. parahaemolyticus*, 18.26% *V. vulnifcus* and 28.44 % *Vibrio* species. In all, 167 confirmed *Vibrio* isolates were randomly selected from a pool of 334 confirmed *Vibrio* isolates for antibiogram profiling. The susceptibility profiles to 20 antimicrobial agents of isolates were typical for the genus, high level of resistance was revealed in AMP^R, ERY^R, NAL^R, SUL^R, TRM^R, SXT^R, TET^R, OTC^R and CHL^R. The percentage of multiple drug resistance of *Vibrio* isolates was 67.6%. The multiple antibiotic resistances (MAR) index mean value of 0.365 of *Vibrio* isolates indicated that vibrios were exposed to high-risk sources of contamination where antibiotics were frequently used.**Conclusions:** The findings in this study revealed aquaculture environment can act as reservoirs for potential spread of multi-resistant bacteria. The presence of resistant *Vibrio* strains could serve as important medium transmitting through the food chain to humans constitute a risk for public health.

Author Disclosure Block:

E.O. Igbinosa: None.

Poster Board Number:

SATURDAY-205

Publishing Title:

Phenotypic and Genotypic Characterization of *Salmonella, E. coli,* and *Enterococcus* Isolated from the Upper Oconee Watershed, Georgia

Author Block:

S. Cho[1], L. M. Hiott[2], C. R. Jackson[2], J. G. Frye[2]; [1]Univ. of Georgia, Athens, GA, [2]USDA-ARS, Athens, GA

Abstract Body:

This study was conducted in order to monitor *Salmonella, E. coli,* and *Enterococcus* found in the Oconee River in Athens, Georgia.Water samples were collected once each season of 2015 at different locations along the Oconee River. The number of water samples varied from 30 to 100, with 222 total samples collected. Cellulose filter powder (Aqua Dew™) was added to 1L of water and filtered onto 47-mm glass fiber filter (Pall Corporation). The filter and powder were incubated overnight (O/N) in buffered peptone (BP) water (BD).*Salmonella* isolation: BP enrichment was added to selective enrichment media (Gram negative Hajna (BD), tetrathionate (BD), Rappaport-Vassiliadis (BD) broths), incubated O/N, and then streaked onto brilliant green sulfa (BD) and xylose–lysine tergitol 4 (BD) agar plates. After O/N incubation, *Salmonella* colonies were assayed with triple sugar iron (BD) and lysine iron agar slants (BD). Positive *Salmonella* isolates were serotyped by PCR.*E. coli* and *Enterococcus* isolation: BP enrichment was streaked onto selective agar plates (CHROMagar ECC (CHROMagar™), m-TEC (HiCrome™) for *E. coli*; and CHROMagar *Enterococcus* (CHROMagar™), Enterococcosel (BD), m-*Enterococcus* (BD) for *Enterococcus*). *E. coli* and *Enterococcus* isolates were genotyped by PCR.260 *E. coli* were isolated, one-third of which belonged to a potentially pathogenic group B2. Enteropathogenic and Shiga toxin genes were detected in 5.0 and 0.4% of the isolates respectively. Few multidrug resistant (MDR) *E. coli* were detected. 405 *Enterococci* were isolated and separated into 8 different species, including *E. avium, E. casseliflavus, E. faecalis, E. faecium, E. gallinarum, E. hirae, E. mundtii,* and *E. pallens.* Almost all the isolates were intermediate or resistant to more than two drugs. Resistances to daptomycin and lincomycin were seen. 315 *Salmonella* were isolated, some of which were serotypes also isolated from humans. MDR *Salmonella* were detected.This study indicates the presence of pathogens, such as *Salmonella, E. faecalis, E. faecium,* and virulent *E. coli* in the Upper Oconee Watershed, many of which are resistant to antimicrobials. Widespread occurrence of *Salmonella* in the surface water suggests that water can serve as a reservoir for *Salmonella* and play a role in its transmission.

Author Disclosure Block:

S. Cho: None. **L.M. Hiott:** None. **C.R. Jackson:** None. **J.G. Frye:** None.

Poster Board Number:

SATURDAY-206

Publishing Title:

Multiple Antibiotic Resistance Phenotypes of *Plesiomonas shigelloides* Recovered from Some Freshwater Environments in Southwest Nigeria

Author Block:

I. M. Adesiyan[1], M. A. Bisi-Johnson[1], A. I. Okoh[2]; [1]Obafemi Awolowo Univ., Ile Ife, Nigeria, [2]Univ. of Fort Hare, Alice, South Africa

Abstract Body:

We evaluated the antimicrobial resistance indexing of *Plesiomonas shigelloides* isolated from some rivers in Osun and Oyo States, Southwestern Nigeria. Water samples were collected over a period of 4 months (May to August, 2015) and analysed using standard procedures. A total of 150 presumptive isolates were recovered and confirmed by polymerase chain reaction technique using PS gene. Thirty-eight (25%) of the isolates were confirmed as *Plesiomonas shigelloides* specie and were screened for *in vitro* antimicrobial resistance profile using disc diffusion assay. All the isolates were resistant to sulphamethoxazole and erythromycin, and variously resistant to other antibiotics in the order: ampicilin (97%), cefuroxine (97%), neomycin (84%), chloramphenicol (74%) and cefotaxine (68%). Overall, antibiotics resistance index obtained across the sampling locations was approximately 0.9 which was by far greater than the 0.2 threshold value, with modal multiple antibiotic resistance pattern TM-E-CTX-NE-C-SMX-KF-NAL-AP-ENR-TS, both suggesting our isolates to be of high risk sources. Our findings signify high prevalence of antimicrobial resistance in the selected water bodies, which could be attributable to excessive antimicrobial usage, thereby necessitating for proper sanitation and hygiene, and enforcement of legislation against indiscriminate use of the conventionally prescribed antimicrobials among inhabitants of the communities

Author Disclosure Block:

I.M. Adesiyan: None. **M.A. Bisi-Johnson:** None. **A.I. Okoh:** None.

Poster Board Number:

SATURDAY-207

Publishing Title:

High Frequency of Isolation of Multi-Resistant Esbl- and Carbapenemase-Producing *Enterobacteriaceae* from Wastewater on the Island of Barbados

Author Block:

E. G. Knaizeh, S. N. Workman; Univ. of the West Indies, Cave Hill Campus, Bridgetown, Barbados

Abstract Body:

ESBL- and carbapenemase-producing *Enterobacteriaceae* have emerged in the community setting in several countries and present a serious public health threat. There is little knowledge of local community reservoirs of these pathogens. This investigation sought to screen local sewage for the presence of ESBL- and carbapenemase-producing enterics, and to determine the susceptibility of isolates to antibiotics commonly recommended for treatment of clinical infections. Influent and effluent sewage samples, collected bi-monthly from the two local wastewater management facilities over a 12-month period, were screened for ESBL- and carbapenemase-producing enterics using selective enrichment and plating techniques, with cefotaxime and meropenem, respectively. The combined disc test and modified Hodge test were used for phenotypic confirmation of ESBL- and carbapenemase-production, respectively. Antimicrobial susceptibility of isolates was determined by disk diffusion assay. ESBL-producers (n = 263) were recovered from 77% of influent and 80% of effluent samples. Carbapenemase-producers (n = 59) were detected in 22% of influent and 27% of effluent samples. For both facilities, isolation rates for ESBL-producers significantly exceeded those for carbapenemase-producers (Fisher's exact, $p < 0.0001$). There was a significant difference in isolation rate of carbapenemase-producers between the two facilities (Fisher's exact, $p = 0.0006$), with more frequent recovery from that receiving sewage from an area inclusive of our public hospital (Fisher's exact, $p = 0.0003$). ESBL-producers were predominantly *E. coli* (67%) and *Klebsiella pneumoniae* (20%). The majority of carbapenemase-producers were *K. pneumoniae* (63%) and *Enterobacter intermedius* (25%). High levels of resistance to cefepime (92%), ciprofloxacin (74%) and sulphamethoxazole-trimethoprim (72%) were observed amongst ESBL-producers. Carbapenemase-producers were generally resistant to amikacin (93%), doxycycline (53%), gentamicin (31%) and tigecycline (53%), but susceptible to fosfomycin (95%). The findings indicate a high frequency of isolation of multi-resistant ESBL- and carbapenemase-producing enterics from wastewater in Barbados and suggest the presence of reservoirs in the community.

Author Disclosure Block:

E.G. Knaizeh: None. **S.N. Workman:** None.

Poster Board Number:

SATURDAY-208

Publishing Title:

Quantitation of Antibiotic Resistance Genes Pollution in Hospital Waste Water Effluent and Urrban River Water

Author Block:

S. K. Walia, A. Kumar; Oakland Univ., Rochester, MI

Abstract Body:

Background: Anthropogenic activities play a major role in the dissemination of bacterial antibiotic resistance genes in the environment. Chemicals of concerns including antibiotics are inadvertently released in the water bodies via hospital waste water effluent, municipal water and storm water. **Methods:** In this study, we assessed the presence of antibiotic resistance genes (ARG) in hospital effluent and urban river water as a potential reservoir of bacteria containing ARG. A total of 156 bacteria isolated from hospital waste water treatment plant were analyzed for ARG including extended spectrum beta-lactamase (ESBL, SHV, TEM and CTX-M-1) genes commonly found in the environment. **Results:** Analysis of antibiotic sensitivity data revealed that 35-60% bacteria were susceptible to cefotaxime, 60-75% to chloramphenicol, 60--70% to tetracycline, 35-75% to ciprofloxacin and 70-50% to gentamicin. Of these 156 bacteria, 34 (22%) were found to be ESBL producing bacteria. The copy number of gene/mL of Hospital Effluent (WBHE) for bla_{SHV}, $bla_{TEM,}$ and $bla_{CTX\text{-}M\text{-}1}$ were 10^2,10^4 and 10^3 respectively. The abundance of bla_{SHV} and $bla_{CTX\text{-}M\text{-}1}$ remained similar in hospital waste water effluent and river water whereas the quantity of bla_{TEM} decreased to an order of 10^2 in Urban Clinton River Water (UCRW).**Conclusions**::The genetic relatedness of ARG among hospital waste water effluent and urban river water bodies is worrisome and cautions the public health authorities for its potential to spread highly resistant "SUPER BUG" in the community.

Author Disclosure Block:

S.K. Walia: None. **A. Kumar:** None.

Poster Board Number:

SATURDAY-209

Publishing Title:

Ges-type Extended-spectrum β-Lactamases in *Aeromonas* spp Isolates from a Hospital Effluent in Southern of Brazil

Author Block:

A. A. Bavaroski[1], J. K. Palmeiro[2], L. S. Rodrigues[1], D. Conte[1], **L. M. Dalla-Costa**[1]; [1]Inst. de Pesquisa Pelé Pequeno Príncipe, Curitiba, Brazil, [2]Hosp. de Clínicas, Curitiba, Brazil

Abstract Body:

Aeromonas spp are ubiquitous bacteria mostly isolated from aquatic environment, reported as emerging human pathogens and important carrier and reservoir of antibiotic resistance genes. We investigated the presence of genus *Aeromonas* in effluents from a pediatric hospital, community health care and from domestic sewage. Matrix assisted laser desorption ionization time of flight (MALDI-TOF) mass spectrometry was used to identify *Aeromonas* spp. To evaluate extended-spectrum β-lactamases (ESBL) production the combined disc methods (ceftazidime and cefotaxime with and without clavulanic acid) were used.. *Aeromonas* spp. representing 18.8% (26/138) of the isolates of the study and out of them 42.3% (11/26) were ESBL producers. PCRs and sequencing performed on the isolates exhibiting an ESBL phenotype using specific primers (*bla*$_{SHV,}$ *bla*$_{TEM,}$ *bla*$_{CTXM,}$ *bla*$_{PER}$ and *bla*$_{GES}$) and resistance genes *bla*$_{TEM}$ (3/27%), *bla*$_{CTXM-2}$ (2/18%) and *bla*$_{GES}$ (3/ 27%) were identified. Nucleotide sequence analysis of *bla*$_{GES}$ showed the occurrence of *bla*$_{GES-7}$, *bla*$_{GES-1}$, and *bla*$_{GES-16,}$ the last two have not been previously described in this genus. These findings suggest a water reservoir of ESBL genes and emphasized the importance of resistance genes search in environmental microorganisms that might represent a potential risk for human health.

Author Disclosure Block:

A.A. Bavaroski: None. **J.K. Palmeiro:** None. **L.S. Rodrigues:** None. **D. Conte:** None. **L.M. Dalla-Costa:** None.

Poster Board Number:

SATURDAY-210

Publishing Title:

Does Water Stress Increase the Incidence of Antibiotic Resistance Genes in Drinking Water Supplies?

Author Block:

M. Sevillano[1], C. W. Knapp[2], S. T. Calus[3], A. J. Pinto[1]; [1]Northeastern Univ., Boston, MA, [2]Strathclyde Univ., Glasgow, United Kingdom, [3]Univ. of Glasgow, Glasgow, United Kingdom

Abstract Body:

Increasing water stress due to population growth, industrial/agricultural demand, and drought has resulted in direct or indirect use of reclaimed water (i.e. treated wastewater) to boost existing drinking water supplies (DWS). This raises concerns about the risk of introduction of anthropogenic contaminants into DWS. To this end, extensive work has been carried out to characterize and quantify chemical contaminants (e.g. pharmaceuticals) in DWS. In contrast, little has been done to evaluate the implications of using reclaimed water for potable use on the exposure to emerging biological contaminants like antibiotic resistance genes (ARGs).We conducted a sampling campaign to characterize the incidence and concentration of ARGs across a gradient of water stress across the United Kingdom in the summer of 2015. 30 samples were collected at drinking water taps from 12 densely populated cities in mainland UK, with variable water stress classification ranging from low to medium, to extremely high. Water quality was assessed by measuring a range of chemical parameters including pH, DO, TOC, inorganic nitrogen species, phosphate, and dissolved metal cations. Samples were filtered to harvest bacterial cells from by filtration through 0.2 μm sterivex filters, followed by DNA extraction. Samples were characterized for a range of clinically relevant ARGs using qPCR. In addition, samples have been submitted for shotgun DNA sequencing for non-targeted detection of a range of ARGs.Preliminary results indicate the presence of a range of ARGs in treated drinking water supplies. Specifically, sul1, acrB, czcA and czcD, and blaTEM were elevated in certain drinking water samples. This work will expand on these preliminary results by providing quantitative information about all ARGs targeted in this study, including assessment of links between ARGs incidence/quantitation and water stress categorization according to sample location. Further, using metagenomic assembly and genome binning we will attempt to place these detected ARGs into genome bins to determine their presence/absence in bacteria that are likely to be relevant from a public health perspective.

Author Disclosure Block:

M. Sevillano: None. **C.W. Knapp:** None. **S.T. Calus:** None. **A.J. Pinto:** None.

Poster Board Number:

SATURDAY-211

Publishing Title:

Multi-State Surveillance Study of Esbl and Carbapenemase-Producing *Enterobacteriaceae* in U.S. Drinking Water

Author Block:

W. D. Tanner[1], A. Gundlapalli, 84047[1], J. Olstadt[2], J. VanDeVelde[3], P. Higgins[4], J. VanDerslice[1]; [1]Univ. of Utah, Salt Lake City, UT, [2]Wisconsin State Lab. of Hygiene, Madison, WI, [3]Illinois Dept. of Hlth., Springfield, IL, [4]Pennsylvania Dept. of Environmental Protection, Harrisburg, PA

Abstract Body:

Background: Community-acquired carbapenemase and extended-spectrum beta-lactamase (ESBL) infections are increasing. Environmental sources of resistant bacteria have been confirmed; however, community drinking water is rarely considered as a source of resistant enteric organisms, especially in the United States. Approximately 1 to 2% of U.S. drinking water samples contain enteric bacteria (1). The purpose of this study was to investigate whether tap water samples containing *Enterobacteriaceae* are also positive for ESBL or carbapenemase genes. **Methods:** Tap water samples testing positive for *Enterobacteriaceae* were acquired from state health laboratories in New York, Illinois, Pennsylvania, Wisconsin, and Utah. Coliform-positive samples were screened for SHV, TEM, and CTX-M ESBL genes and OXA-48, KPC, NDM, and VIM genes by multiplex PCR. PCR products from positive samples were sequenced to confirm the specific genes present. ESBL and carbapenemase-producing bacteria were subsequently isolated, identified, and tested for susceptibility to various antibiotics.**Results:** Of 150 coliform-positive samples tested to date, more than 20 were confirmed to have ESBL genes. The most predominant ESBL-producers were Serratia fonticola and Klebsiella oxytoca, although these were primarily intrinsic ESBL genes with some corresponding ESBL phenotypes. One CTX-M-producer and four SHV-producing enteric isolates were also identified. Sequencing results of SHV PCR products produced multiple GeneBank matches with ESBL and non-ESBL types. Two samples tested positive by PCR for OXA-48 genes and sequencing of the PCR products showed 99% and 100% GeneBank matches to OXA-48-like gene sequences.**Discussion:** While *Enterobacteriaceae* detection in treated U.S. tap water is uncommon, some of these bacteria do carry ESBL and carbapenemase genes, indicating that there is a potential for ingestion of these organisms via U.S. tap water. Although many of the detected ESBL genes were intrinsic, many environmental bacterial species serve as the original source of ESBL and carbapenemase genes of clinical concern (2).

Author Disclosure Block:

W.D. Tanner: None. **A. Gundlapalli:** None. **J. Olstadt:** None. **J. VanDeVelde:** None. **P. Higgins:** None. **J. VanDerslice:** None.

Poster Board Number:

SATURDAY-212

Publishing Title:

Analysis of Surface Longevity of *Klebsiella pneumoniae carbapenemase* (Kpc)-Producing Enterobacteriaceae on an Environmental Surface

Author Block:

R. P. Orr, A. Cheruvanky, W. Chai, M. Denecke, J. Carroll, A. Mathers; Univ. of Virginia Hlth.System, Charlottesville, VA

Abstract Body:

Background: KPC-producing Enterobacteriaceae have been identified as an urgent threat to hospitalized patients around the world. However, our understanding of nosocomial transmission of these organisms is incomplete and may involve the environment. Little is known about the surface longevity on inanimate objects of different species that carry KPC and cause nosocomial infections. This would be critical information for targeting infection control measures. We examined the difference in surface survival between the two most common KPC-positive nosocomial pathogens *Klebsiella pneumoniae* (KPC-Kp) and *Enterobacter cloacae* (KPC-Ec). **Methods:** Three independent 30-day trials of clinical strains of KPC-Kp and KPC-Ec were performed using a bleach-sterilized surface outlined with a grid design. Each cell was inoculated with $5X10^7$ colony forming units (CFU)s. Sterilized, pre-soaked cotton swabs were applied approximately every 24 hours to a new cell along with a swab from a paired surface without added organisms as a negative control. Collected samples were incubated overnight in tryptic soy broth with a 10µg ertapenem disk. If growth was visually detected, the sample was plated on KPC selective chromogenic agar and pigmented colonies consistent with KPC-Kp or KPC-Ec were considered positive.**Results:** A total of 129 samples were processed throughout the duration of the experiment. Both KPC-Kp and KPC-Ec had some samples that survived the entirety of the 30-day trial. Surface samples were 100% (21/21) and 100% (21/21) week 1, 52% (11/21) and 100% (18/18) week 2, 54% (13/24) and 91% (22/24) weeks 3 and 4 for KPC-Kp and KPC-Ec, respectively. Comparison of surface survival from day 8 to 30 demonstrated KPC-Ec had more persistence then KPC-Kp (40/42 versus 24/45; p=0.001 by Fischer exact).**Conclusion:** We conclude that KPC-Kp and KPC-Ec have the capacity to survive on an environmental surface for prolonged periods of time. This indicates that the hospital environment could play a larger role in KPC-positive Enterobacteriaceae transmission than currently recognized. KPC-Ec persisted slightly longer in the environment than KPC-Kp, which may point to a larger role of the environment in hospital transmission of this species and will require further investigation.

Author Disclosure Block:

R.P. Orr: None. **A. Cheruvanky:** None. **W. Chai:** None. **M. Denecke:** None. **J. Carroll:** None. **A. Mathers:** None.

Poster Board Number:

SATURDAY-213

Publishing Title:

Isolation and Antibiotic Screening of Halophilic Actinomycetes from the Cabo Rojo Solar Salterns

Author Block:

L. Casillas, Valentin, L., E. Bayala, X. Alejandro, M. Claudio,L.M. Casillas, I. Garcia; UPR-Humacao, Humacao, Puerto Rico

Abstract Body:

As microbial resistance has been rising at alarming rates in the last decades, there is an urgent need to find new antibiotic to fight against such resistant pathogens. Extremophiles have unique genetic and chemical characteristics, thus they are attractive targets for drug discovery. Microbes inhabiting the Cabo Rojo solar salterns live in an extreme environment (high salinity, temperature and UV radiation) composed of a series of salt ponds surrounded by microbial mats. In order to isolate for halophylic Actinobacteria, we initially set up enrichments with samples from the salt ponds and microbial mats using commercially available Actinomyces broth supplemented with 4-20% NaCl. More than twenty morphologically distinctive *Actinomyces* fungus like-colonies were identified with differential antibiotic resistance patterns after traditional Kirby Bauer tests. As *Actinomyces sp*. are known to produce a variety of antibiotics, we tested six of these isolated strains for their ability to inhibit the growth of five clinically relevant pathogens (*Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae, Salmonella typhimurium,* and *Escherichia coli*). Isolates 18, 41, 72, 78, 87, and 88 were sub-cultured into Marine broth (MB) and incubated for 48 hours at 37ºC prior to the bioassay. The filtered supernatant was deposited in wells within each pathogen inoculated in the agar plates. Antibiotic activity was determined after 48-72 hours. Ampicillin (c = 0.0125 mg/mL) was used as positive control for antibiotic activity. By using the agar well diffusion assay we detected antibiotic activity in four of the six tested strains. Our results indicate that isolates 18 and 41 produced antibiotics active against *S. aureus*, *P. aeruginosa* and *K. pneumoniae* with inhibition zones in a range of 11-20 mm. Interestingly, Isolate 87 exhibited broad antibacterial activity inhibiting all pathogens tested but resulting in smaller inhibition zones (7-10 mm). Crude extracts from Isolate 72 began to show antibacterial activities only after 72 hours of incubation. Our next steps are to chemically identify the components in these crude extracts by HPLC-MS and phylogenetically identify these halophylic *Actinomyces*.

Author Disclosure Block:

L. Casillas: None.

Poster Board Number:

SATURDAY-214

Publishing Title:

Methicillin-resistant *Staphylococcus aureus* Isolated from Surfaces and Mobile Devices at a Caribbean Medical School

Author Block:

J. C. Harrington; Univ. of Med. and Hlth.Sci., Basseterre, Saint Kitts and Nevis

Abstract Body:

Background: The island of Saint Kitts is home to a medical school, University of Medical and Health Sciences, which include rotations at JNF Hospital in the curriculum. Students frequently interact with patients with diabetes mellitus (adult population incidence = 13.2%), a condition associated with opportunistic and life-threatening microbial infections. Previous studies have identified campus surfaces and mobile devices as transient fomites for *Staphylococcus aureus* contamination, thus medical student may be acting as a transmission vector to compromised patients. The objective of this study was to characterize methicillin susceptibility of *S. aureus* isolates present on campus surfaces and mobile devices from medical students at UMHS-St Kitts campus, and to determine if alcohol swabs are an effective means for microbial reduction. **Methods:** A campus survey was conducted to culture non-fastidious fungal and bacteria isolates with the use of sterile swabs, tryptic soy agar plates, and standard microbiological and biochemical assays for identification. 100 hard surfaces were sampled on campus. 60 mobile devices of students were sampled pre- and 24-hour post-disinfection with 70% isopropyl alcohol wipes. Putative *S. aureus* isolates were confirmed with BactiStaph agglutination and assayed for oxacillin susceptibility with Oxoid disks.**Results:** For the campus surfaces sampling, 23 surfaces (23%) were positive for *S. aureus* contamination, predominantly from commonly touched surfaces. Of the confirmed bacterial colonies cultured from surface samples, 6 isolates (26%) demonstrated resistance to oxacillin. For the mobile device sampling, 31 (52%) phones were positive for *S. aureus* prior to disinfection, from which 3 isolates (9%) displayed resistance to oxacillin. After 24 for hours post-cleaning with 70% alcohol, 35 phones (58%) were positive for *S. aureus* and 5 (14.8%) of the isolates were oxacillin resistant. All test isolates were susceptible to vancomycin.**Conclusions:** Methicillin-Resistant *S. aureus* is prevalent on the UMHS campus, including majority of the student mobile devices tested. Alcohol swabs were ineffective at reducing the *S. aureus* on the mobile devices; thus, alternative disinfectant methods should be explored.

Author Disclosure Block:

J.C. Harrington: None.

Poster Board Number:

SATURDAY-215

Publishing Title:

Susceptibility of 194 *Salmonella* Isolates to 17 Chemicals Used as Sanitizers and Biocides

Author Block:

S. B. Humayoun, L. Hiott, C. R. Jackson, J. G. Frye; USDA, Athens, GA

Abstract Body:

Salmonella is a major cause of foodborne illnesses in the United States. Meat processing facilities use a wide variety of antimicrobial interventions to reduce *Salmonella* contamination. The objective of this study was to determine the minimum inhibitory concentration (MIC) of sanitizers and biocides against *Salmonella*. The biocide used in this study were: Acidified sodium chlorite (ASC), Acetic acid (AA), Arsenite (ARI), Arsenate (ARA), Benzalkonium chloride (BC), Cetylpyridinium chloride (CC), Citric acid (CA), 1,3 Dibromo, 5,5 dimethylhydantoin (DBH), Dodecyltrimethylammonium chloride (DC), Hexadecyltrimethylammonium bromide (HB), Hexadecyltrimethylammonium chloride (HC), Lactic acid (LA), Peroxyacetic acid (PXA), Sodium hypochlorite (SHB), Sodium metasilicate (SM), Trisodium phosphate (TP), Chlorhexidine (CH). 194 animal associated *Salmonella* isolates representing 84 serotypes were assayed for susceptibility. Isolates were grown on Blood Agar plates. Colonies were picked and suspended in Mueller Hinton broth (MHB), and inoculum concentration was adjusted to 10^5 cells/ml following protocols for Sensititre^{TM} (ThermoFisher, Pittsburgh, PA). MIC assay was done in 96 well plates using two-fold serial dilutions of chemicals in MHB. Plates were incubated at 37ºC for 24 hrs and turbidity was used as indicator of bacterial growth. Results were recorded using the Sensititre vision system. The lowest concentration at which no growth was observed was reported as the MIC to the chemical in µg/ml. The median MIC (µg/ml) for 194 isolates was: DC =256, CC=40, LA=7556, CA=6316, BC =40, AA =1640, TP =37952, HB=82, HC =78, ARI =30, ARA 408, SM =120640, SHB=55500, PXA=884, ASC = 1272, DBH =1248, and CH =3.5. Most of the isolates showed similar susceptibilities to each chemical with MICs within +/- one two-fold dilution from the median MIC. However, 16 *Salmonella* isolates had a four-fold higher MIC than the median MIC to Arsenate and 19 isolates had a four-fold higher MIC to Arsenite. Additionally, 5 isolates had a four-fold lower MIC to Arsenate. This variability in MIC may indicate reduced susceptibility or hyper susceptibility in some *Salmonella* isolates to these compounds.

Author Disclosure Block:

S.B. Humayoun: None. **L. Hiott:** None. **C.R. Jackson:** None. **J.G. Frye:** None.

Poster Board Number:

SATURDAY-216

Publishing Title:

A Survey of Microbial Contamination of Antiseptics and Disinfectants in Lagos, Nigeria

Author Block:

A. E. OMOTAYO, M. S. Sanusi, C. J. Nsofor, S. A. Adesida; Univ. of Lagos, Lagos, Nigeria

Abstract Body:

Background: Antiseptics and disinfectants have been widely used as a germicidal agents, they are believed to be highly effective against microorganisms though they are prone to microbial contamination. This study was carried out to assess the microbial contamination of antiseptics and disinfectants.**Materials:** Fifty (50) samples were purchased from different pharmacies, supermarkets, local markets and hawkers in Lagos State. The samples were pre-enriched in Tryptone Soya Broth and Sabouraud Dextrose Broth. Isolation of bacteria and fungi were done by culturing on Tryptone Soya Agar, Mannitol Blood Agar, Centrimide Agar, MacConkey Agar and Sabouraud Dextrose Agar using the spread plate method. The bacterial isolates were identified by standard biochemical test, the use of Analytical Profile Index (API) kit and Microbact kit. The fungal isolates were identified by cultural and morphological characteristics. The antifungal and antibiotic sensitivity test of the isolates were determined by the disk diffusion method using commercially available antimicrobial agents.**Results:** Bacterial and fungal contamination rate of 80% and 86% were observed respectively. The isolates identified were *Acinetobacter baumanii, Bacillus* spp., *Corynebacterium* spp., *Paenibacillus macerans, Pseudomonas* spp. *and Staphylococus* spp. All the isolates showed variable sensitivity to antibiotics with *Pseudomonas aeruginosa, P. fluorescens and A. baumanii* having the highest Multiple Antibiotics Resistance (MAR) Index of 0.69 and *S. aureus* with the lowest MAR index of 0.07. The fungi isolated were *Penicillium chrysogenum, Aspergillus flavus, Chrysosporium tropicum, Alternaria solani, Fusarium solani, Penicillium marneffei* and *Aspergillus niger.* The isolates were resistant to the three antifungal agents (Nystatin, Voricinazole and Fluconazole) used, with some having MAR Index of up to 1.00.**Conclusion:** This study revealed the high level of microbial contamination of antiseptics and disinfectants including some potentially pathogenic organisms that could have serious medical consequences.

Author Disclosure Block:

A.E. Omotayo: None. **M.S. Sanusi:** None. **C.J. Nsofor:** None. **S.A. Adesida:** None.

Poster Board Number:

SATURDAY-218

Publishing Title:

Prevalence of Multiple Antibiotic Resistant Bacteria in University of Ibadan Library Books and Library Indoor Air

Author Block:

H. G. Johnsons; Univ. of Ibadan, Ibadan, Nigeria

Abstract Body:

Resistance to antimicrobials by bacteria has become worrisome worldwide specially in developing nation like Nigeria, threatening the ability to treat infections. Poorly ventilated closed settings like libraries in our institution may provide occasions for the transmission of multiple antibiotic resistant bacteria between immune-compromised and normal individuals. Although, studies have shown the presence of pathogenic bacteria in formites like toys and magazines in hospitals, little or nothing is documented about pathogenic bacteria in library books especially in Nigeria. Thus this study not only analyzed the possibility of pathogenic bacteria on books and air in these libraries but also considered the possibility of multidrug resistance among the bacteria and also assessed if these media can serve as latent reservoirs of multiple drug resistant bacteria.The settle plate method was used to collect indoor air samples while surfaces of books were swabbed. Sensitivity of 40 out of the isolated bacteria to broad spectrum antibiotics was done using the Kirby-Bauer agar disk diffusion method.Bacteria counts were statistically significant at $p < 0.05$ with mean count from air (using the Commission of European Communities (1993) standard) and books ranging from $0.23 \times 10^3 \pm 0.065$ CFU/m^3 to $1.99 \times 10^3 \pm 0.655 \times$ CFU/m^3 and $5.83 \times 10^4 \pm 0.0247$ CFU/ml to $1.32 \times 10^4 \pm 0.0283$ CFU/ml. Species of *Bacillus, Staphylococcus*, *Proteus, Micrococcus*, *Yersenia*, *Erwinia*, *Klebsiella Serratia, Pseudomonas* and *Providencia* were isolated. *Bacillus* spp. were the most (27.5%) followed by *Staphylococcus* spp. (22.5%), *Erwinia* and *Providencia* spp. (2.5%) each. Resistance ranged between 17.5% for ciprofloxacin to 75% for tetracycline. Irrespective of species, 46.25% of the isolates were resistant to all the drugs while 42.5% were susceptible to all drugs. Also, 100% were resistant to at least one or more antibiotics while 82.5% were multidrug resistant. *Staphylococcus aureus, Bacillus subtilis* and *Staphylococcus cohnii* were 100% resistant to all the antibiotics.The high counts of multiple drug resistant bacteria in these libraries showed that air and books in these libraries serves as reservoirs of multidrug resistant bacteria and thus indicating a treat in health of the library staff and other users.

Author Disclosure Block:

H.G. Johnsons: None.

Poster Board Number:

SATURDAY-219

Publishing Title:

***Staphylococcus aureus* and Methicillin-Resistant *Staphylococcus aureus* in Nursing Homes**

Author Block:

S. Cheatham, D. Thapaliya, M. Taha, K. Milliken, M. Dalman, J. Kadariya, D. Grenier, T. C. Smith; Kent State Univ., Kent, OH

Abstract Body:

Staphylococcus aureus is a common pathogen that can cause a wide range of infectious diseases. For the past few decades, methicillin-resistant *S. aureus* (MRSA) has become a deadly nosocomial infection in many institutions such as hospitals, community centers, and nursing homes. The bacterium can be transmitted directly person-to-person, or via contact with contaminated fomites. Nursing homes (NH) can be a major risk when it comes to nosocomial infections due to transporting residents to and from appointments, the rate of discharges per day including discharges home and to the hospital, the number of new admits on a weekly basis, and transferring patients from room to room. The purpose of this study was to examine the prevalence of both *S. aureus* and MRSA in NHs and obtain molecular and epidemiological information in order to implement new ways of infection control within. A total of 259 environmental cultures were obtained from 7 different nursing homes within 3 geographical areas in Northeast Ohio; rural, urban, and suburban, from July 7, 2015 to July 12, 2015. Samples were processed within 24 hours of collection. DNA amplification and PCR was run to identify the presence of the *mec*A and PVL genes for all *S. aureus* isolates. Finally, *spa* and multi-locus sequence typing (MLST) were performed in order to identify strain types. The overall prevalence of *S. aureus* was 29.0% (75/259). Of positive isolates, 64.0% (48/75) were MRSA. The presence of the PVL gene was 32.0% (24/75). Of 75 positive isolates, there were 18 different *spa* types with t002 the most common and found in urban and suburban NHs When comparing urban, rural, and suburban, the urban area had the highest prevalence of *S. aureus* (47.2%) with 80% being MRSA. It also had the highest frequency of the PVL gene at 42.9%. Suburban NHs had 27.0% positive for *S. aureus* with 53.3% of those MRSA and 26.7% positive for PVL. Lastly, rural NHs only had 10.8% prevalence of *S. aureus* with 37.5% MRSA and none that were PVL-positive. Part of these differences may result from transfers from hospitals; the urban NHs had anywhere between 4-15 hospitals nearby whereas suburban and rural had 1-3 hospitals within the area. The results of this study indicate that infection control in both hospitals and nursing homes could possibly contribute to the high prevalence of environmental MRSA in urban nursing homes.

Author Disclosure Block:

S. Cheatham: None. **D. Thapaliya:** None. **M. Taha:** None. **K. Milliken:** None. **M. Dalman:** None. **J. Kadariya:** None. **D. Grenier:** None. **T.C. Smith:** None.

Poster Board Number:

SATURDAY-220

Publishing Title:

Pilot Study to Explore MSSA and MRSA Nasal Carriage in Female College Students

Author Block:

S. Donovan, G. Chiumento, J. Richards, M. O'Seaghdha, S. M. Duty, E. Scott; Simmons Coll., Boston, MA

Abstract Body:

Background: *Staphylococcus aureus (S. aureus)* is a Gram-positive bacterium that causes localized and systemic infections. Methicillin-resistant *S. aureus* (MRSA) is a strain of *S. aureus* that has developed resistance to beta-lactam antibiotics. According to CDC, MRSA infects approximately two million people per year and accounted for 11,285 deaths in the USA in 2013.[1] Methicillin-sensitive *S. aureus* (MSSA) can be carried and transmitted from person to person. Approximately 33% of people are *S. aureus* carriers [1] and up to 7% are colonized with MRSA.[2]**Methods:** The aim of this research is to explore potential predictors of nasal carriage rates for MSSA and MRSA including factors such as face touching frequency, hormonal contraceptive use, public transport use, hospital work, roommates, URT symptoms, antibiotic use, and other variables obtained by survey.This study comprised 111 female microbiology students. Subjects were directed to swab their own nares. Samples were tested for the presence of MSSA and MRSA by analysis of growth on Baird Parker agar. Positive isolates were examined by Gram's stain and assayed for coagulase and catalase production. Finally, putative isolates were grown on Oxacillin agar and CHROMagar™ before being classified as MSSA or MRSA.**Results:** Preliminary data indicated that 18 (16.2%) subjects were *S. aureus* positive and that 7 (6.3%) were MRSA positive in at least one nostril. Survey data showed that 29.7% of subjects have had hospital work experience within the past year, 73% of subjects used public transportation more than once a week, and 98.1% of subjects lived with at least one roommate. Nearly half of all subjects had recently used, or were using hormonal contraceptives at the time of the study. 18.2% of subjects were suffering from URT symptoms at the time of nasal swabbing and 9.3% were using an antibiotic. More than 95% of subjects frequently touched their faces.**Conclusion:** At this juncture no significant predictors for MSSA or MRSA nasal carriage in the student population have been identified.

Author Disclosure Block:

S. Donovan: None. **G. Chiumento:** None. **J. Richards:** None. **M. O'Seaghdha:** None. **S.M. Duty:** None. **E. Scott:** None.

Poster Board Number:

SATURDAY-221

Publishing Title:

Determination of Sensitivity Pattern of Microorganisms Isolated from Hospital Environment (One Center Study)

Author Block:

n. afshan; Jinnah Univ. for Women, karachi, Pakistan

Abstract Body:

Hospital-acquired infections are a major challenge to patient safety that is most commonly associated with invasive medical devices or surgical procedures. Multidrug resistant especially Methicillin resistant *S.aureus* (MRSA) as well as gram-ve bacteria become a growing problem in many parts of the developing world. So according to severity of hospital acquired infection. We here at microbiology department in Jinnah university for women, Karachi design a study project in which we evaluate the resistivity pattern of different microorganisms isolated from Intensive care Unit, Neonate Intensive care unit, general ward, labor room, operation theater and kitchen All samples were collected aseptically and platted right after the collection. Identification had done by standard microbiological methods, such as Gram staining, colony morphology, biochemical tests.Antibiograms were done on Mueller-Hinton agar plates with disk diffusion according to Kirby Bauer method .Disks were tested: (Oxoid Ltd., England) The results were interpreted according to the Guidelines of the Clinical and Laboratory Standards Institute.(CLSI).Result showed the presence of *Staph aureus* in 44%, S.epidermidis 7% *Neisseria* in 15%, *Klebsiella 6%, Pseudomonas 6%*, *E.coli* 7% *Aspergillus* in 6.% and *Bacillus* in 9%. All *staph* isolates were resistant to Methicillin and sensitive to Vancomycin , all *Neisseria* isolates were resistant to ceftriaxone and sensitive to ofloxacin, *Bacillus* were sensitive to Chloromphenicol and Doxycyclin where as resistant to Gentamycin and *Klebsiella* sensitive to Chloromphenicol and Doxycyclin and resistant to Penicillin, *E.coli* and *Pseudomonas* are resistant to penicillin,Gentamycin & cephalosporin groups. Presence of multidrug resistant organisms in Hospital environment is a challenge for infection control authorities.Key words:*Multidrugresistant,infection,CLSI*

Author Disclosure Block:

N. afshan: None.

Poster Board Number:

SATURDAY-222

Publishing Title:

Detection of ESBL, AmpC and MBL among the Clinical Bacterial Isolates

Author Block:

R. Anandan, D. Lakshmipriya, V. Nithyakalyani, C. Banurekha, S. Rajkumar; Dr MGR Janaki Coll. of Arts and Sci. for, Chennai, India

Abstract Body:

Background: Emergence multi drug resistant (MDR) bacterial clones among the clinical isolates complicates the clinical outcomes. This extent the patient hospital stay thereby adds considerable burden on the public health system and jeopardize the patient prognosis. This assumes greater importance in developing and underdeveloped countries where the clinical outcome is further marred by the socio-economic status of the patient and overcrowded hospitals. These factors ensures the persistence of pathogens and facilitate horizontal transfer of drug resistance trait. More surveillance data especially in third world countries is required to facilitate Governments and public health agencies to take informed policy decisions. In this background, we screened various clinical specimens from a semi-urban private hospital for multi-drug resistant bacterial isolates and further differentiated their resistant profile for various drugs.**Methods:** A total of 50 isolates were isolated from a semi-urban private hospital that included blood (n=30), sputum (n=10), endotracheal aspirate (n=6), pus and other fluids (n=4) using standard bacteriological isolation techniques. Conventional antibiotic disc diffusion technique was used for preliminary screening of these clinical isolates and isolates manifesting resistance to $\geq$3 antibiotics were segregated as MDR and proceeded for targeted analyses to delineate them into ESBL, AmpC and MBL clones.**Results:** Among the clinical Gram negative bacterial isolates 36% (18/50) were found to be MDR as they exhibited resistance to over 3 antibiotics tested by conventional disk diffusion technique. While 16/50 isolates were ESBL producers, 12/50 isolates were found to borne the AmpC phenotype. Interestingly, all isolates were found resistant to imipenem.**Conclusions:** Incidence of MDR bacterium in various clinical specimens is indeed a cause of concern. Stringent measures that includes, better hospital and personal hygiene practices, discriminate and prudent use of antibiotics guided by laboratory tests need to be enforced to address this growing public health burden.

Author Disclosure Block:

R. Anandan: None. **D. Lakshmipriya:** None. **V. Nithyakalyani:** None. **C. Banurekha:** None. **S. Rajkumar:** None.

Poster Board Number:

SATURDAY-223

Publishing Title:

Companion Animals Are Reservoirs of High-Risk Human Esbl and Ampc-Producing *Escherichia coli* Lineages

Author Block:

A. Belas, C. Marques, A. Franco, **C. F. Pomba**; Faculty of Vet. Med., Univ. of Lisbon, LISBON, Portugal

Abstract Body:

Background: The increase of ESBL and AmpC β-lactamases in companion animals is a great public health concern as they may act as reservoirs to humans. This study aimed to detect and characterize the *E. coli* lineages resistant to 3rd cephalosporin (3GC) causing urinary tract infection (UTI) in companion animals over 17 years in Portugal.**Methods:** Antimicrobial susceptibility of 324 uropathogenic *E. coli* isolated from dogs and cats (1999 to April 2015) was studied by the disk diffusion method. The phylogenetic groups were determined for all strains by PCR. 3GC-resistant *E. coli* strains were typed by MLST and screened for 8 pathogenicity islands markers and 8 virulence genes.**Results:** *E. coli* isolates were resistant to ampicillin 48% (n=154/324), 14.0% (n=45/324) to amoxicillin/clavulanate and 9.6% (n=31/324) to 3GC. *E. coli* B2 group was the most common (57.4%, n=186/324). 3CG-resistant *E. coli* group B2 and D strains had pathogenicity profiles and virulence-associated genes belonging mostly to $I_{536}II_{J96}IV_{536}II_{CFT0T3,}$ $I_{536}II_{J96}IV_{536}I_{CFT0T3}II_{536}$, *ecpA-hly*A-*cnf1-sfaDE-papEF*, and $II_{536}II_{J96}IV_{536}I_{CFT0T3,}$ *ecp*A-*pap*, respectively. In 2004, the first CTX-M-15-producer *E. coli* belonged to O25b:H4-ST131 human pandemic clone. About 33% (n=15/45) from group D *E. coli* were 3GC-resistant. Moreover, 29% (n=13/45) group D *E. coli* were pAmpC producers (harbouring bla_{CMY-2}) and 2.2% were pAmpC and ESBLs producers (harbouring bla_{CMY-2} and $bla_{CTX-M-9}$). From 2010 group D 3GC-resistant *E. coli* belonged mostly to ST648 (n=9), followed by ST1775 (n=3), ST405 (n=1) and ST354 (n=1) lineages. Among group B1 strains, 6.8% (n=3/44) were 3GC-resistant. Group B1 strains belonged to ST539 (n=1), ST533 (n=1), ST224 (n=1) and were found to harbour bla_{CMY-2}, $bla_{CTX-M1group}$ and $bla_{CTX-M-32}$ respectively. Group A 3GC-resistant *E. coli* (16.3%, n=8/49) harboured, $bla_{CTX-M-15}$ (n=1), $bla_{CTX-M-32}$ (n=1) and $bla_{CTX-Mgroup}$ (n=2) and belonged to ST23 (n=1), ST48 (n=1), ST88 (n=1) and ST609 (n=1).
Conclusions: The detection of *E. coli* lineages highly important for humans causing UTI in companion animals raises great public-health concerns regarding their role as reservoirs and the risk of transfer to humans.

Author Disclosure Block:

A. Belas: None. **C. Marques:** None. **A. Franco:** None. **C.F. Pomba:** None.

Poster Board Number:

SATURDAY-224

Publishing Title:

Molecular Epidemiology of Carbapenem Non-Susceptible (Carb-Ns) Clinical Isolates in Detroit

Author Block:

M. Veve[1], S. L. Davis[2], R. J. Tibbetts[1]; [1]Henry Ford Hosp., Detroit, MI, [2]Wayne State Univ., Detroit, MI

Abstract Body:

Background: The emergence and spread of carbapenem resistant organisms (CRO) is a significant burden to healthcare systems worldwide. Bacteria that possess enzymatic resistance mechanisms to carbapenems are associated with poor patient outcomes. We describe the molecular *bla*-characterization of CARB-NS isolates found in our institution.**Materials/Methods:** The molecular epidemiology and mechanisms of resistance for CARB-NS clinical blood, urine, respiratory tract and other isolates were determined at a single center institution in Detroit from 1/2013 to 12/2015. Whole genomic DNA from CARB-NS *Pseudomonas aeruginosa* (PA) and Enterobacteriaciae (EB) was purified before undergoing real-time polymerase chain reaction using SYBR Green (Bio-Rad). Primers and control organisms (CDC, Georgia) for the following *bla* sequences (NDM, KPC, VIM, SME, GES, IMP) were confirmed using melt-curve analysis relative to control organisms. Clinically relevant PA isolates were tested for susceptibility to ceftolozane/tazobactam (TOL/TAZ) and EB to ceftazidime/avibactam (CAZ/AVI).**Results:** 142 isolates screened positive for potential carbapenemase production; PA 67 (47%), EB 76 (54%). 34 (24%) were found to harbor bla_{KPC} (EB 32, 94%; PA 2, 6%). Isolate characteristics are shown in Table 1. TOL/TAZ and CAZ/AVI susceptibility was 67% and 100%, respectively.**Conclusions:** The mechanisms of CARB-NS in Detroit are not commonly related to carbapenemases. The only *bla*-sequence isolated was KPC. An understanding of the regional genomic sequence of CARB-NS is beneficial for clinicians to target appropriate antibiotic therapy and to improve infection control procedures.Table 1. Characteristics of clinical isolates

Bacterial strains, *n* = 142 (*n*, %)	Culture site (*n*)				Percentage bla_{KPC}, *n* = 142
	Urine	Respiratory	Blood	Other	
K. pneumoniae (29, 20%)	15	4	9	1	13%
E. coli (20, 14%)	12	1	4	3	5%
C. freundii (2, 1%)	2	0	0	0	1%

E. cloacae (16, 12%)	8	2	3	3	1%
P. aeruginosa (67, 47%)	1	64	1	1	1%
*M. morganii (*1, 1%)	1	0	0	0	1%
S. marcescens (4, 3%)	2	0	2	0	1%
P. mirabilis (3, 2%)	2	0	1	0	0%

*PCR for NDM, VIM, SME, GES and IMP were not confirmatory

Author Disclosure Block:

M. Veve: None. **S.L. Davis:** None. **R.J. Tibbetts:** None.

Poster Board Number:

SATURDAY-225

Publishing Title:

Characterization of Type I-F of CRISPR-Cas System in Strains of Serratia spp. from the Mosquito Gut

Author Block:

D. Pei, W. Yu, 88003, J. Xu, 88003; New Mexico State Univ., Las Cruces, NM

Abstract Body:

The mosquito Anopheles gambiae midgut ecosystem accommodates a diverse and dynamic microbiome. Within this microbiome, Serratia is an abundant genus. CRISPR-Cas system is an adaptive immune system in bacteria and archaea, which targets different types of invaders. In this study, two strains of Serratia spp., Ag1 and Ag2, were isolated from the mosquito midgut. Draft genome sequences were determined and annotated. There are two subtypes of type I CRISPR-Cas system, I-E and I-F, present in the genome. The I-F subtype consists of six cas genes and two CRISPR arrays, which includes 28 spacers and 15 spacers, respectively. I-E subtype consists of seven cas genes and one CRISPR array with 19 spacers. Possible origins of the spacer sequences of the CRISPR systems were examined by comparing spacers against the databases of phages and conjugative plasmids. Phage or conjugative plasmid derived spacers were identified. Based on phylogenetic analysis, I-F cas3 of Serratia was closer to the cas3 of Yersinia than to the cas3 of E. coli and Pseudomonas. Interestingly, there is an insertion in the I-F cas3 gene in Ag2 strain. The inserted DNA fragment was 1.5 kb with three coding regions. The inserted fragment is 96% identical to plasmid pYAB, carried by a strain of Yersinia pseudotuberculosis. The type I-F system of both Serratia strains was functional. It was able to degrade the plasmids that carried engineered protospacers. Apparently, in the strain Ag2, the insertion in cas3 did not affect its function. In Serratia spp., we found one self-targeting spacer that hit to a prophage tail protein coding sequence in its own genome. Since Serratia is predominant in the microbial community in the mosquito gut. It will be interesting to know if the CRISPR system plays a role in shaping and stabilizing of the mosquito microbiome.

Author Disclosure Block:

D. Pei: None. **W. Yu:** None. **J. Xu:** None.

Poster Board Number:

SATURDAY-226

Publishing Title:

The Ecology of Algal Polysaccharide Utilization: Novel *Verrucomicrobia* Isolates Efficiently Degrade Fucoidan

Author Block:

C. H. Corzett[1], J-H. Hehemann[2], E. J. Alm[1], M. F. Polz[1]; [1]Massachusetts Inst. of Technology, Cambridge, MA, [2]Max Planck Inst. for Marine Microbiol., Bremen, Germany

Abstract Body:

Marine macroalgae are vital players in the global carbon cycle, and polysaccharides represent a significant output of their primary production. Identifying the microbes and metabolic pathways responsible for degrading these sugars is not only crucial to understanding marine carbon flow, but also offers potential for biofuel production using seaweed feedstocks. Fucoidans are an important class of structurally heterogeneous sulfated polysaccharides found in brown seaweeds, yet few organisms have been shown to metabolize this abundant carbohydrate. Enrichment cultures from coastal samples yielded numerous isolates from diverse genera (*Vibrio, Stappia, Neptunomonas, Alteromonas, Tenacibaculum*) implicated in the breakdown of fucoidan polysaccharides, and novel *Verrucomicrobia* isolates performing an especially critical role in facilitating this process were sequenced. Draft genomes reveal Polysaccharide Utilization Loci (PULs) enriched with numerous and diverse Carbohydrate-Active Enzymes (CAZymes), with some isolates encoding as many as 60 genes with homology to established fucosidases. Differences in the dynamics and extent of fucoidan utilization among closely related isolates suggest variation in enzymatic capabilities and may reflect resource partitioning. Specific combinations of natural isolates appear to complement one another and yield greater overall biomass accumulation, suggesting engineered organisms or communities with a full repertoire of enzymatic machinery may facilitate the efficient conversion of algal biomass.

Author Disclosure Block:

C.H. Corzett: None. **J. Hehemann:** None. **E.J. Alm:** None. **M.F. Polz:** None.

Poster Board Number:

SATURDAY-227

Publishing Title:

Discovery of a Novel Prodigiosin Producing *Vibrio* from the Gulf of Mexico

Author Block:

S. F. Morgan[1], E. Warrick[2], B. J. Gasper[3], K. Walstrom[4]; [1]New Coll. of Florida, Bradenton, FL, [2]State Coll. of Florida, Bradenton, FL, [3]Florida Southern Coll., Bradenton, FL, [4]New Coll. of Florida, Sarasota, FL

Abstract Body:

Prodigiosins are a group of often brightly colored pigment molecules produced by a variety of bacterial organism as secondary metabolites. Most notably, prodigiosin is known as the pigment responsible for both the bright red appearance and antibiotic activity of *Serratia marcescens.* The biomolecules consist of a pyrrolylpyrromethene core with varying alkyl substituent groups. Prodigiosin compounds have been studied for their antibacterial, anti-malarial and anti-cancer properties. The compounds also have potential applications in industry, as their brightly colored appearance makes them ideal candidates for natural dyes.Environmental samples taken from a salt flat in Bradenton, FL led to the discovery of a novel halophillic bacteria capable of producing a bright pink compound. Full genomic sequencing of the isolate and analysis of the genomic sequence utilizing the genome browser Artemis, NCBI's BLAST and Clustal Omega was performed. Additionally, the pigment compound was isolated using a multistep purification procedure and analyzed with UV-Visible spectroscopy, IR spectroscopy, nuclear magnetic resonance spectroscopy and liquid chromatography mass spectrometry.The bacterial isolate was identified as a member of the *Vibrio* genus most closely related to *Vibrio rhizosphaerae* and *Vibrio gazogenes.* A crude extract of cultured bacterial cells contained a broad spectrum antibiotic capable of inhibiting growth of ESKAPE safe relatives: *Staphylococcus epidermidis, Bacillus subtilis, Enterobacter aerogenes, Citrobacter* f*reundii* and *Chromohalobacter salexigens.* A prodigiosin biosynthesis gene cluster was found within the genome similar in sequence and structure to the prodigiosin gene cluster in *S. marcescens.* Spectroscopy of the compound(s) shows similar chemical properties to the prodiginine family.

Author Disclosure Block:

S.F. Morgan: None. **E. Warrick:** None. **B.J. Gasper:** None. **K. Walstrom:** None.

Poster Board Number:

SATURDAY-228

Publishing Title:

Complete Genome Analysis of *Vibrio parahaemolyticus* FORC_023, Isolated from Storage Water for Raw Fish

Author Block:

H. Chung[1], S. Kim[1], B. Lee[1], E. Na[1], J-H. Lee[2], S. Choi[1]; [1]Seoul Natl. Univ., Seoul, Korea, Republic of, [2]Kyung Hee Univ., Yongin, Korea, Republic of

Abstract Body:

Vibrio parahaemolyticus is a Gram negative halophilic bacterium and consumption of seafood contaminated with this bacterium can cause gastroenteritis. *V. parahaemolyticus* FORC_023 strain was isolated from the storage water for raw fish, and its whole genome sequence was determined using two different platforms of PacBio RS II platform and Illumina MiSeq platform. The genome FORC_023 is composed of two circular chromosomes without plasmid. Chromosomes consist of 4,227 predicted open reading frames (ORFs), 131 tRNA genes, and 37 rRNA genes. The FORC_023 strain does not include major virulence factors such as thermo-stable direct hemolysin (TDH) and TDH-related hemolysin (TRH). However, the genome of FORC_023 contains various genes related to hemolysin, secretion, and iron uptake. Among various *V. parahaemolyticus*, FORC_023 shows the highest average nucleotide identity (ANI) value with UCM-V493 which is one of the most virulent strains, indicating that FORC_023 is virulent as well. Furthermore, comparative genome analysis of the two strains revealed that FORC_023 carries an additional genomic region encoding strong virulence factors such as repeats-in-toxin (RTX) and type II secretion, which could lead to host cell death. In addition, *in vitro* cytotoxicity testing suggests that FORC_023 exhibits a higher level of cytotoxicity toward the INT-407 human epithelial cells as determined by lactose dehydrogenase (LDH) release. All these results suggest that the FORC_023 isolated from storage water for raw fish is one of the most virulent strains of *V. parahaemolyticus*, providing new insights into the necessity for rapid detection as well as epidemiological investigation to prevent food-borne outbreaks by *V. parahaemolyticus.*

Author Disclosure Block:

H. Chung: None. **S. Kim:** None. **B. Lee:** None. **E. Na:** None. **J. Lee:** None. **S. Choi:** None.

Poster Board Number:

SATURDAY-229

Publishing Title:

Complete Genome Analysis of *Vibrio parahaemolyticus* FORC_018, Isolated from Sea Bass

Author Block:

E. Na[1], S. Kim[1], H. Chung[1], B. Lee[1], J-H. Lee[2], S. Choi[1]; [1]Seoul Natl. Univ., Seoul, Korea, Republic of, [2]Kyung Hee Univ., Yongin, Korea, Republic of

Abstract Body:

Vibrio parahaemolyticus is a Gram negative halophilic pathogen that causes gastroenteritis following consumption of contaminated seafood. Ten strains of the species were obtained from Korean Ministry of Food and Drug Safety to help characterize *V. parahaemolyticus* isolated from foodborne outbreaks in South Korea. To select the most virulent strain for genome sequencing, gDNA samples were prepared from the ten strains and subjected to virulence gene-specific PCR. The results indicated that they all have the genes encoding a trans-membrane protein involved in regulating virulence genes (ToxR), a component of putative type III secretion system (VopS), and a collagenase (ColA). Furthermore, the cytotoxicity of *V. parahaemolyticus* strains was evaluated by measuring the activity of cytoplasmic lactate dehydrogenase (LDH) that was released from the INT-407 human epithelial cells. Because the cytotoxicity of *V. parahaemolyticus* #331 was either comparable to or higher than that of *V. parahaemolyticus* KCTC 2471 (positive control), the #331 isolate, which is from sea bass, was selected for genome sequencing and designated as FORC_018. The FORC_018 genome consists of two circular chromosomes of 3,273,522 bp (chromosome I) and 1,772,038 bp (chromosome II), with a G+C content of 45.37 and 45.53, respectively. The entire genome contains 4,498 open reading frames, 37 rRNAs, and 132 tRNA genes. Virulence factors of the *V. parahaemolyticus* FORC_018 were identified using BLAST against Virulence Factor Database (VFDB), demonstrating that *V. parahaemolyticus* FORC_018 has genes encoding thermolabile hemolysin (TLH) and type III secretion system, and lacks genes encoding thermo-stable direct hemolysin (TDH) and TDH-related hemolysin (TRH). This report provides a deeper understanding of *V. parahaemolyticus* and will help future studies on rapid detection, epidemiological investigation, and prevention of foodborne outbreaks of the pathogen.

Author Disclosure Block:

E. Na: None. **S. Kim:** None. **H. Chung:** None. **B. Lee:** None. **J. Lee:** None. **S. Choi:** None.

Poster Board Number:

SATURDAY-230

Publishing Title:

The Complete Genome Analysis of *Confluentimicrobium naphthalenivorans* Ns6 and Its Naphthalene Degradation Property

Author Block:

H. Jeong, K. Kim, C. Jeon; Chung-Ang Univ., Seoul, Korea, Republic of

Abstract Body:

Confluentimicrobium naphthalenivorans NS6, responsible for naphthalene degradation, was isolated from a tidal flat and naphthalene degradation tests using ^{13}C-naphthalene showed that strain NS6 completely mineralized naphthalene via the gentisate pathway that *Ralstonia* U2 and *Polaromonas naphthalenivorans* CJ2 utilize. In this study, the whole genome of strain NS6 was sequenced for better understanding of its biodegradation properties of polycyclic aromatic hydrocarbons. The complete genome sequencing of strain NS6 is characterized by a circular chromosomal genome of 3.65-Mb with a 64.5% G+C content and three plasmids with 184.7 kb, 157.3 kb, and 156.1 kb sizes. The informatics genome analysis revealed that the genome harbors genes encoding diverse oxygenases, indicating a broad range of degradation ability toward polycyclic aromatic hydrocarbons of strain NS6. However, we could not find out a naphthalene dioxygenase responsible for naphthalene degradation from the genome of strain NS6 by the bioinformatics genome analysis. Therefore, we constructed a phylogenetic tree using Rieske domain sequences of all hydroxylase alpha subunits found from the genome and eventually chose four putative naphthalene dioxygenase genes. To investigate the substrate ranges of the four putative naphthalene dioxygenase genes, they were knock-outed using a double cross-over recombination approach, respectively and the biodegradation abilities of the four knock-out mutant strains were tested against various aromatic hydrocarbons. Eventually, we found complete naphthalene-degrading genes from the genome and we will discuss these in more details in the poster section.

Author Disclosure Block:

H. Jeong: None. **K. Kim:** None. **C. Jeon:** None.

Poster Board Number:

SATURDAY-231

Publishing Title:

Genomic Comparisons And Pathogenic Potential Of *campylobacter* Isolates

Author Block:

A. M. Weis, D. B. Storey, C. C. Taff, A. K. Townsend, B. A. Byrne, S. A. Woutrina, B. C. Weimer; Univ. of California, Davis, Davis, CA

Abstract Body:

Campylobacter jejuni is the leading cause of gastroenteritis in humans worldwide with recent studies indicating wild birds as zoonotic vectors. American crows, abundant in urban, suburban, and agricultural settings, carry *C. jejuni* as a commensal organism. Studies demonstrating that the crow is a *Campylobacter* zoonotic reservoir are lacking, and no studies have investigated these *Campylobacter* genomes that are distributed among the crow population. To examine pathogenic potential, zoonotic exchange and genomic phylogeny, we sequenced 160 *Campylobacter* genomes from crow, chicken, cow, sheep, goat, and non-human primate origin from the Sacramento Valley, CA. Many genomes displayed high similarity to sequences of isolates implicated in human disease, suggesting these isolates as potential pathogens of public health importance. Tetracycline resistance gene *tetO* was present in 21.9% of the isolates, and 22 isolates (8 *C. jejuni*, 11 *C. coli*, 3 *C. lari*) contained mutations in *gyrA* indicative of Fluoroquinolone resistance. Cytolethal Distending Toxin genes were present in 95% of isolates, 100% contained invasion and adherence genes, and 20% of genomes contained Type IV SS genes. Although resistance and virulence genes were broadly distributed throughout these isolates, specific genotypes were associated with individual host species and a sub-set of isolates were associated with several hosts (i.e. crows, primates, sheep, humans), suggesting that these genotypes may be from zoonotic exchange. This study, utilizing whole genome sequencing (WGS), associated specific *Campylobacter* genotypes that have high potential zoonotic transmission opposed to host adapted genotypes in the samegeographic area to show antibiotic resistance, virulence potential, and zoonotic potential.

Author Disclosure Block:

A.M. Weis: None. **D.B. Storey:** None. **C.C. Taff:** None. **A.K. Townsend:** None. **B.A. Byrne:** None. **S.A. Woutrina:** None. **B.C. Weimer:** None.

Poster Board Number:

SATURDAY-232

Publishing Title:

Partial Characterization of the Novel Plasmid Pec6.2 Isolated from *Enterococcus casseliflavus* in Puerto Rico

Author Block:

C. Muriel Mundo, N. Rodriguez-Bonano; Univ. Del Este, Carolina, PR

Abstract Body:

Plasmids are extrachromosomal DNA elements associated with the acquisition of new bacterial metabolic and virulent traits. It is well known that these elements contribute significantly to gene flux, evolution and adaptation of bacteria in the environment. Recently, we recovered an Enterococcus casseliflavus isolate harboring a 6.2 kb plasmid (pEC6.2) from a recreational water sample in Puerto Rico. The isolate exhibited susceptibility to 18 antibiotics tested including tetracycline, vancomycin and penicillin. It is unknown if pEC6.2 encodes for antibiotic resistance to other antibiotics besides the ones tested in the laboratory. However, it is also unknown whether pEC6.2 is related to any metabolic traits in E. casseliflavus. The aim of this project was to characterized pEC6.2 and determine which traits are encoded by the plasmid. Prolonged subculturing without selective pressure, DNA extraction and agarose gel electrophoresis demonstrated that pEC6.2 is a stable and high copy number plasmid. Based on sequence analysis, we have determined that there is no similarity between pEC6.2 and other known and characterized E. casseliflavus plasmids, like pEC30. We have not identified antibiotic resistant traits in pEC6.2. Yet, we have identified the replication region (rep) and it exhibits similarity with rep regions from other enterococcal plasmids. Well characterized plasmids from other Enterococcus spp. do not share the same size, stability and copy number with pEC6.2. For these reasons, we believe that pEC6.2 is a new enterococcal plasmid. More recently, we have perform next generation sequencing for pEC6.2 and de novo assembly of pEC6.2 is currently in progress.

Author Disclosure Block:

C. Muriel Mundo: G. Member; Self; Chris Muriel. **N. Rodriguez-Bonano:** B. Collaborator; Self; nydia Rodriguez.

Poster Board Number:

SATURDAY-233

Publishing Title:

Community Stability and Dynamics, Novel Organisms, and Draft Genomes from an Anode-Respiring Microbial Consortium

Author Block:

T. J. Arbour[1], B. Gilbert[2], J. F. Banfield[1]; [1]Univ. of California - Berkeley, Berkeley, CA, [2]Lawrence Berkeley Natl. Lab., Berkeley, CA

Abstract Body:

Understanding soil microbial communities is a grand challenge due to the vast number of co-occurring species and strains. To obtain a system of reduced complexity and focus on organisms capable of extracellular respiration using insoluble electron acceptors (as well as their syntrophic partners), we used a bioelectrochemical system to enrich for a subset of the total soil community present in subsurface aquifer at the DOE field site at Rifle, CO. Our main aims were to discover potential novel iron(III)-oxide reducers and to examine the stability and dynamics of the microbial community over a range of timescales. In our bioelectrochemical system, a graphite anode poised at a voltage to mimic the redox potential of iron(III) oxide minerals serves as the only added electron acceptor, and acetate served as electron donor. We sampled anode-biofilm and planktonic communities over a period of almost three years under varying conditions of acetate abundance or limitation. We used next-generation metagenomic sequencing and genome assembly to profile the microbial community present in each sample. We recovered ~130 near-complete and partial genomes across more than 10 phyla/classes, including that of a novel Deltaproteobacteria that was the most abundant organism in anode biofilm samples. Outside of the Deltaproteobacteria, several other organisms not known to perform extracellular electron transfer have an abundance of multiheme *c*-type cytochromes that suggests this ability. The stability of the community through time, species co-occurrence patterns, and new metabolic insights will be presented.

Author Disclosure Block:

T.J. Arbour: None. **B. Gilbert:** None. **J.F. Banfield:** None.

Poster Board Number:

SATURDAY-234

Publishing Title:

Physiology Of *Pseudomonads* isolated From Groundwater That Proliferate More Effectively In Co-Culture Than In Monoculture Under Denitrifying Conditions

Author Block:

A. B. Aaring[1], A. Pettenato[1], A. M. Deutschbauer[1], M. W. W. Adams[2], **R. Chakraborty**[1];
[1]Lawrence Berkeley Natl. Lab., Berkeley, CA, [2]Univ. of Georgia, Athens, GA

Abstract Body:

As part of the Ecosystems and Networks Integrated with Genes and Molecular Assemblies (ENIGMA) consortium, we study the microbial community at the U.S. Department of Energy's Field Research Center (FRC) in Oak Ridge. The groundwater at this site contains plumes of nitrate with concentrations up to 14,000mg/L among other contaminants. Several strains of *Pseudomonas* were isolated from the same background groundwater sample. These isolates utilized diverse carbon sources ranging from acetate to glucose while growing under denitrifying conditions. Analysis of their genomes revealed that each strain contains circa 1000 genes distinct from each other. Full genome sequencing supported by metabolite analysis showed that some strains lacked any genes for nitrite reduction, leading to buildup of nitrite. The strains were also screened for nitrate tolerance and a couple of them were shown to be tolerant to 300-375 mM nitrate under anaerobic conditions. In the field site the bacteria live in consortia rather than in isolation, therefore we hypothesized that growth of these strains will be more robust in co-culture, as the denitrification pathway was segmented between the species. Three of the isolates (*Pseudomonas fluorescens* strains N1B4, N2E2, N2E3) were selected for in-depth analysis based on growth in pairwise co-cultures relative to monocultures, and the availability of the relevant genetic tools, such as transposon mutant libraries. Our results show strain N1B4 (with truncated denitrifying pathway) grows more quickly and to greater density in co-culture than in monoculture. Utilizing RB-TnSeq libraries of our strains, it was also found that some genes involved in nitrate reduction, sulfate permeability, molybdenum utilization, and anaerobic reduction are important for growth under these conditions. In addition, a few uncharacterized genes were also shown to be positively correlated to growth. These data help to broaden our understanding of how bacteria interact and grow in conditions that are relevant to the environment and enable us to probe the ways in which bacterial communities interact and overcome genetic deficiencies of individual strains to deal with environmental conditions.

Author Disclosure Block:

A.B. Aaring: None. **A. Pettenato:** None. **A.M. Deutschbauer:** None. **M.W.W. Adams:** None. **R. Chakraborty:** None.

Poster Board Number:

SATURDAY-235

Publishing Title:

Mechanisms of the Antagonistic Interaction between *Phaeobacter inhibens* and Fish Pathogenic Vibrios

Author Block:

B. Rasmussen, C. Phippen, K. Nielsen, L. Gram, M. Bentzon-Tilia; Technical Univ. of Denmark, Kgs. Lyngby, Denmark

Abstract Body:

Bacteria of the prevalent marine *Roseobacter* clade, e.g. *Phaeobacter inhibens*, are capable of antagonizing fish pathogenic bacteria such as *Vibrio anguillarum* and *V. harveyi*. Adding *P. inhibens* as a probiotic in fish larvae and live feed have congruently been shown to inhibit the proliferation of fish pathogenic vibrios and to increase survival of the larvae. One antibiotic compound, tropodithietic acid (TDA), is important in this interaction. It is, however, not exclusively responsible for the observed antagonism as TDA deficient mutants only exhibit reduced inhibitory activity. Hence, the mechanisms behind the antagonistic interaction between *P. inhibens* and vibrios are not fully understood and the purpose of the present study was to investigate if other bioactive compounds could potentially be involved. By combining genome mining, bioassays and UHPLC-DAD-QTOFMS analysis we examined closely related TDA producing strains of which some do, and some do not show TDA-independent inhibition of vibrios. We identified several gene clusters encoding potential bioactive compounds, which could be attributed to TDA-independent inhibition, including a siderophore cluster, a linaridin-like cluster, and a putative nonribosomal peptide synthetase cluster. Extracts of wild type strains and TDA-negative mutants were made to isolate potential bioactive compounds. The strains were pre-cultured under different conditions prior to testing in inhibition assays. Inhibition zones only emerged when strains were grown on plates with vibrio embedded before extraction and only if they were pre-cultured in nutrient rich medium. We were able to extract one or more bioactive compounds directly from *P. inhibens* biomass from vibrio-embedded plates, but not from liquid culture, or the plates themselves. Hence, it is likely that multiple secondary metabolites are involved in the antagonism of vibrios by *P. inhibens*.

Author Disclosure Block:

B. Rasmussen: None. **C. Phippen:** None. **K. Nielsen:** None. **L. Gram:** None. **M. Bentzon-Tilia:** None.

Poster Board Number:

SATURDAY-236

Publishing Title:

Physiological and Molecular Characterizations of the Microbial Interactions in Cocultures for Methane Production from Cellulose

Author Block:

H. Lu, P. Lee; City Univ. of Hong Kong, Hong Kong, Hong Kong

Abstract Body:

Biomethanation can be harnessed for both effective waste treatment and sustainable energy production. This process in nature is driven by a complex microbial community mainly consisting of three metabolic groups of anaerobes: fermentative, acetogenic and methanogenic microorganisms. The interspecies interactions in the biomethanation community play a vital role in biomass degradation and methane formation. However, the physiological and molecular mechanisms of how these microbes interact remain poorly understood due to the lack of an experimentally tractable model system. In this study, we successfully established two experimentally and genetically tractable models combining the cellulose-degrading bacterium *Clostridium cellulovorans* 743B (*CC*) with *Methanosarcina barkeri* str. Fusaro (*MB*) and *Methanosarcina mazei* Gö1 (*MM*) respectively for the direct conversion of cellulose to methane. Physiological characterizations of these models revealed that the methanogens were able to efficiently utilize hydrogen, formate and acetate produced from *CC* during cellulose degradation, for methanogensis through the CO_2 reduction pathway and aceticlastic pathway. In return, the consumption of hydrogen, formate and acetate helped to maintain a thermodynamically favorable condition and a neutral pH for *CC* in the coculture systems, resulting in enhanced cellulose degradation. Interestingly, although formate consumption was observed in cocultures of *MB* and *CC*, *MB* monocultures were not able to utilize formate for growth. Furthermore, next-generation sequencing was used to query the global transcriptomic responses of these models to obtain a more comprehensive understanding of the interspecies interactions. Overall, the combination of physiological and molecular characterizations of these two models deepens our understanding on interspecies interactions for methane production from cellulose, providing useful insights for better industrial biomethanation process design.

Author Disclosure Block:

H. Lu: None. **P. Lee:** None.

Poster Board Number:

SATURDAY-237

Publishing Title:

Metatranscriptomic and Metaproteomic Insights into Long-Term Cellulose and Xylan Enrichments from Anaerobic Digesters

Author Block:

Y. Jia, P. K. H. Lee; City Univ. of Hong Kong, Hong Kong, Hong Kong

Abstract Body:

A number of amplicon 16S rRNA gene and metagenomic studies have previously investigated cellulosic biomass anaerobic digestion microbial communities. However, genome-based study alone cannot provide information related to the activity of the microbial populations. In contrast, transcriptomics sequencing and mass-spectrometry based proteomics can provide comprehensive information on the active metabolic pathways and enzymes within a microbial community.In this study, based on our previous 16S rRNA gene sequencing and metagenomic results, metatranscriptomics and metaproteomics were applied to five enrichment cultures at the mid-exponential phase of growth in batch systems. The enrichments originated from two different anaerobic digesters and have been fed cellulose or xylan as the sole carbon source at 35 or 55 ^{o}C for over 3 years. A 15-days time course experiment was also carried out to reveal the dynamics of the microbial activities in two mesophilic cellulose cultures. Results of one of the time course mesophilic cellulose cultures showed that, transcripts assigned to the order *Clostridiales* had a relative abundance of 74% by day 5 and then gradually decreased to ~45%, indicating *Clostridiales* were one of the key functional populations. Reads belonging to *Methanobacteriales* did not make up a significant portion (~2.7% by day 15) of the entire transcriptome. Functional analysis of the metatranscriptomes indicated that protein metabolism, motility and chemotaxis, and carbohydrates were the three most highly transcribed subsystems. From the metaproteomics analysis, more than 900 unique proteins were identified against a customized database constructed using reads from both the metagenomes and metatranscriptomes. Besides basic central metabolism, proteins involved in carbohydrate and membrane transport were highly expressed, which is consistent with the cellulose-digestion functions in the enrichments. Overall, multi-omics tools have shed new insights into the cellulose- and xylan-digestion enrichments, and such detailed understanding could aid the development of cellulosic biofuels.

Author Disclosure Block:

Y. Jia: None. **P.K.H. Lee:** None.

Poster Board Number:

SATURDAY-238

Publishing Title:

Revealing the Complexity of Electrode Respiration Using Tnseq in *Geobacter sulfurreducens*

Author Block:

C. Chan, C. Levar, F. Jimenez, D. Bond; Univ. of Minnesota Twin Cities, St. Paul, MN

Abstract Body:

G. sulfurreducens grows with poised graphite electrodes as terminal electron acceptors by transmitting electrons through a system of membrane and surface proteins. Thus far, the genes important for sensing and responding to this environment have been identified by first screening mutants defective in Fe(III) reduction, or from predictions based on expression analyses. To directly identify mutants impaired in electrode respiration, we constructed a *mini*Himar RB1 transposon library in *G. sulfurreducens* using a transposon modified to contain Type IIS MmeI recognition site, enabling Illumina adapter ligation for mass sequencing to identify genomic insertion sites. About 45,000 insertional mutants were pooled and grown on soluble acceptors or poised electrodes. By comparing the frequency of insertions in each library after the same number of doublings, transposon insertions in 126 genes were identified that resulted in at least a calculated growth rate decrease of 25%. We categorize these broadly in five groups- cytochromes (5 genes), protein secretion and lipoprotein maturation (14), regulation and sensing (41), exopolysaccharides and attachment (38) and hypotheticals (28). Unexpectedly, after reconstruction of mutants identified from this analysis, many of these mutants are defective in electrode reduction, but retain wild type Fe(III) reduction activity. These include a cytochrome conduit cluster, and genes encoding cell signaling pathways. This suggests that *G. sulfurreducens* possesses dedicated pathways for sensing and colonizing surfaces that poised electrode mimic, and that electrodes do not always simulate Fe(III) as an electron acceptor. Additionally, genes encoding extracellular cytochromes were not detected in our analysis, suggesting these cytochromes could be shared when growing on electrodes. Analysis of the parent *G. sulfurreducens* TnSeq library also revealed essential genes for growth on the soluble acceptor fumarate, many of of which were not predicted in previous modeling studies due to insufficient data or having multiple homologs on the chromosome. Future work will focus on these new molecular mechanisms and regulatory networks specific for electrode respiration.

Author Disclosure Block:

C. Chan: None. **C. Levar:** None. **F. Jimenez:** None. **D. Bond:** None.

Poster Board Number:

SATURDAY-239

Publishing Title:

A Comprehensive Comparative Transcriptomic Analysis of *Penicillium chrysogenum* Treated with Two Enantiomers of 1-Octen-3-ol

Author Block:

G. Yin[1], Y. Zhang[2], K. Pennerman[1], G. Wu[3], J. Yu[4], W. Jurick II[4], S. Lee[1], L. Bu[5], Q. Huang[2], J. Bennett[1], A. Guo[2]; [1]Rutgers, The State Univ. of New Jersey, New Brunswick, NJ, [2]Chinese Academy of Tropical Agricultural Sci., Haikou, China, [3]US Dept. of Energy, Oak Ridge, TN, [4]Dept. of Agriculture, Beltsville, MD, [5]Dept. of Biology, Albuquerque, NM

Abstract Body:

Fungal volatile organic compounds have different roles in many biological processes. Well-known for its "mushroom" odor, the aliphatic alcohol 1-octen-3-ol is gaining attention because of its irritant and Parkinsonism neurodegenerative effects. The R and S enantiomers of 1-octen-3-ol have distinguishable influences on the health and behavior of diverse organisms. However, the molecular basis for these effects is poorly understood. Therefore, a comprehensive comparative transcriptomic analysis of *Penicillium chrysogenum* was performed via treatment with the two enantiomers. Altogether, about 80% of clean reads could be mapped to the reference genome and the transcriptome data were annotated to 11,396 genes. The top three active pathways were metabolic (978 transcripts), biosynthesis of secondary metabolites (420 transcripts), and microbial metabolism in diverse environments (318 transcripts). With respect to the control, treatment with R affected the transcription levels of 91 transcripts, while S affected only 41 transcripts. Additionally, 136 transcripts were up-regulated and 3 were down-regulated in fungi treated with R compared to S enantiomer (FDR < 0.05). Go functional enrichment analysis demonstrated that most differential expressed genes (DEGs) were annotated to be involved in biological process. Comparing S to R treatment, it was determined that transport, establishment of localization, transmembrane transport were significantly altered (*p*-value < 0.001). Alternative splicing and SNPs analyses indicated that R could impose greater effects on *Penicillium* than S. The qRT-PCR analyses of DEGs such as ABC transporter, MFS transporter, PPos *etc*., confirm the transcriptome data. This study represents the first step in elucidating the global molecular mechanisms by which 1-octen-3-ol enantiomers differentially affect fungal growth and development. Data from this pioneering investigation provide foundational information to target specific genes for functional analysis to better understand fundamental processes of volatile signaling in fungi.

Author Disclosure Block:

G. Yin: None. **Y. Zhang:** None. **K. Pennerman:** None. **G. Wu:** None. **J. Yu:** None. **W. Jurick II:** None. **S. Lee:** None. **L. Bu:** None. **Q. Huang:** None. **J. Bennett:** None. **A. Guo:** None.

Poster Board Number:

SATURDAY-240

Publishing Title:

Unraveling Lanthanide-Dependent Methylotrophy

Author Block:

N. M. Good, C. Suriano, **N. Martinez-Gomez**; Michigan State Univ., East Lansing, MI

Abstract Body:

The recent finding that rare earth elements like lanthanides are essential cofactors for many methylotrophic bacteria provides opportunities to investigate discoveries surrounding biological acquisition and biochemistry with potential changes in carbon distribution during methylotrophic growth. The role of methylotrophs in global carbon cycling and global warming is pivotal, as methane and methanol are the two most abundant organic compounds in the atmosphere and methane is a potent green-house gas.[1] Metabolism of both methane and methanol require the oxidation of methanol, which can be catalyzed by methanol dehydrogenases (MeDHs). Recently, it was shown that some types of MeDHs, specifically XoxF-MeDHs, are induced by and use lanthanides as cofactors.[2,3] Our preliminary Illumina-based transcriptome data (RNA-seq) from *M. extorquens* grown in methanol medium in the presence and absence of La^{3+} has allowed us to predict additional components involved in La^{3+}-dependent methylotrophic growth. Of special interest among the La^{3+}-upregulated genes are those encoding a quinoprotein ethanol dehydrogenase (ExaF). Purified ExaF was found to contain PQQ and La^{3+}, and have relatively poor methanol dehydrogenase activity but high catalytic efficiency with formaldehyde. Both phenotypic and biochemical analyses have demonstrated that ExaF directly produces formate from methanol. Such oxidation has important consequences for metabolism including the benefit of decreasing toxic formaldehyde production, but also the disadvantage of bypassing a formate production pathway which produces NAD(P)H for carbon assimilation. Also of interest, upregulation of genes involved in oxalate and formate metabolism such as formate dehydrogenase (Fdh4), formyl-CoA transferase (Frc12), and oxalyl-CoA reductase (Oxr) was found in our RNAseq studies. It has been suggested that Fdh4 is the most crucial Fdh in methanol metabolism although it is the one we know the least about;[4] it may be that Fdh4 activity can only be measured in cells grown with lanthanides. Our preliminary HPLC analyses corroborate increased accumulation of formate and oxalate in extracts from cells grown on methanol with La^{3+}. Our results confirm that the lanthanide-dependent metabolic network includes novel methanol oxidation systems and changes in carbon assimilation suggesting that the methylotrophic bacterial community may be underestimated.

Author Disclosure Block:

N.M. Good: None. **C. Suriano:** None. **N. Martinez-Gomez:** None.

Poster Board Number:

SATURDAY-241

Publishing Title:

***Vibrio fischeri* as a Biosensor of Emerging Contaminants**

Author Block:

N. Rosario-Meléndez, M. Tolentino, D. M. García, J. Pérez, Z. Y. Mercado, L. Díaz, Z. Flores-Cruz; Univ. of Puerto Rico-Rio Piedras, San Juan, PR

Abstract Body:

Fertilizers, pesticides, pharmaceuticals, and personal care products are used in everyday life by millions of people. However, the disposal of these substances is not regulated; therefore they are not being removed in treatment facilities, which cause them to end up in the ocean. The effect these chemicals might have on living organisms, or their combination is not known and there are no efficient ways to detect them. As an alternative to these problems we used the marine bioluminescent bacterium *Vibrio fischeri* as a biosensor. *V. fischeri* is a well studied bacteria known for its ability to emit bioluminescence. For this purpose we grew the bacteria in the presence of various emerging contaminants. The growth of *V. fischeri* was not affected by the tested contaminants, with the exception of known antibiotics. In contrast, bioluminescence changed significantly in presence of caffeine (dietary supplement) and amlodipine besylate (prescription medication). These results suggest that *V. fischeri* could be used for the detection of emerging contaminants. Future work includes testing other emerging contaminants, and a metabolomic analysis to observe how *V. fischeri* is metabolizing the contaminants.

Author Disclosure Block:

N. Rosario-Meléndez: None. **M. Tolentino:** None. **D.M. García:** None. **J. Pérez:** None. **Z.Y. Mercado:** None. **L. Díaz:** None. **Z. Flores-Cruz:** None.

Poster Board Number:

SATURDAY-242

Publishing Title:

Effects of Water Temperature and Toc Leached from Pex Pipes on the Occurrence of Bacterial in Household Water Distribution Systems

Author Block:

M. Wang, M. E. Salehi, A. J. Whelton, Z. Zhou; Purdue Univ., West Lafayette, IN

Abstract Body:

Background: Although tap water is treated in drinking water treatment plants before it reaches our faucets, bacteria may still grow in the household water distribution systems and pose potential health risks. However, studies on the factors that may affect the occurrence of bacteria in drinking water pipes are still limited. The objective of this study was to examine the effects of water temperature and total organic carbon (TOC) leached from PEX pipes on the occurrence of bacteria in domestic water distribution systems. **Methods**: Tap water samples from eight locations in a three-story domestic residential house in Indiana were collected in this study. Culture-based heterotrophic plate count (HPC) method was used to measure the numbers of heterotrophic bacteria in tap water, and culture-independent real-time PCR assays were used to quantify gene copy numbers of bacteria in tap water. Samples were collected at day 0, 3, 15, 30, 60 and 90 after a 30-minute flushing before sample collection. **Results**: During the 90 days, the bacterial gene copy numbers increased from 2.82×10^{3}-1.5×10^{6} to 2.41×10^{4} -1.67×10^{7} per liter. The results showed that the occurrence of bacteria in hot water pipes in the drinking water distribution systems was 55.49 times higher than those detected in cold water pipes. A positive correlation between TOC levels and bacterial gene copy numbers was detected ($R^2 = 0.61$, $p = 0.065$) in the hot water pipe in the basement. **Conclusion**: The results suggested that the temperature and TOC leached from PEX pipes may promote bacterial growth in drinking water distribution systems.

Author Disclosure Block:

M. Wang: None. **M.E. Salehi:** None. **A.J. Whelton:** None. **Z. Zhou:** None.

Poster Board Number:

SATURDAY-243

Publishing Title:

Response of Bacteria Isolated from Segment of River Asa to Linear Alkylbenzene Sulfonate (LAS)

Author Block:

K. I. T. Eniola[1], T. M. Kayode-Isola[2]; [1]Joseph Ayo Babalola Univ., Ikeji-Arakeji, Ilesa, Osun State, Nigeria, [2]Adeniran Ogunsanya Coll. of Ed., Otto-Ijanikin, Lagos, Nigeria

Abstract Body:

Responses of bacterial species isolated from segments of river Asa in Ilorin Nigeria to linear alkylbenzene sulfonate (LAS) were assessed in terms of ability or failure of the organisms to grow in the presence of varying concentrations of LAS. A total of twenty two (22) bacterial strains belonging to eight (8) species: *Alcaligenes odorans*(3 strains), *Bacillus subtilis* (3strains), *Citrobacter diversus*(3 strains) ,*Escherichia coli* (3 strains), *Klebsiella* spp (3 strains), *Micrococcus luteus* (2 strains), *Pseudomonas* spp (3 strains), and *Staphylococcus* spp (2 strains) were isolated. The isolates varied in their responses to different concentrations of LAS and were categorized as LAS-sensitive bacteria (LSB), LAS-tolerant bacteria (LTB) and LAS-utilizing bacteria (LUB). Fifteen (15) of the strains were tolerant of LAS (0.1% w/v) (LTB) and Seven (7) isolates were sensitive to LAS (0.1% w/v) (LSB). Seven (7) of the LAS tolerant bacteria showed potential to degrade LAS. Strains of the same species isolated from sediment (biofilm) were more tolerant of LAS than their planktonic counterparts, this difference could be genetic, and hence a detailed comparative study of the genome of the organisms would be desirable. The pattern of their responses may be relied upon in assessing water bodies for detergent pollution and some of the strains may be explored for bio-treatment of detergent bearing waste.

Author Disclosure Block:

K.I.T. Eniola: None. **T.M. Kayode-Isola:** None.

Poster Board Number:

SATURDAY-244

Publishing Title:

Screening of Algicidal Bacteria and Development of Bio-Eco Ceramic System for Controlling Harmful Algal Blooms (*Cochlodinium polykrikoides*) in Fish-Raising Farm

Author Block:

S. Moon[1], J. Kim[1], S. Kim[1], J. Bang[2], T. You[3], T-K. Lee[4], S-S. Lee[1]; [1]Kyonggi Univ., Suwon, Korea, Republic of, [2]North Carolina Central Univ., Durham, NC, [3]Campbell Univ., Buies Creek, NC, [4]Korea Inst. of Ocean Sci. & Technology, Geoje, Korea, Republic of

Abstract Body:

Harmful algal blooms (HABs) have made massive economic losses and marine environmental disturbances. Normally, loess was scattered to control HABs in Korea, but it arises secondary contamination problems. Therefore, development of highly effective and eco-friendly strategies are required to control the HABs. In the present study about 300 bacterial strains were screened which kills the *Cochlodinium polykrikoides*, a major kind of HABs in Korea. From the screening result, strain 2R1 was selected for further study which showed 98.9% algicidal activity against *C. polykrikoides* at their optimal growth conditions (modified marine broth with 1% NaCl at 28℃). Moreover, selective algicidal activity of strain 2R1 was also tested against few more HABs in Korea such as *Chatonella marina, Heterocapsa triquetra, Heterosigma akashiwo, Scrippsiella trochoidea, Skeletonema costatum* and *Prorocentrum minimum*. Strain 2R1 did not showed any activity against these algal blooms. To supply and preserve strain 2R1 continuously, an algicidal reactor with bio-eco ceramics was developed consisting of the strain and multi-pore ceramics. This system resulted in more than 94.0% of algicidal activity when the bacteria grown on their optimal conditions. From this study, it was determined that the strain 2R1 have selective algicidal activity against *C. polykrikoides* and the strain had no efficiency about other six kinds of HABs. The extracted algicidal activity materials were further analyzed by GC-MSD which indicated them as 7-Methyl-8-hydroxyquinoline and 1-Methyl-β-carboline. In conclusion, the present research have devised an innovative bioreactor system which have very high potential to control HABs. In addition, two promising algicidal compounds are also identified having HABs removal activity.

Author Disclosure Block:

S. Moon: None. **J. Kim:** None. **S. Kim:** None. **J. Bang:** None. **T. You:** None. **T. Lee:** None. **S. Lee:** None.

Poster Board Number:

SATURDAY-245

Publishing Title:

Enrichment and Isolation of Lead-Resistant Bacteria from Saint Claire River Sediments

Author Block:

N. Bowman, M. Fenner, R. Gismondi, D. Patel, A. Oest, D. Azzopardi, S. Tiquia-Arashiro; Univ. of Michigan-Dearborn, Dearborn, MI

Abstract Body:

One of the most common heavy metals present in polluted waters is lead (Pb). Certain bacteria have the ability to resist and reduce lead's toxic effects and play a role in bioremediation. Two enrichment experiments using LB and R2A broths containing 10g of sediments and increasing concentrations of Pb (NO3)2 were carried out to isolate Pb-resistant strains. The enrichments were incubated at room temperature and shook at 250 rpm; aliquots were transferred to fresh media after 48 hours three times. Cells that grew in LB (1500 mg/L) and R2A (1250 mg/L) were serially-diluted and plated. Colonies on the plates were isolated, purified, and the resulting 92 pure cultures were transferred onto slants. Optical density (550 nm) was used to measure cell viability and Pb-resistance. The specific growth rates in LB and R2A enrichments were 0.604h-1 and 0.426h-1, respectively. Relative growth rates of cells in high Pb concentration enrichments did not grow as much as lower concentration enrichments, however they grew well compared to the control. Bio-flocculation assay was performed on each isolate using Kaolin solution and CaCl to assess their ability for extracellular Pb sequestration. For R2A, seven out of 20 isolates produced optimal flocculent product, ranging from 23-40%. For LB, eight out of 21 isolates produced optimal flocculent product, ranging from 25-57%. To continue the research, various lead resistant mechanisms such as efflux mechanism, extracellular sequestration, biosorption, precipitation, enhanced siderophore production and intracellular lead bioaccumulation must be tested on these isolates to determine the best strains for Pb bioremediation.

Author Disclosure Block:

N. Bowman: None. **M. Fenner:** None. **R. Gismondi:** None. **D. Patel:** None. **A. Oest:** None. **D. Azzopardi:** None. **S. Tiquia-Arashiro:** None.

Poster Board Number:

SATURDAY-246

Publishing Title:

Influence of Natural Coagulants and Biosand Filtration on the Characteristics of Cassava Starch Processing Wastewater and Its Reuse in Irrigation

Author Block:

J. O. OLAITAN, Dr, S. B. AKINDE, Dr, S. A. Adebisi, I. O. AWONIYI, O. O. OLUWAJIDE, O. R. AKANGBE, C. O. BABATUNDE; Osun State Univ., Osogbo, Nigeria

Abstract Body:

This study evaluated the effectiveness of natural coagulants and biosand filter in the reduction of physico-chemical and microbiological hazards in wastewater from cassava starch processing plant. Cassava wastewater was collected fresh from a large-scale cassava starch-processing factory in Ogbese, Southwest Nigeria. The experimental setup was conducted at the Biology Laboratory of Osun State University, Osogbo, Nigeria. The natural coagulants used include *Moringa oleifera* seeds, activated charcoal of *Manihot esculenta* (cassava) peel, activated charcoal from *M. oleifera* husk and laterite. Jar test experiment was used to determine the effective coagulation-flocculation dosage of the natural coagulants. The physico-chemical parameters and microbiological analysis were determined in accordance with internationally acceptable methods. The result showed high improvement in the turbidity and cyanide contents and high reductions in heavy metals concentration after each treatment. A significant improvement on the BOD, COD and TSS was also observed after each treatment. The BOD value was reduced within the range of 19.17 and 7.43mg/l while COD was within 96.23mg/l and 38.4mg/l. The total suspended solid reduced drastically between 151.66mg/l and 19.00mg/l. In terms of the microbial characteristics, the total coliform count was greatly reduced compared to reduction in both THB and THF. It was observed that the use of biosand filter was highly effective and economically friendly in the treatment of cassava wastewater compared to the natural coagulants thus significantly reducing the potential hazard to the environment.

Author Disclosure Block:

J.O. Olaitan: None. **S.B. Akinde:** None. **S.A. Adebisi:** None. **I.O. Awoniyi:** None. **O.O. Oluwajide:** None. **O.R. Akangbe:** None. **C.O. Babatunde:** None.

Poster Board Number:

SATURDAY-247

Publishing Title:

A Bacterium *Nocardioides kribbensis* Isolated from Sekoto River Produces a Thermostable Glycoprotein Flocculant

Author Block:

M. Agunbiade; Univ. of Free State, Qwaqwa, South Africa

Abstract Body:

Background: Chemical flocculants have been widely used in waste water treatment, potable water production and fermentation industries. However, despite the low cost and high efficiency, there usage has posed a negative threat on aquatic life and environment. Hence there is a need to replace synthetic flocculants with environmentally friendly flocculants.**Methods:** Twenty five bioflocculant producing actinomyces strains were isolated from the Sekoto River in Eastern Free State Province of South Africa. Strain SR7 exhibited the highest flocculating activity out of these strains. The 16S ribosomal DNA confirmed the bacteria to have 100% similarity to *Nocardioides kribbensis* strain DS4-2B and the nucleotide sequence was deposited in GenBank as *Nocardioides kribbensis* accession number KM891548.1.**Results:** Optimum flocculating activity was attained at 48 h using galactose, yeast extract and Ca^{2+} as sole sources of carbon, nitrogen and cations respectively at an alkaline pH of 11. Inoculum size of 3% (v/v) supported the efficiency of bioflocculant production and 3.97 g was recovered from 1 Litre of the culture broth. The chemical composition of the purified bioflocculant revealed 78.5% sugar and 20.2% protein content. Energy Dispersive X- ray analysis confirmed elemental composition of the purified bioflocculant in mass proportion (%w/w): carbon (7.13), oxygen (40.6), sodium (30.58), phosphorous (20.86) and potassium (0.82).**Conclusions:** The purified bioflocculant exhibited highest flocculating activity when compared with other conventional flocculants. Thus, its application could serves as a replacement for chemical flocculants in the treatment of waste and drinking water.

Author Disclosure Block:

M. Agunbiade: None.

Poster Board Number:

SATURDAY-248

Publishing Title:

Characterization of Sulfate Reducing Bacteria for Removing Selenate from Flu Gas Desulfurization Wastewater

Author Block:

T. Wu; Georgia Southern Univ., Statesboro, GA

Abstract Body:

Selenate, SeO_4^{2-}, is the most oxidized form of selenium. It is water soluble and highly toxic. Selenate is currently found at high levels in coal power plant Flu Gas Desulfurization (FGD) wastewater; the United States Environmental Protection Agency (EPA) requirement for selenate is up to10 ppb. This study involves selecting a biological method to reduce selenate, selecting selenate reducing bacteria, and determining the carbon source affecting the selenate reduction rate. Sulfate reducing bacteria are able to reduce selenate, and are obtained from activated sludge. The bacteria were cultured using Sulphate Reducing HiVeg Medium. The carbon sources tested were sodium lactate and methanol. DNA was extracted and partially nested Polymerase Chain Reaction (PCR) approach was used to amplify *dsrB* gene (350bp). Primer sets used for amplification include DSRmixF/DSRmixR and DSRp2060-GC/DSR4R. The *dsrB* gene contains the sequence for enzymatic activity known to reduce sulfate. Denaturing Gradient Gel Electrophoresis (DGGE) was applied to detect the presences of *dsrB* gene in samples. Samples were analyzed by inductively coupled plasma triple quadrupole mass spectrometry (ICP-QQQ-MS) for selenate reductions. DGGE confirmed the presences of *dsrB* gene in samples and presented a community outlook of sulfate reducing bacteria based on band intensity. Selenate reduction was significant in sodium lactate medium. Dissolved selenium decreased 73 % at day five and additional 56% at day ten. In the samples of methanol as carbon source, dissolved selenium decreased 58% at day five and additional 67% at day ten. At day ten there is no significant difference between methanol and sodium lactate. Biological components to selenate removal utilizing microbial agents have been found to be the key entities in the biogeological cycling of selenium in the environment and have a great potential for the removal of selenate from FGD wastewater.

Author Disclosure Block:

T. Wu: None.

Poster Board Number:

SATURDAY-249

Publishing Title:

Development of Microorganisms Based Effluent Treatment System to Remove Nitrogen and Phosphorus from Land Fish Farms

Author Block:

K. Kim[1], J. Kim[1], S. Kim[1], J. Bang[2], T. You[3], T-K. Lee[4], S-S. Lee[1]; [1]Kyonggi Univ., Suwon, Korea, Republic of, [2]North Carolina Central Univ., 1801 Fayetteville St, NC, [3]Campbell Univ., 143 Main st., NC, [4]Korea Inst. of Ocean Sci.&Technology, Geoje, Korea, Republic of

Abstract Body:

Background: Red tide blooms have made massive economic losses and marine environmental disturbances in coastal region of Korea. This research was focused to develop an effective biological Anaerobic/Anoxic/Oxic (A^2O) treatment system for Land Fish-farm with highly efficient nitrogen and phosphorus removing bacteria and sediments.**Methods:** High efficient bacteria were isolated from the emerging algal blooms sites at Southern coastal of Korea. A total of 960 strains were determined by their nitrogen and phosphorus removal efficiency.**Results:** From screening, *Bacillus aryabhattai* strain KGN1 and *Vibrio neocaledonicus* strain KGP1 showed more than 86.0% nitrogen and 99.9% phosphorus removal efficiency in 10 h, respectively. Interestingly, when consortia of strain KGN1 and KGP1 were made they were able to remove both nitrogen and phosphorus up to 98.0% for 10 h in a batch culture. Furthermore, both strains with sediments were employed to lab-scale A^2O biological treatment system and then operated for 7 months with various operating factors such as different HRT, F/M ratio, aerobic/anaerobic time, volumetric ratio. The optimal conditions for highest effluent treatment were as HRT 6 h, F/M ratio 0.27, intermittent aeration for 30min, volumetric ratio of 1:0.95:1.4 and internal recycle of 1 Q. The reactor showed T-N of 95.6%, NH_3-N of 97.3%, T-P of 97.6% and PO_4^{3-}-P of 96.0% removal efficiency, respectively. The kinetic parameters of the optimal operating condition were 0.17 d^{-1} for specific growth rate (μ) and 2.26 $mgMLVSS/mgSCOD_{Cr}$ for yield (Y). At the optimal operating condition, 20% of both the strains in the microbial community of eco-friendly sludge were maintained and confirmed by NGS analysis. **Conclusions:** In conclusion, this study will be contributed on study of efficient saline wastewater treatment system of land fish farms and near costal area. Finally, the study could be one of helpful tools to solve the cause of red tide blooms in a long-term aspect.

Author Disclosure Block:

K. Kim: None. **J. Kim:** None. **S. Kim:** None. **J. Bang:** None. **T. You:** None. **T. Lee:** None. **S. Lee:** None.

Poster Board Number:

SATURDAY-250

Publishing Title:

Saline Wastewater Treatment from Land Fish-Farm by Biological Sequencing Batch Reactor (Sbr) by High Efficiency Nitrogen and Phosphorus Removing Bacteria

Author Block:

S. Cho[1], J. Kim[2], S. Kim[2], J. Bang[3], T. You[4], T-K. Lee[5], S-s. Lee[6]; [1]kyonggi, Suwon, Korea, Republic of, [2]kyonggi Univ., Suwon, Korea, Republic of, [3]Earth and Geospatial Sci., North Carolina Central Univ., north carolina, NC, [4]kyonggi, Buies Creek, NC, [5]Korea Inst. of Ocean Sci.&Technology,, Geoje,, Korea, Republic of, [6]94-6 Iui-dong, Suwon, Korea, Republic of

Abstract Body:

Research has been rarely reported to develop saline wastewater from land fish-farm. Unfortunately, several systems require very high costs to operate and treat saline wastewater from land fish-farm and coastal area. Thus, there are urgent requirements to develop the low cost and eco-friendly systems for land fish farms. This study was conducted to apply eco-friendly sediment with high efficient bacteria to lab-scale biological SBR system for saline wastewater from land fish-farms. Nitrogen and phosphorus high removal efficient bacteria were screened of 960 strains isolated from seawater and sediment. Nitrogen removal high efficient bacteria *Bacillus aryabhattai* strain KGN1 and phosphorus removal high efficient bacteria *Vibrio neocaledonicus* strain KGP1 were designed as 86.0% nitrogen removal efficiency and 99.9% phosphorus removal efficiency in 10 h, respectively. High efficient bacteria were applied to marine sediment as eco-friendly material, and adapted to make eco-friendly sludge. The eco-friendly sludge was applied to lab-scale SBR system and operated for 7 months with various operating 7 conditions. Lab-scale biological SBR system showed nitrogen and phosphorus removal efficiency with 87.0% and 76.6%, respectively in optimal operating condition (3h/cylcle): aeration for 1 hour, settle for 1 hour and idle for 1 hour at the total volume of 40 L. The optimal kinetic parameters were 0.33 of food-to-mass ratio (F/M ratio), 0.41 of cell yield coefficient (Y), 0.64 of specific growth coefficient (μ) and 0.63 of sludge volume index (SVI), respectively. In addition, NGS (next generation sequencing) and qPCR analysis showed the abundance of *Vibrio neocaledonicus* strain KGP1 was maintained and adapted up to 32% of total microbial community of activated sludge in the lab-scale SBR reactor. From the study, our findings will give possible solution to develop biological treatment of saline wastewater from land fish-farm eco-friendly.

Author Disclosure Block:

S. Cho: None. **J. Kim:** None. **S. Kim:** None. **J. Bang:** None. **T. You:** None. **T. Lee:** None. **S. Lee:** None.

Poster Board Number:

SATURDAY-251

Publishing Title:

Carbapenemases among *Acinetobacter* spp. Isolated from NICU of a Tertairy Care Hospital in Karachi

Author Block:

F. uddin[1], A. Bux[1], M. A. Durrani[1], M. Sohail[2]; [1]Basic Med. Sci. Inst., Jinnah Postgraduate Med. Ctr., Karachi, Pakistan, [2]Univ. of Karachi, Karachi, Pakistan

Abstract Body:

Background: Resistance to multiple classes of antibiotics and especially to the carbapenem is an increasing global problem. The gravity of the problem is ever increasing in the developing counteries where infection control practices are limited. Carbapenem-resistant Acinetobacter spp. has emerged in healthcare facilities and considered as just a tip of an ice berg. The resistance to carbapenem is mainly due to loss of porin or carbapenemases. The current study was designed to determine the carbapenemases in carbapenem-resistant Acinetobacter spp. **Methods:** Study was carried out at the Department of Microbiology, Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre. The carbapenem-resistant *Acinetobacter* strains were collected from NICU of a tertairy care hospital of Karachi. The *Acinetobacter* at species level were identified by using the API 20NE and screening of carbapenem resistance was performed by meropenem and imipenem discs and MICs, by IMP 32 µg/ml Etest strips. The carbapenemase production was detected by RAPIDEC® CARBA NP (Biomeriux, France). The Class B and A carbapenemase production was detected by phenotypic EDTA and boronic acid double disc diffusion test respectively. **Results:** A total of 100 *Acinetobacter* species were collected from the clinical speciemens of neonates. The samples were blood and tracheal aspirates. The patients age ranged from 1-28 days. The main species (95%) was *Acinetobacter calcoaceticus baumannii complex* (ABC), followed by *Acinetobacter lwoffii (*5%). The overall resistance to carbapenem was 95% but higher (100%) in *Acinetobacter lwoffii* in comparision to *Acinetobacter calcoaceticus baumannii complex* (ABC) which was 94.7%. Phenotypic characterization revealed that 93.6% of both the species were class D carbapenemase producers, of which 2.10% were MBLs and 4.2% were non-producers. **Conclusions:** The study revealed that amongst Carbapenem-resistant *Acinetobacter spp*. the class D carbapenemases are the main mode of resistance to carbapenems.

Author Disclosure Block:

F. uddin: None. **A. Bux:** None. **M.A. Durrani:** None. **M. Sohail:** None.

Poster Board Number:

SATURDAY-252

Publishing Title:

Comparison of Maldi-Tof MS Speciation of *Acinetobacter* with Antibiotic Susceptibility Results at a Tertiary Healthcare Facility

Author Block:

P. DeMoss[1], M. Roiko[2], B. H. Schmitt[3]; [1]Univ. of Mississippi Med. Ctr., Jackson, MS, [2]Altru Hlth.System, Grand Forks, ND, [3]Indiana Univ. Sch. of Med., Indianapolis, IN

Abstract Body:

Background: *Acinetobacter baumannii* can be an highly resistant nosocomial infection, with other Acinetobacters reported as less resistant. Differentiation of *Acinetobacter* spp. has been difficult historically. Other Acinetobacters, thought to be less pathogenic, are reported as possibly incorrect species, due to practical laboratory limits. MALDI-TOF MS, shown capable of unambiguously identifying many organisms to the species level, provided fidelity in speciation of Acinetobacter. Here we investigate whether *Acinetobacter* spp. identified by MALDI-TOF MS have differing antibiotic susceptibility profiles, supporting the clinical utility of further speciation of *Acinetobacter* spp. **Methods:**223 clinical isolates of *Acinetobacter* species were compared. Due to previous reporting standards, MALDI-TOF results were initially reported to the genus level, with O/F glucose tests used to further classify as *A. baumannii* or *A. lwoffii*. with some isolates identified by Vitek-2. Subsequently, only five species of *Acinetobacter* were reported during the study timeframe. Antimicrobial susceptibility testing was performed on all isolates. **Results:**Analysis of MALDI-TOF MS results alone identified 13 different species. 148 *A. baumannii* species complex and 68 *A. lwoffii* were identified, among others. 57% of reported *A. baumannii* species were identified as *A. baumannii* by MALDI-TOF and 29% identified as *A. pittii*. For reported *Acinetobacter lwoffii* species, isolates were identified as *A. ursingii* (32%), *A. lwoffii* (26%), *A. radioresistens* (18%), and *A. junii* (15%). MALDI-TOF MS identified *A. baumannii* displayed a much higher level of resistance compared with other members of the *A. baumannii* complex ($p<0.005$), with 70% non-susceptible to five or more antibiotics (compared to none for other members of *A. baumannii* complex). No statistical difference was seen in susceptibiltiy between *A. lwoffii* and others previously identified as *A. lwoffii*.**Conclusions:**Analysis of MALDI-TOF identification in conjunction with susceptibility testing demonstrated that true *A. baumannii* demonstrated significantly less susceptibility than other complex members, while *A. lwoffii* members did not show a statistical difference in non-susceptibility.

Author Disclosure Block:

P. DeMoss: None. **M. Roiko:** None. **B.H. Schmitt:** None.

Poster Board Number:

SATURDAY-253

Publishing Title:

Susceptibility of Carbapenem-Resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa* to Cephalosporin and Beta-Lactamase Inhibitor Combinations

Author Block:

C. Quarshie[1], K. Bobbili[1], J. Lawhorn[2], J. Koirala[1]; [1]Southern Illinois Univ Sch. of Med., Springfield, IL, [2]Mem. Med. Ctr., Springfield, IL

Abstract Body:

Background: There are limited options available for treatment of carbapenem-resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa*. We evaluated activity of ceftazidime-avibactam, ceftolozane-tazobactam and colistin against carbapenem-resistant *A. baumannii* and *P. aeruginosa*. **Methods:** We obtained 41 clinical isolates of MDR *A. baumannii* and 20 isolates of MDR *P. aeruginosa* from our local hospitals. We subcultured of these isolates and plated on MHA agar (Mueller-Hinton agar, Sigma-Aldrich, St. Louis, MO) for susceptibility testing. We evaluated susceptibility against imipenem, colistin, ceftazidime-avibactam, and ceftolozane-tazobactam using E-test method following instructions from the manufacturer (bioMérieux) and guidelines provided by the Clinical and Laboratory Standards Institute (CLSI document M100-S25).**Results:** Of the 41 selected isolates of *A. baumannii* and 20 isolates of *P. aeruginosa*, 33 isolates of *A. baumannii* and 18 isolates of *P. aeruginosa* were resistant to imipenem. Among the imipenem-resistant *A. baumannii* (MIC50 >32 µg/mL), all 33 (100%) isolates were susceptible to colistin (MIC ≤2 µg/mL), whereas only 2 (6%) were susceptible to ceftazidime-avibactam (MIC ≤8 µg/mL), and none to ceftolozane-tazobactam (MIC ≤2 µg/mL). MIC50 of colistin, ceftazidime-avibactam, and ceftolozane-tazobactam for these isolates were 0.25, 48 and 16 µg/mL, respectively. Among the imipenem non-susceptible *P. aeruginosa* (MIC50= 24 µg/mL), all 18 (100%) isolates were susceptible to colistin (MIC ≤2 µg/mL) and ceftolozane-tazobactam (MIC ≤4 µg/mL), whereas only 9 (50%) were susceptible to ceftazidime-avibactam (MIC ≤8 µg/mL). MIC50 of colistin, ceftazidime-avibactam, and ceftolozane-tazobactam for these isolates were 1, 10 and 1.5 µg/mL, respectively.**Conclusions:** Newer antibiotics with combination of cephalosporins and beta-lactamase inhibitors were found active only against carbapenem-resistant isolates of *P. aeruginosa*, but not against *A. baumannii*. Ceftolozane-tazobactam performed better than ceftazidime-avibactam against *P. aeruginosa*. Colistin remains highly active against all carbapenem-resistant isolates of *A. baumannii* and *P. aeruginosa* at our institution.

Author Disclosure Block:

C. Quarshie: None. **K. Bobbili:** None. **J. Lawhorn:** None. **J. Koirala:** None.

Poster Board Number:

SATURDAY-254

Publishing Title:

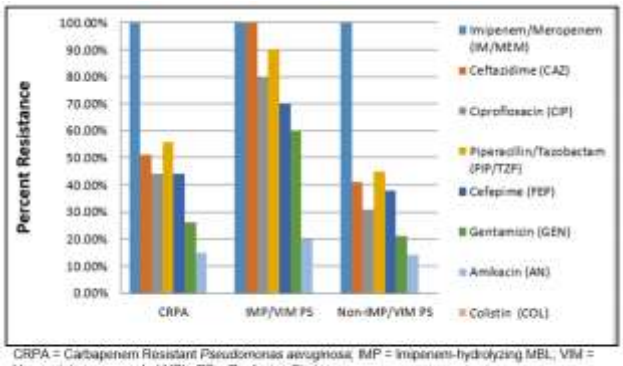
Antibiotic Profiling and Detection of *bla*VIM, *bla*IMP, and *bla*SPM in Carbapenem-Resistant *Pseudomonas aeruginosa* in Selected Tertiary Hospitals in Metro Manila, Philippines

Author Block:

S. Tesalona[1], E. Lagamayo[2], A. Cabal[3]; [1]De La Salle Hlth.Sci. Inst., Cavite, Philippines, [2]Univ. of Santo Tomas, Manila, Philippines, [3]Adamson Univ., Manila, Philippines

Abstract Body:

Antibiotic resistance profile and the presence of several metallo-β-lactamase (MBL) genes conferring resistance to carbapenem were investigated in this study. Previously identified carbapenem-resistant Pseudomonas aeruginosa (CRPA) were tested for the presence of MBL enzyme using E-test. Amplification of blaVIM, blaIMP, and blaSPM was done using conventional polymerase chain reaction (PCR). Antibiotic resistance pattern of each antibiotic tested among MBL and non-MBL producing strains were statistically tested using Fisher exact test. Any $p \leq 0.05$ were considered significant. Of the 39 CRPA tested, 10 MBL producing strains were detected, 9 (23.1%) carried the *bla*IMP and 1 (2.5%) carried the *bla*VIM gene, whereas *bla*SPM was not observed. Increased resistance against ceftazidime, piperacillin/tazobactam, and ciprofloxacin in *bla*IMP and *bla*VIM-producing strains showed significant difference compared to non-*bla*IMP and non-*bla*VIM producing strains ($p<0.05$). On the other hand *bla*IMP and *bla*VIM producing strains with increased resistance to cefepime, and gentamicin has no significant difference, ($p>0.05$). Colistin appeared to be the most effective antimicrobial agent, with 0% resistance to both *bla*IMP and *bla*VIM producing and non-producing strains (Figure 1). The *bla*IMP and *bla*VIM gene were mostly detected from strains isolated from respiratory specimens, 6 (15.4%), followed by urine specimens, 4 (10.2%). These study suggest the presence of *bla*IMP and *bla*VIM-producing *P. aeruginosa* in the Philippines represented by the selected tertiary hospitals in Metro Manila.



CRPA = Carbapenem Resistant Pseudomonas aeruginosa; IMP = Imipenem-hydrolyzing MBL; VIM = Verona-integron encoded MBL; PS = Producing Strains

Figure 1. Antibiotic Resistance Profile of 39 CRPA isolates, *bla*IMP and *bla*VIM Producing and non-Producing Strains Interpreted Based on CLSI, 2014.

Author Disclosure Block:

S. Tesalona: None. **E. Lagamayo:** None. **A. Cabal:** None.

Poster Board Number:

SATURDAY-255

Publishing Title:

Comparison of the *In Vitro* Activity of Ceftolozane-Tazobactam and Ceftazidime-Avibactam against Ceftazidime Non-Susceptible *Pseudomonas aeruginosa*

Author Block:

D. Sneed, K. G. Van Horn; Kindred Hosp., Rancho Cucamonga, CA

Abstract Body:

Multi-drug resistance in *P. aeruginosa* has increased, including strains resistant to ceftazidime, an anti-pseudomonal cephalosporin. Two newer antimicrobials, ceftolozane-tazobactam (CEF-TAZ) and ceftazidime-avibactam (CAZ-AVI) have both been recently FDA cleared with indications for use against *P. aeruginosa* in select infections. CEF is a newer beta-lactam and TAZ is a penicillanic acid sulfone beta-lactamase inhibitor. CAZ is an established cephalosporin combined with AVI, a non-beta-lactam beta-lactamase inhibitor that inactivates select beta-lactamases and protects ceftazidime from degradation. This study compares the in vitro activity of CEF-TAZ and CAZ-AVI against 87 recent clinical isolates from a variety of sources (respiratory 65, urine 12, wound 9, blood 1) of *P. aeruginosa* that have tested previously as intermediate (I, 16 mcg/mL) or resistant (R, ≥32 mcg/mL) to CAZ by the VITEK 2 system and obtained from selected Southern California hospitals. CEF-TAZ and CAZ-AVI were tested by the Etest method according to manufacturer's instructions. A 0.5 McFarland suspension of pure organism was rolled with a swab onto the surface of a Mueller-Hinton II agar plate then incubated at 37ºC for 16-24 h and the MIC read as the point where the ellipse of inhibition met the Etest strip. Quality control was performed with *P. aeruginosa* ATCC 27853 and results were acceptable according to the FDA ranges. There were 31 CAZ intermediate strains and 56 resistant strains tested. 76 (87%) of the isolates were susceptible to CEF-TAZ and 61 (70%) isolates were susceptible to CAZ-AVI. CEF-TAZ isolates were 87% S (27/31 if I and 49/56 if R) for both CAZ I and R isolates. However, CAZ-AVI showed 77% S (24/31) if CAZ was I and 66% S (37/56) if the *P. aeruginosa* isolates were CAZ R. No isolates were S to CAZ-AVI when the CEF-TAZ was R.CEF-TAZ appears to have better in vitro activity than CAZ-AVI against ceftazidime non-susceptible *P. aeruginosa*.

Author Disclosure Block:

D. Sneed: None. **K.G. Van Horn:** None.

Poster Board Number:

SATURDAY-256

Publishing Title:

Hypermutable *Pseudomonas aeruginosa* (PA) Linked with Antibiotic Resistance in Australian Cystic Fibrosis Patients

Author Block:

V. E. Rees[1], J. B. Bulitta[2], A. Y. Peleg[1], T. Kotsimbos[1], R. L. Nation[1], A. Oliver[3], C. B. Landersdorfer[1]; [1]Monash Univ., Melbourne, Australia, [2]Univ. of Florida, Orlando, FL, [3]Hosp. Son Espases, Palma, Spain

Abstract Body:

Background: Hypermutable Pa isolates have a greatly increased mutation rate and have been found in chronic respiratory infections. They have an enhanced ability to become resistant and are associated with reduced lung function in CF-patients. This study aimed to provide the first characterization of clinical Pa isolates from Australian CF-patients, and to determine the prevalence of hypermutation and antibiotic susceptibility. **Methods:** 59 clinical Pa isolates from Australian CF-patients, PAO1 and PAOΔ*mutS* (hypermutable) were characterized. Mutation frequencies (MF) on 300 mg/L rifampicin-containing agar plates were assessed in triplicate. Hypermutation was defined by the rifampicin MF being ≥20-fold greater compared to PAO1. MICs were performed by Etest and susceptibility judged by EUCAST breakpoints.**Results:** Of all clinical Pa isolates 22% were hypermutable. Multidrug-resistance (MDR; resistance to ≥1 agent from ≥3 different antimicrobial categories) was significantly higher in hypermutable (38%) than non-hypermutable isolates (13%; Chi-square test, $p<0.05$). Hypermutable isolates were more commonly resistant to 2 or more antibiotics in comparison to non-hypermutable isolates (62% *vs.* 28%; Chi-square test, $p<0.05$). Among hypermutable isolates, resistance was 77% for ciprofloxacin, 46% for ceftazidime, and 38% for meropenem and tobramycin. In non-hypermutable isolates, resistance was common for ciprofloxacin (52%), but less frequent for tobramycin (22%), ceftazidime (20%) and meropenem (13%).**Conclusions:** The prevalence of hypermutable isolates in Australian CF-patients was similar to that in European CF-patients. Hypermutable isolates had a significantly higher proportion of MDR and were more often resistant to all tested antibiotics in comparison to non-hypermutable isolates. Therefore, hypermutable Pa are playing a critical role in the antibiotic resistance problem in Australian CF-patients.

Author Disclosure Block:

V.E. Rees: None. **J.B. Bulitta:** E. Grant Investigator; Self; Trius, Cempra. H. Research Contractor; Self; Pfizer, Cubist. **A.Y. Peleg:** None. **T. Kotsimbos:** None. **R.L. Nation:** None. **A. Oliver:** None. **C.B. Landersdorfer:** None.

Poster Board Number:

SATURDAY-257

Publishing Title:

Susceptibility of *Pseudomonas aeruginosa* Bloodstream Isolates to Ceftazidime-Avibactam in Northeastern United States

Author Block:

M. Wungwattana[1], S. D. Mahlen[2], A. M. Casapao[3]; [1]Maine Med. Ctr., Portland, ME, [2]Affiliated Lab., Inc., Bangor, ME, [3]Husson Univ., Bangor, ME

Abstract Body:

Background: Ceftazidime-avibactam (CAZ-AVI) has *in vitro* activity against certain Gram-negative and Gram-positive bacteria. Avibactam (AVI), a novel beta-lactamase inhibitor, is unique from all other currently available beta-lactamase inhibitors due to its diaza-bicyclo octane structure and allows ceftazidime (CAZ) to regain activity in the presence of class A and class C beta-lactamase enzymes. Reductions in the minimum inhibitory concentrations (MICs) of CAZ with the addition of AVI have ranged from 4- to 1024-fold among Enterobacteriaceae and 4-fold for *Pseudomonas aeruginosa*. The aim of this study was to analyze the susceptibility of ceftazidime-avibactam against *P. aeruginosa* bloodstream isolates obtained from patients in the northeastern region of the United States.**Methods:** Clinical *P. aeruginosa* isolates obtained from blood cultures from multiple sites in Maine between 2011 and 2015 were tested against CAZ-AVI E-test strips. Demographics and MICs from automated susceptibility testing were also collected and analyzed.**Results:** We report the results of 20 *P. aeruginosa* bloodstream isolates that were tested against CAZ-AVI. The MIC_{50} and MIC_{90} were 1 mg/L and 2 mg/L, respectively [0.75 - 6 mg/L]. The MIC_{50} and MIC_{90} to CAZ alone were 1 mg/L and 4 mg/L, respectively [≤0.5 - >32 mg/L]. Of the 16 patients who received empiric antibiotic therapy, 15/16 (93.8%) received an agent with anti-pseudomonal activity and 3/16 (18.8%) received combination therapy. Patients were bacteremic for a median time of 2 days [1-6 days] and received antibiotic therapy for a median time of 14.5 days [5-51 days]. For the isolate displaying CAZ MIC >32 mg/L, the CAZ-AVI MIC was 6 mg/L.**Conclusions:** Ceftazidime-avibactam displayed good activity against *P. aeruginosa* bloodstream isolates. The MIC_{50} between CAZ and CAZ-AVI did not differ. However, we detected a 1-fold dilution decrease in the MIC_{90} between CAZ (2 mg/L) and CAZ-AVI (4 mg/L). Based on the Clinical & Laboratory Standards Institute breakpoint of ≤8/4 mg/L, all of our *P. aeruginosa* isolates were susceptible to CAZ-AVI. The results from our study show that ceftazidime-avibactam may be a therapeutic option for patients infected with *P. aeruginosa* displaying elevated ceftazidime MICs.

Author Disclosure Block:

M. Wungwattana: None. **S.D. Mahlen:** None. **A.M. Casapao:** C. Consultant; Self; ♣ Cerexa subsidiary of Activis. E. Grant Investigator; Self; ♣ Cerexa subsidiary of Activis, ♣ Cubist

Pharmaceuticals, wholly owned subsidiary of Merck, ♣ Michigan Department of Community Health, ♣ Forest Laboratories subsidiary of Activis, ♣ Astellas Pharmaceuticals Inc.. J. Scientific Advisor (Review Panel or Advisory Committee); Self; ♣ The Medicine's Company.

Poster Board Number:

SATURDAY-258

Publishing Title:

High Prevalence of FosA Among Clinical *Pseudomonas aeruginosa* Isolates Accounts for Decreased Fosfomycin (FOF) Susceptibility

Author Block:

E. B. Hirsch[1], P. C. Zucchi[1], A. Chen[1], A. Phung[1], C. L. Emery[2], T. Bias[3]; [1]Northeastern Univ., Boston, MA, [2]Drexel Univ. Coll. of Med., Philadelphia, PA, [3]Hahnemann Univ. Hosp., Philadelphia, PA

Abstract Body:

Background: FOF is often used to treat urinary or systemic infections caused by multidrug-resistant (MDR) pathogens for which few treatment options remain. It has gained traction in treatment of *P. aeruginosa* infections though limited data regarding susceptibility or resistance mechanisms among clinical isolates have been reported. We determined the in vitro activity of FOF and identified resistance mechanisms among a set of recent clinical *P. aeruginosa* isolates. **Methods:** Thirty-one clinical *P. aeruginosa* isolates (1 per patient episode) from two US centers were included. Isolates were collected between 2013-2015 from urine (n = 27), sputum (n = 2), or wound (n = 2) and were screened for the presence of *fosA* gene and mutations in the *glpT* gene via PCR and gene sequencing. FOF MICs were determined in duplicate, on separate days, by both the reference method of agar dilution (AD) and Etest (ET). The CLSI susceptibility breakpoint for *Escherichia coli* (≤ 64 mg/L) was used for interpretation. **Results:** Twelve (39%) isolates were considered MDR (non-susceptible to ≥ 1 agent in at least 3 antibiotic classes). All but one isolate was positive for the plasmid-mediated enzyme *fosA* by PCR. Sixteen of 30 FosA+ isolates had wild-type (WT) copies of the gene while the other 15 contained mutations resulting in truncated proteins. MICs by AD were higher for the WT FosA group, ranging from 32-256 (MIC_{50}=64) mg/L compared to the non-WT FosA group (range, 4-128; MIC_{50}=16 mg/L). Nine (56%) of 16 isolates with WT FosA were considered susceptible when applying the CLSI interpretive criteria for *E. coli*. Essential agreement of ET with AD was 77%; in 4 of 8 isolates without agreement, WT FosA was present and ET more accurately classified them as resistant (≥ 256 mg/L). Preliminary screening of 4 isolates revealed no mutations in the glpT transporter necessary for FOF uptake. **Conclusion:** In this small collection of clinical *P. aeruginosa* isolates, the presence of FosA was common and contributed to increased FOF MICs. Application of the CLSI *E. coli* breakpoints for *P. aeruginosa* may be inappropriate and result in false susceptibility. Identification of additional resistance mechanisms and susceptibility determinations among a larger group of isolates are ongoing.

Author Disclosure Block:

E.B. Hirsch: J. Scientific Advisor (Review Panel or Advisory Committee); Self; Theravance Biopharma. L. Speaker's Bureau; Self; The Medicines Company. **P.C. Zucchi:** None. **A. Chen:** None. **A. Phung:** None. **C.L. Emery:** None. **T. Bias:** E. Grant Investigator; Self; Merck, Actavis/Allergan. L. Speaker's Bureau; Self; The Medicines Company.

Poster Board Number:

SATURDAY-259

Publishing Title:

Evaluation of Cefolozane/Tazobactam Susceptibilities of *Pseudomonas aeruginosa* Blood Isolates at Two Major Tertiary Referral Medical Centers

Author Block:

C. Wilson[1], J. Graffam[1], M. Wungwattana[2], S. D. Mahlen[3], A. M. Casapao[1]; [1]Husson Univ, Bangor, ME, [2]Maine Med Ctr, Portland, ME, [3]Affiliated Lab Inc., Bangor, ME

Abstract Body:

Background: Ceftolozane/tazobactam (TOL/TAZ) is a newly approved antimicrobial indicated to treat Gram-negative bacteria that cause complicated intra-abdominal infections (IAI) & complicated urinary tract infections (UTI), including *Pseudomonas aeruginosa* (PSAR). The aim of this study was to evaluate the minimum inhibitory concentrations (MICs) of TOL/TAZ in PSAR blood isolates from patients at two major medical centers in Maine. **Methods:** A retrospective, epidemiological study that included patients whom had at least one positive blood culture of PSAR admitted to multiple sites in Maine. Isolates stored at -20ºC were grown on Mueller-Hinton blood agar media and TOL/TAZ Etest MICs were recorded. PSAR isolate data was collected through medical records from 2011 to 2015. Data collected included demographics and antimicrobial therapy. PSAR is considered susceptible to TOL/TAZ at MICs ≤4/4 mg/L & resistant at MICs ≥16/4 mg/L. **Results:** Total of 50 patients had PSAR bacteremia; of these patients, 41 (82%) were treated at one of the top two major medical centers in Maine. The median Pitt bacteremia score was 2 (range 0-9) and 43.9% were admitted to the critical care unit. A total of 38/41 (93%) of patients were treated with one empiric antipseudomonal agent and 3/41 (7%) patients received an antipseudomonal empiric agent that was resistant. The most common antimicrobial included cefepime (48%) & piperacillin/tazobactam (35%). Median total hospital duration of therapy was 7 days (1-23). Outpatient therapy was continued in 61% of patients were discharged. Median length of stay was 11 days (3-92). Hospitalization mortality was 29.3%. A total of 50 (100%) isolates were susceptible to TOL/TAZ. TOL/TAZ MICs ranged from 0.125-1.5 mg/L with MIC50 of 0.38mg/L and MIC90 of 1mg/L. PSAR resistance rates were highest with aztreonam (12%), piperacillin/tazobactam (8%), meropenem (8%), ciprofloxacin (6%), and cefepime (4%).**Conclusions:** TOL/TAZ is a potent antipseudomonal agent and our PSAR MIC90 value is two-fold lower than the upper susceptible breakpoint. TOL/TAZ may be a good option for UTI and IAI resulting from PSAR. TOL/TAZ may potentially be used as empiric therapy especially if caused by PSAR*;* although follow up susceptibilities are still warranted.

Author Disclosure Block:

C. Wilson: None. **J. Graffam:** None. **M. Wungwattana:** None. **S.D. Mahlen:** None. **A.M. Casapao:** C. Consultant; Self; Cerexa subsidiary of Activis. E. Grant Investigator; Self; Cerexa

subsidiary of Activis, Cubist Pharmaceuticals, wholly owned subsidiary of Merck, Michigan Department of Community Health, Forest Laboratories subsidiary of Activis, Astellas Pharmaceuticals Inc.. J. Scientific Advisor (Review Panel or Advisory Committee); Self; The Medicine's Company.

Poster Board Number:

SATURDAY-260

Publishing Title:

Activity of Ceftazidime-Avibactam against Resistant *Enterobacteriaceae* and *Pseudomonas aeruginosa*

Author Block:

K. G. Van Horn; Kindred Hosp., Rancho Cucamonga, CA

Abstract Body:

Resistance to β-lactams including carbapenems continues to increase in Gram negative bacilli. The new antimicrobial combination of ceftazidime with avibactam (CEF-AVI) a non-β-lactam β-lactamase inhibitor, inactivates β-lactamases and protects ceftazidime from degradation. In vitro activity has been demonstrated against Enterobacteriaceae (ESBL, AmpC, KPC) and *Pseudomonas aeruginosa* (AmpC and some OprD) producing isolates. We studied the susceptibility of CEF-AVI to organisms isolated from long-term acute care hospital (LTACH) patients. A total of 304 recent clinical isolates collected from March to December 2015 were obtained from various Southern California LTACHs and tested against CEF-AVI by the Etest method according to manufacturer instructions. A 0.5 McFarland suspension of pure organism was rolled with a swab onto the surface of a Mueller-Hinton II agar plate then incubated at 37ºC for 16-24 h and the MIC read as the point where the ellipse of inhibition met the Etest strip. Quality control with *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603, and *P. aeruginosa* ATCC 27853 was performed and results were acceptable according to the CEF-AVI FDA ranges. All 29 ESBL-producing Enterobacteriaceae (carbapenem-susceptible) were susceptible to CEF-AVI. There were 245 carbapenem-resistant Enterobacteriaceae (232 *Klebsiella pneumoniae*) tested and 228 were susceptible (98.3%). The MIC_{50} and MIC_{90} for carbapenem-resistant *K. pneumoniae* respectively were 2/4 (CEF-AVI) mcg/mL and 4/4 mcg/mL. The susceptible breakpoint value for Enterobacteriaceae is ≤ 8/4 mcg/mL. There were a total of 30 *P. aeruginosa* strains tested. All 6 ceftazidime susceptible (MIC ≤ 8 mcg/mL) isolates were also susceptible to CEF-AVI. Of the 24 ceftazidime intermediate/resistant strains (MIC >8 mcg/mL), 19 (79.2%) were susceptible to CEF-AVI . Ceftazidime-avibactam appears to be an active agent in vitro against carbapenem-resistant Enterobacteriaceae and ceftazidime resistant *P. aeruginosa*.

Author Disclosure Block:

K.G. Van Horn: None.

Poster Board Number:

SATURDAY-261

Publishing Title:

***In Vitro* Activity of Three Antibiotic (Abx) Combination (Combi) against *Stenotrophomonas maltophilia* (Sm) Isolates in Singapore**

Author Block:

T-P. Lim[1], C-X. Gao[2], J. Teo[1], Y. Cai[1], S-X. Tan[1], W. Lee[1], T-H. Koh[1], N. Tee[3], T-T. Tan[4], A-L. Kwa[1]; [1]SINGAPORE GEN. HOSP., SINGAPORE, Singapore, [2]Natl. Univ. of Singapore, SINGAPORE, Singapore, [3]KK Women's & Child. Hosp., SG, Singapore, [4]SINGAPORE GEN. HOSP., SG, Singapore

Abstract Body:

Background: The prevalence of SM with reduced susceptibilities to trimethoprim-sulfamethoxazole (TS) is increasing in Singapore, leaving few treatment options available. Combi therapy may be the only therapeutic option till new abx are available. We previously found that only levofloxacin (LEV) + polymyxin B (PB) were inhibitory against XDR SM. This study aimed to evaluate the bactericidal activity of 3-abx combi against SM. **Methods:** Non-repeat SM isolates were collected from Oct 14 - Dec 15. Aztreonam (AZT), LEV, PB, tigecycline (TG), ticarcillin-clavulanic acid (TC), TS & ceftazidime (CFZ) MICs were determined using Etest. Presence of L1, L2 & Sul genes were identified using PCR. Time-kill studies (TKS) were conducted with approximately 10^5 CFU/ml at baseline with maximally clinical achievable concentrations (mg/L) of AZT (24), LEV (8), PB (2), TG (2), TC (60/2), TM (2/38) & CFZ (35), in 3-abx combi. **Results:** 160 SM isolates were collected. 64% were from respiratory cultures (73/160 from upper respiratory & 29/160 from lower respiratory cultures), 8% were from blood cultures, 4% were from urine cultures & the remaining were tissue cultures. MIC50/MIC90 (mg/L) of AZT, LEV, PB, TG, TC, TS & CFZ were >=512/>=512, 0.75/8, 1.5/16, 1.5/4, 32/>=512, 0.125/4 & 4/>=512, respectively. Four XDR SM isolates were included in TKS. 35 3-abx combi were evaluated against each isolate. The 4 selected XDR SM isolates had MICs (mg/L) of AZT, LEV, PB, TG, TC, TS & CFZ as >=512 (for all), 12/2/8/>=64, 8/1/2/0.5, 2/3/6/12, 16/16/32, >=64 (for all) & 12/4/12/16, respectively. In TKS, LEV+TG+TC was synergistic against SM1; PB+TG+TC was synergistic against SM2; AZT+PB+TC was synergistic against SM4 while PB+CFZ+TS was bactericidal against SM4. PCR confirmed the presence of L1, L2 & Sul genes in all isolates. **Conclusions:** Potential abx combi as pre-emptive therapy for XDR SM infections are highly strain-specific. 3 Abx combi may be useful for XDR SM in our institution.

Author Disclosure Block:

T. Lim: None. **C. Gao:** None. **J. Teo:** None. **Y. Cai:** None. **S. Tan:** None. **W. Lee:** None. **T. Koh:** None. **N. Tee:** None. **T. Tan:** None. **A. Kwa:** None.

Poster Board Number:

SATURDAY-262

Publishing Title:

Antimicrobial Susceptibility to β-Lactam Antibiotics and the Production of BRO β-Lactamase in Clinical Isolates of *Moraxella catarrhalis* from a Japanese Hospital: Does BRO Inactivate Ceftriaxone?

Author Block:

K. Yamada[1], K. Arai[1], R. Saito[2]; [1]Tokyo Metropolitan Hlth.and Med. Treatment Corp. Toshima Hosp., Itabashi-ku, Japan, [2]Tokyo Med. and Dental Univ., Bunkyo-ku, Japan

Abstract Body:

Background: *Moraxella catarrhalis* is an important respiratory pathogen. However, there is little information regarding the susceptibility of this organism to β-lactam antibiotics. In this study, we investigated the antimicrobial susceptibility to β-lactam antibiotics and the BRO β-lactamase production of clinical *M. catarrhalis* isolates.**Methods:** In total, 233 *M. catarrhalis* clinical isolates obtained between January 2013 and July 2015 in Tokyo were examined. Phenotypic and genotypic production of BRO β-lactamase were tested using cefinase disk and PCR analyses, respectively. Antimicrobial susceptibility was tested using the Etest.**Results:** Among the 233 clinical isolates tested, 232 (99.4%) were BRO producers, while only one (0.6%) was BRO-negative. Among the 232 BRO-producers, there were 224 BRO-1-producing strains (96.6%) and eight BRO-2-producing strains (3.4%). The geometric mean minimum inhibitory concentration (MIC), MIC_{50}, and MIC_{90} values of amoxicillin, amoxicillin clavulanate, cefaclor, and ceftriaxone for BRO-1 producers were significantly higher than those for BRO-2 producers ($P < 0.01$). Notably, four of the BRO-1-producing isolates exhibited elevated ceftriaxone MIC values (2.0 mg/mL), but only the cefaclor MIC breakpoint was exceeded. Based on the results of ceftriaxone inactivation tests, ceftriaxone decomposition activity was confirmed against BRO-1 producers. However, ceftriaxone inactivation activity was not investigated in BRO-2 producers.**Conclusions:** Our results indicated that *M. catarrhalis* infections should be treated with amoxicillin clavulanate instead of cephalosporin antibiotics. The number of *M. catarrhalis* isolates with acquired resistance to β-lactam antibiotics is expected to increase in the near future. Therefore, we plan to continue our study of antimicrobial susceptibility in clinical *M. catarrhalis* isolates.

Author Disclosure Block:

K. Yamada: None. **K. Arai:** None. **R. Saito:** None.

Poster Board Number:

SATURDAY-263

Publishing Title:

Plasmid Profile of Resistant (MAR) Bacteria from Leachate in Some Rural Communities of Ekiti State, Nigeria

Author Block:

A. T. Odeyemi; Ekiti State Univ., Ado-Ekiti, Ekiti State, Nigeria, Ado-Ekiti, Nigeria

Abstract Body:

profile of bacteria isolated from leachate samples of some rural communities in Ekiti State, Nigeria were analyzed. Antibiotics sensitivity and plasmid profile were assayed using disk diffusion and gel electrophoresis techniques respectively; while the plasmids were cured using acridine orange. The total bacterial, total coliform and total enteric bacteria counts of the leachate samples ranged from 0.64×10^8 to 0.85×10^9CFU/ml, 0.78×10^8 to 0.76×10^9CFU/ml and 1.90×10^8 to 2.30×10^9CFU/ml respectively. *Escherichia coli* with 29.2% had the highest frequency of occurrence while *Enterobacter aerogenes* had the lowest occurrence of 7.5%. The antibiotics sensitivity test showed that the least percentage (3%) of the isolates were resistant to ofloxacin while the highest percentage (99%) of the isolates were resistant to cefuroxime. Multiple Antibiotics Resistant bacteria were subjected to plasmid analysis showing that nine (9) were devoid of plasmid while other six (6) isolates carry plasmid with high molecular weight, ranging from 9.41kbp to 23.1kpb. Three MAR isolates (*Escherichia coli*, *Pseudomonas aeruginosa* and *Enterococcus faecalis*) were selected for curing. The three (3) retained resistance pattern to all antibiotics used aside *Escherichia coli* and *Pseudomonas aeruginosa* that became susceptible to ofloxacin after curing. Incidence of such MAR bacterial isolates in leachate samples is however an indication of possible environmental hazard.

Author Disclosure Block:

A.T. Odeyemi: None.

Poster Board Number:

SATURDAY-264

Publishing Title:

Multidrug-Resistant Bacteria in Postoperative Wound Infection at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia

Author Block:

M. Hailu; Addis Ababa Univ., Coll. of Hlth.Sci., Addis Ababa, Ethiopia

Abstract Body:

Background: Postoperative wound infection is a major problem throughout the world even in hospitals with most modern facilities. Infections get worsen due to Multidrug resistance (MDR) which is the challenge for controlling now days. Hence the aim of this study was to determine the prevalence of multidrug resistant bacteria in postoperative wound infection.**Method:** A cross sectional study was conducted from March 30 to August 28/2015. A total of 197 surgical patients who developed postoperative wound infections were included. Wound swabs were collected using sterile cotton swab and dipped into sterile transport media. All wound swabs were cultured on Blood, MacConkey and Mannitol salt agar. All culture positive samples were characterized by gram stain and standard biochemical tests. Antimicrobial susceptibility test was performed on Muller-Hinton agar using Kirby-Bauer method. Data was analyzed using SPSS version 20.**Results:** The overall prevalence was 75.6% (n=149/197) and the predominant bacteria isolated were *S. aureus33.3%* (n=56/168) and *E. coli 14.3% (n=24/168.* Double infections 11.4% (n=17/149) were seen in which *S. aureus* and *Pseudomonas species* 23.5% (n=4/17) showed common association. Highest proportion of bacteria 28.2% (n=42/149) were isolated from orthopedics followed by General Surgery 24.8% (n=37/149). Multidrug resistances (MDR) were recorded in 65.5% (n=110/168) of all bacterial isolates. Gram positive and Gram negative bacteria showed 55.3% (n=42/76) and 73.9% (n=68/92) MDR respectively. Among the antibiotics tested amoxicillin (93.5%), Ceftriaxone (85.3%), Penicillin (84.5%) and Cefotaxime (82.7%) showed high level of resistance. Clindamycin (7.9%) and Amikacin (1.1%) showed high sensitivity for Gram Positive and Gram negative respectively**Conclusion:** Choice of drugs for treatment of bacterial isolates from postoperative wound infections is quite narrow today due to the wide scale resistance to common drugs. Hence rational use of antibiotics and regular monitoring of antimicrobial resistance patterns are essential and mandatory to prevent further emergence and spread of multidrug resistance bacterial pathogens.

Author Disclosure Block:

M. Hailu: None.

Poster Board Number:

SATURDAY-265

Publishing Title:

Molecular Characteristics of Extended-spectrum β-Lactamase-Producing *Escherichia coli* Isolates from Retail Food Products, Healthy Workers and Patients: Horizontal Transfer of Ctx-M-55 Plasmids

Author Block:

Q. P. Le[1], S. Ueda[2], T. N. H. Nguyen[1], T. T. N. Tran[1], T. A. V. Hoang[1], T. N. D. Nguyen[1], T. V. K. Dao[1], M. T. Tran[1], T. T. T. Le[1], T. L. Le[1], I. Hirai[2], T. Nakayama[3], T. H. Do[1], Q. M. Vien[1], Y. Yamamoto[4]; [1]Nha Trang Pasteur Inst., Nha Trang, Viet Nam, [2]Univ. of Ryukyus, Okinawa, Japan, [3]Osaka Univ., Osaka, Japan, [4]Osaka Prefectural Inst. of Publ. Hlth., Osaka, Japan

Abstract Body:

Background: There is widespread dissemination of extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* worldwide, with the emergence of CTX-M type ESBLs. In particular, dissemination in communities in developing countries is an urgent public health issue. However, the dissemination mechanisms are not clear. Therefore, the current study was conducted to determine the molecular characteristics of ESBL-producing *E. coli* isolated from both food and humans.**Methods:** Retail food (pork meat) samples (n = 147), fecal samples of healthy workers selling retail food products at a market (n = 156), and *E. coli* clinical isolates from patients with infection (n = 86) were collected from Nha Trang, a central province of Vietnam, from 2013 to 2015. ESBL-producing *E. coli* was isolated, and ESBL genotypes, phylogroups, plasmidomes, and antibiotic-resistance profiles were determined.**Results:** The prevalence of ESBL-producing *E. coli* in samples from workers (71.8%) was significantly higher than that for retail food products (32.0%). A CTX-M type *E. coli* strain was the dominant strain in ESBL-producing *E. coli* isolates obtained from food (43/47, 91.5%), workers (115/117, 98.3%), and patients (35/36, 97.2%); PCRs and DNA sequencing revealed that CTX-M-55, CTX-M-27, and CTX-M-15, respectively, were dominant in these sample types. Phylogroups B2 and D were common in isolates from workers and patients, whereas B1 and A were predominant in food isolates. S1-PFGE and southern blotting showed variation in the size of plasmids with CTX-M-55 (105-240 kb). However, several isolates obtained from food, workers, and patients showed a similar size for the plasmid with the CTX-M-55 gene, indicating possible horizontal transfer of the plasmid with the ESBL-encoding gene.**Conclusions:** The results indicate that ESBL-producing *E. coli* disseminates not only in retail food products but also in communities and nosocomial settings. Furthermore, such dissemination may be caused by transfer of a plasmid with CTX-M genes.

Author Disclosure Block:

Q.P. Le: None. **S. Ueda:** None. **T.N.H. Nguyen:** None. **T.T.N. Tran:** None. **T.A.V. Hoang:** None. **T.N.D. Nguyen:** None. **T.V.K. Dao:** None. **M.T. Tran:** None. **T.T.T. Le:** None. **T.L.**

Le: None. **I. Hirai:** None. **T. Nakayama:** None. **T.H. Do:** None. **Q.M. Vien:** None. **Y. Yamamoto:** None.

Poster Board Number:

SATURDAY-266

Publishing Title:

Molecular Characterization of Extended Spectrum Betalactamase Producing Genes in *E. coli* from Zaria, Nigeria

Author Block:

J. A. Onaolapo; Ahmadu Bello Univ., Zaria, Zaria, Nigeria

Abstract Body:

Background: Poor therapeutic outcome and high economic burden have been associated with extended spectrum betalactamase (ESBL) producing *E. coli* due to the expression of multidrug resistance (MDR) to penicillins, cephalosporins and monobactams, which are commonly prescribed in the hospitals. **Methods:** This study evaluated the presence of ESBL in *E. coli* isolated from urinary tract infection (UTI) and diarrhea patients in Zaria, Nigeria using double disc diffusion method and molecular techniques.**Results:** Using Microgene identification kit 87 of the 132 presumptive *E. coli* isolates from UTI and diarrhea patients in selected hospitals in Zaria, Nigeria were confirmed as *E. coli* and subsequently evaluated for the presence of ESBL. The result showed that 70.6% (51) of the isolates produced ESBL phenotypically while the PCR characterization of the 21 most resistant isolates showed that 90.5% (19) harbored the $CTXM_{15}$ gene, 81% (17) of the isolates encoded the TEM gene, 38.1% (8) harbored the OXA gene while one percent (1%) had the SHV gene. The susceptibility profile of the isolates to some selected betalactam antibiotics showed that 93.1% of the isolates were resistant to amoxicillin, 90.8% to cefpirome, 77% to cefotaxime, 69% to cefpodoxime, 36.8% to azetreonam while 26.4% were resistant to ceftriaxone.**Conclusions:** This study showed that among the commonly prescribed betalactam antibiotics, ceftriaxone (cephalosporin) and azetreonam (monobactam) are the most effective antibiotics and this calls for the need for review of the use of betalactam in hospitals, as most of the clinical isolates of *E. coli* evaluated in this study harbored gene(s) that encode for resistance to betalactams.

Author Disclosure Block:

J.A. Onaolapo: None.

Poster Board Number:

SATURDAY-267

Publishing Title:

Antibiotic Susceptibility Pattern of Organisms Isolated from Gall Bladder

Author Block:

A. Ghazal, A. Hannan, **S. Saleem**, S. Sarwar, U. Arshad; Univ. of Hlth. Sci.s Lahore, Lahore, Pakistan

Abstract Body:

Background: Pyogenic cholecystitis and cholangitis are common causes of cholestectomy. Culture reports are worthy for the planning of treatment with antibiotics and prophylaxis. There is very limited data available on it from Pakistan.**Methods:** In our study we collected 60 samples of Gallbladder with its bile. All the clinical samples were cultured directly on blood and MacConkey agar and also inoculated in RCM (Robertsom cooked medium) broth to support anaerobic organisms. The isolated organisms were identified by the standard morphological, cultural and biochemical profile. Antibiotic susceptibility was done by Kerby beur disc diffusion method and organisms were also screened for ESBL production.**Results:** Out of sixty patients, forty (66%) were positive for bacteria either in bile or gall bladder wall or in both gall bladder wall and bile. Gram negative microorganisms were most frequently isolated from 40 culture positive patients. The commonest gram negative bacteria were Pseudomonas aeruginosa (39% n=30), E.coli (25% n=19), Klebsiella pneumonia (13% n=10), Enterobacter clocae (12% n=09), Enterococcus fecalis (4% n=03), Enterobacter sakazakki (3% n=02), Enterobacter aerugenes (1.3% n=01), Citrobacter fereuddii (1.3% n=01), Proteus mirabullus (1.3% n=01). Organisms were mostly resistant to all cephalosporin and Augmentin due to ESBL production while Carabapanum, aminoglysides and combination drugs were found to be most effective.**Conclusions:** Significant culture positive rate shows the importance of obtaining cultures from Gall bladder wall and bile at the time of cholecystectomy so that appropriate antibiotics could be administrated and necessary changes could be made according to the antibiotic sensitivity tests to prevent serious complications of resistant pathogens and septicemia .

Author Disclosure Block:

A. Ghazal: None. **A. Hannan:** None. **S. Saleem:** None. **S. Sarwar:** None. **U. Arshad:** None.

Poster Board Number:

SATURDAY-268

Publishing Title:

Detection And Characterization Of Extended Spectrum Beta-Lactamase And Cabapenemase Genes Among Enterobacteriaceae In Lagos, Nigeria

Author Block:

K. O. AKINYEMI[1], B. A. Iwalokun[2], A. O. B. Oyefolu[1], C. O. Fakorede[1]; [1]Lagos State Univ., Ojo, Lagos, Lagos, Nigeria, [2]Natl. Inst. of Med. Res.,, Lagos, Nigeria

Abstract Body:

Background: The worldwide spread of extended spectrum beta -lactamase (ESBL) and cabapenemase producing gram negative bacteria in the family enterobacteriaceae remains a serious public health challenge in the light of the poor chemotherapeutic outcomes due to increasing multiple drug resistant (MDR) pathogens encountered in our hospitals and community in recent times. This study evaluated the presence of ESBL and Cabapenemase genes among enterobacteriaceae isolates and detected the genetic basis for production**Methods:** A total of one-hundred and twenty-seven enterobacteriaceae isolates (*Escherichia coli* = 57, *Klebsiella pneumoniae* = 43, *Salmonella* spp =27) were isolated from clinical samples by standard methods and were subjected to *in vitro* susceptibility test by disk diffusion methods. Isolates that were resistant to cefoxitin and third generation cephalosporin (3GC) were screened for ESBL {Double Disc Synergy Test Method (DDST). ESBL genes were detected by multiplex PCR and random amplified polymorphic DNA (RAPD) analysis was performed on the isolates by standard procedures.**Results:** In all over 60% of the enterobacteriaceae isolates produced ESBL and were MDR positive Specifically, 37/57 (64.9%) and 31/43 (69.8%) of *E.coli* and *K. pneumoniae* strains were ESBL positive respectively. Over 60% of *E. coli* and *K. pneumoniae* were MDR with with 15 and 11 resistant patterns observed respectively and were found to contain at least one of the three markers Bla-TEM, Bla-SHV, Bla-CTX. Three strains of *K. pneumoniae* developed resistant to carbapenem. None of the strains of Salmonella spp produced cabapenemase as done in one strain of *E. coli* in this study.**Conclusions:** The study revealed circulation of multiple drug resistant ESBL producing pathogens among enterobacteriaceae. It also indicated the emergence in patients of MDR *K. pneumoniae* with ESBL and cabapenemase production, thus poses further and serious challenges for the possibility of spread to other members of enterobacteriaceae in hospital and community.

Author Disclosure Block:

K.O. Akinyemi: None. **B.A. Iwalokun:** None. **A.O.B. Oyefolu:** None. **C.O. Fakorede:** None.

Poster Board Number:

SATURDAY-269

Publishing Title:

Colistin Resistance in *Enterobacter cloacae* and *Enterobacter aerogenes* Isolates from South Korea

Author Block:

Y-K. Hong, J-Y. Lee, K. Ko; SungKyunKwan Univ. Sch. Of Med., Suwon, Korea, Republic of

Abstract Body:

Background: *Enterobacter* spp. are common pathogens responsible for nosocomial infections, especially blood stream infections in intensive care units. Colistin is often the only therapeutic option to treat infections caused by multidrug-resistant (MDR) Gram-negative bacterial pathogens. Colistin resistance is rarely described in *Enterobacter* spp. compared with other pathogens.**Methods:** Antimicrobial susceptibility testing was performed for 211 *E. cloacae* and 143 *E. aerogenes* isolates. For genotyping, multilocus sequence typing (MLST) and AP typing were performed for colistin-resistant *E. cloacae* and *E. aerogenes* isolates, respectively. *pmrAB* and *phoPQ* genes, which are known to be associated with colistin resistance in other Gram-negative bacterial species, were sequenced and their amino acid alterations were identified. The isolates showing skip-well phenomenon were compared colistin-resistant and -susceptible isolates by time-kill assay and population analysis.**Results:** A total of 50 *E. cloacae* (23.7%) and six *E. aerogenes* (4.2%) isolates were resistant to colistin, and eight *E. cloacae* (3.8%) and three *E. aerogenes* (2.1%) isolates showed skip-well phenomena against colistin. The 50 colistin-resistant *E. cloacae* isolates showed diverse STs, and ST53 were the most common (eight isolates, 16.0%). In addition, the AP typing showed that six colistin-resistant *E. aerogenes* belonged to different clones. While the isolates of skip-well phenomenon showed similar to colistin-resistant isolates in time-kill assay, they were similar to colistin-susceptible isolates in population analysis. Most of colistin-resistant *E. cloacae* isolates showed amino acid alterations in *pmrB*, Thr295Ile.**Conclusions:** In this study, we report high colistin resistance rates especially in *E. cloacae*, and their diverse genotypes, indicating that colistin resistance occurred independently. In addition, we identified that PmrAB alteration was the common mechanism of colistin resistance in *E. cloacae.*

Author Disclosure Block:

Y. Hong: None. **J. Lee:** None. **K. Ko:** None.

Poster Board Number:

SATURDAY-270

Publishing Title:

Antibiotic (Abx) Combinations (Combi) for Ndm-Producing *Providencia rettgeri* & *Citrobacter spp.* Clinical Isolates

Author Block:

J. Teo[1], K. Lin[2], T-P. Lim[1], Y. Cai[1], W. Lee[1], T-H. Koh[1], T-T. Tan[1], A-L. Kwa[1]; [1]Singapore Gen. Hosp., SG, Singapore, [2]Natl. Univ. of Singapore, SG, Singapore

Abstract Body:

Background: Carbapenem resistance is increasing in Singapore. Aside from occurring in common Enterobacteriaceae like *E.coli*, *Klebsiella* & *Ente*robacter species, it has emerged in less common Enterobacteriaceae locally. We aim to identify effective abx combi for these highly resistant isolates.**Methods:** 3 clinically significant isolates were selected [*P. rettgeri* (EC1) from foot tissue, *C. sedlakii* (EC2) from urine, *C. freundii* (EC3) from blood]. All 3 harbored TEM/CTXM, AmpC & NDM. MICs were tested using CLSI broth dilution, except for fosfomycin (Etest). 24-h time-kill studies (TKS) were performed with approximately 5 log CFU/ml at baseline using maximally achievable clinical, unbound concentration (mg/L) of levofloxacin (L) (8), aztreonam (A) (24), cefepime (C) (50), piperacillin/tazobactam (Taz) (35/7), meropenem (M) (20), ertapenem (E) (15), rifampicin (R) (2), tigecycline (T), polymyxin B (P) (2) & fosfomycin (F) (160) alone & in combi. 34 M-, Az-, R-, Taz-, P- & F-based combis were tested.**Results:** EC1 was pandrug-resistant. EC2 & 3 remained susceptible to T, P & F. The MICs of Az, C, Taz, M & R were ≥32 mg/L for all isolates. L/E/T/P/F MICs were ≥32/≥32/16/≥32/128, ≥32/≥32/2/0.25/1 & 4/16/0.5/1/0.5 mg/L for EC1, 2, 3 respectively. In single Abx TKS, all drugs were not bactericidal (>3 log reduction from baseline) for all 3 isolates at 24h, including those with low MICs (P, T, F). AzE was the only bactericidal combi for EC1. For EC2, P-based combis (Az, Taz, M, R, T, F) were bactericidal. For EC3, P-based combis (L, Taz, E, M, R, F) & ME were bactericidal. The following combinations were synergistic - EC1 (AzTaz, AzF); EC2 (CP, CF); EC3 (MC, MAz, MTaz, MR).**Conclusions:** No one combi was bactericidal for all 3 isolates, in spite of similar beta-lactam resistance mechanisms. Selected P-based combis are effective if P MIC is ≤ 1 mg/L. E as a sacrificial drug may allow beta-lactams like Az & M to be used. Low MIC results in P,T,F susceptibility testing may not be sufficient to predict clinical success as a lack of bactericidal activity was observed in *in vitro* TK. While P & T heteroresistance has been reported, further work on emergence of resistance to F is warranted. In addition, profiling of other resistance mechanisms might be required to understand why select combinations were bactericidal.

Author Disclosure Block:

J. Teo: None. **K. Lin:** None. **T. Lim:** None. **Y. Cai:** None. **W. Lee:** None. **T. Koh:** None. **T. Tan:** None. **A. Kwa:** None.

Poster Board Number:

SATURDAY-271

Publishing Title:

Antimicrobial Activity of Tigecycline and Colistin Against Multi-drug Resistant Isolates of *Enterobacteriaceae*

Author Block:

V. Khare, F. Haider; Era's Lucknow Med. Coll. and Hosp., Lucknow, India

Abstract Body:

Background: In view of the increasing prevalence of multi-drug resistance in *Enterobacteriaceae*, there is a need to keep an eye on the sensitivity pattern of limited drugs available for their treatment. Carbapenems are being frequently prescribed to treat infections by Extended Spectrum Beta Lactamase (ESBL) producers and this has lead to an increased isolation of Carbapenem Resistant Enterobacteriaceae (CRE) isolates in our hospital. Tigecycline and Colistin are two important treatment options. This study was undertaken to evaluate the in-vitro activity of Tigecycline and Colistin against multi-drug resistant *Enterobacteriaceae* isolates.**Methods:** The study was carried out at Era's Lucknow Medical College and Hospital, a 1000 beded tertiary care hospital in Lucknow.The duration of the study was from January to December 2015. Antimicrobial susceptibility was done for all isolates of *Enterobacteriaceae* by disk diffusion (Kirby-bauer) as per CLSI 2015 guidelines and all suspected ESBL producers showing resistance to third generation Cephalosporins were confirmed by combined disk testing. Tigecycline was tested by using 15ug disk (Hi-Media) and Colistin was tested by using E test (Ezy MIC TM CL strips , Hi Media). Quality control was done using *E.coli* ATCC 25922. In view of the lack of CLSI guidelines for disk diffusion for Tigecycline, EUCAST 2015 guidelines were used.**Results:** 297 multi-drug resistant *Enterobacteriaceae* were isolated from various samples including pus, sputum, endotracheal aspirates etc. (excluding urine) from patients admitted in ICUs, HDU, surgical and medical wards of the hospital. These included 128 *Escherichia coli* , 108 *Klebsiella spp.* and 61 Others (*Enterobacter spp.* and *Citrobacter spp.*). Of these, Carbapenem resistance was 64% and 57.4% in *E.coli*, and *Klebsiella spp.* respectively with an overall resistance of 63.4%. Overall Tigecycline was resistant in 12 (4%) isolates, mostly *Klebsiella spp*. (8/12). When CRE were considered, Tigecycline resistance was 6.3%. None of the isolates showed resistance to Colistin.**Conclusions:** There is a need to test Tigecycline routinely in *Enterobacteriaceae* and we need to keep in mind the threat of emerging resistance.

Author Disclosure Block:

V. Khare: None. **F. Haider:** None.

Poster Board Number:

SATURDAY-272

Publishing Title:

Antimicrobial Resistance in Shiga Toxin- producing *Escherichia coli* (Stec) and Non-typhoidal *Salmonella* in Michigan

Author Block:

S. Mukherjee[1], P. Singh[1], R. Mosci[1], J. T. Rudrik[2], S. D. Manning[1]; [1]Michigan State Univ., East Lansing, MI, [2]Michigan Dept. of Hlth.and Human Services, Lansing, MI

Abstract Body:

Foodborne pathogens such as STEC and non-typhoidal *Salmonella* are a serious health burden in the US, with the CDC reporting approximately 265,000 cases and 1.2 million cases per year, respectively. Treatment of infections caused by these pathogens is hindered due to the emergence of resistance and the possibility of lateral transfer of resistance genes to other bacteria adds to the threat of antibiotic resistance. An accurate representation of the prevalence and mechanisms of resistance in Michigan is not present because antibiotic resistance has not been widely researched among STEC and systematic collection of susceptibility data for non-typhoidal *Salmonella* has not been carried out. Here, clinical isolates of STEC (N=398) and non-typhoidal *Salmonella* (N=107) were obtained from MDHHS and the frequency of resistance was determined using disk diffusion and broth microdilution respectively. Overall, 8.02% (15) of the STEC O157 isolates (N=187) and 10.4% (22) of the STEC non-O157 isolates (N=211) were resistant to at least one antimicrobial. Our study showed higher percentages of resistance to ampicillin and trimethoprim-sulfamethoxazole in STEC O157 isolates in Michigan, compared to national levels reported by NARMS. Genotypic studies showed that resistance to ampicillin for most isolates was not associated with chromosomal *ampC* while SNP genotyping of the O157 isolates demonstrated that the resistant isolates belonged to separate clades. Resistance to important antimicrobials was also observed in non-typhoidal *Salmonella*, with 10.27% of all isolates resistant to at least one antimicrobial. The frequency of resistance varied by serovar and 5.6% of all isolates were multidrug-resistant with four distinct resistance patterns. Future studies should focus on analyzing resistance trends for larger populations and identifying genotypic factors associated with resistance. Factors such as geographical location, serotypes & serovars, source of isolates etc. determine resistance frequencies and hence continuous monitoring of antibiotic resistance in enteric pathogens is important from a public health perspective to combat resistance.

Author Disclosure Block:

S. Mukherjee: None. **P. Singh:** None. **R. Mosci:** None. **J.T. Rudrik:** None. **S.D. Manning:** None.

Poster Board Number:

SATURDAY-273

Publishing Title:

Mepm and Cl Heteroresistance in *Enterobacteriaceae* Isolates in Japan

Author Block:

K. UECHI[1], T. TADA[2], K. SHIMADA[2], I. NAKASINE[1], T. KIRIKAE[2], J. FUJITA[1]; [1]Univ. Hosp. of the Ryukyus, Okinawa, Japan, [2]Natl. Ctr. for Global Hlth.and Med., Tokyo, Japan

Abstract Body:

Background: The rapid spread of carbapenem- resistant *Enterobacteriaceae* (CRE), with the emergence of colistin (CL)- resistant CRE, has become an urgent health concern worldwide. We described here that the first identification of two isolates, a MEPM- heteroresistant *Klebsiella pneumoniae* and a meropenem (MEPM)- resistant and CL- heteroresistant *Enterobacter cloacae*.**Methods:** We conducted active surveillance to find patients colonized with CRE in a university of Ryukyu hospital in Okinawa Japan. Rectal swabs were screened for CRE using ESBLs selective agar (in house), and CHROMagar KPC (CHROMagar: France) according to Laboratory Protocol for Detection of Carbapenem-Resistant or Carbapenemase-Producing, *Enterobacteriaceae*. As for the phenotype check, Modified Hodge Test (MHT) and CarbaNP test were performed. Drug susceptibility tests were done by both a two-fold dilution method and disk method / Etest according to CLSI guidelines. Antibiotics heteroresistance was defined as a phenomenon where subpopulations of presumed isogenic bacteria exhibited a range of susceptibility to MEPM or CL. The whole genomes of CRE isolates were determined by sequencing with Illumina's MiSeq.**Results:** Of 20 *Enterobacteriaceae* isolates, 8 showed MICs of MEPM (2-32 ug/ml). However, MHT and CarbaNP test were negative. of them, a *K. pneumoniae* isolate showed heteroresistance to MEPM, and an *E. cloacae* isolate did heteroresistance to CL as determined by using a disk method, but both did resistance to MEPM or CL, when determined by using a microdilution method. Whole genome sequence analyses revealed that the *K. pnemoniae* isolate harbored $bla_{\text{CTX-M-55}}$, $bla_{\text{TEM-1}}$ and $bla_{\text{SHV-11}}$ but not any carbapenemase- encoding genes, and that the *E. cloacae* did $bla_{\text{TEM-1}}$, $bla_{\text{SHV-12}}$ and $bla_{\text{ACT-2}}$ but not any carbapenemase-encoding genes. The CL- heteroresistant *E. cloacae* isolate had mutations in encoding genes of two-component systems *(pmrABC* and *phoPQ*) associated with CL resistance.**Conclusions:** This is the first identification of MEPM- heteroresistant *K. pneumoniae* and CL-heteroresistant *E. cloacae* in Japan.

Author Disclosure Block:

K. Uechi: None. **T. Tada:** None. **K. Shimada:** None. **I. Nakasine:** None. **T. Kirikae:** None. **J. Fujita:** None.

Poster Board Number:

SATURDAY-274

Publishing Title:

US Gulf-Like Toxigenic 01 *Vibrio cholerae* Causing Sporadic Cholera Outbreaks in China

Author Block:

Y. Luo[1], S. Octavia[2], D. Jin[1], J. Ye[1], Z. Miao[1], T. Jiang[1], S. Xia[1], R. Lan[2]; [1]Zhejiang Provincial Ctr. for Disease Control and Prevention, Hangzhou, China, [2]Univ. of New South Wales, Sydney, Australia

Abstract Body:

Background: Toxigenic O1 *Vibrio cholerae* causes pandemic cholera with the current 7th pandemic clone still causing epidemic or endemic cholera in developing countries. China has had no epidemic cholera reported in recent years.**Methods:** In this study we identified and characterised 76 non-7th pandemic O1 toxigenic *V. cholerae* isolates from diarrhoeal patients from 2009 to 2014 in Zhejiang Province, China. These isolates were analysed by multilocus sequence typing (MLST) and pulsed field gel electrophoresis (PFGE) to determine their overall genetic relatedness and their relationship to other O1 toxigenic strains.**Results:** By MLST, 19 sequence types (STs) were found. Thirty three isolates belonged to ST75 which also contains the US Gulf coast clone. The genomes of 13 isolates were also sequenced. PFGE and genome sequencing revealed multiple linked cases suggesting small outbreaks. Genome sequencing further showed that the US Gulf coast clone and the Chinese ST75 isolates can be separated into two distinct lineages, ST75a and ST75b. ST75b was the predominant O1 in our survey of O1 isolates collected from 2009 to 2014 in Zhejiang, China. The ST75b lineage carried unique features differing from the ST75a lineage with a CTX prophage identical to the 7th pandemic clone and carriage of a VSP-1. However, the VPI-1 encoding the toxin-coregulated pilus is very similar between ST75a and ST75b strains with only six SNP differences.**Conclusions:** ST75b strains may pose significant threat to public health and epidemiological surveillance is required to further understand their epidemic potential.

Author Disclosure Block:

Y. Luo: None. **S. Octavia:** None. **D. Jin:** None. **J. Ye:** None. **Z. Miao:** None. **T. Jiang:** None. **S. Xia:** None. **R. Lan:** None.

Poster Board Number:

SATURDAY-275

Publishing Title:

Whole Genome Sequence Analysis of Antimicrobial Resistant *E. coli* Isolated from Irish Retail Meats

Author Block:

C. Brehony[1], B. Mahon[1], S. Kavanagh[1], M. Cormican[1], R. H. Madden[2], C. Kelly[2], L. Moran[2], C. Carroll[1], J. Bray[3], K. A. Jolley[3], M. C. J. Maiden[3], D. Morris[1]; [1]Natl. Univ. of Ireland, Galway, Ireland, [2]Food Sci. Branch, Agri-Food & BioSci.s Inst., Belfast, United Kingdom, [3]Univ. of Oxford, Oxford, United Kingdom

Abstract Body:

Background: The appropriate and inappropriate use of antimicrobial agents in human and veterinary medicine and agriculture, for several decades has resulted in the emergence and dissemination of antimicrobial resistant bacteria. Such antimicrobial resistance is recognised globally as a major public health concern. This study examined the role of food in the dissemination of antimicrobial resistant bacteria. **Methods:** 600 raw meat samples were purchased from retail outlets throughout the island of Ireland Nov 2013 - Sept 2014. All samples were tested for antimicrobial resistant E. coli (AREC) and 496 AREC isolates were obtained. All AREC isolates were characterised by a series of phenotypic and genotypic tests and based on these results 96 isolates were selected for whole genome sequencing. Isolate genomes were hosted in and analysis was performed using a local installation of BIGSdb. The O25b:H4-ST131 EC958 reference genome was used for whole genome pairwise comparisons amongst the genomes.**Results:** 54 isolates were extended spectrum beta lactamase (ESBL) producers. 33 of these had blaCTX-M, 19 blaTEM, 18 blaSHV, and none had blaOXA. There were 46 7 locus sequence types (STs) and 12 clonal complexes. There were 61 ribosomal sequence types (rSTs) with the most common, rST-1544, accounting for 15% of isolates. There was an association with this rST and source as 92.9% were from chicken samples (13/14). The majority of rSTs were diverse but there were some groupings and, the largest of which, rST-1544, consisted of two groups: ST162 (469 Cplx) and ST1431 (Unassigned). 4981 loci were compared for all genomes and the fewest differences (n=39) were found in 2 chicken meat isolates within the rST-1544 grouping. None of the rST-1544 group were ESBL producers.**Conclusions:** AREC found in Irish retail meats were relatively diverse. Although blaCTX-M predominated amongst ESBL E. coli other ESBL genes were also common. Interrogation of whole genome databases for emerging antimicrobial resistance determinants provided a rapid low cost approach to evaluate the extent of dissemination prior to recognition and will become a more powerful tool as databases expand.

Author Disclosure Block:

C. Brehony: None. **B. Mahon:** None. **S. Kavanagh:** None. **M. Cormican:** None. **R.H. Madden:** None. **C. Kelly:** None. **L. Moran:** None. **C. Carroll:** None. **J. Bray:** None. **K.A. Jolley:** None. **M.C.J. Maiden:** None. **D. Morris:** None.

Poster Board Number:

SATURDAY-276

Publishing Title:

Plasmid Mediatedcolistin Resistance Encoding Gene Mcr-1 Not Detected in *E. coli* Isolated inIreland from Retail Meats and People

Author Block:

C. Brehony[1], B. Mahon[1], S. Kavanagh[1], M. Cormican[1], R. H. Madden[2], C. Ludden[1], C. Kelly[2], L. Moran[2], C. Carroll[1], J. Bray[3], K. A. Jolley[3], M. C. J. Maiden[3], D. Morris[1]; [1]Natl. Univ. of Ireland, Galway, Ireland, [2]Food Sci. Branch, Agri-Food & BioSci.s Inst., Belfast, United Kingdom, [3]Univ. of Oxford, Oxford, United Kingdom

Abstract Body:

Background: The plasmid-mediated colistin resistance gene, mcr-1, was first described in November 2015 by Liu et al in E. coli isolated from food, animals and humans. Colistin resistance is a major cause for concern as it is one of the very few antimicrobial agents available for treatment of infection associated with carbapenemase producing Enterobacteriaceae. Subsequently, others have isolates E. coli harbouring mcr-1 from meat and human specimens, as well as in Salmonella Typhimurium isolated from food samples. Prior to these findings, colistin resistance was found to be mediated by mutations in chromosomally encoded genes and the dissemination of such resistance required the spread of carrier organisms. However, the plasmid can be carried by many different genera of bacteria, raising concerns of the rapid and widespread dissemination of colistin resistance. **Methods:** Whole genome sequences of 96 E. coli isolates collected from retail meats in Ireland and Northern Ireland (November 2013 - September 2014) and 96 E. coli isolates collected (2005 - 2011) primarily from residents of long term care facilities were examined. Genomes were hosted in and analysis was performed using a local installation of BIGSdb. The mcr-1 sequence of Liu et al was used to conduct a BLASTN search against all 192 Irish human and food E. coli genomes.**Results:** No significant matches were returned indicating the absence of the gene in this set of genomes.**Conclusions:** The absence of mcr-1 in this limited collection of food and human genomes from Ireland suggests that it has not yet been disseminated widely in food animals or humans in this region though further testing including retrospective will be required to confirm this. Use of colistin and related compounds in human health care on the island of Ireland is very limited. The recent finding of a transferable colistin resistance mechanism in China and more recently in Europe and North America is of major concern and underlines the necessity of continuous surveillance.

Author Disclosure Block:

C. Brehony: None. **B. Mahon:** None. **S. Kavanagh:** None. **M. Cormican:** None. **R.H. Madden:** None. **C. Ludden:** None. **C. Kelly:** None. **L. Moran:** None. **C. Carroll:** None. **J. Bray:** None. **K.A. Jolley:** None. **M.C.J. Maiden:** None. **D. Morris:** None.

Poster Board Number:

SATURDAY-277

Publishing Title:

Emerging Biofilm Forming Enteroinvasive *E. coli* (Bf-Eiec): Analysis of Whole Genome Sequencing

Author Block:

J. Iqbal, O. G. Gomez-Duarte; Vanderbilt Univ., Nashville, TN

Abstract Body:

Emergent intestinal *E. coli* pathogens pose a serious threat to human health. We have recently identified an emergent biofilm forming enteroinvasive *E. coli* (BF-EIEC) from Colombian children suffering from severe diarrhea. EIEC are closely related to *Shigella* spp. and cause Shigellosis-like disease. Biofilm is a unique feature to this BF-EIEC emergent pathotype and we believe that it may be involved in pathogenesis. The objective of this study was to identify known EIEC and emergent virulence genes and pathogenicity islands in this BF-EIEC strain by whole genome analysis. Genome sequencing was performed on the BF-EIEC 52.1 strain using NextGen Illumina and Pacific Biosciences (PacBio) platforms. Data was analyzed using different publically available software and databases. BF-EIEC genome consists of 5,193,449 bp long chromosomal DNA, 269589 bp large invasion plasmid and 45997bp long additional plasmid. BF-EIEC genome analysis identified 57 genomic islands, 26 pathogenicity islands and 12 prophage integrations. Virulence associated genes identified in the genome included: i) type three secretory system (TTSS), which plays a central role in EIEC/*Shigella* cell invasion and cell spread; ii)cell adhesion and colonization, iii) biofilm formation, iv) survival in host cell and v) cytotoxicity. Other putative gene function detected included, iron chelation, stress response, evasion of immune response, inflammation and proteolytic degradation of host factors. Antibiotic resistance-associated genes were also detected including efflux pumps and antibiotic degradation enzymes genes among others. These studies indicate that BF-EIEC 52.1 strain contains virulence genes known to be associated with *Shigella* and EIEC pathogenesis, especially the TTSS genes. The identification of virulence genes and multiple episomal elements may provide evidence of horizontal transfer from other pathogens. Sequencing of BF-EIEC genome not only shed light on the evolution and pathogenesis of this emerging *E. coli* but may also open opportunities for exciting new discoveries.

Author Disclosure Block:

J. Iqbal: None. **O.G. Gomez-Duarte:** None.

Poster Board Number:

SATURDAY-278

Publishing Title:

Phylodynamics of *Vibrio cholerae* O1 Isolated in Rural Coastal Area of Bangladesh

Author Block:

S. M. Rashed[1], S. Y. Choi[2], N. A. Hasan[2], D. Ceccarelli[1], M. Alam[3], D. A. Sack[4], R. B. Sack[4], A. Huq[1], R. R. Colwell[1]; [1]Univ. of Maryland, College Park, MD, [2]CosmosID Inc, Rockville, MD, [3]icddr,b, Dhaka, Bangladesh, [4]Johns Hopkins Bloomberg Sch. of Publ. Hlth., Baltimore, MD

Abstract Body:

Vibrio cholerae, the causative agent of cholera, is autochthonous to the aquatic environment worldwide. Cholera remains a serious threat to millions in several countries of Asia, Africa, and South America. Microevolution of the bacterium in the aquatic reservoir and human host enables emergence of new clones, which contribute significantly to the dynamics of cholera. Cholera outbreaks in the coastal areas of Bangladesh are triggered by seasonal influences annually. In this study, 23 clinical and environmental isolates of *V. cholerae* O1 collected during 2010 - 2011 in Mathbaria, Bangladesh, were characterized by genotyping, antimicrobial susceptibility, and whole-genome sequencing and compared with outbreak isolates from Asia, Africa, and South America. All *V. cholerae* O1 isolates possessed the virulence genes *tcpA*, *ctxA*, *hlyA*, *ace*, and *zot*. Of 23 isolates, 17 had classical cholera toxin gene (*ctxB1*), whereas six had Haiti type cholera toxin (c*txB7*). Resistance to streptomycin (S), nalidixic acid (NA), and trimethoprim/sulfamethoxazole (SXT) was uniformly present in the isolates, whereas tetracycline (TE) and erythromycin (E) resistance varied. Multidrug resistant isolates from Bangladesh showed three distinct patterns:(1) S^R, NA^R, SXT^R (65.21%); (2) S^R, NA^R, TE^R, SXT^R (30.43%); (3) S^R, NA^R, E^R, TE^R, SXT^R (4.35%), and all contained SXT integrase gene int_{SXT}. Minimum inhibitory concentration (MIC) for ciprofloxacin was 0.25 - 0.75 µg/mL (MIC_{50} = 0.38 µg/mL and MIC_{90} = 0.5 µg/mL), suggesting reduced susceptibility to ciprofloxacin. Phylogenetic analysis based on single nucleotide polymorphisms (SNPs) showed *V. cholerae* O1 isolates of Bangladesh belong to two different clusters in the seventh pandemic clade, correlating with cholera toxin genes, *ctxB1* or *ctxB7*. The six *V. cholerae* O1 isolates from Bangladesh harboring *ctxB7* were closely related to Haitian strains isolated during 2010 - 2012, whereas those possessing *ctxB1* showed clonal relatedness to *V. cholerae* O1 altered El Tor from Asia and Africa.

Author Disclosure Block:

S.M. Rashed: None. **S.Y. Choi:** None. **N.A. Hasan:** None. **D. Ceccarelli:** None. **M. Alam:** None. **D.A. Sack:** None. **R.B. Sack:** None. **A. Huq:** None. **R.R. Colwell:** None.

Poster Board Number:

SATURDAY-279

Publishing Title:

Emergence of a Highly Virulent *Shigella sonnei* Clone in California

Author Block:

V. Kozyreva[1], G. Jospin[2], A. Greninger[1], J. Eisen[2], V. Chaturvedi[1]; [1]California Dept. of Publ. Hlth., Richmond, CA, [2]Univ. of California, Davis, CA

Abstract Body:

Background: *Shigella sonnei* has caused large outbreaks in California recently. We constructed and analyzed *S.sonnei* whole genome sequencing (WGS) database to probe public health significance of these outbreaks. **Methods:** Sixty one CA *S.sonnei* human isolates (1980-2015), were identified by standard methods. WGS of paired-end libraries was performed using Illumina MiSeq Sequencer. *De novo* assembly was done on CLCbio GW 8.0.2. Genomes were annotated with prokka v1.1, CGE online tools, and JGI IMG database. *In silico* MLST was performed with CGE tool. Phylogenetic analysis was performed based on SNP calling with min coverage 30x and min base quality 200; and using PhyloSift pipeline, COG- and Pfam-based clustering. **Results:** All *S.sonnei* CA isolates except one were assigned to sequence type 152 (ST152) and clustered together based on PhyloSift phylogeny. In both historical and contemporary outbreak isolates, following resistance markers were identified: *bla*$_{TEM1}$*; bla*$_{OXA2}$; *aph(3'')-Ib; aph(6)-Id; aac(3)-IId; aadA1/2*; *mphA*; *sul1/2*; *catA1*; *dfrA1/8/12*; *tetA/B.* A single amino acid substitution in DNA-gyrase *gyrA* gene conferring a low-level FQ resistance was present in few isolates prior to 2008. However, recent isolates had a combination of double mutation of *gyrA* and one mutation of topoisomerase IV *parC* genes, mediating high-level FQ resistance. Significantly, most recent isolates carried Shiga toxin gene *stx1* in association with a novel lambdoid phage. The phage is homologous with the phage found in *E.coli* O104:H4 outbreak strain from 2011 German outbreak. Hierarchical clustering based on COG and Pfam profiles shows that CA *S.sonnei* isolates cluster together with non-O157 *E.coli*, particularly with European O104:H4 STEC, and are distinct from other *S.sonnei* isolates. **Conclusions:** *S.sonnei* ST152, a historical clone in CA, has increased its antibiotic resistance and virulence. The data suggests a high potential for more future *S.sonnei* ST152 outbreaks with serious consequences.

Author Disclosure Block:

V. Kozyreva: None. **G. Jospin:** None. **A. Greninger:** None. **J. Eisen:** None. **V. Chaturvedi:** None.

Poster Board Number:

SATURDAY-280

Publishing Title:

Population Genomics of *Campylobacter jejuni* Associated with Sheep Abortion

Author Block:

Z. Wu[1], B. Periaswamy[2], S. Chen[2], O. Sahin[1], Q. Zhang[1]; [1]Iowa State Univ., Ames, IA, [2]Genome Inst. of Singapore, Singapore, Singapore

Abstract Body:

Campylobacter infection is a leading cause of ovine abortion worldwide. Outbreaks of *Campylobacter*-associated ovine abortion are traditionally caused by multiple species and strains of this zoonotic organism. However, a highly-pathogenic and tetracycline-resistant clone of *C. jejuni* (clone SA for sheep abortion) has recently emerged as the persistently predominant cause of sheep abortions in the USA. This finding is quite surprising and indicates that clone SA is ecologically well adapted and pathologically hypervirulent in sheep as a result of its increased fitness. To ascertain the evolution and the adaptive genetic changes responsible for the emergence and predominance of clone SA, we analyzed the whole genomes of 99 *C. jejuni* isolates from a historical collection encompassing clinical isolates from sheep abortion cases collected from geographically distinct regions since early 1990s. A whole-genome phylogeny confirmed the clonal expansion of clone SA, with a monophyletic origin close to ST50 *C. jejuni* strains in clonal complex 21, a reduced recombination subsequent to the emergence and a reduced haplotype diversity, and led to an estimate of its emergence in the 1970s. However, the non-clone SA abortion isolates are paraphyletic and genetically indistinguishable from non-abortion isolates. Population genetic tests suggested that mutations of an outer membrane protein and integration of *tet(O)* into the genomes might have favored the expansion of clone SA. These findings provide significant insights into the molecular genetic events underlying its emergence, evolutionary history and increased fitness.

Author Disclosure Block:

Z. Wu: None. **B. Periaswamy:** None. **S. Chen:** None. **O. Sahin:** None. **Q. Zhang:** None.

Poster Board Number:

SATURDAY-281

Publishing Title:

Clade-Specific Production Of Hemolysin Bl And Nonhemolytic Enterotoxin Nhe By *bacillus Cereus* Group Dairy Isolates

Author Block:

J. Kovać, R. A. Miller, L. M. Carroll, J. Jian, D. J. Kent, M. Wiedmann; Cornell Univ., Ithaca, NY

Abstract Body:

Background: The *Bacillus cereus* Group comprises the human pathogens *B. cereus* (BC), *B. cytotoxicus* and *B. anthracis* (BA), the insect pathogen *B. thuringiensis* (BT), the psychrotolerant food spoilage organism *B. weihenstephanensis,* probiotic *B. toyonensis* and environmental *B. mycoides* and *B. pseudomycoides*. *B. cereus* Group isolates often contaminate rice, meat, and dairy products and can present food poisoning and food spoilage risks. Due to the high genetic similarity of different BC group species, identification at the species level and prediction of the risk an isolate poses for human health remains a challenge.**Methods:** We have sequenced 22 dairy-associated *B. cereus* Group isolates on Illumina HiSeq platform (2x100 bp) and compared their draft genomes with 47 closed reference genomes acquired from NCBI Genome to i) identify 22 dairy-associated isolates at the species level based on maximum likelihood phylogeny (RaxML version 8) constructed using core genome SNPs (kSNP v2), ii) identify virulence gene profiles specific for individual species using protein BLAST, and iii) investigate the correlation of *hblACD* an *nheABC* gene presence/absence and sequence variability with hemolysin BL and nonhemolytic enterotoxin NHE production at 37°C, as determined using Duopath Cereus Enterotoxin kit.**Results:** A maximum likelihood tree revealed nine distinct clades and whole-genome sequence (WGS) data enabled the identification of monophyletic *B. weihenstephanensis* and *B. toyonensis*, but failed to identify polyphyletic BC, BA and BT. Production of two diarrheal toxins, hemolysin BL (HblC) and nonhemolytic enterotoxin (NheB) revealed evidence for differences in hemolysin BL production among phylogenetic clades. Among the 4 BC/BT clades, one clade was negative for toxin production due to the gene absence, two were positive, and one showed variable production among isolates. Isolates from the *B. weihenstephanensis* clade carried atruncated variant of hemolysin BL binding component (*hblA*) and did not produce hemolysin BL toxin.**Conclusions:** Results of our analyses suggest that WGS data has the potential to be used to predict the diarrheal toxin production and pathogenicity of *B. cereus* Group isolates.

Author Disclosure Block:

J. Kovać: None. **R.A. Miller:** None. **L.M. Carroll:** None. **J. Jian:** None. **D.J. Kent:** None. **M. Wiedmann:** None.

Poster Board Number:

SATURDAY-282

Publishing Title:

Toward Diagnosis of Diarrheal Disease Using 16s Rrna Taxonomic Profiling and Metagenomics

Author Block:

A. V. Pena Gonzalez[1], J. K. Hatt[1], G. Trueba[2], W. Cevallos[3], J. N. S. Eisenberg[4], K. Levy[5], K. T. Konstantinidis[1]; [1]Georgia Inst. of Technology, Atlanta, GA, [2]Univ. San Francisco de Quito, Quito, Ecuador, [3]Univ. Central de Ecuador, Quito, Ecuador, [4]Univ. of Michigan, Ann Arbor, MI, [5]Emory Univ., Atlanta, GA

Abstract Body:

Diarrheal disease continues to be a significant cause of mortality around the world. Consequently, better characterization of the causative agents and the compositional alterations that occur in the gut microbiome during the pathological state continue to be critical. In this study we used a 16S rRNA taxonomic profiling combined with shotgun metagenomic sequencing to investigate several cases of human diarrheal disease of undefined etiology in individuals from Northern Coastal Equador, and compare them with diarrheal cases where the etiological agent was determined by PCR. By sequencing the V4 region of the 16SrRNA gene, we assessed changes in OTU richness, evenness and diversity in both diseased and healthy samples from the same individual and determined the effects of additional demographic variables such as age, race, and geographical distance. In addition, we developed bioinformatics approaches to characterize the pathogens at the strain level in the cases where the etiological agent was identified. We observed a significant reduction in OTU richness and diversity in individuals with diarrhea of undefined origin when compared with their matched healthy samples, and higher species richness in individuals from more remote communities (Pearson's R= 0.30756). Despite these differences, multidimensional analysis failed to separate healthy from diseased samples. When the causative agent was specified, such as in acute rotavirus or E. coli+ infections, we were able to distinguish healthy from diarrheal samples and detect significant differences in the taxonomic composition and metabolic potential in the gut microbiome. Together, these results highlight the potential of using sequence-based methods to understand better the impact of diarrheal diseases in the diversity of the gut microbiota.

Author Disclosure Block:

A.V. Pena Gonzalez: None. **J.K. Hatt:** None. **G. Trueba:** None. **W. Cevallos:** None. **J.N.S. Eisenberg:** None. **K. Levy:** None. **K.T. Konstantinidis:** None.

Poster Board Number:

SATURDAY-283

Publishing Title:

Differential Evolution in Mitochondrial Metabolism and Invasion-related Proteins in Cryptosporidium

Author Block:

S. Liu[1], D. M. Roellig[2], Y. Guo[1], N. Li[1], M. A. Frace[2], K. Tang[2], L. Zhang[3], **Y. Feng**[1], L. Xiao[2]; [1]East China Univ. of Sci. and Technology, Shanghai, China, [2]CDC, Atlanta, GA, [3]Henan Agricultural Univ., Zhengzhou, China

Abstract Body:

Cryptosporidium spp. are important apicomplexan parasites causing diarrhea in humans and animals. They differ from each other in host specificity and predilection sites. The genomes of three *Cryptosporidium* species, including *C. parvum*, *C. hominis* and *C. muris*, have been sequenced and are available in public databases. In this study, we sequenced the genomes of *C. ubiquitum* and *C. andersoni* and conducted a comparative genomic analysis of *Cryptosporidium* spp. All *Cryptosporidium* species share common genomic features such as gene content and organization. Major metabolic differences among *Cryptosporidium* spp. are in mitochondrial energy metabolism. Comparing to *C. parvum* and *C. hominis*, *C. andersoni* possesses more aerobic metabolic pathways, whereas *C. ubiquitum* has further reduction in mitochondrial metabolism. Minor metabolic differences among *Cryptosporidium* spp. were seen in the biosynthesis of *N*-glycan and GPI-anchor precursors and inter-conversion of amino acids and nucleotides. In addition to these metabolic differences, the comparative genomic analysis has shown distinct host cell invasion-related proteins among *Cryptosporidium* species. In agreement with the previously observed reduction in the number genes of secreted MEDLE and insulinase-like proteins in the subtelomeric regions of chromosomes 5 and 6 in *C. hominis*, these genes are lost in *C. ubiquitum* and *C. andersoni*. Mucin-type glycoproteins associated with host cell adhesion and invasion are highly divergent between the gastric *C. andersoni* and intestinal *Cryptosporidium* species analyzed. Thus, rapidly evolving mitochondrial metabolism and secreted invasion-related proteins could be responsible for the distinct host specificity and tissue tropism among *Cryptosporidium* spp.

Author Disclosure Block:

S. Liu: None. **D.M. Roellig:** None. **Y. Guo:** None. **N. Li:** None. **M.A. Frace:** None. **K. Tang:** None. **L. Zhang:** None. **Y. Feng:** None. **L. Xiao:** None.

Poster Board Number:

SATURDAY-284

Publishing Title:

Wgs Analysis of *Salmonella enterica*, Isolated from Food Animals to Identify Antibiotic, Metal, and Biocide Resistance Genes

Author Block:

S. K. Gupta[1], C. R. Jackson[1], P. T. Desai[2], M. McClelland[2], J. G. Frye[1]; [1]United States Natl. Poultry Res. Ctr., Athens, GA, [2]Univ. of California, Irvine, CA

Abstract Body:

Background: Whole-genome sequencing (WGS) has superior sensitivity to conventional genetic techniques for the investigation of infections and outbreaks. WGS data of *Salmonella enterica* isolated from food animal sources were analyzed to identify resistance genes (RG) for various antibiotics, metals, and biocides.**Methods:** *Salmonella enterica* (n = 194) with different patterns of antibiotic resistance isolated from food animals (chicken, swine, etc.) were selected and subjected to WGS using an Illumina HiSeq 2500. Sequences were assembled using A5-miseq assembler and annotated with Prokka. Contigs were analyzed using ARGANNOT, BacMet metal and BacMet biocide RG databases, to detect known antibiotic, metal, and biocide RG, respectively.**Results:** A total of 883 antibiotic RG from nine different antibiotic classes (aminoglycosides (Agly), beta-lactamase, tetracycline, phenicol, sulfonamide, macrolide (Mls), fosfomycin, rifampicin (Rif), trimithoprim) were detected in the *S. enterica* isolates. Approximately 36% of the 194 isolates had at least one antibiotic RG and 3% had 14 antibiotic RG detected. Ninety-nine percent of the isolates were resistant to Agly. Rif (*arr*2) and Mls (*ere*A/*mef*A/*mph*) RG, which are rarely reported in *Salmonella,* were detected in 1% and 3% of isolates, respectively. Major metal and biocide RG were detected in 100% of the strains: *mdt*A/B/C:zinc; *sit*A/B/C:arsenic; *mod*A/B/C/E:tungsten/molybdenum; *teh*A/B:Quaternary ammonium compounds/EtBr; *ydd*g:methyl viologen; and *sod*A:Se/H_2O_2. Gold (*ges*A/B/C), copper (*pco*A/B/D/R/S) and silver (*sil*A) RG were detected in 94%, 22% and 21% of isolates, respectively. The *bae*S/R-*tol*C genes were detected in all the isolates and may confer multidrug and metal resistance.**Conclusions:** WGS can be implemented as a powerful tool to detect different RG. Our findings suggest that the selection or maintenance of resistance in *Salmonella* isolated from food animal sources could be due to exposure to metals in addition to antibiotics. WGS data can improve our ability to predict resistance patterns, emerging outbreaks, and detect markers for molecular diagnostics.

Author Disclosure Block:

S.K. Gupta: None. **C.R. Jackson:** None. **P.T. Desai:** None. **M. McClelland:** None. **J.G. Frye:** None.

Poster Board Number:

SATURDAY-285

Publishing Title:

Development of a Genome Sequence Database for Foodborne Pathogenic Bacteria in Hong Kong

Author Block:

H. Kwan; The Chinese Univ. of Hong Kong, Shatin, Hong Kong

Abstract Body:

Background: In Hong Kong, *Salmonella enterica* serovars Enteritidis and Typhimurium and *Vibrio parahaemolyticus* causes most of outbreaks. Foodborne diseases demand medical attention and reduce productivity. Besides, the emerging multidrug-resistant (MDR) *S.* Typhimurium strains has complicated treatments, posing a serious threat to the public. Here we constructed the first genome sequence database for Hong Kong foodborne pathogens *S.* Typhimurium (ST) and *V. parahaemolyticus* (VP).**Methods:** We seqeunced genomes of 20 Hong Kong clinical isolates of ST and 10 VP Hong Kong isolates using Roche 454 FLX-Titanium pyrosequencer. Sequencing reads were assembled using GS De Novo Assembler (Roche Diagnostics). Nucleotide-level variations, such as single nucleotide polymorphism (SNP), insertion and deletion (indel) , and inversion among local isolates and several foreign strain sequences were identified using GS Reference Mapper (Roche Diagnostics). Protein genes were predicted from the assemblies using GeneMark and other prediction tools.**Results:** VP genome assemblies have 130 to 1205 contigs of N50 contig length from 7kb to 305kb whereas the assembly of ST yielded 32 to 90 contigs of N50 contig length between 192kb and 458kb. The foodborne pathogen genome database was constructed based on the Ensembl genome annotation system using Perl scripts. Genome DNA sequences can be input into the platform via a user-friendly web-based interface. A suite of useful computational tools was added for comparative sequence analysis and data mining such as multi-locus sequence typing (MLST), restriction enzyme typing and detection of genetic variations using local and foreign typed VP and ST genome data. Genome-wide comparison based on 2151 conserved genes SNPs to show the relationship between local and foreign VP O3:K6 isolates of MLST sequence type ST3. We used 7962 SNPs to differentiate local ST isolates from the foreign ST isolates with MLST sequence type ST19. We have identified 184 orthologs as candidate multi-drug resistance determinants in ST.**Conclusions:** We have developed the first genome sequence databases of Hong Kong foodborne pathogenic bacteria as a user-friendly platform to analyze the genome sequences and annotations of the two pathogens, *Vibrio parahaemolyticus* and *Salmonella enterica* serotype Typhimurium. The platform will be used to monitor trends of foodborne outbreaks.

Author Disclosure Block:

H. Kwan: None.

Poster Board Number:

SATURDAY-286

Publishing Title:

Comparative Genomics Reveals *Cyclospora cayetanensis* Possesses Coccidia-Like Metabolism and Invasion Components but Unique Surface Antigens

Author Block:

S. Liu[1], L. Wang[1], H. Zheng[2], Z. Xu[1], D. M. Roellig[3], N. Li[1], M. A. Frace[3], K. Tang[3], L. Zhang[4], Y. Feng[1], **L. Xiao**[3]; [1]East China Univ. of Sci. and Technology, Shanghai, China, [2]Chinese Natl. Human Genome Ctr. at Shanghai, Shanghai, China, [3]CDC, Atlanta, GA, [4]Henan Agricultural Univ., Zhengzhou, China

Abstract Body:

Background: *Cyclospora cayetanensis* is an apicomplexan that causes diarrhea in humans. The investigation of foodborne outbreaks of cyclosporiasis has been hampered by a lack of genetic data and poor understanding of pathogen biology. In this study we sequenced the genome of *C. cayetanensis* and compared it with genomes of other apicomplexans. **Results:** The genome organization, metabolic capabilities and potential invasion mechanism of *C. cayetanensis* are very similar to those of *Eimeria tenella*. Propanoyl-CoA degradation, GPI anchor biosynthesis, and N-glycosylation are some apparent metabolic differences between *C. cayetanensis* and *E. tenella*, suggesting that metabolism within monoxenous coccidian parasites is highly conserved. The similar repertoire of host cell invasion-related proteins possessed by all coccidia suggests that *C. cayetanensis* has an invasion process similar to the one in *T. gondii* and *E. tenella*. However, the significant reduction in the number of rhoptry protein kinases, phosphatases and serine protease inhibitors indicates that the capabilities of host cell regulation especially the interruption of host cell nuclear activities are limited in monoxenous coccidia, especially *C. cayetanensis*. *C. cayetanensis* does not possess any SAG1 related proteins seen in *T. gondii*, has only a few homologs of the TA4-type SAG surface antigens seen in *E. tenella*, and may use a different family of surface antigens in host cell recognition and attachment. **Conclusions:** Our findings indicate that *C. cayetanensis* possesses coccidia-like metabolism and invasion components but unique surface antigens. Amino acid metabolism and post-translation modifications of proteins are some major differences between *C. cayetanensis* and other apicomplexans. *C. cayetanensis* likely has only limited in modulating host cell signaling pathways. The whole genome sequence data of *C. cayetanensis* improve our understanding of the biology and evolution of this major foodborne pathogen and facilitate the development of intervention measures and advanced diagnostic tools.

Author Disclosure Block:

S. Liu: None. **L. Wang:** None. **H. Zheng:** None. **Z. Xu:** None. **D.M. Roellig:** None. **N. Li:** None. **M.A. Frace:** None. **K. Tang:** None. **L. Zhang:** None. **Y. Feng:** None. **L. Xiao:** None.

Poster Board Number:

SATURDAY-287

Publishing Title:

Clinical Metagenomics for Rapid Detection of Diarrheagenic Pathogens and Characterization of Intestinal Microbiome in Health and Disease

Author Block:

N. A. Hasan[1], K. Moffat[2], P. Subramanian[2], R. Isom[2], S. Choi[2], S. M. Rashed[1], J. Li[2], H. Li[2], A. Huq[3], G. Nair[4], T. Ramamurthy[4], R. R. Colwell[1]; [1]Univ. of Maryland, College Park, MD, [2]CosmosID, Rockville, MD, [3]Univ. of Maryland, College park, MD, [4]Natl. Inst. of Cholera and Enteric Diseases, Kolkata, India

Abstract Body:

Versatility, reduced cost, and faster turnaround time of sequencing suggest high-throughput, and high-resolution metagenomic analysis can be used as a diagnostic tool for clinical management of intestinal and extra-intestinal infectious diseases. A retrospective case-control study of 65 fecal samples was done that included 45 patients (17 known and 28 unknown disease etiology) and 20 healthy individuals. Samples were collected during a systematic surveillance at the National Institute of Cholera and Enteric Diseases, Calcutta, India. Samples were sequenced using Illumina GAIIx and results analyzed by CosmosID MetaGenID bioinformatics package and curated GenBook® databases. Metagenomic analyses identified pathogens, *i.e., Vibrio cholerae, Shigella, Escherichia coli,* and *Campylobacter,* in samples of known etiology, whereas samples of unknown etiology contained predominantly members of the *E. coli* super family, namely pathogenic *E. coli*, *Shigella,* and *Salmonella enterica,* as well as *Aeromonas caviae*, *C. jejuni,* and *Cryptosporidium*. Multiple pathogens were detected. Patients were differentiated from healthy individuals based on organism abundance and diversity. Patients exhibiting profound watery diarrhea carried pathogens primarily of the *Escherichia coli* complex. The antibiotic resistome that was detected, comprised major classes of antibiotics, including beta-lactam, aminoglycoside, tetracycline, and trimethoprim. Microbiomes of healthy individuals of Indian descent were markedly different from those of Western descent and contained low numbers of pathogens. This study showed the intestinal microbiome could differentiate healthy, diseased, asymptomatic carriers, and individuals in early stages of disease. Clinical metagenomics potentially will revolutionize diagnostics, prophylactics, and therapeutics of infectious disease.

Author Disclosure Block:

N.A. Hasan: D. Employee; Self; CosmosID. **K. Moffat:** D. Employee; Self; CosmosID. **P. Subramanian:** D. Employee; Self; CosmosID. **R. Isom:** D. Employee; Self; CosmosID. **S. Choi:** D. Employee; Self; CosmosID. **S.M. Rashed:** None. **J. Li:** D. Employee; Self;

CosmosID. **H. Li:** D. Employee; Self; CosmosID. **A. Huq:** None. **G. Nair:** None. **T. Ramamurthy:** None. **R.R. Colwell:** A. Board Member; Self; CosmosID.

Poster Board Number:

SATURDAY-288

Publishing Title:

Genomic Insight Into A Sustained Multi-Centre Outbreak Of Yersinia Pseudotuberculosis

Author Block:

S. Baines, A. Gonçalves da Silva, T. Seemann, G. Carter, T. Stinear, B. Howden, D. Williamson; The Univ. of Melbourne, Melbourne, Australia

Abstract Body:

Yersinia pseudotuberculosis (Yptb) is a zoonotic pathogen and the second most common cause of human yersiniosis after *Y. enterocolitica*. Although most infections with Yptb are thought to be sporadic, outbreaks of foodborne infection have been reported. Between August 14th and November 1st 2014 a sustained outbreak of yersiniosis due to Yptb occurred across all major cities in New Zealand (NZ), with a total of 220 laboratory-confirmed cases.In addition to an epidemiological investigation, a detailed genomic examination of the outbreak was undertaken, with whole genome sequencing (WGS) being performed on 128 NZ isolates of Yptb. This collection represented isolates recovered during the outbreak period (n=95), as well as 10 years prior (n=28), and one year following (n=5) the outbreak. These isolates were further supplemented with the publicly available genome sequences of 52 global Yptb.Reconstruction of the population structure for these 180 Yptb isolates identified that the outbreak was caused by a single clade of Yptb belonging to the globally prominent multi locus sequence type 42. This extremely genetically restricted clade comprised 82 of the 95 outbreak isolates; the population differing by only 13 core genome single nucleotide polymorphisms. Furthermore, WGS enabled the exclusion of isolates that were genetically distinct from the outbreak clade but thought to be associated based on epidemiological classification. Amongst the outbreak isolates no temporal, geographic, or genomic signals were detected to identify potential transmission networks, instead supporting a single point source contamination of the food chain, with subsequent distribution of contaminated produce across NZ.This outbreak of Yptb in NZ represents, to date, the largest laboratory-confirmed outbreak of Yptb globally. The use of WGS enabled robust classification of outbreak and non-outbreak isolates, and provided strong support for a single point-source contamination event as the cause of this outbreak. WGS is particularly useful in investigating outbreaks of yersiniosis, for which existing typing methods are non-discriminatory and should be used routinely as a first-line epidemiological tool in investigating outbreaks of yersiniosis.

Author Disclosure Block:

S. Baines: None. **A. Gonçalves da Silva:** None. **T. Seemann:** None. **G. Carter:** None. **T. Stinear:** None. **B. Howden:** None. **D. Williamson:** None.

Poster Board Number:

SATURDAY-289

Publishing Title:

Molecular Epidemiology and Resistome Analysis of Toxigenic *Clostridium difficile* in Asia-Pacific Countries

Author Block:

K. Aoki, Y. Ishii, K. Tateda; Toho Univ., Ota-ku,, Japan

Abstract Body:

Background: *C. difficile* epidemiology is well studied in North America and Europe, whereas in Asia-Pacific countries is less often performed. In this study, we analyzed *C. difficile* isolated from diarrhea stool samples collected prospectively in 12 Asia-Pacific countries using next-generation sequencing (NGS). **Methods:** Four hundred and fourteen *C. difficile* were isolated from diarrhea stool samples between April, 2014 and February, 2015. To determine the genome-wide draft sequence, all isolates were analyzed by MiSeq (Illumina). Multilocus sequence typing (MLST), the presence of toxin encoding genes(*tcdA*, *tcdB* and *tcdC*), the binary toxin encoding genes (*cdtA* and *cdtB*) and antimicrobial resistance genes including substitution of quinolone-resistance determining regions (QRDRs) in *gyrA* and *gyrB* and Rifamycin-resistance determining regions (RRDRs) in *rpoB* were analyzed from the draft genome contigs.**Results:** The 378 isolates were classified into 53 STs. Thirty-six isolates were assigned novel STs (ST numbers not yet assigned). ST37 (associated with PCR ribotype[RT]017) was the most dominant ST with 62 isolates (15.0%), followed by ST17(53 isolates, 12.8%), ST2(38 isolates, 9.2%) and ST8(30 isolates, 72%). Characteristically, ST37 isolates were *tcdA*(-), *tcdB*(+), *cdtA*(-),*cdtB*(-) and had no mutations in *tcdC*. Among the six countries with over 38 *C. difficle* isolated, the dominant STs were ST2(Australia[20/50 isolates]), ST8(Taiwan[21/88]), ST17(Korea[34/88] and Japan[15/46]), ST35(China[6/45]) and ST37(Thailand[19/38]), respectively. Hypervirulent clones belonging to ST1(3 isolates[RT027]) , ST5(5 isolates[RT023]), ST11(6 isolates[RT078]), ST35(1 isolates[RT002]) and ST42(1 isolates[RT106]) had an insertion, a deletion or a point mutation in *tcdC* resulting in frameshift or nonsense mutation. Isolates belonging to ST1, ST5, ST11 and ST228 were *cdtA* and *cdtB* positive. The QRDR mutations of GyrA Thr82Ile, GyrB Asp426Asn/Val were harbored in 150(36.2%) and 32(7.7%) of all isolates, respectively. Two hundred (48.3%), 147(35.5%) and 87(21.0%) of all isolates possessed *erm(B)*, *tet(M)* and *aac(6')-aph(2")*, respectively. There were no mutations in RRDRs.**Conclusions:** Compared with North America and Europe, Asia-Pacific countries had major differences in hypervirulent clones. Interestingly, ST37 lacking *tcdA* was the most common clone.

Author Disclosure Block:

K. Aoki: None. **Y. Ishii:** None. **K. Tateda:** None.

Poster Board Number:

SATURDAY-290

Publishing Title:

Missed Opportunities for Treatment: Implementation of a Molecular Diagnostic for Pediatric Acute Gastroenteritis (Ge): The Filmarray Gi Panel Impact Study

Author Block:

K. M. Bourzac[1], GI IMPACT Study Team, A. T. Pavia[2], K. C. Chapin[3]; [1]BioFire Diagnostics, Salt Lake City, UT, [2]Univ. of Utah, Salt Lake City, UT, [3]Rhode Island Hosp., Providence, RI

Abstract Body:

Background: The FilmArray Gastrointestinal (GI) Panel (BioFire Diagnostics) is a fully automated ~1hr PCR-based test for identification of 22 different pathogens from stool specimens. Previous studies have demonstrated very good analytical sensitivity and specificity. Here, we present preliminary results of a pre/post intervention study to measure patient outcomes following implementation of the FilmArray GI Panel for children with gastroenteritis (GE) presenting to Emergency Departments (ED). **Methods:** Children with chief complains of GE were recruited at five US EDs. Data collection included pre- and post-enrollment questionnaires and medical chart abstraction. Stool specimens were collected at the time of presentation to the ED or within two days. During the pre-intervention period, FilmArray GI Panel testing was performed but results were not reported to clinicians or families. Clinicians ordered standard of care (SOC) GI testing as they determined appropriate. The number of additional healthcare encounters, total healthcare costs, antibiotic prescription rates and appropriateness, lost time from school/work/daycare, and subject satisfaction with management of the illness were analyzed.**Results:** To date, 310 subjects have been enrolled in the pre-intervention period and returned stool for analysis. FilmArray identified 222 specimens positive for one or more potential pathogens (222/310; 72%). In contrast, SOC testing was ordered in only 56 subjects, yielding 17 positives (17/310; 5%). Significantly, the FilmArray identified treatable pathogens in 63/310 (20%) subjects that were not diagnosed by clinicians including *Campy* (N=8), ETEC (N=10), *Shigella* (N=25), *Crypto* (N=10), and *Giardia* (N=9). Other organisms identified only by FilmArray and missed by SOC that could potentially modify care included *Salmonella* (N=9) and STEC (N=10). Viruses were identified in 101.**Conclusions:** These preliminary data suggest that clinicians in EDs may under-diagnose treatable infections in pediatric patients with GE. Data collected during the pre- and post-intervention periods will measure whether the availability of this information will lead to improved patient outcomes.

Author Disclosure Block:

K.M. Bourzac: D. Employee; Self; BioFire Diagnostics. **A.T. Pavia:** B. Collaborator; Self; BioFire Diagnostics. **K.C. Chapin:** B. Collaborator; Self; BioFire Diagnostics.

Poster Board Number:

SATURDAY-291

Publishing Title:

Increased Incidence of Infections Due to *Streptococcus anginosus* between 2006 and 2015

Author Block:

E. O. Hand[1], B. Duhon[1], C. Pickard[1], S. Hallowell[1], K. Traugott[2]; [1]The Univ. of Texas at Austin Coll. of Pharmacy, San Antonio, TX, [2]Univ. Hlth.System, San Antonio, TX

Abstract Body:

Background: Recently a rise in infections due to *Streptococcus anginosus* was noted at our institution, particularly a rise in bacteremias. It was unclear whether this increased incidence was purely anecdotal or part of a larger trend in changing epidemiology. The purpose of this study was to evaluate the rates and types of infections caused by *S. anginosus* at our institution over the preceding decade.**Methods:**A query of the microbiology laboratory database was performed to identify all positive cultures growing *S. anginosus* from January 2006 to October 2015. This list was then organized by year and site of infection.**Results:**A total of 1667 separate cultures were identified, corresponding to 1129 unique patients. In 2006, 76 patients accounted for 107 positive cultures. The number of individual patients with a positive culture for *S. anginosus* doubled by 2014 (n=158) and number of cultures increased to 235. The most common site of infection in all years studied was described as “body fluid” or “exudate” (n=1031/1667, 62%) with the number of positive cultures increasing from 72 in 2006 to 140 in 2014. Respiratory infections were relatively uncommon (n=50/1667, 3%) but increased from three cultures to 14 between 2006 and 2014. Notably, the number of positive blood cultures increased from 8 in 2006 to 24 in 2014. When 2015 data were extrapolated to a full calendar year, both number of patients (n=190) and number of positive cultures (n=279) were project to be the highest values in any year studied.**Conclusions:**Infections caused by *S. anginosus* have continually risen at our institution between 2006 and 2015. The cause of this increase has yet to be fully elucidated, but further investigations will focus on changes in antimicrobial susceptibility over the time frame as well as predisposing factors that may put patients at increased risk of *S. anginosus* infections.

Author Disclosure Block:

E.O. Hand: None. **B. Duhon:** None. **C. Pickard:** None. **S. Hallowell:** None. **K. Traugott:** None.

Poster Board Number:

SATURDAY-292

Publishing Title:

Characterization of *S. pyogenes* and *S. dysgalactiae* Isolated from Rheumatic Heart Disease Patients

Author Block:

N. Gishen[1], D. Asrat[2], Y. Woldeamanuel[3], A. Habte[4], T. Riaz[5], S. Frye[5], E. Gedlu[2], T. Tønjum[5], A. Aseffa[4]; [1]Debre Berhan Univ., Debre Berhan, Ethiopia, [2]Addis Ababa Univ., Addis Ababa, Ethiopia, [3]Addis Ababa Univ., Debre Berhan, Ethiopia, [4]Armauer Hansen Res. Inst., Addis Ababa, Ethiopia, [5]Oslo Univ. Hosp., Oslo, Norway

Abstract Body:

Background: Among children diagnosed to have chronic rheumatic valvular heart disease many were observed to develop recurrence of rheumatic fever despite the secondary prophylaxis. This study determined *emm* gene type and protein profile of *S. pyogenes* and *S. dys*galactiae isolated from children having rheumatic heart disease who were receiving on-going prophylaxis.**Methods:** The *emm* gene typing of four *S. pyogenes* and another four *S. dysgalactiae subsp.* Equisimilis was determined by PCR sequencing. Protein of these strains was analysed by Q-exactive mass spectrometry.**Results:** Six different *emm* gene types were identified including one newly discovered subtype (*stGrobn.1*) associated with an *S. dysgalactiae subsp.* Equisimilis isolate which possess Lancefield group A. The streptococcal glycosyltransferase in strain *S. pyogenes* emm 68.2 had N-linked glycosylation carrying a unique N-acetylhexoseamine (HexNAc)-deoxyhexose; a novel post-translational modification (PTM) not previously recognized. **Conclusions:** The strains from S. pyogenes and S. dysgalactiae assessed here had different emm gene types and PTM patterns, enabling distinction of subgroups of these closely related strains with pathogenic potential in rheumatic fever disease.

Author Disclosure Block:

N. Gishen: None. **D. Asrat:** None. **Y. Woldeamanuel:** None. **A. Habte:** None. **T. Riaz:** None. **S. Frye:** None. **E. Gedlu:** None. **T. Tønjum:** None. **A. Aseffa:** None.

Poster Board Number:

SATURDAY-293

Publishing Title:

Serotype Distribution of *Streptococcus pneumoniae* in Clinical Isolates after the Introduction of Pneumococcal Vaccines in Japan

Author Block:

H. Miyazaki[1], N. Midorikawa[1], R. Shibuya[2], A. Haque[1], R. Kobayashi[1], K. Ohkusu[1], T. Oguri[3], T. Matsumoto[1]; [1]Tokyo Med. Univ., Tokyo, Japan, [2]Saiseikai Yokohama Tobu Hosp., Yokohama, Japan, [3]Tokyo Hlth.Care Univ., Tokyo, Japan

Abstract Body:

In Japan, introduction of pneumococcal vaccines has reduced invasive diseases caused by *S. pneumoniae*. For children, the seven-valent pneumococcal conjugate vaccine (PCV7) was licensed in 2009, which was replaced by PCV13 in 2013. For adults, the 23-valent pneumococcal polysaccharide vaccine (PPSV23) is being used and PCV13 was licensed in June, 2014. However, relative increase in non-vaccine serotypes has been reported since the introduction of these vaccines. To assess the dynamic seroepidemiology of this pathogen, capsular serotyping using multiplex PCR was performed on 150 pneumococcal isolates obtained from a hospital in Yokohama, Japan during October 2014 to March 2015. One hundred ten isolates (73.3%) were obtained from sputum, and others from samples like; bronchial lavage, nasopharyngeal discharge, blood, cerebrospinal fluid, and otorrhea. Seventy-nine strains (52.6%) were isolated from children under 5 years of age, forty strains (26.7%) were from patients ≧65 years old, and others were from age group between 5 to 64 years. The strains from under 5 years included 1 (1.2%) PCV7-targeted serotype, 13 (16.4%) PCV13 serotypes, and 48 (60.7%) non-PCV13 serotypes. Predominant serotypes in isolates from children were 35B (non-PCV13 serotype), serogroup 15 (non-PCV13 serotypes), and 19A (PCV7 but non-PCV13). These results indicate replacement of the vaccine types by non-vaccine types. Although increasing trend of serotype 19A isolates in the post-PCV7 period was still observed, this serotype is predicted to decrease. Whereas, the strains isolated from ≧65 years included 7.5% PCV7 serotypes, 30.0% PCV13 serotypes, 37.5% PPSV23 serotypes, and 17.5% non-PPSV23 serotypes. These results suggest emergence of indirect effects following introduction of PCV7 for children. PPSV23 serotypes in the ≧65 years population are predicted to decrease, because immunization program of the age based recommendation has been launched in 2014. Predominant serotypes in the strains from age group between 5 to 64 years were 3 and 19A, which are both PCV13 and PPSV23 serotypes.Continuous monitoring of pneumococcal serotypes is necessary to follow the changing epidemiology of pneumococcal diseases.

Author Disclosure Block:

H. Miyazaki: None. **N. Midorikawa:** None. **R. Shibuya:** None. **A. Haque:** None. **R. Kobayashi:** None. **K. Ohkusu:** None. **T. Oguri:** None. **T. Matsumoto:** None.

Poster Board Number:

SATURDAY-294

Publishing Title:

A Rapid, Reference-Free Tool for Simplifying Whole Genome Strain Typing

Author Block:

K. E. Simmon[1], M. A. Fisher[2]; [1]ARUP Lab., Salt Lake City, UT, [2]Univ. of Utah, Salt Lake City, UT

Abstract Body:

Background: Comparing bacterial strains is important for outbreak investigation. Whole genome sequencing (WGS) has the potential to provide more clinically actionable information than current methods, however the data analysis and interpretation are often more complex. WGS strain typing is typically performed by alignment to a reference genome, but some strains may have DNA regions not present in the reference, leading to sub-optimal alignment and strain comparison. We developed a tool called StrainTypeMer (STM) that performs rapid, reference-free comparison of strains using kmers. STM can compare multiple strains in minutes and generates easy to interpret similarity tables displaying percent identity between strains. **Methods:** Raw sequence files were processed to remove adapters and poor quality reads. Rarefaction analysis to determine optimal sequence coverage was performed by calculating the number of reads required to obtain the predicted kmer quantity. STM was developed using python and requires jellyfish [github.com/gmarcais/Jellyfish]. We assessed STM's performance for typing >125 isolates from both internal and previously published data comparing PFGE and WGS using a reference-based approach. **Results:** Rarefaction curves show that nearly all kmers were observed when average sequence coverage is ≥25X. Strain comparisons are performed in ~30 min for ~20 strains, with a rapid mode allowing comparisons in <5 min. Dendrograms created from kmer distance matrices show strain relationships very similar to those derived from reference genome alignment. Mean kmer identity was 99.8% for replicates. Mean kmer identity for PFGE categories of identical, closely/possibly related, and different were, 99.2%, 94.6%, and 58.3%, respectively. **Conclusions:** Unlike current methods, STM does not require a reference genome, allowing comparison of sequences present in strains of interest but not in the reference. Additional features include the: 1) Ability to filter kmers to allow separate comparison of the pan- and core-genomes; 2) Creation of compact data files that can be used to create screenable strain libraries; 3) Ability to directly correlate with MLST. STM demonstrates that kmer-based approaches can facilitate rapid and simple reporting for strain typing, which will ultimately allow for wider adoption of WGS epidemiological techniques in diagnostic laboratories.

Author Disclosure Block:

K.E. Simmon: None. **M.A. Fisher:** None.

Poster Board Number:

SATURDAY-295

Publishing Title:

First Report of *Candida auris* in the American Continent: Clinical and Microbiological Data of 18 Episodes of Candidemia

Author Block:

B. Calvo[1], **A. S. A. Melo**[2], M. Hernandez[1], E. C. Francisco[2], J. Meis[3], A. L. Colombo[2];
[1]SAHUM, Maracaibo, Venezuela, Bolivarian Republic of, [2]EPM-UNIFESP, Sao Paulo, Brazil, [3]Dep Med Microbiol Radboudumc, Nijmegen, Netherlands

Abstract Body:

Background: *Candida auris* has been described as an emerging fungal human pathogen that may cause invasive infections in risk populations including critically ill and eventually cancer patients exposed to broad-spectrum antibiotics and invasive medical procedures. Nosocomial *C. auris* infections has been recently described in Korea, India and South Africa. The real prevalence of this pathogen may be underestimated once *C. auris* has been misidentified as *C. famata* or *C. haemulonii* when tested by phenotypic commercial systems, instead of molecular methods. Here we first describe in America the isolation of *C. auris* from 18 critically ill patients admitted in a single institution.**Methods:** We studied 18 blood stream isolates of *C. auris* from different patients admitted at a tertiary medical center in Maracaibo: "Servicio Aotónomo Hospital Universitario de Maracaibo" from March, 2012 and July, 2013. Species identification was initially obtained by the Vitek 2C system and further by ITS rDNA sequencing. Susceptibility tests were performed by using the CLSI broth microdilution method and 5 antifungal drugs.**Results:** All cases were critically ill pediatric (13) and adult (5) patients (26D median age), who had been previously exposed to antibiotics and multiple invasive medical procedures including central venous catheter (100%) and surgery (10 out of 18). Clinical management included catheter removal and antifungal therapy. Thirteen patients (72%) survived up to 30 days after developing candidemia. The isolates were identified as *C. haemulonii* by Vitek 2C, but the molecular method identified all of them as *C. auris.* All strains were considered resistant to FLC and VOR, but were susceptible to AMB, 5-FC and AND. **Conclusions:** This is the first report of *C. auris* in the American continent enrolling adult and pediatric critically ill patients. All isolates were resistant to triazoles but exhibited high susceptibility to candin. Crude mortality rate was lower than previously reported in the literature due to the large participation of children as well as prompt CVC removal and early therapy with appropriate antifungal therapy.

Author Disclosure Block:

B. Calvo: None. **A.S.A. Melo:** None. **M. Hernandez:** None. **E.C. Francisco:** None. **J. Meis:** None. **A.L. Colombo:** None.

Poster Board Number:

SATURDAY-296

Publishing Title:

Notable Trend of Increase in Azole Non-Susceptible *Candida tropicalis* Causing Invasive Candidiasis in China, August 2009 to July 2014

Author Block:

X. Fan, M. Xiao, H. Wang, Y-C. Xu; Peking Union Med. Coll. Hosp., Beijing, China

Abstract Body:

Background: Worldwide, *C. tropicalis* has become the first to fourth leading cause of invasive candidiasis (IC). Here we report a notable and continuous increase in azole non-susceptibility among *C. tropicalis* causing IC in China in the past half-decade.**Methods:** Between August 2009 and July 2014, 585 *C. tropicalis* isolates were collected from 10 hospitals from the China Hospital Invasive Fungal Surveillance Net (CHIF-NET) study. The *in vitro* susceptibility of isolates to nine antifungal drugs - fluconazole, voriconazole, itraconazole, posaconazole, caspofungin, micafungin, anidulafungin, amphotericin B and 5-flucytosine - was determined by Sensititre YeastOne YO10 methodology. Current available species-specific clinical breakpoint or epidemiological cut-off values were used for interpretation of results.**Results:** Overall, 22.1% and 19.5% of isolates were non-susceptible to fluconazole and voriconazole, respectively, with 10.4% of the isolates showing cross-resistance to both. Over five years, there was a significant decrease in azole susceptibilities, particularly during the last two years. As shown in the Figure, the non-susceptible rate of *C. tropicalis* isolates to fluconazole and voriconazole continuously increased from 10.4% to 42.6% for fluconazole, and from 10.4% to 39.1% for voriconazole. The prevalence of fluconazole-voriconazole cross-resistant isolates also increased from 6.6% to 21.7%. Moreover, although all isolates remained of wild-type phenotype to itraconazole and posaconazole, the GM MIC and MIC_{50} values for these two drugs also rose by over two fold, and the MIC_{90} values had a 4-fold increase in the study period. In comparison, all *C. tropicalis* isolates included in the present study were of wild-type to amphotericin B, and only 0.7% of isolates were of non-wild type phenotype to 5-flucytosine. Over 99% of the isolates remained susceptible to all three echinocandin agents tested. In addition, during the five years of surveillance, there were no significant changes (within ±1 dilution) in MIC_{50}, MIC_{90}, and GM MIC values for these drugs.**Conclusions:** Our findings show an unusual high-level of fluconazole and voriconazole resistance, and a significant trend of decreasing azole susceptibility. Further study is needed to identify the contributing factors.

Author Disclosure Block:

X. Fan: None. **M. Xiao:** None. **H. Wang:** None. **Y. Xu:** None.

Poster Board Number:

SATURDAY-297

Publishing Title:

Seroepidemiologic Study of Canine Leptospirosis in Northern Taiwan During 2008-2015

Author Block:

C-H. Hsu, C-S. Lin; Natl. Taiwan Univ., Taipei, Taiwan

Abstract Body:

Background: Leptospirosis is an important infectious disease in dogs and humans with worldwide distribution. In Taiwan, canine leptospirosis is easily ignored and underreported because of its variable clinical manifestations and difficulty of diagnosis. The microscopic agglutination test (MAT) is the most common diagnosis method for this disease. Our aim is to investigate the serovars of canine leptospirosis in northern Taiwan from 2008-2015 and to compare the characteristics of different MAT titers.**Methods:** Data were extracted and analyzed from medical records at the National Taiwan University Veterinary Hospital.**Results:** Among 159 suspected cases, 78 serum positive cases (MAT titer≧100) and 81 serum negative cases were identified. The positive rate of MAT titer≧400, which was considered as acute infection, was 24.4% (19/78). Twelve pathogenic serovars of serum positive cases were identified, including Canicola, Icterohaemorrhagiae, Shermani, Lyme, Pyogenes, Australis, Bataviae, Inadai, Javanica, Pomona, Tarassovi, and Grippotyphosa; besides, 21 cases were multiple serovars infection. The most common serovars among these were Icterohaemorrhagiae (23.6%), Shermani (20.0%) and Canicola(18.2%). The mean value of serum concentrations of liver enzymes (ALKP, ALT and AST), total bilirubin, blood urea nitrogen (BUN), creatinine and white blood cell count were elevated in most serum positive cases. Among serum positive cases, there was also no statistically significant difference in age, breed, gender, location and serum biochemistry parameters between titer ≧400 and titer <400 while the mean value of platelet count was lower than normal range in titer≧400.**Conclusions:** We concluded that serovar Shermani should be the candidate for vaccine because of its high seroprevalence in northern Taiwan. Since the clinical features and the severity of the disease showed a similar pattern in the two positive titer groups, it is suggested not to underestimate the care and treatments for the patients with titer <400. Moreover, dogs can used as sentinels or indicators for human pathogenic Leptospira spp. , knowing the seroprevalence, risk factors, CBC and biochemical data may improve the prevention of canine leptospirosis and public health.

Author Disclosure Block:

C. Hsu: None. **C. Lin:** None.

Poster Board Number:

SATURDAY-298

Publishing Title:

Polio Legacy on Epidemiology of Polio Case Based Surveillance in Ethiopia, from 2007-2014

Author Block:

F. HAILEMARIAM, **F. HAILEMARIAM**, A. ADEM; WHO, ADDIS ABABA, Ethiopia

Abstract Body:

Background: Polio was one of the most dreaded childhood disease mainly affecting under five years of age, one in 200 infection leads to irreversible paralysis among those paralyzed, 5%-10% die when their respiratory muscles become paralyzed. Since the global polio eradication initiative was launched in 1988 the number of cases was fallen by 99% (from 350,000 to 359 cases) only two counties in the world remain epidemic Pakistan & Afghanistan. The disease is targeted to eliminate by 2018. This study was conducted to determine the prevalence, age distribution, seasonal variation and regional distribution of Polio Cases in Ethiopia, 2007-2014. **Method:** Any person with acute flaccid paralysis was suspected Polio case. Stool sample for each suspected AFP case and demographic information was collected from suspected cases throughout the country, 2007-2014 onset of paralysis. Stool samples were tested for the presence of poliomyelitis by PCR to confirm. Demographic data and results were entered to data base and analysed by Epi Info software. **Results:** Among a total of 17359 stool samples collected and tested for Polio Suspected, 676 were suspected positive. The highest positivity rate 173 was seen in 2013, and the lowest 46 in 2011 From the Suspected Polio case 98% are all Sabin type and 2%(15) Wild type 1 Polio have been found Mostly in 2013 (9 Cases) and last case been in January 2014 and NEPNT rate highest in 2007 11.4% and lowest 4.5% in 2012. **Conclusion:** Ethiopia is Free from Polio for the past 2 years. To maintain zero Polio Improved routine and campaign Polio immunization and cross border vaccination and strong ongoing active case surveillance are recommended for communicable diseases. And Document this Polio Legacy so as it will be also helpful for other vaccine preventable disease like Measles which is endemic in Ethiopia.

Author Disclosure Block:

F. Hailemariam: None. **F. Hailemariam:** None. **A. Adem:** None.

Poster Board Number:

SATURDAY-299

Publishing Title:

Surveillance for West Nile Virus in Southwestern Nigeria

Author Block:

W. F. Sule[1], D. Oluwayelu[2], R. Adedokun[2], N. Rufai[3], L. Hernandez-Triana[4], K. Mansfield[4], L. Thorne[4], F. McCracken[4], N. Johnson[4]; [1]Osun State Univ., Osogbo, Nigeria, [2]Univ. of Ibadan, Ibadan, Nigeria, [3]Lagos Polo Club, lagos, Nigeria, [4]Animal and Plant Hlth. Agency, Surrey, United Kingdom

Abstract Body:

Background: To investigate role of West Nile virus in undifferentiated fevers (UF) in Southwestern Nigeria we undertook surveillance for the virus in humans, horses and mosquitoes.**Methods:** Blood samples were collected from consecutively selected unvaccinated horses (159) and humans, including horse grooms (213), at polo clubs and health care facilities, respectively in Lagos and Ibadan, southwest Nigeria. Mosquitoes were collected from around stables within polo clubs. Seroprevalence was assessed in horse and human sera by WNV IgG/IgM using ELISA and PRNT while human blood samples were screened for malaria and typhoid fever. Mosquitoes were sorted and analyzed by pan-flavivirus real-time RT-PCR (qRT-PCR).**Results:** WNV IgM was not detected in any horse sera. However, 143 (89.9% [95% CI: 85.3-94.6]) had WNV IgG. Group-specific seroprevalence rates were high and associated with study location (p=0.02) and horse breed (p=0.001). A similar situation was observed for human sera with 156 (73.2% [95% CI: 67.3-79.2]) being positive for WNV antibody. Being clinically ill was a covariate (p=0.001) of WNV seropositivity. "Active" and "non-active" participants had comparable (p=0.21) prevalence of 74.6% and 62.5%, respectively. Forty-five percent (18/40) of febrile participants had antibodies to only WNV suggesting an association with UF. Participants aged 18-66 years had higher (p=0.008) prevalence (75.8%) than those ≤ 17 years (47.4%). Participants showed an increased frequency of being WNV positive (75.5%) than *Plasmodium* (33.5%) and *S. typhi/paratyphi* (39.9%). PRNT confirmed that 50.0% and 10.5% of tested horses and humans respectively had neutralizing antibody. A total of 4,112 female mosquitoes (*Culex,* 98.98% and *Aedes,* 1.02%) were tested but no WNV RNA was detected in any sample.**Conclusions:** There is evidence for WNV in southwestern Nigeria with high seroprevalence observed in horses and humans. Seroprotection was higher among horses than humans with evidence that WNV infection contributed to UF in the study area. No evidence of WNV infection in tested mosquitoes, predominance of a competent vector - *Culex quinquefasciatus* - suggests a source of West Nile virus transmission.

Author Disclosure Block:

W.F. Sule: None. **D. Oluwayelu:** None. **R. Adedokun:** None. **N. Rufai:** None. **L. Hernandez-Triana:** None. **K. Mansfield:** None. **L. Thorne:** None. **F. McCracken:** None. **N. Johnson:** None.

Poster Board Number:

SATURDAY-300

Publishing Title:

Risk Factors for Long-Term Central Venous Catheter Infection Failure among Cancer Patients

Author Block:

M. P. Freire[1], P. Figueiredo[2], A. Zerati[1], K. Ibrahim[1], D. Peixoto[1], P. Bonazzi[1], A. Kono[1], M. Vieira[1], L. Souza[1], P. Hoff[1], E. Abdala[1], L. C. Pierrotti[1]; [1]Inst. do Câncer do Estado de São Paulo, São Paulo, Brazil, [2]São Paulo Univ., São Paulo, Brazil

Abstract Body:

Infections related to long-term central venous catheter (LTCVCI) have a great impact on cancer patients' survival.The goal of this study is to analyze the risk factors for failure in treatment of LTCVCI in an oncology population. **Methods:** We analyzed all LTCVCI episodes identified through active surveillance from January/2009 to December/2014. Patients who did not receive 24 hours of effective treatment or who lost follow up before the end of LTCRI treatment were excluded. Failure was defined as persistence of positive culture or persistence of clinical signs of infection after 7 days of effective therapy, evolution to complications (embolization or endocarditis), death during therapy or recurrence of LTCVCI by the same microorganism. Independent variables analyzed were: type of tumor, gender, age, totally implantable or semi-implantable catheter, site of insertion, presence of neutropenia, type of infection, infection by multidrug resistant (MDR) bacteria, agent of infection, time lapsed before the start of effective therapy, outpatient treatment, catheter removed, lock-therapy, and catheter thrombosis. Univariate statistical analysis was performed by Chi-square or Mann-Whitney, while logistic regression was used for multivariate analysis. **Results:** A total of 268 LTCVCI were identified, 9 were excluded. The types of infections were: incisional in 26 cases (10.0%), pocket in 45 (17.4%) and bloodstream infection in 188 (72.5%). Failure of initial treatment occurred in 64 (24.7%) cases and recurrence in 17 (6.6%); deaths related to LTCVCIs occurred in 40 (15.4%) patients. Multivariate analysis for failure treatment of all types of LTCVCI identified infections by *P. aeruginosa (PA)* or *A. baumannii (AB)* (*P* 0.02, OR 2.41 (1.12-5.18)) as risk, and incisional infection as protective factor (*P* 0.05, OR 0.30 (0.09-0.89)). Multivariate analysis for failure of catheter related bloodstream infection identified infection by *PA* or *AB* (P 0.06, OR 2.27 (0.96-5.41)) as risk and lock-therapy (P 0.01, OR 0.39 (0.18-0.83)) as protective factor.**Conclusions:** The use of lock-therapy has a positive impact on outcome of LTCVCI. Not only *PA* but also *AB* as agent of LTCRI may lead to a worse outcome of cancer patients with LTCVCI.

Author Disclosure Block:

M.P. Freire: None. **P. Figueiredo:** None. **A. Zerati:** None. **K. Ibrahim:** None. **D. Peixoto:** None. **P. Bonazzi:** None. **A. Kono:** None. **M. Vieira:** None. **L. Souza:** None. **P. Hoff:** None. **E. Abdala:** None. **L.C. Pierrotti:** None.

Poster Board Number:

SATURDAY-301

Publishing Title:

Clinical Scoring System to Differentiate *Pneumocystis pneumonia* versus Rituximab-Induced Interstitial Lung Disease in Lymphoma Patients Receiving Rituximab-Containing Chemotherapy

Author Block:

S. Park, M. Kim, W. Choi, Y. Jang, T. Kim, S. Hong, J. Jung, Y. Chong, D. Yoon, H. Sung, S-O. Lee, S-H. Choi, Y. Kim, C. Suh, J. Woo, S-H. Kim; Asan Med. Ctr., Seoul, Korea, Republic of

Abstract Body:

Background: It is difficult to differentiate *P. jirovecii* pneumonia (PCP) from rituximab-induced interstitial lung disease (RILD) in lymphoma patients with diffuse pulmonary infiltrates who are receiving rituximab-containing chemotherapy. Invasive diagnostic work-up often permits definite diagnosis, but this is not always feasible or without risk in critically ill patients. **Methods:** We reviewed the medical records of lymphoma patients who had received rituximab-containing chemotherapy between 2012 and 2015 in a tertiary hospital. PCP was diagnosed from a positive result in an immunohistochemical antibody assay for *P. jirovecii* in BAL fluid from patients with respiratory symptoms and radiologic findings compatible with PCP. Patients were classified as having RILD if (1) diffuse bilateral pulmonary infiltrates were seen on CT scan, (2) there was no evidence of other respiratory infection, and (3) if they experienced clinical improvement without receiving any drug active against *P. jirovecii*.**Results:** Among 613 lymphoma patients receiving rituximab-containing chemotherapy, 97 (16%) had diffuse pulmonary infiltrates. Of these, 16 (16%) with an alternative diagnosis and 22 (23%) with an indeterminate diagnosis were excluded. Finally, 21 (22%) patients were classified as having PCP and the remaining 38 (39%) as having RILD. Fever, short duration of symptoms (≤5 days), systemic inflammatory response syndrome (SIRS), and severe extent of disease on CT scan (>75%) were more common in patients with PCP than in those with RILD. Clinical scores were determined using the following system: SIRS=score 1, symptom duration ≤5 days = score 1, extent of disease on CT >75% = score 4. A score of ≥2 differentiated PCP from RILD with 91% sensitivity (95% CI, 70-99) and 71% specificity (95% CI, 54-84).**Conclusions:** A clinical scoring system based on presence of SIRS, short duration of symptoms, and severe extent of disease on CT scan appears to be useful in differentiation of PCP from RILD.

Author Disclosure Block:

S. Park: None. **M. Kim:** None. **W. Choi:** None. **Y. Jang:** None. **T. Kim:** None. **S. Hong:** None. **J. Jung:** None. **Y. Chong:** None. **D. Yoon:** None. **H. Sung:** None. **S. Lee:** None. **S. Choi:** None. **Y. Kim:** None. **C. Suh:** None. **J. Woo:** None. **S. Kim:** None.

Poster Board Number:

SATURDAY-302

Publishing Title:

***Pneumocystis jirovecii* Pneumonia at Major Risk of Death in Chronic Obstructive Pulmonary Disease: A Retrospective Analysis on 113 Cases**

Author Block:

E. Gentilotti, **A. Ricciardi**, L. Coppola, G. Maffongelli, C. Cerva, V. Malagnino, A. Di Veroli, A. Mari, F. Berrilli, F. Apice, D. Di Cave, M. Andreoni, L. Sarmati; Tor Vergata Univ., Rome, Italy

Abstract Body:

Background: *Pneumocystis jirovecii* is an opportunistic fungus causing PCP, a severe lung infection mostly affecting immunocompromised patients. Adverse outcome and more acute and severe course of PCP has been reported in non-HIV compared to HIV-infected. We present a retrospective analysis of 113 patients diagnosed with PCP at "Tor Vergata" University Hospital of Rome.**Methods:** Data on 113 PCP inpatients from different units (Infectious Diseases, Hematology, Transplant, Respiratory Diseases) were retrospectively collected from 2011 to 2015. Demographic, clinical, radiological and genotype characteristics were considered. Statistical analysis was performed using IBM SPSS. **Results:** Sixty-seven out of 113 patients (59.3%) were male aged >50 years old (mean 54.3 years old, range 20-87); 36 (31.9%) were onco-hematologic patients (receiving chemotherapy or haematological stem cell transplantation); 23 (20.3%) had COPD; 22 (19.5%) were HIV-positive; 6 (5.3%) had solid cancer; 4 (3.5%) underwent a solid organ transplant; and the remaining 22 (19.5%) had other immunocompromising conditions. At univariate analysis, a negative PCP outcome statistically correlated with age>50 (p=0.04), pneumonia severity at the onset (p=0.01) and type of pathology (p=0.01). The outcome was available in 100 out of 113 patients: 19 out of 100 (19%) died due to PCP related respiratory failure. Of these, 9 (47.4%) were affected by COPD, while 5 (26,3%) by immunodeficiency illnesses, 3 (15,8%) by onco-hematologic neoplasms, 2 (10, 5%) by solid cancer. A significant higher number of patients with COPD died compared to the other groups (p=0.003). No adverse PCP outcome was observed in HIV-positive and solid organ transplanted subjects.**Conclusions:** A number of published data demonstrated an increase in *Pneumocystis* colonization in COPD which is linked to small airways dysfunction in this category of patients. The higher risk of death for PCP in COPD demonstrated by our retrospective study, leads to carefully consider PCP in the differential diagnosis of worsening lung function in patients with COPD. More study is needed in order to move towards a change of current clinical procedures regarding prophylaxis among patients affected by COPD.

Author Disclosure Block:

E. Gentilotti: None. **A. Ricciardi:** None. **L. Coppola:** None. **G. Maffongelli:** None. **C. Cerva:** None. **V. Malagnino:** None. **A. Di Veroli:** None. **A. Mari:** None. **F. Berrilli:** None. **F. Apice:** None. **D. Di Cave:** None. **M. Andreoni:** None. **L. Sarmati:** None.

Poster Board Number:

SATURDAY-303

Publishing Title:

Beta-D-Glucan as an Early Marker of Clinical Response in Patients with Candidemia

Author Block:

K. Nakajima, Y. Takesue, T. Ueda, K. Ichiki, Y. Wada, A. Doita, T. Tsuchida; Hyogo Coll. of Med., Nishinomiya, Japan

Abstract Body:

Background: beta-D-glucan (BG) is used as a standard of the empiric treatment of invasive candidiasis. Recently it is reported that the change of BG between initial and end of treatment.In this study, we investigated the corelation of the curative effect with the change of BG between initiation and early after treatment start.**Methods:** BG (WAKO) values >=11 pg/ml were interpreted as being positive. The change of BG at 7 days after the start of treatment of candidemia was assessed in patients with positive for initial BG was measured before treatment. Treatment response was evaluated at 7th days after start of treatment.**Results:** 44 episodes of candidemia (echinocandins 60.9%, azoles 32.6%, liposomal amphotericin B + flucytosine 6.5%) were evaluated. Treatment success was obtained in 78.3%. 28 days mortality rate was 4.5 %. BG decreased significantly at 7 days of therapy in patients with success (baseline 187.0±207.4 vs. 142.6±152.1, P<0.015). In contrast, there was no significant change in clinical failure/indeterminate (249.7±257.8 vs.244.1±258.4, p=0.47). BG remained positive at the end of treatment in 25 of 29 evaluable patients (86.2%) with successful treatment. Cutoff point of declining rate of BG to detect success was determined by receiver operating characteristic (ROC) curve. The area under ROC curve was 0.61, and best cutoff point of BG reduction rate to predict clinical success was 11.9%. Based on this definition, BG reduction was achieved in 66.7% of patients with success, and there was a significant difference when compared to clinical failure/indeterminate case (30.0%, p=0.037). A sensitivity, specificity, positive predictive value, and negative predictive value were 36.8%, 88.9%, 70.0% and 66.7%, respectively. In univariate analysis serum albumin (<=2.5g/dl), APACHIIscore (>=20) and BG reduction were associated with treatment success. In multivariate analysis, however, the change of BG was not independent factor associated with treatment success. **Conclusions:** In this study, we could not confirm that the early BDG change is a marker to clinical efficacy of in candidemia. The measurement of BG after start of treatment in patients with candidemia might be supportive manner.

Author Disclosure Block:

K. Nakajima: None. **Y. Takesue:** None. **T. Ueda:** None. **K. Ichiki:** None. **Y. Wada:** None. **A. Doita:** None. **T. Tsuchida:** None.

Poster Board Number:

SATURDAY-304

Publishing Title:

Spectrum of Candidemia in Icus at a Tertiary Referral Center

Author Block:

R. K. Shields, C. J. Clancy, M. H. Nguyen; Univ. of Pittsburgh, Pittsburgh, PA

Abstract Body:

Background: Early antifungal (AF) treatment reduces mortality for candidemia. Optimal strategies for identifying ICU populations that benefit from empiric or pre-emptive AFs are not defined. Incorporating prediction scores or non-culture diagnostics into rational strategies will require better understanding of invasive candidiasis (IC) in specific ICU settings. The objective of this study was to describe the spectrum of candidemia in ICUs at our center. **Methods:** Retrospective review of patients (pts) in medical (MICU), surgical (SICU), or transplant (TICU) ICUs at the onset of candidemia from 2009 - 2015. **Results:** 53 MICU, 49 TICU and 32 SICU pts were included. Pts were admitted from outside hospitals (45%), medical wards (26%), emergency dept. (17%), or long-term care facilities (12%). Median time from ICU admission to candidemia was 4d (range: 0 - 175). 44% developed candidemia within 48 hours of ICU admission. Mean age was 61 yrs; 58% of pts were men. Charlson Comorbidity Index (median = 3 [0 - 14]) and Pitt Bacteremia Scores (median = 6 [0 - 14]) did not vary by ICU location. Median SOFA scores ranged from 7.5 in the SICU to 13 in the TICU (P=0.006). Septic shock was present at the time of candidemia in 58%, 59%, and 37% of pts in the MICU, TICU, and SICU, respectively (P=0.06). 17% died prior to AFs; among the remaining pts, median time to AFs was 55 hours (1 - 205) and 55% received AFs >48 hours after blood cultures. 34% and 54% died within 7 and 30 days, respectively. Common risk factors included vascular catheters (96%), receipt of antibiotics (85%), and mechanical ventilation (82%). Less common factors varied by ICU, including ICU admission > 4 days (33-51%; P=0.07), hemodialysis (17-42%; P=0.03), recent surgery (9-67%; P<0.01), solid-organ transplant (3-24%; P=0.01), and hepatic insufficiency (10-41%; P<0.01). Using criteria from recent trials for empiric AFs, only 26% and 27% of pts met eligibility for MSG-01 and EMPIRICUS, respectively. **Conclusions:** Published criteria based on one-size-fits-all strategies are too insensitive to be useful in guiding empiric AFs against candidemia in ICUs. Strategies that incorporate non-culture diagnostics should be devised and tailored to pt populations, risk factors and types of IC within specific ICUs. In MICUs, strategies should be directed against candidemia. In SICUs and TICUs, however, strategies must also target intra-abdominal and necrotizing pancreatitis.

Author Disclosure Block:

R.K. Shields: I. Research Relationship; Self; Astellas, Merck. **C.J. Clancy:** I. Research Relationship; Self; Astellas, Merck, Pfizer, T2 Biosystems. **M.H. Nguyen:** I. Research Relationship; Self; Astellas, Merck, T2 Biosystems.

Poster Board Number:

SATURDAY-305

Publishing Title:

Microbiological & Clinical Characteristics of Blood Versus Abdominal *Candida* Infections

Author Block:

J. Teo, S. Lee, H. Leck, J. Aw, C-A. Chua, H. Neo, K. Leow, A-L. Tan, Y. Cai, T-P. Lim, W. Lee, A-L. Kwa; Singapore Gen. Hosp., Sg, Singapore

Abstract Body:

Background: In our previous Candidemia surveillance, we detected the emergence of echinocandin resistance. Abdominal (ABD) candidiasis has been suggested to be a hidden reservoir for echinocandin resistance. This study aimed to describe the microbiological & clinical characteristics of blood (BLD) & ABD candida infections. **Methods:** Non-duplicate BLD & ABD *Candida* isolates from a large tertiary Singapore hospital obtained between Oct 2014 - Nov 2015 were included. MICs were tested by the Sensititre YeastOne method. **Results:** 272 isolates (163 ABD, 109 BLD) from 200 cases were included. *C. albicans* (32%) were commonest, followed by *C. glabrata* (27%), *C. tropicalis* (22%) & *C. parapsilosis* (9%). *C. albicans* (40%) is predominant in ABD cultures, while *C. glabrata* (29%) was commonest in BLD. 15 cases involved both ABD & BLD cultures - only 2 had the same species type(s) with similar susceptibilities in both ABD & BLD cultures. Typically, only 1 species was isolated from BLD, while >1 species may be isolated from ABD. MICs are shown in Figure 1. Susceptibilities were similar among BLD & ABD isolates. More than 30% of *C. tropicalis* were azole non-susceptible. Echinocandin resistance was detected in 2 *C. glabrata* (1 ABD, 1 BLD). Median (range) age of patients were 68 years (22 - 101), with 24% from ICU. Most were surgical patients (62%). 30-day mortality rate was 30%. **Conclusions:** Species distribution differs between culture sites, even in cultures isolated from the same patients. High azole resistance in *C. tropicalis* & emerging echinocandin resistance in *C. glabrata* is a concern. Susceptibility patterns may no longer be sufficiently predicted by speciation in our institution. Relying on blood culture alone may not be enough in treatment of abdominal candidiasis.

Author Disclosure Block:

J. Teo: None. **S. Lee:** None. **H. Leck:** None. **J. Aw:** None. **C. Chua:** None. **H. Neo:** None. **K. Leow:** None. **A. Tan:** None. **Y. Cai:** None. **T. Lim:** None. **W. Lee:** None. **A. Kwa:** None.

Poster Board Number:

SATURDAY-306

Publishing Title:

The Presence of *Candida* in Bronchoalveolar Lavage Specimens in Immunocompromised Patients

Author Block:

K. Trevino, T. Davis; Indiana Univ., Indianapolis, IN

Abstract Body:

The presence of *Candida* in bronchoalveolar lavage (BAL) specimens is both common and commonly ignored due to its association with oral contamination. Invasive candiasis has an incidence of 10-14 per 100,000 individuals and an even higher incidence in immunocompromised patients. Whether the presence of *Candida* in a BAL specimen is significant in an immunocompromised patient population is unknown, leaving clinicians to interpret this reported finding based on the clinical scenario.Infectious disease pathology consults performed at Indiana University were reviewed from 2013-2015. 139 cases had yeast consistent with *Candida* in their BAL specimens. 124 of the cases underwent chart review, and culture, antigen testing, treatment, and outcomes data were collected for the 4 weeks surrounding the consult. The cases were sorted based on the presence or lack of treatment with an antifungal and the reason for treatment. Data was analyzed using chi square to assess the significance of outcome differences among those treated and those not treated for candidiasis.5 of 124 cases reviewed demonstrated invasive candidiasis by blood culture. None of these cases had corresponding antigen testing performed. 8 other cases had positive antigen testing but blood cultures remained negative. Overall, 61 of the 124 patients received treatment with an antifungal. 51 of the 124 patients had a poor outcome (death or discharged to hospice). There was no significant difference in outcome between patients who were treated with an antifungal and those who were not (poor outcomes in 28/61 and 23/63 patients respectively). There was also no significant difference in outcomes between groups based on the reason for treatment.Incidence of invasive candiasis in this population was higher than the general population (4% vs. 0.014%). However, we did not compare this rate to an immunocompromised population that did not demonstrate *Candida* in their BAL specimens. This further study is needed to assess whether this incidence is higher than a similarly immunocompromised population without the presence of *Candida* in their BAL. Nearly 50% of patients were treated with an antifungal during their hospitalization, with 12% treated solely based on their BAL result. However, no difference was seen in the overall outcome of the patients based on this treatment.

Author Disclosure Block:

K. Trevino: None. **T. Davis:** None.

Poster Board Number:

SATURDAY-307

Publishing Title:

Yeast-Like Organisms In The Blood In Solid Organ Transplant (Sot) Recipients: Choosing Echinocandins Or Fluconazole

Author Block:

H-Y. Sun[1], Y-S. Huang[2], A. Cheng[2], C-M. Ho[1], R-H. Hu[1], S-S. Wang[1], C-C. Hung[1], Y-C. Chen[1], S-C. Chang[1]; [1]Natl. Taiwan Univ. Hosp., Taipei, Taiwan, [2]Natl. Taiwan Univ. Hosp., Hsin-Chu Branch, Hsin-Chu City, Taiwan

Abstract Body:

Background: An echinocandin is recommended as initial therapy for candidemia, but it is not active against Cryptococcus. Both mycosis cause significant mortality in SOT recipients, and it is critical to distinguish the two mycosis to optimize the empirical treatment. The present study aimed to characterize the differences between the two mycosis in SOT recipients. **Methods:** From Jan 2001 to Dec 2014 at National Taiwan University Hospital, a total of 37 SOT recipients with *Candida* species and 7 SOT recipients with *Cryptococcus neoformans* isolated from the blood were included for analysis to identify factors to distinguish these two mycosis in SOT recipients. The date of fungemia onset and diagnosis was the date of blood sampling. No anti-fungal prophylaxis was given post-transplant. **Results:** Candidemia developed in 10 heart, 14 liver, 9 kidney, and 4 lung transplant recipients while cryptococcocemia in 5 heart, 2 liver transplant recipients. The mortality within 2 weeks of fungemia onset was 32% and 29%, respectively. Fungemia developed within 3 months post-transplant in 21.6% of SOT recipients with candidemia and 42.9% of SOT recipients with cryptococcocemia (p=0.341). Patients with cryptococcocemia were more likely to presented with meningitis (28.6% vs. 0%) and was diagnosed sooner after admission than those with candidemia (median duration after admission 14 vs. 24 days) while candidemia developed more frequently in intensive care unit (ICU) than cryptococcemia (72.2% vs. 28.6%) (all P<0.05).**Conclusions:** In SOT recipients, cryptococcemia was community onset and presented frequently with meningitis while candidemia developed after prolonged hospital stay and in ICU setting.

Author Disclosure Block:

H. Sun: None. **Y. Huang:** None. **A. Cheng:** None. **C. Ho:** None. **R. Hu:** None. **S. Wang:** None. **C. Hung:** None. **Y. Chen:** None. **S. Chang:** None.

Poster Board Number:

SATURDAY-308

Publishing Title:

***Scopulariopsis brevicaulis*: A Case of Mistaken Takayasu Arteritis**

Author Block:

T. B. Walls, R. F. Relich, T. E. Davis, B. H. Schmitt; Indiana Univ. Sch. of Med., Indianapolis, IN

Abstract Body:

Background: *Scopulariopsis brevicaulis*, a hyaline mold, is generally associated with onychomycosis, but in rare instances, it can cause deep tissue infections, otomycosis, and infections of the brain, lungs, and bone. This report describes a case of *Scopulariopsis* endocarditis secondary to iatrogenic immunosuppression. **Case:** A 37-year-old Japanese woman with a past medical history of aortic valve replacement secondary to a congenital aortic aneurysm and presumed Takayasu arteritis treated with prednisone presented to the hospital with left-sided back and lower abdominal pain. The patient had a 9-month recent history of repeated emboli with fevers. A CTA of the chest was obtained and showed a moderately-sized pseudoaneurysm from the ascending aortic graft and filling defects concerning for thrombi in the distal ascending aorta/proximal aortic arch near the graph anastomosis. Portions of thrombus from the thrombectomy were sent for histopathology and culture. **Results:** Histopathologic examination of the left iliac artery thrombus revealed branching, septate hyphae; however, blood cultures were negative. Subsequently, voriconazole was added to the patient's empiric antimicrobial regimen to cover suspected aspergillosis. The diagnosis of Takayasu arteritis was dismissed as the etiology of the patient's aneurysm and the patient's regimen of prednisone was discontinued. Fungal cultures of the tissue grew *S. brevicaulis* and amphotericin B was added to the antifungal regimen pending susceptibility data. **Discussion:** The isolate was later determined to be susceptible to posaconazole and micafungin, which prompted treatment with these drugs exclusively. After a 78-day hospital stay, the patient was discharged on long-term antifungal therapy with posaconazole. *Scopulariopsis* species are a rare cause of infective endocarditis. Less than 50% of cases of fungal endocarditis yield positive blood cultures, rendering this diagnostic method suboptimal. A combination of culture and histopathology are necessary to effectively detect this organism, and susceptibility testing of isolates is necessary to optimize therapy.

Author Disclosure Block:

T.B. Walls: None. **R.F. Relich:** None. **T.E. Davis:** None. **B.H. Schmitt:** None.

Poster Board Number:

SATURDAY-309

Publishing Title:

Detection of the Dimorphic Phases of *Mucor circinelloides* in a Blood Specimen

Author Block:

M. A. Arroyo, B. Schmitt, T. E. Davis, R. F. Relich; Indiana Univ. Sch. of Med., Indianapolis, IN

Abstract Body:

Background: *Mucor circinelloides* is a dimorphic zygomycete that exhibits hyphal growth in aerobic conditions and multi-budded yeast growth under anaerobic or high-CO_2 conditions. To date, very few cases of fungemia associated with *M. circinelloides* have been reported. Here, we describe a case of fungemia caused by *M. circinelloides* that demonstrated both dimorphic phases during Gram-stain examination of two sets of blood cultures.**Case:** A 38-year-old female presented to the Emergency Department with shortness of breath and cough, sinus congestion, fever of 103 °F, nasal drainage, sore throat, and yellow-green sputum production. The patient's medical history included sarcoidosis, IgG deficiency, and sinus infection for which she completed treatment with cefdinir 1 week before admission. As part of the initial diagnostic workup, blood specimens were obtained for culture and other laboratory testing.**Results:** Peripheral blood specimens obtained at the time of initial presentation demonstrated leukocytosis and low glucose, potassium, CO_2, total protein, and magnesium. An X-ray of her chest was unremarkable. The aerobic bottles from both sets of blood cultures turned positive within 24 hours of incubation. Gram stains revealed pleomorphic yeast forms and wide hyphae with right-angle branching. The isolate recovered from the blood-broth was a fast-growing mold that was initially identified as a *Mucor* species. The identity of the isolate as *M. circinelloides* was supported by its microscopic morphology and its ability to convert into yeast forms under anaerobic conditions.**Discussion**: Following diagnosis of fungemia based on initial blood culture results, the patient was started on therapy with liposomal amphotericin B. Subsequent blood and sinus cultures were negative. In addition, surgical pathology sinus biopsy results and CAT scan of the chest and sinuses did not show any clear evidence of fungal involvement. As a consequence, antifungal treatment was discontinued after 6 days and the patient was discharged home without further complications. At a follow-up assessment 1 week after discharge, the patient had no signs of *Mucor* infection. In conclusion, based on our patient's disease course and clinical outcome, this organism is presumably of low virulence.

Author Disclosure Block:

M.A. Arroyo: None. **B. Schmitt:** None. **T.E. Davis:** None. **R.F. Relich:** None.

Poster Board Number:

SATURDAY-310

Publishing Title:

Prognosis and Predictors of Death in Cryptococcal Meningitis - A Portuguese Cohort

Author Block:

A. Silva-Pinto, R. Serrão, A. Sarmento, L. Santos; Centro Hosp.ar São João, Porto, Portugal

Abstract Body:

Background: Cryptococcal meningitis usually occurs in people with immunodeficiency problems and has a considerably high mortality rate. The aim of this study is to evaluate the prognosis and outcome' predictors of patients with cryptococcal meningitis.**Methods:** We performed a retrospective study of cryptococcal meningitis patients admitted to our Infectious Diseases Unit between 2000 and 2014. We used the most appropriate measure of central tendency, of distribution and statistic test. The significance level used was 0.05.**Results:** We included 61 patients (55 men; age: median 37 and interquartile range (IQR) 15). The median symptoms time until hospitalization was 7 days (IQR 12.5). The most common symptoms reported were headache (n=53), fever (n=39), nausea (n=31), dizziness (n=27) and behavioral impairment (n=21). Only one aged patient had no known immunosuppression; the immunosuppression reported was HIV infection (n=55; median CD4 count 29/mm^3, IQR 42), hematologic neoplasia (n=2), chronic corticotherapy (n=1), tacrolimus (n=1) and hyper IgM symdrome (n=1). The serum cryptococcal antigen was positive in 44 patients (median 1/512). The cerebrospinal fluid (CSF) revealed in median 41 cells/mm^3, with hyperproteinorrhachia (median 56 mg/dL) and hypoglycorrhachia (mean 33% of the serum value). The CSF India ink was positive in 38 (62%) patients and the CSF cryptococcal antigen in 52 (median 1/1024). Fifty (82%) patients had a positive CSF culture and 24 positive blood culture. The quickest symptom to improve was fever (median 4 days IQR 3), followed by headache (median 6 days IQR 2,5) and behavioral impairment (median 7 days IQR 7). Nine patients died (15%) due to the cryptococcosis and nine patients had a relapse. The majority (n=31) cured the infection without sequel and 8 patients had significant morbidity. Predictors of death were as follows: hypoglycorrhachia (independent t test, p=0.040), higher CSF cryptococcal antigen (Mann Whitney U, p=0.037) and higher neutrophil count at admission (Mann Whitney U, p=0.050).**Conclusions:** Cryptococcosis is still a frequent disease specially associated with immunosuppression. Death rate is not negligible. This study addresses hypoglycorrhachia, high CSF cryptococcal antigen and higher neutrophil count at admission as predictors of death.

Author Disclosure Block:

A. Silva-Pinto: None. **R. Serrão:** None. **A. Sarmento:** None. **L. Santos:** None.

Poster Board Number:

SATURDAY-311

Publishing Title:

Laryngeal Cryptococcus Infection

Author Block:

O. A. M. Saeed[1], T. Walls[1], G. Ward[1], D-J. Summerlin[1], T. E. Davis[1], T. C. Huntley[2]; [1]Indiana Univ. Sch. of Med., Indianapolis, IN, [2]Ctr. for Ear Nose Throat & Allergy, Carmel, IN

Abstract Body:

Background: Laryngeal Cryptococcosis is a rare disease that mimics laryngeal carcinoma. There are only 18 cases reported in the literature. In this case report we describe the findings of a 61-year-old male who had laryngeal Cryptococcus Infection. **Methods:** case report and review of relevant English language literature**Results:** The usage of inhaled and oral steroids was described on 31.5 % and 26.3 % of the laryngeal cryptococcosis cases respectively. The gross description of the laryngeal lesion in the reported cases varied from as mild as congestion and redness to an irregular mass suspicious of laryngeal carcinoma. Over half of the cases were described as having granulomatous inflammation in the histological examination. Our patient was found to have a laryngeal mass on induction of general anesthesia. He was on prolonged long-term oral steroid. Direct laryngoscopy revealed a mass extending from the anterior aspect of the right arytenoid to the right false vocal fold. H & E histopathological examination showed diffuse granulomatous inflammation with fungal elements. GMS, PAS and Mucicarmine stain revealed Cryptococcus microorganisms. Blood Cryptococcus antigen test was positive. HIV screening was negative. The patient was treated with fluconazole 400 mg daily for 6 months.**Conclusion:** Prolonged usage of oral steroids is an important risk factor for laryngeal Cryptococcus infection. The laryngoscopic findings of the lesion can mimic laryngeal neoplasms. The use of fungal stains can usually confirm the diagnosis. Oral fluconazole is the most commonly used treatment modality.

Author Disclosure Block:

O.A.M. Saeed: None. **T. Walls:** None. **G. Ward:** None. **D. Summerlin:** None. **T.E. Davis:** None. **T.C. Huntley:** None.

Poster Board Number:

SATURDAY-312

Publishing Title:

Osteoarticular Mycoses - Comparative Analysis of the Epidemiology, Pathogenesis, Clinical, Diagnostic, and Therapeutic Characteristics of 1011 Cases

Author Block:

M. Gamaletsou, B. Rammaert, M. Bueno, B. Brause, D. Denning, M. Henry, D. M. Kontoyiannis, A. Miller, R. Petraitiene, V. Petraitis, E. Roilides, N. V. Sipsas, S. Taj-Aldeen, V. Zeller, O. Lortholary, T. Walsh; Intl. Consortium for Study of Osteoarticular Mycoses, New York, NY

Abstract Body:

Background: Osteoarticular mycoses (OM) are chronic debilitating infections that require extended courses of antifungal therapy and may warrant expert surgical intervention. To our knowledge, there is no systematic analysis of the different forms of osteoarticular mycoses. **Methods:** Using a database of 1011 cases fulfilling prespecified criteria, we studied the comparative epidemiology, pathogenesis, clinical manifestations, inflammatory biomarkers, treatment and outcome of six major groups of OM: *Candida* osteomyelitis (CO), *Aspergillus* osteomyelitis (AO), *Candida* arthritis (CA), *Aspergillus* arthritis (AA), dimorphic fungal osteomyelitis (DFO), and non-Aspergillus mould osteomyelitis (NAMO).**Results:** The age range extended from neonates to older adults most widely in CO and CA. For all OM, M:F ratio= 2.1. CO and CA, were associated with significantly greater events of hematogenous dissemination in 67% and 81%, respectively, than that of AO (45%) and than that of NAMO (39%) ($P<0.001$). There were significantly more immunocompromised patients with CO, CA, AO, AA, and NAMO than with DFO ($p<0.001$). Traumatic inoculation was more commonly associated with AO, AA, and NAMO ($p<0.01$). Values of inflammatory biomarkers overlapped considerably among groups. Synovial fluid cultures were highly sensitive in detection of CA and AA. Relapsed infection, possibly related to inadequate duration of therapy, developed among 32% with CA, significantly exceeding that of AO, AA, and CA ($p<0.05$). Overall mortality was greater for *Penicillium (Talaromyces) marneffei* (38.5%) osteomyelitis ($p<0.05$).**Conclusions:** OM constitute distinct clinical entities with a high representation of males, greater hematogenous infection in CA and CO, significantly more traumatic inoculation in AO, AA, and NAMO, and a high propensity for relapse in CO.

Author Disclosure Block:

M. Gamaletsou: None. **B. Rammaert:** None. **M. Bueno:** None. **B. Brause:** None. **D. Denning:** None. **M. Henry:** None. **D.M. Kontoyiannis:** None. **A. Miller:** None. **R. Petraitiene:** None. **V. Petraitis:** None. **E. Roilides:** None. **N.V. Sipsas:** None. **S. Taj-Aldeen:** None. **V. Zeller:** None. **O. Lortholary:** None. **T. Walsh:** None.

Poster Board Number:

SATURDAY-313

Publishing Title:

Dandruff Aetiology and the Effects of Edible Lipids on the Growth of Isolates from Lesions in Calabar, Nigeria

Author Block:

O. M. Ogba, L. N. Abia-Bassey, N. Biobelemoye; Univ. of Calabar, Calabar, Nigeria

Abstract Body:

Background: Dandruff is caused by *Malassezia* species, a lipophilic fungus.Information on the aetiology of the fungi associated with this condition and profile of affected persons are sparse in our locality. This prospective study was designed to determine the aetiology of dandruff causing fungi and to assess the effects of some edible lipids on the growth of predominant isolates in our locality.**Materials and methods:** A total of 245 subjects were enrolled for the study; 145 with dandruff-like lesions and 100 without lesions (controls). Ethical approval was obtained. Questionnaires were administered for biodata. Scalp scrapings were obtained from subjects visiting hair and beauty salons into sterile paper envelopes and transported to the Microbiology Laboratory, UCTH, for analysis. Samples were subjected to culture, microscopy and physiological tests. The effect of lipids on the growth of *Malassezia* species was assessed using common edible oils including groundnut oil.**Results:** Out of the 145 subjects with dandruff-like lesions, 90.3% were positive for different *Malassezia* species. The most prevalent *Malassezia* species among subjects with dandruff lesions was *M. furfur* (70.2%) while *M. globosa* (51.9%) was the most prevalent among the controls. *Malassezia pachydermatis* was the least prevalent species among the two groups. *M. furfur* grew exceptionally on SDA overlaid with groundnut oil.**Conclusion:** Groundnut oil could be used as an alternative for the cultivation of *M. furfur* especially in a resource poor setting like ours and should not be used in the preparation of hair ointment or creams.

Author Disclosure Block:

O.M. Ogba: None. **L.N. Abia-Bassey:** None. **N. Biobelemoye:** None.

Poster Board Number:

SATURDAY-314

Publishing Title:

***Candida parapsilosis* (*cp*) Intra-abdominal Candidiasis (Iac) May Stem From Endogenous Gastrointestinal (Gi) Sources**

Author Block:

P. Vergidis, M. H. Nguyen, C. J. Clancy; Univ. of Pittsburgh, Pittsburgh, PA

Abstract Body:

Background: IAC is at least as common as candidemia, but it is less well studied. *Cp,* generally considered an exogenous pathogen that is introduced via indwelling catheters, has been a rare cause of IAC. We reviewed *Cp* IAC at our center in an era of increased use of intra-abdominal drains and stents. **Methods:** We performed a retrospective study of adult patients in 2012-13. IAC was defined by clinical evidence of intra-abdominal infection and isolation of *Candida* from an intra-abdominal sample collected under sterile conditions. We excluded patients whose samples were collected from drains that were in place >24 hours. In univariable analysis, we compared patients with mono-infections caused by *Cp* and other *Candida* species. The log-rank test was used for survival analysis.**Results:** A total of 180 isolates were recovered from 163 patients with IAC (*C. albicans* 57%, C. *glabrata* 25%, *Cp* 10%). *Cp* was isolated in 18 patients. In 5 patients, co-infection with another *Candida* species occurred. Mean age was 58 years, 67% were men. Infection was caused by GI tract perforation (8), enteric wall inflammation (2), complicated pancreatitis (4), liver/pancreas allograft infection (2), cholecystectomy complication (1), and peritoneal dialysis catheter infection (1). Intra-abdominal abscesses developed in half of the cases. Infection due to *Cp* more commonly resulted in septic shock compared to other *Candida* species (46% *vs* 15%, p=0.02). Among subjects who underwent prior surgery, IAC occurred at a mean time of 11 days post-operatively for *Cp* compared to 53 days for other *Candida* species (p=0.01). Infection was controlled with percutaneous drainage in 22% and surgery in 78%. Antifungal treatment was administered in 78%. 100-day mortality was similar between patients with IAC due to *Cp* and other *Candida* spp. (15% *vs* 29%, p= 0.35).**Conclusion:** In this cohort, *Cp* was the third leading cause of IAC. Most cases were not associated with intra-abdominal drains or stents, but rather disruption to GI tract mucosal integrity. Therefore, *Cp* may be an endogenous pathogen within the abdominal cavity, consistent with emerging microbiome data that the species can be found as part of GI flora. Outcomes of *Cp* IAC were comparable to other *Candida* species and septic shock was more common; this experience is in contrast to *Cp* candidemia, for which outcomes are better than with other species.

Author Disclosure Block:

P. Vergidis: None. **M.H. Nguyen:** None. **C.J. Clancy:** None.

Poster Board Number:

SATURDAY-315

Publishing Title:

***Candida albicans* and *Klebsiella pneumoniae* Interact Synergistically During Intra-Abdominal Infection (IAI)**

Author Block:

P. Vergidis, C. J. Clancy, S. Cheng, M. H. Nguyen; Univ. of Pittsburgh, Pittsburgh, PA

Abstract Body:

Background: *C. albicans* and *K. pneumoniae* are human gastrointestinal tract commensals, and leading causes of IAIs. We hypothesized that *C. albicans* and *K. pneumoniae* interact synergistically during IAI.**Methods:** Interactions between *C. albicans* SC5314 and a *K. pneumoniae* clinical isolate were studied *in vitro* and during mouse infections. Non-immunosuppressed, 6-8 wk old ICR mice were injected intraperitoneally with *C. albicans*, *K. pneumoniae*, or both organisms (10 mice/group; inocula of $5 \bullet 10^7$ and $5 \bullet 10^6$ CFU for survival and tissue burden studies, respectively). RT-PCR for select *C. albicans* genes was performed on liver homogenates (SYBR Green).**Results:** *C. albicans* growth was decreased by 1.2 $\log_{10}$ in YPD medium at 72h in the presence of *K. pneumoniae*. *K. pneumoniae* growth was not affected when the organisms were co-incubated. In survival studies, 7-day mortality rates among mice with *C. albicans* and *K. pneumoniae* mono-infections were 60% and 0%, respectively. Mortality during *C. albicans*-*K. pneumoniae* co-infections was 90% (p=0.06 compared to *C. albicans* mono-infection). In tissue burden studies, *C. albicans* concentrations were higher for co-infections than mono-infections in peritoneal fluid (median, 4.1 *vs* 2.0 $\log_{10}$ CFU/ml, p<0.001) and liver (5.5 *vs* 4.9, p=0.02) at 24h. At 72h, there was no significant difference in peritoneal fluid during co-infections (p=0.35), but there was a trend toward higher *C. albicans* burdens in liver (4.7 *vs* 3.3, p=0.09). At 24h *K. pneumoniae* burdens were higher in peritoneal fluid (6.1 *vs* 4.5, p<0.001) and liver (6.5 *vs* 3.3, p=0.02) during co-infections. Similarly, at 72h *K. pneumoniae* burdens were higher in peritoneal fluid (3.0 *vs* 1.5, p<0.001) and liver (6.2 *vs* 1.9, p<0.001) during co-infections. Expression of hyphal-specific *C. albicans* genes *RBT1*, *ECE1* and *HWP1* was 9-, 3- and 2-fold higher, respectively, in liver during co-infections than mono-infections at 24h. *RBT1*, *NRG1* and *HWP1* expression was 3-, 3- and 4-fold higher at 72h, respectively.**Conclusion:** *C. albicans* and *K. pneumoniae* exhibit synergistic interactions during IAI, which are not observed *in vitro*. Induction of hyphal-specific genes during co-infections suggests that *C. albicans* filamentation may facilitate *K. pneumoniae* invasion and persistence at sites of IAI.

Author Disclosure Block:

P. Vergidis: None. **C.J. Clancy:** None. **S. Cheng:** None. **M.H. Nguyen:** None.

Poster Board Number:

SATURDAY-316

Publishing Title:

A Comparative Study of Mixed Microbial Biofilms of *Aspergillus fumigatus, Scedosporium apiospermum* and *Lomentospora prolificans* Formed with *Pseudomonas aeruginosa*

Author Block:

E. Manavathu, S. Wakade, J. Vazquez; Med. Coll. of Georgia/Augusta Univ., Augusta, GA

Abstract Body:

Background: *Aspergillus fumigatus* (*Af*), *Scedosporium apiospermum* (*Sa*) and *Lomentospora prolificans* (*Lp*) are the most commonly isolated filamentous fungi from the airways of cystic fibrosis (CF) patients either alone or with *Pseudomonas aeruginosa* (*Pa*). In the CF airways *Pa* interacts with fungi to form polymicrobial (PM) biofilm highly tolerant/resistant to antimicrobial drugs. The primary objective of this study was to research the structure, development and antimicrobial susceptibility of *Af-Pa*, *Sa-Pa* and *Lp-Pa* PM biofilms. **Methods:** *Pa* PA01, 27853, MJK8, CF39wt, CF39s, *Sa*3634, *Lp*201214, *Af*43004 and *Af*43135 were used. Structure and development of PM biofilm were examined by SEM. Mixed microbial interaction was studied by coculture experiments and agar plate inhibition assays. Antimicrobial susceptibility was studied by exposing biofilms to various concentrations of the drug(s) individually and in combination. The effectiveness of the drug treatment was determined by CFU or MTT assays. **Results:** *Pa* formed PM biofilm with *Af, Sa* and *Lp*. The structure and development of *Pa-Af*, *Pa-Sa* and *Pa-Lp* biofilms were similar. The *Pa* cells were firmly attached to the fungal hyphae by surface appendages exploiting the extracellular matrix (ECM). In mature PM biofilm the bacterial cells were fully encased or trapped in the ECM made up of bacterial and fungal cellular components. The interaction of *Pa* and *Af* cells in mixed microbial cultures resulted in the growth inhibition and death of *Af* cells. In contrast, *Sa* and *Lp* cells were tolerant to the killing effect of *Pa* in cocultures, although growth was substantially inhibited. The antimicrobial drug susceptibility of the fungal cells in PM and monomicrobial (MM) biofilms was similar. In contrast, the *Pa* cells in PM showed tolerance/resistance to cefepime and imipenem (0.5-1.5 logs CFU reduction at 64µg/ml) compared to that in MM biofilm (2-3 logs CFU reduction at 64µg/ml). The susceptibility to ciprofloxacin and tobramycin was similar (4-5 logs CFU reduction at 64 µg/ml) for both MM and PM biofilms. **Conclusions:** The structure, development and antimicrobial drug susceptibility of *Pa-Af, Pa-Sa* and *Pa-Lp* biofilms are similar. However, the interaction of *Pa* with *Af* leads to the death of the fungal cells, whereas the fungicidal effect of *Pa* against *Sa* and *Lp* was minimal.

Author Disclosure Block:

E. Manavathu: None. **S. Wakade:** None. **J. Vazquez:** None.

Poster Board Number:

SATURDAY-317

Publishing Title:

Mixed Microbial Biofilms of *Aspergillus fumigatus* with Methicillin-Susceptible and Methicillin-Resistant *Staphylococcus aureus*: Structure, Development and Antimicrobial Drug Susceptibility

Author Block:

E. Manavathu, J. Vazquez; Med. Coll. of Georgia/Augusta Univ., Augusta, GA

Abstract Body:

Background: *Staphylococcus aureus* (*Sa*) and *Aspergillus fumigatus* (*Af*) are commonly isolated pathogens from the airways of cystic fibrosis (CF) patients. For CF patients, with methicillin-resistant *Sa* (MRSA) the outcome is poor compared to those patients with methicillin-susceptible *Sa* (MSSA). In chronically infected CF airways, the interaction of *Af* and *Sa* may lead to the formation of *Af-Sa* biofilm showing high level tolerance to antimicrobial therapy. The objective of this work was to study the structure, development and antimicrobial drug susceptibility of monomicrobial (MM) and polymicrobial (PM) biofilms of MSSA and MRSA.**Methods:** *Sa*25923, *Sa*59395 (MSSA), *Sa*43300, *Sa*56694 (MRSA), *Af*43004 and *Af*43135 were used. Biofilms were grown in brain heart infusion (BHI) broth in 24-well cell culture dishes. The *Af-Sa* biofilm structure and development were studied by SEM. The antibiosis activity of *S*a on *Af* was examined by coculturing the organisms followed by CFU assay. Biofilm susceptibility to cephalexin (CPH), ceftaroline (CFT) ciprofloxacin (CIP) and linezolid (LIN) was studied by using various concentrations followed by a CFU assay.**Results:** The development of *Af-Sa* biofilm was dependent on fetal calf serum (FCS) in the growth medium. The rank order of *Af-Sa* biofilm production in BHI with and without FCS was BHI+10% FCS >100% FCS >BHI alone. SEM images showed that the *Sa* cells were firmly adhered to *Af* hyphae and encased by the extracellular matrix. Without FCS few *Sa* cells were adhered to *Af* hyphae. Coculture experiments showed no *Sa* antibiosis activity against *Af* cells. The MM and PM biofilms of MSSA and MRSA were approximately 300-1000 fold more tolerant to antibacterial drugs than the planktonic cells (5-7 logs CFU reduction). The MM and PM biofilms of MSSA were more susceptible to CPH (4-5 logs CFU reduction at 64µg/ml). The biofilms with MRSA were susceptible to CFT (2.5-3 logs CFU reduction at 64µg/ml). In contrast, the MM and PM of MSSA and MRSA showed similar susceptibility (1.5-3 logs CFU reduction at 64µg/ml) to CIP and LIN.**Conclusions:** *Af-Sa* biofilm formation is dependent on serum proteins. *Sa* in cocultures showed no antibiosis against *Af*. The *Sa* cells in MM and PM biofilms of MSSA and MRSA showed antibiotic dependent variation in drug susceptibility.

Author Disclosure Block:

E. Manavathu: None. **J. Vazquez:** None.

Poster Board Number:

SATURDAY-318

Publishing Title:

The Tolerability of Low-Dose Pentamidine as the 2nd-Line Treatment for HIV-Related Pneumocystis Pneumonia Patients

Author Block:

Y. Mutoh, S. Shibata, T. Aoki, K. Teruya, Y. Kikuchi, H. Gatanaga, S. Oka; Natl. Ctr. for Global Hlth.and Med., Tokyo, Japan

Abstract Body:

Background: The current understanding is that 1st choice of treatment for Pneumocystis pneumonia (PCP) among HIV-1 patients is Sulfamethoxazole-trimethoprim (SMX-TMP). However, several cases have to change treatment to another agent, such as pentamidine or atovaquone, because of their adverse events.In several cases, physicians treating with pentamidine (4 mg/kg/day IV daily) for PCP experience some adverse events such as neutropenia, kidney injury, angialgia, or hypoglycemia. Hence, some experts recommend using reduced-dose pentamidine (3 mg/kg/day IV daily) in order to avoid adverse events. However, its tolerability is not well known. Here, we assessed the tolerability of reduced-dose pentamidine for HIV-related PCP.**Methods:** In this single center, retrospective cohort study, 108 patients who were clinically diagnosed HIV-related PCP and admitted to the National Center of Global Health and Medicine, Japan, between January 2004 and December 2015 were included. All of their treatments were initiated with SMX-TMP, and intravenous reduced-dose pentamidine was used as the 2nd-line regimens. The primary outcome is the tolerability of reduced-dose pentamidine.**Results:**In total 108 patients received 2nd-line reduced-dose pentamidine treatment, male were 103 of 108 (95.4%), median age was 37 years old (IQR: 31.8-44.3), median CD4 count on admission was 35.5cells/mm^3 (IQR: 18.5-67.0). The median duration of reduced-dose pentamidine use was 7days (IQR: 5.0-10.0). Adjuvant glucocorticoids were used for 75 patients (69.4%). The number of patients who were able to complete the treatment with 2nd line reduced-dose pentamidine was 62 patients (57.4%). No patient discontinued pentamidine due to treatment failure. The three month mortality rate was 2.8% (3/108); however, the causes of death for all three patients were not PCP. The reasons of discontinuing treatment were fever (n=18, 16.7%), kidney injury (n=11, 10.2%), elevated liver enzyme (n=9, 8.3%), angialgia (n=8, 7.4%), neutropenia, (n=5, 4.6%), and hypoglycemia (n=1, 0.9%).**Conclusions:**Reduced-dose pentamidine is as much tolerable as conventional dose of pentamidine treatment for HIV-related PCP as the 2nd-line treatment. These results provide new insight into our understanding for 2nd line treatment of HIV-related PCP.

Author Disclosure Block:

Y. Mutoh: None. **S. Shibata:** None. **T. Aoki:** None. **K. Teruya:** None. **Y. Kikuchi:** None. **H. Gatanaga:** None. **S. Oka:** None.

Poster Board Number:

SATURDAY-319

Publishing Title:

The Novel Species *Paenibacillus* sp. VT-400 as a Potentially Pathogenic Spore-Forming Bacteria in the Oral Cavity of Patients with Hematological Malignancies

Author Block:

V. Tetz, G. Tetz; Inst. of Human Microbiol., New York, NY

Abstract Body:

Background: Acute leukemia accounts more than 10,000 deaths annually. The major cause of morbidity in patients with leukemia, is opportunistic infection due to immunosuppression. In this study, we describe *Paenibacillus* sp. VT-400, a novel spore-forming bacterium (complete genome is deposited in GenBank No. LELF01000000), which has never been detected in humans before, isolated from the saliva of children with lymphoblastic leukemia, and investigate its potential to cause pneumonia. **Methods:** We used a mouse model of pneumonia to study virulence *in vivo*. C57BL/6 mice aspirated 50 µL *Paenibacillus* sp. VT-400, for a total dose of 5.0×10^7 - 5×10^9 CFU/mouse. Bacterial load was measured in the lungs and spleen of surviving mice 48 h post infection. Susceptibility to antibiotics was determined by the disc diffusion method. Whole-genome analysis was annotated using RAST and the NCBI Pipelines.**Results:** Analysis revealed a large number of virulence genes like proteases, ureases, chitinases, hemolysin D, superantigen CD4+ T-cell-stimulating antigen. Mice intranasally challenged with *Paenibacillus* sp. VT-400 exhibited typical signs of acute pneumonia within 24 h with direct correlation between severity of symptoms and dose. The isolate caused mortality in mice infected with 5.0×10^8 or more CFU due to acute pneumonia. Genome analysis revealed that *Paenibacillus* sp. VT 400 harbors antibiotic resistance genes: MFS, ABC, MATE transporters, SMR proteins *vanZ*, fosmidomycin, *TetA*, β-lactamases; genes encoding resistance to chemotherapeutics like tunicamycin and bleomycin. Expression of these genes revealed the resistance to erythromycin and azithromycin, chloramphenicol and trimethoprim.**Conclusions:** Detection, of a novel spore-forming *Paenibacillus* sp. VT 400 that harbors virulence factors and genes that confer resistance to antibiotics and to chemotherapeutic drugs in patients with acute leukemia is a critical result, especially in light of *in vivo* studies in which mice challenged with isolate died from pneumonia, with dissemination of the infection. The presence of identified pathogenic spore-forming bacteria with numerous antibioticresistance genes that may trigger a life-treatening respiratory infection of immunocompromised patients warrants special attention.

Author Disclosure Block:

V. Tetz: None. **G. Tetz:** None.

Poster Board Number:

SATURDAY-320

Publishing Title:

Sepsis Biomarkers in the Initial Assessment of Fever in Children with Cancer and Neutropenia

Author Block:

F. Carlesse[1], O. Araujo[2], D. C. B. Silva[1], M. Brunialti[3], A. Senerchia[1], A. Petrilli[1], R. Salomão[3]; [1]Pediatric Oncology Inst.(IOP/GRAACC) Univ.e Federal de Sao Paulo, Sao Paulo, Brazil, SAo Paulo, Brazil, [2]Pediatric Oncology Inst. (IOP/GRAACC), Univ.e Federal de Sao Paulo, Sao Paulo, Brazil, SAo Paulo, Brazil, [3]Univ.e Federal de Sao Paulo, Sao Paulo, Brazil, SAo Paulo, Brazil

Abstract Body:

Background: Biomarkers are potentially useful to discriminate between febrile neutropenic (FN) children with cancer that will develop a favorable clinical outcome from those that will develop sepsis and need more aggressive management.**Methods:** We tested 10 cytokines by Citometric Bead Array (interleukins 8, 6, 17, 21, 10, 1β, TNF-α, IL-12/23p40, G-CSF and GM-CSF), C-reactive protein (immunoturbidimetry) and procalcitonin (PCT ELISA) in the first 24 hours of fever in 35 patients with FN (median age 4 y-o), as potential discriminators of sepsis.**Results:** Thirteen Patients (37%) developed sepsis, with 10 severe sepsis (4 with shock) within 72 h of diagnosis of FN. Out of the 13 sepsis, 10 had bacteria isolated in cultures (8 in blood cultures: 3 coagulase-negative Staphylococci, 1 Enterococcus raffinosus, 2 E. coli, 1 Enterobacter sp., 1 Pseudomonas oryzihabitans; and 2 K. pneumoniae in urine cultures). IL-6, IL-8, IL-10 and TNF-α showed significant differences between septic and non-septic by Mann-Whitney test. For discrimination of sepsis, IL-8 showed an area under the ROC curve (AUC) of 0.86 (95% CI 0.72 - 1, P <0.0001); AUC was 0.87 for IL-6 (95% CI 0.75-0.99; P <0.0001) and 0.89 (95% CI 0.78 to 0.99; P <0.0001) for PCT. The optimal cutoff points were 240, 170 and 190 pg/mL for IL-8, IL-6 and PCT, according to Youden's J statistic. The C-reactive protein showed no power of discrimination. **Conclusions:** IL-6, IL-8 and PCT on the first day of fever may be useful markers to identify neutropenic patients who will develop sepsis in up to 72 h. The combination of two markers (IL-6 or IL-8 + PCT), with lower cutoffs, can improve the performance of the tests.

Author Disclosure Block:

F. Carlesse: None. **O. Araujo:** None. **D.C.B. Silva:** None. **M. Brunialti:** None. **A. Senerchia:** None. **A. Petrilli:** None. **R. Salomão:** None.

Poster Board Number:

SATURDAY-321

Publishing Title:

Microevolution of *Campylobacter* and *Helicobacter* Species within Two Chronically Infected Patients with X-Linked Agammaglobulinemia

Author Block:

R. A. Weingarten[1], S. Conlan[2], I. Fuss[3], W. Strober[3], S. K. Datta[3], S. M. Holland[3], R. Agarwala[4], J. A. Segre[2], K. M. Frank[1]; [1]Clinical Ctr., NIH, Bethesda, MD, [2]Natl. Human Genome Res. Inst., NIH, Bethesda, MD, [3]Natl. Inst. of Allergy and Infectious Diseases, NIH, Bethesda, MD, [4]Natl. Library of Med., NIH, Bethesda, MD

Abstract Body:

Patients with the primary immune disorder X-linked agammaglobulinemia (XLA) have abnormal development of B-cells and are prone to frequent, persistent viral and bacterial infections beginning at a young age. Despite immunoglobulin (Ig) replacement therapy, there are many case reports that document recurrent infections caused by bacteria including *Campylobacter* and *Helicobacter* species. We present a retrospective study to track the genomic evolution of *C. jejuni* and *H. bilis* isolated from blood and stool samples of two patients with XLA while undergoing long-term antibiotic treatment and Ig therapies. DNA was extracted from 17 *C. jejuni* isolates cultured between 2008 and 2015 in Patient 1 and five *H. bilis* isolates cultured between 1998 and 2013 from Patient 2. Illumina sequencing analysis was used to track single nucleotide variants (SNVs) and insertions/deletions of the genomes. Sequence analysis is consistent with chronic infections, rather than reinfection with new organisms, for both patients. There was limited *in vivo* evolution of the bacteria despite the long infection timeline. A number of SNVs were identified in both *C. jejuni* and *H. bilis* isolates and were mapped to efflux and transport systems, flagellin, and chemotaxis genes. *C. jejuni* iron-associated genes including FUR also contained SNVs. *C. jejuni* isolates developed high tinidazole resistance within months of tinidazole therapy initiation for Patient 1. *In vitro* experiments are currently underway to further examine specific SNVs identified *in silico*. Significantly altered genes identified by sequence analysis may offer insight into the role that *C. jejuni* and *H. bilis* play in maintaining these long-term bacteremic infections in patients with XLA.

Author Disclosure Block:

R.A. Weingarten: None. **S. Conlan:** None. **I. Fuss:** None. **W. Strober:** None. **S.K. Datta:** None. **S.M. Holland:** None. **R. Agarwala:** None. **J.A. Segre:** None. **K.M. Frank:** None.

Poster Board Number:

SATURDAY-322

Publishing Title:

Concordance of Pathology and Microbiology Testing in Identifying Pathogens in Immunosuppressed Individuals: A Case Report Series

Author Block:

E. McDonald[1], R. Relich[2], B. Schmitt[2], T. Davis[2]; [1]St. Vincent Hosp., Indianapolis, IN, [2]Indiana Univ. Sch. of Med., Indianapolis, IN

Abstract Body:

Immunocompromised patients are susceptible to infection from a wide variety of pathogens and identification of the causative organism(s) is crucial to choosing appropriate antimicrobial treatment. To meet the challenges these cases often present, our institution has established an infectious disease pathology (IDP) protocol. The IDP combines a battery of STAT DiffQuick, Giemsa, Gram, GMS, Calcofluor, and Modified Acid Fast stains and examination by pathologists with a panel of traditional microbiological tests. It is uncertain whether the microbiology test results generally confirm the STAT pathology findings or whether the two laboratory modalities provide discrete information. We wanted to determine 1) the overall positivity of pathological versus microbiological testing 2) the concordance rates of the two testing modalities and 3) do discordant results provide any significant clinical value. 513 IDP reports from specimens sent to IU Health in 2013 were compiled. Cases with equivocal pathological interpretations and with inaccessible microbiological results were excluded, reducing the number of cases analyzed to 442. Based upon pathological interpretation, 20.8% of cases were positive. Microbiology testing produced 25.8% positive cases by bacterial culture, 8.8% by fungal culture, and 4.8% by AFB culture. The overall concordance rate between the results was 70.4%. When the initial pathology result was negative, the concordance with microbiological testing increased to 75.4%. However, when the initial pathology result was positive, the concordance rate decreased significantly to 51.1%. Analyzing the discordant results with a positive pathology interpretation showed 55.6% of cases identified a fungal pathogen, most commonly Pneumocystis jirovecii, which cannot be identified by fungal culture. Discordant results with a negative pathology interpretation revealed 70.5% of cases grew a bacterial pathogen by culture, most commonly Pseudomonas aeruginosa and Staphylococcus aureus. Thus, the two testing modalities should be viewed as complimentary rather than confirmatory, as both the pathological results and microbiological results provide distinct information that will directly influence clinical treatment decisions.

Author Disclosure Block:

E. McDonald: None. **R. Relich:** None. **B. Schmitt:** None. **T. Davis:** None.

Poster Board Number:

SATURDAY-323

Publishing Title:

Evaluation of a Specific *Nocardia* Genus Pcr on Clinical Samples for the Diagnosis of Nocardiosis

Author Block:

C. Rouzaud[1], V. Rodriguez-Nava[2], E. Catherinot[3], F. Mechaï[4], E. Bergeron[2], E. Farfour[5], A. Scemla[6], F. Suarez[7], O. Lortholary[1], D. Lebeaux[1]; [1]Ctr. d'Infectiologie Necker Pasteur, Paris, France, [2]Université Lyon 1, UMR CNRS 5557 Ecologie microbienne, Laboratoire de Mycologie Microbiologie, Lyon, France, [3]Hôpital Foch, Pneumologie, Suresnes, France, [4]Hôpital Avicenne, Maladies Infectieuses et Tropicales, Bobigny, France, [5]Hôpital Foch, Bactériologie, Suresnes, France, [6]Hôpital Necker Enfants Malades, Transplantation Rénale, Paris, France, [7]Hôpital Necker Enfants Malades, Hématologie, Paris, France

Abstract Body:

Background: The diagnosis of nocardiosis, a severe opportunistic infection, is challenging. This study aimed to: i) assess the sensibility and specificity of a specific 16S-based *Nocardia* PCR performed on clinical samples ii) describe cases of nocardiosis diagnosed with PCR. **Methods:** French prospective study conducted between January 2014 and April 2015. Inclusion criteria were: i) underlying condition favoring nocardiosis; ii) clinical and/or radiological signs compatible with nocardiosis. Patients were retrospectively classified as:Negative control (NC): negative culture for *Nocardia* and proven alternate diagnosis or improvement at 6 months without any anti-*Nocardia* treatment Positive control (PC): positive culture for *Nocardia* Probable nocardiosis (PN): positive *Nocardia* PCR from clinical samples and negative culture for *Nocardia* and no alternative diagnosis.**Results:** 67 patients were included. At the end of the follow up period, 47 were classified as NC, 9 as PC, 11 as NC. Mean age was 51(+/-19) years. Patients had a mean of 2 nocardiosis risk factors, most frequent being: immunosuppressive treatment (n=51), pulmonary disease (n=27), organ (n=14) or hematopoietic stem cell transplantation (n=17). Among the 47 NC, PCR was negative in 74% (35/47) of the cases. All patients with a false-positive PCR had respiratory tract samples. Moreover, patients with a false-positive PCR more frequently had chronic pulmonary disease: 67% (8/12) VS 31% (11/35), p=0.044. PCR was positive for 8/9 PC (88%). We also describe 11 cases of PN: 5 disseminated, 7 pulmonary and 7 extrapulmonary diseases.**Conclusions:** In our study, specific *Nocardia* PCR had a specificity and sensibility of 74% and 88%. Nocardiosis might be underestimated but the interpretation of PCR on respiratory samples is difficult, due to the possible detection of colonization.

Author Disclosure Block:

C. Rouzaud: None. **V. Rodriguez-Nava:** None. **E. Catherinot:** None. **F. Mechaï:** None. **E. Bergeron:** None. **E. Farfour:** None. **A. Scemla:** None. **F. Suarez:** None. **O. Lortholary:** None. **D. Lebeaux:** None.

Poster Board Number:

SATURDAY-324

Publishing Title:

Genotypic and Phenotypic Diversity Among Clinical Isolates of *Granulibacter bethesdensis*, an Emerging Pathogen in Chronic Granulomatous Disease

Author Block:

J. Chu[1], L. Ding[1], A. Pettinato[1], K. Barbian[2], S. Kramer[2], C. Martens[2], E. Dahlstrom[2], H. Hong[1], J. Neves[3], L. Rogge[1], E. L. Falcone[1], D. E. Greenberg[4], A. M. Zelazny[5], S. F. Porcella[2], S. M. Holland[1], J. I. Gallin[1], **K. A. ZAREMBER**[1]; [1]NIAID, Bethesda, MD, [2]NIAID, Hamilton, MT, [3]Hosp. Dona Estefania, Lisbon, Portugal, [4]Univ. of Texas Southwestern Med. Ctr., Dallas, TX, [5]Clinical Ctr., Bethesda, MD

Abstract Body:

Frequent life-threatening infections associated with Chronic Granulomatous Disease (CGD) occur due to deleterious mutations in genes encoding the reactive oxygen species-generating phagocyte NADPH oxidase (NOX2). Commonly, *Staphylococcus*, *Burkholderia*, and *Aspergillus* cause infections in CGD, however, over the past decade ≥9 CGD patients had serious infections (2 being fatal) caused by *G. bethesdensis*, a facultative methylotroph and member of the *Acetobacteraceae*. Serology of NIH CGD patients suggests that exposure is even more common and, in some cases, long-term sub-clinical infections may occur. Previous studies utilizing the type strain demonstrated NOX2-dependent killing by human myeloid cells as well as dramatic transcriptional adaptation of *G. bethesdensis* to its intracellular niche. To better understand why some cases were lethal and others were relatively mild, we compared the genotypes and phenotypes of 7 NIH isolates to a lethal Portuguese isolate. Total genome sequencing of these strains demonstrates that despite ≥99.7% identity in the 16s rRNA gene, unique genetic material accounts for up to 11% of each isolate's genome indicating promiscuous gene exchange in this genus. Phenotypically, the Portuguese isolate grew faster than NIH isolates *in vitro*, was a significantly less potent activator of human neutrophils and bound less complement but had intermediate sensitivity to normal neutrophils. On the other hand, it resisted killing by normal monocytes. Challenge of CGD mice demonstrated that the Portuguese isolate is significantly more virulent than 2 different NIH isolates. Further studies are underway to identify specific genetic determinants of virulence in the Portuguese and NIH isolates to better understand microbial pathogenesis by this emerging and sometimes lethal CGD pathogen.

Author Disclosure Block:

J. Chu: None. **L. Ding:** None. **A. Pettinato:** None. **K. Barbian:** None. **S. Kramer:** None. **C. Martens:** None. **E. Dahlstrom:** None. **H. Hong:** None. **J. Neves:** None. **L. Rogge:** None. **E.L. Falcone:** None. **D.E. Greenberg:** None. **A.M. Zelazny:** None. **S.F. Porcella:** None. **S.M. Holland:** None. **J.I. Gallin:** None. **K.A. Zarember:** None.

Poster Board Number:

SATURDAY-325

Publishing Title:

Comparison of Clinical Characteristics and Outcomes of Spontaneous Bacterial Peritonitis and Culture Negative Neutrocytic Ascites in Patients with Liver Cirrhosis

Author Block:

S. Na, N. Kim, K-H. Song, P. Choe, W. Park, J-H. Bang, E. Kim, S-W. Park, H. Kim, M-d. Oh; Seoul Natl. Univ. Coll. of Med., Seoul, Korea, Republic of

Abstract Body:

Background: Ascitic fluid infection (AFI) in cirrhotic patients can be classified into two groups: spontaneous bacterial peritonitis (SBP) and culture-negative neutrocytic ascites (CNNA). The aim of this study is to compare the clinical characteristics and prognosis of patients with SBP or CNNA. **Methods:** We retrospectively reviewed the medical records of cirrhotic patients with AFI. We evaluated demographic data, clinical presentation, laboratory findings, including ascites and/or blood culture, liver function defined by Child-Pugh score, Model for End-stage Liver Disease (MELD) score, and mortality rates.**Results:** Between January, 2006, and December, 2014, 565 patients with AFI were evaluated; 249 (44.1%) of those patients had SBP while 316 (55.9%) had CNNA. *Escherichia coli* (35.7%) was the most common isolated pathogen, followed by *Klebsiella pneumoniae* (17.7%), and Viridans streptococci (8.0%). Although the prevalence of fever (57.8% in SBP vs. 56.6% in CNNA, p=0.777), portal systemic encephalopathy (20.9% vs. 18.0%, p=0.395) and gastrointestinal variceal bleeding (10.0% vs. 10.4%, p=0.876) did not differ between the two groups, abdominal pain was significantly more frequently observed in patients in the SBP group (69.5% vs. 46.8%, p<0.001). Ascites neutrophil counts (4,378/mm3 vs. 1,091/mm3, p<0.001) and blood culture positive rate (37.3% vs. 20.3%, p<0.001) were significantly higher in the SBP group. The SBP group had a significantly worse MELD score with a mean value of 24.26 ± 8.84, compared with the CNNA group (21.67 ± 9.12) (p=0.001). The 7-day mortality rate was significantly higher in the SBP group (10.2% vs. 4.5%, p=0.010), but the 30-day mortality rate (24.3% vs. 18.0%, p=0.085) and 90-day mortality rate (40.1% vs. 36.1%, p=0.382) showed no significant difference.**Conclusions:** Patients in the SBP group had a higher MELD score, ascites neutrophil counts, and positive blood culture rate. Although 7-day mortality was significantly higher in the SBP group, 30-day and 90-day mortality rates were similar between the two groups.

Author Disclosure Block:

S. Na: None. **N. Kim:** None. **K. Song:** None. **P. Choe:** None. **W. Park:** None. **J. Bang:** None. **E. Kim:** None. **S. Park:** None. **H. Kim:** None. **M. Oh:** None.

Poster Board Number:

SATURDAY-326

Publishing Title:

Impact of Antibacterial Prophylaxis in Refractor or Relapse Acute Myeloid Leukemia

Author Block:

B. Ganti, B. Marini, **J. Nagel**, A. Perissinnotti; Univ. of Michigan, Ann Arbor, MI

Abstract Body:

Background: Antibacterial prophylaxis with fluoroqinolones or trimethoprim/sulfamethoxazole in neutropenic patients is associated with decreased rates of infection. However, several publications report increasing rates of infection with nosocomial pathogens and antibiotic resistant pathogens. Significant heterogeneity in both the degree of immunosuppression and incidence of infection exists among all neutropenic patients. There are minimal published data evaluating the impact of antibacterial prophylaxis in profoundly immunosuppressed patients with relapsed or refractory acute myeloid leukemia (AML). **Methods:** This retrospective, single-center cohort study evaluated the impact of levofloxacin prophylaxis in neutropenic patients with relapsed or refractory AML from November 2006 to December 2015. A formal institutional protocol recommending antibacterial prophylaxis for relapsed or refractory AML was implemented in December 2013. The primary and secondary outcomes evaluated include incidence of bacteremia, incidence of overall infections, and clinical outcomes for patients receiving levofloxacin prophylaxis versus those not receiving prophylaxis. **Results:** A total of 145 patients were included; 97 in the control group and 48 in the prophylaxis group. Antibacterial prophylaxis significantly reduced the incidence of gram-negative bacteremia (21.6% vs. 2.1%, p=0.001) and the incidence of gram-negative infections from all sites (32.0% vs. 14.6%, p=0.028). Antibacterial prophylaxis was associated with a non-significant reduction in bacteremia from any pathogen (53.6% vs. 37.5%, p=0.079), a reduction in overall infections from any cultured pathogen (69.1% vs. 47.9%, p=0.081), and a numeric reduction in all-cause mortality during admission (14.4% vs. 10.4%, p=0.61). Antibacterial prophylaxis did not increase the incidence of *C. difficle* infection (13.4% vs. 10.4%, p=0.79). **Conclusion:** Bacteremia and other bacterial infections are common in relapsed or refractory AML patients. Antibacterial prophylaxis was associated with a reduction in gram-negative bacteremia, and gram-negative infections from all sites. A numeric, non-significant reduction in all-cause mortality and infection from any pathogen was also noted in patients receiving antibacterial prophylaxis.

Author Disclosure Block:

B. Ganti: None. **B. Marini:** None. **J. Nagel:** None. **A. Perissinnotti:** None.

Poster Board Number:

SATURDAY-327

Publishing Title:

Use of the Filmarray Respiratory Panel as Part of a Rapid Diagnostic Protocol for Critically Ill, Immunosuppressed Patients

Author Block:

B. H. Schmitt, T. E. Davis, R. F. Relich; Indiana Univ. Sch. of Med., Indianapolis, IN

Abstract Body:

Background: Our institution serves a very large immunosuppressed patient population. To help guide their therapy, our practice offers a rapid microscopic analysis of bronchoalveolar lavage fluid (BALF) and other specimen types using a customized protocol. The infectious disease pathology (IDP) protocol includes a battery of six stains that permit detection of bacterial, fungal, and some parasitic pathogens, as well as visualization of host cell populations and detection of viral cytopathology. In a previous case series, we identified that up to 26.3% of IDP cases provided clinically-useful information based on rapid microscopic examination alone. Traditional staining, however, does not permit detection of many viral and atypical bacterial respiratory pathogens. As a result, we used a relatively lengthy PCR method that provided results sometimes 24 hours following specimen collection. In an effort to provide information more rapidly, we recently implemented the FilmArray Respiratory Panel (RP) as part of the IDP. This study analyzes the types and frequency of additional pathogens rapidly identified from critically ill, immunosuppressed patients using the RP as an adjunct to microscopy.**Methods:** Between implementation of the assay and time of this data analysis, 302 patients received IDP's with the RP as a component. The data was assessed for types of and frequency of pathogens identified.**Results:** In total, 60/302 (19.9%) patient specimens yielded pathogens detected by the RP that were otherwise missed by microscopy, with 65 pathogens identified in total. These included: rhino/enterovirus (33); adenovirus (6); respiratory syncytial virus (6); parainfluenza virus type 1 (3), type 3 (9), and type 4 (1); coronavirus NL63 (1); human metapneumovirus (1); *Bordetella pertussis* (1); and *Mycoplasma pneumoniae* (1). In 5 patients, 2 pathogens were detected.**Conclusions:** The RP allowed for detection of non-microscopically identifiable pathogens in nearly 20% of additional patients. Coupled with the microscopic component of the IDP, approximately 45% of cases examined during this timeframe would likely provide an actionable result. The RP panel can potentially provide a useful adjunct rapid diagnostic method in the critically ill, immunosuppressed population.

Author Disclosure Block:

B.H. Schmitt: None. **T.E. Davis:** None. **R.F. Relich:** None.

Poster Board Number:

SATURDAY-328

Publishing Title:

***Cryptococcus neoformans* Infections in Patients with Lymphoproliferative Disorders**

Author Block:

M. V. Dioverti, S. A. Parikh, A. J. Tande, D. R. Osmon; Mayo Clinic, Rochester, MN

Abstract Body:

Background: Cryptococcosis is an opportunistic infection reported with increasing frequency in those with hematologic malignancies. While several cases of cryptococcosis in patients with lymphoproliferative disorders (LPD) have been reported, the clinical course and outcomes remain undefined. We performed a retrospective review to describe cryptococcosis in these patients.**Methods:** Adult patients with LPD and cryptococcosis from 1/2000 to 7/2015 were identified from an existing database. Data was obtained via retrospective chart review and descriptive statistics were used for analysis.**Results:** We identified 34 patients. The median age was 69 years (62 - 75) and patients were predominantly male (n= 29, 85%). The hematologic diagnosis was chronic lymphocytic leukemia (CLL) in 56% (n=19) while the rest had other LPD. There were 9 patients (26%) who had not received chemotherapy prior to infection. Infectious syndromes included disseminated infection 38% (n=13), pneumonia 29% (n=10), central nervous system (CNS) infection 15% (n=5), asymptomatic antigenemia 9% (n=3) or pulmonary colonization 9 % (n=3). Based on EORTC/MSG definition, 82% of patients had proven infection and 18% had probable infection, excluding asymptomatic antigenemia or colonization. Diagnosis was made via both culture and antigen in 59% (n=20), culture alone in 26% (n=9) and antigen alone in 12% (n=4). None were receiving antifungal prophylaxis. There was 1 death due to cryptococcal infection. Mortality at 30-days and 1 year was 18% and 46% respectively. We also stratified those with disseminated/CNS infection (DCI) (n=18, 53%) versus other (n=16, 47%). The diagnosis of malignancy preceded the infection by a median of 57 months (0-202 months) in the first group versus 18 months (0 - 131 months) in the latter (p=0.07). There were 16 patients (89%) with DCI and 8 patients (50%) without who received prior chemotherapy. 30 day mortality was 17% in the DCI group versus 19% in other.**Conclusions:** Cryptococcal infection in LPD patients is associated with poor outcomes, with 30 day mortality of 18% in our cohort. Further research to identify those at highest risk of infection is warranted, to define whether targeted antifungal prophylaxis would be beneficial.

Author Disclosure Block:

M.V. Dioverti: None. **S.A. Parikh:** None. **A.J. Tande:** None. **D.R. Osmon:** None.

Poster Board Number:

SATURDAY-329

Publishing Title:

Multiple Sclerosis and Infection: Microorganisms and Resistance Patterns

Author Block:

A. Silva-Pinto, A. Costa, M. Sá, P. Abreu, R. Serrão, A. Sarmento; Centro Hosp.ar São João, Porto, Portugal

Abstract Body:

Background: Infections requiring hospital admission and microorganisms' resistance patterns are poorly characterized in Multiple Sclerosis (MS). Our aim was to identify the most frequent site of infections, which microorganisms often cause infection in MS patients and relate resistance patterns with severity of infection, Intermediate/Intensive care Units (ICU) admission and death.**Methods:** We performed a retrospective study of MS patients admitted to our hospital between 2006 and 2014 due to infections. We used the most appropriate measure of central tendency, of distribution and statistic test. The significance level used was 0.05.**Results:** From the 608 MS patients's admissions, 60 hospital admissions occurred due to infections (34 patients). The patients had a median age of 56.5 and 18 were female. Most common locations of infections were higher (n=21) and lower urinary tract infections (n=11) and pneumonia (n=11).Other less frequent sites of infections were higher airway (n=5) and gastrointestinal infections (n=2). Thirty-six patients had sepsis; 3 severe sepsis and 8 septic shock. Eleven patients were admitted to ICU. Cultural isolation was obtained in 34 hospital admissions: *K. pneumoniae* (n=13) and *E. coli* (n=7) were the most frequently isolated microorganisms. Eleven had multiple susceptible organisms, 18 multidrug resistant (MDR) and 5 extensively drug-resistant (XDR). Patients infected with MDR/XDR microorganisms were more often admitted to the hospital (Mann-Whitney U, p=0.015). We found a statistically significant association between the presence of XDR microorganism and death (χ^2, p=0.05). Full recovery was obtained in most patients (n=57), 1 patient partially recovered and 2 patients died due to devastating infections.**Conclusions:** Urinary tract infections are the most common location of infection requiring hospitalization in MS patients. The majority of isolated microorganisms had antibiotic resistance and five were extensively-drug resistant. Drug resistance was associated with more hospital admissions and death.

Author Disclosure Block:

A. Silva-Pinto: None. **A. Costa:** None. **M. Sá:** None. **P. Abreu:** None. **R. Serrão:** None. **A. Sarmento:** None.

Poster Board Number:

SATURDAY-330

Publishing Title:

Clinical Significance of Identifying *Corynebacterium* Species and Other Coryneform Gram-positive Bacilli in Blood Bb Maldi-Tof Mass Spectrometry - A Retrospective Chart Review

Author Block:

A. Mushtaq, D. J. Chen, B. L. Dylla, N. C. Cole, G. J. Strand, J. Mandrekar, R. Patel; Mayo Clinic, Rochester, MN

Abstract Body:

Background: With the advent of matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS), most Gram-positive bacilli (GPB) are readily identified, but their clinical relevance in blood cultures may be unclear. Herein, we assessed the clinical significance of identifying coryneform GPB in blood cultures by MALDI-TOF MS.**Methods:** A retrospective chart review of patients who presented to the Mayo Clinic, Rochester, MN from January 1, 2013 to October 13, 2015 was performed. We included all instances of blood cultures positive for *Corynebacterium* species or coryneform GPB. We assessed the number of bottle sets positive for a given isolate, time to positivity of blood cultures, patient age, medical history, interpretation of culture results by the healthcare team and whether an infectious diseases consultation was performed.**Results:** During the study period, 181 patients had a total of 246 GPBs isolates from blood cultures. 55.8% (n=101) of positive patient cultures were determined to be contaminants by the healthcare team and were not treated; 32.6% (n=59) were clinically determined to represent true bacteremia and were treated; 7.7% (n=14) were considered to be of uncertain significance, with patients prescribed treatment regardless; and, 3.9% (n=7) of patients had been discharged, were deceased or were transferred to another facility at culture positivity. Patient characteristics associated with an isolate being treated included younger age ($p=0.019$), identification to the species level ($p=0.02$), higher number of positive blood culture sets ($p<0.0001$), shorter time to positivity ($p<0.0001$), immunosuppression ($p=0.02$), and recommendation made by an infectious disease specialist ($p=0.0005$).**Conclusions:** This study highlights the role of MALDI-TOF MS in identification of GPB in blood and its implication on patient management, as well as the role of patient age, full species identification, number of positive blood culture sets, time-to-positivity of culture, immunosuppression, and infectious diseases consultation in healthcare teams' decisions to treat.

Author Disclosure Block:

A. Mushtaq: None. **D.J. Chen:** None. **B.L. Dylla:** None. **N.C. Cole:** None. **G.J. Strand:** None. **J. Mandrekar:** None. **R. Patel:** None.

Poster Board Number:

SATURDAY-331

Publishing Title:

Outcomes and Safety of De-Escalation Therapy in Cancer Patients Receiving Empiric Meropenem

Author Block:

K. Shah[1], E. Fay[2], M. Kleinberg[1], E. Heil[2], J. T. Bork[3]; [1]Univ. of Maryland Sch. of Med., Baltimore, MD, [2]Univ. of Maryland Med. Ctr., Baltimore, MD, [3]Johns Hopkins Univ., Baltimore, MD

Abstract Body:

Background: Meropenem is frequently used for suspected infections in febrile cancer patients, and is standard empiric treatment in patients receiving fluoroquinolone (FQ) prophylaxis at our institution. Carbapenem-sparing regimens may prevent development of resistance and preserve broad-spectrum antibiotics. The purpose of this study is to analyze outcomes of meropenem de-escalation in febrile cancer patients. **Methods:** This is a retrospective study performed at the University of Maryland Greenebaum Cancer Center. Patients were identified from an existing antimicrobial prescription database from June 2014 to August 2015. Inclusion criteria were age ≥18 y and first febrile episode for which meropenem was given within 48 h of admission. Descriptive and outcome data were collected. A comparison was performed between: Group 1) de-escalated from meropenem, and Group 2) maintained on meropenem. **Results:** Eighty-seven patients met inclusion criteria. Mean age was 59 y, 64% had acute leukemia, and 95% had received chemotherapy. The main indication for meropenem was use of FQ prophylaxis (60%). 22% of patients had positive cultures, of which 37% were GN, and none had ceftriaxone or meropenem resistance. Fifty-four patients (62%) were de-escalated from meropenem an average of 66.2 h after first dose, mostly to cefepime or piperacillin-tazobactam (TZP). In Group 1, 26% of patients had positive cultures (vs. 15% in Group 2), and of these 36% were GN (vs. 20% in Group 2). Only three resistant GNs were isolated, two in Group 1 (FQ and TZP), and one in Group 2 (FQ). There was no breakthrough bacteremia in either group. Patients in Group 1 had fewer multidrug-resistant (MDR) GNs after admission (7% vs. 9%), and were also more likely to be discharged home (82% vs. 76%). Group 1 had a higher mortality than Group 2 (9% vs 6%), however, none of these deaths were attributed to untreated infection. **Conclusions:** De-escalated patients were more likely to have positive cultures and less likely to have MDR GNs isolated after hospitalization. No breakthrough MDR GN bacteremia was observed. This study suggests that febrile cancer patients with negative cultures can be safely de-escalated to narrow-spectrum antibiotics. Larger scale studies are needed.

Author Disclosure Block:

K. Shah: None. **E. Fay:** None. **M. Kleinberg:** None. **E. Heil:** None. **J.T. Bork:** None.

Poster Board Number:

SATURDAY-332

Publishing Title:

Evolving Linezolid Resistance Mechanisms in a Worldwide Collection of Enterococcal Clinical Isolates: Results from the Sentry Antimicrobial Surveillance Program

Author Block:

R. E. Mendes, L. M. Deshpande, M. Castanheira, R. N. Jones; JMI Lab., North Liberty, IA

Abstract Body:

Background: Linezolid (LZD) has been in clinical use for several years and large surveillance studies have reported low and stable resistance rates among Gram-positive clinical pathogens. The LZD resistance mechanisms are well known and mostly comprised of G2576T alterations. However, a new transferable determinant (*optrA*) was recently reported in enterococcal isolates from human and animal origins in China. This study evaluated the LZD resistance mechanisms among a global collection of enterococcal clinical isolates.**Methods:** Isolates from the SENTRY Antimicrobial Surveillance Program (2008-2015) were selected. Identification and MIC testing were performed by MALDI-TOF and broth microdilution (CLSI), respectively. Isolates with LZD MICs at ≥4 μg/ml were screened for *cfr*, *optrA* and mutations in the 23S rRNA-, L3 and L4-encoding genes. *optrA*-carrying isolates were submitted to whole genome sequencing for characterization of genetic context.**Results:** A total of 26 *E. faecalis* (EF) and 60 *E. faecium* (EFM) had LZD MICs at ≥4 μg/ml (0.35% of surveillance isolates received). EF had a LZD MIC range of 2 - 16 μg/ml ($MIC_{50/90}$, 8/16 μg/ml), while EFM displayed higher values (4 - 64 μg/ml; $MIC_{50/90}$, 8/32 μg/ml). *optrA* was detected in 13 (50.0%) EF and nine (33.3%) isolates had G2576T mutations in the 23S rRNA. The remaining EF isolates had *cfr* (1 isolate) or L4 alterations (2) alone. Most (53.8%) *optrA*-carrying EF were from the Asia-Pacific region (China [5], Malaysia [1] and Thailand [1]), but other isolates originated from the USA (2), Ireland (2), Panama (1) and Ecuador (1). Two *optrA*-carrying EF (Thailand and Panama) also produced Cfr. All EFM had G2576T mutations, while two isolates each had 23S rRNA alternations with concomitant presence of *cfr* (USA) or *cfr*(B) (USA). *optrA*-carrying enterococci (13 EF) exhibited high MICs for chloramphenicol, retapamulin and tiamulin. *optrA* was plasmid-located in most isolates and genetic context varied greatly.**Conclusions**: LZD resistance mechanisms differ between EF and EFM isolates. 23S rRNA alterations remain the main resistance mechanism in EFM, while the ABC transporter *optrA* prevails in EF. Plasmid-borne *optrA* was detected in isolates from countries other than China and showed a diverse genetic context.

Author Disclosure Block:

R.E. Mendes: None. **L.M. Deshpande:** None. **M. Castanheira:** None. **R.N. Jones:** None.

Poster Board Number:

SATURDAY-333

Publishing Title:

Tigecycline Activity against Multi-Drug Resistant (MDR) *Acinetobacter baumannii* Collected from the Test Program: A Global Perspective 2012-2015

Author Block:

D. Hoban[1], **D. Biedenbach**[1], D. Sahm[1], H. Leister-Tebbe[2]; [1]IHMA, Inc., Schaumburg, IL, [2]Pfizer, Inc, Collegeville, PA

Abstract Body:

Background: *Acinetobacter baumannii* (ACB) causes infections including wound, lower respiratory tract, intra-abdominal and bacteremia among seriously ill hospitalized patients and is associated with high levels of resistance to multiple antimicrobial classes including carbapenems. Resistance patterns for this species can vary geographically. In this study analysis of global data from the Tigecycline Evaluation Surveillance Trial (TEST) was done to evaluate regional differences in ACB resistance patterns.**Methods:** Hospitals in Asia, Africa, Europe (EU), North America (NA), Latin America (LA) and Middle East (ME) contributed 4674 ACB isolates from multiple specimen sources in 2012-2015. Susceptibility testing was performed at each site by broth microdilution following CLSI guidelines and MIC results were interpreted using current CLSI and FDA (tigecycline) breakpoints. For this study MDR was defined as resistance to three or more drug classes.**Results:** The activity of seven agents against ACB are shown in the table.

Antibiotics	**% Susceptible/MIC$_{90}$ (mcg/ml)**							
	All ACB (N=4674)	**MDR ACB (N=2946)**	**MDR Asia (N=82)**	**MDR Africa (N=166)**	**MDR EU (N=1800)**	**MDR NA (N=502)**	**MDR LA (N=224)**	**MDR ME (N=172)**
Amikacin	54.5/>64	27.7/>64	13.4/>64	21.1/>64	24.3/>64	47.6/>64	23.2/>64	24.4/>64
Cefepime	33.4/>32	1.9/>32	1.2/>32	1.2/>32	2.2/>32	2.0/>32	0.9/>32	0/>32
Ceftazidime	35.0/>16	4.6/>16	1.2/>32	3.6/>16	4.9/>16	5.4/>16	3.1/>16	2.9/>16
Levofloxacin	33.6/>8	2.4/>8	7.3/>8	6.6/>8	1.6/>8	2.2/>8	1.8/>8	7.0/>8
Meropenem	37.6/>16	3.4/>16	0/>16	1.8/>16	4.1/>16	3.9/>16	0.9/>16	0.6/>16
Pip-Tazo	33.7/>128	1.0/>128	2.2>128	0/>128	0.8/>128	2.0/>128	0.5/>128	0/>128
Tigecycline	NB[a]/2	NB/2	NB/2	NB/1	NB/2	NB/2	NB/1	NB/2

[a]NB, no breakpoint criteriaAmong the 4674 ACB isolates collected 63.0% were MDR in all regions combined. MDR rates were higher in ME, Asia, Africa and LA (85-92%) compared to

EU (63.3%) and NA (47.7%).**Conclusions:** The percent susceptibility to each drug tested dropped substantially with the MDR phenotype. Tigecycline was the only drug, based on MIC_{90} data, that maintained a potent level of in vitro activity against ACB overall, and this level of activity did not appear to be affected by the MDR phenotype. Although tigecycline is not indicated for the treatment of infections caused by ACB, it was the only drug to maintain good in vitro activity and this activity should continue to be monitored over time and on a regional basis.

Author Disclosure Block:

D. Hoban: M. Independent Contractor; Self; IHMA, Inc. **D. Biedenbach:** M. Independent Contractor; Self; IHMA, Inc. **D. Sahm:** M. Independent Contractor; Self; IHMA, Inc. **H. Leister-Tebbe:** D. Employee; Self; Pfizer, Inc..

Poster Board Number:

SATURDAY-334

Publishing Title:

***In vitro* Activity of Tigecycline and Comparators against a North American Collection of *Enterobacteriaceae* - Update of the Test Program**

Author Block:

D. Biedenbach[1], H. Leister-Tebbe[2], D. Sahm[1]; [1]IHMA, Inc., Schaumburg, IL, [2]Pfizer, Inc, Collegeville, PA

Abstract Body:

Background: Several species of Enterobacteriaceae are problematic to treat empirically due to intrinsic and/or acquired resistance mechanisms that are in a steady state of flux. MIC data collected from the Tigecycline Evaluation Surveillance Trial (TEST) were analyzed to provide updated susceptibility results for five commonly prescribed empiric agents used to treat infections caused by five common Enterobacteriaceae species or species groups. **Methods:** Isolates were identified and susceptibility tested using broth microdilution according to CLSI guidelines at each participating institution in North America during The TEST program 2011-2015. CLSI breakpoint criteria were applied to define susceptibility rates. FDA breakpoints were used for tigecycline. **Results:** Susceptibility to agents by organism are provided in the following table.

	Organism (n)/ MIC_{90}/%S					
Drug	***Citrobacter* spp. (52)**	***Enterobacter* spp. (4134)**	***E. coli* (4772)**	***Klebsiella* spp. (4670)**	***Serratia* spp. (1751)**	
Cefepime	4/86.5	4/89.9	4/89.1	2/90.5	≤0.5/96.3	
Levofloxacin	8/86.5	0.5/95.4	>8/67.8	4/89.0	1/95.1	
Meropenem	0.25/92.5	0.25/98.9	≤0.06/99.5	0.12/96.5	0.25/97.9	
Piperacillin-tazobactam	64/76.9	64/84.2	8/95.4	16/90.5	8/95.8	
Tigecycline	1/100	1/96.0	0.25/99.9	1/96.1	2/96.9	

ESBL rates were 9.8%, 8.6%, and 3.7% for *E. coli*, *K. pneumoniae*, and *K. oxytoca*, respectively.**Conclusions:** Tigecycline and meropenem were the only agents that provided >90% susceptibility against the five organism groups examined in this study. Variability among drug activity against certain species was observed, including lower levofloxacin susceptibility among *E. coli* and reduced activity of piperacillin-tazobactam activity against intrinsic AmpC-producing

Citrobacter spp. and *Enterobacter* spp. The TEST program will continue to monitor changes in the activity of these agents against Enterobacteriaceae.

Author Disclosure Block:

D. Biedenbach: M. Independent Contractor; Self; IHMA, Inc. **H. Leister-Tebbe:** D. Employee; Self; Pfizer, Inc. **D. Sahm:** M. Independent Contractor; Self; IHMA, Inc..

Poster Board Number:

SATURDAY-335

Publishing Title:

Activity of Telavancin against a Global Collection of *Staphylococcus aureus* Causing Bacteremia (2011-2014)

Author Block:

R. E. Mendes[1], H. S. Sader[1], D. J. Farrell[1], J. I. Smart[2], R. K. Flamm[1], R. N. Jones[1]; [1]JMI Lab., North Liberty, IA, [2]Theravance Biopharma US, Inc, South San Francisco, CA

Abstract Body:

Background: Telavancin (TLV) vs standard intravenous therapy is under investigation for the treatment of subjects with *S. aureus* (SA) bacteremia, including endocarditis in a Phase 3 trial. TLV activity was assessed against a global collection of SA bacteremia isolates, including those responsible for endocarditis.**Methods:** 4,191 SA bacteremia isolates from a global network of hospitals were included. Isolates were submitted to a central laboratory as part of a surveillance program (2011-2014). Identification was confirmed and susceptibility (S) testing performed by CLSI methods. MIC interpretation of TLV results used the USA FDA, CLSI and EUCAST approved criteria.**Results:** Overall, SA isolates (100.0% TLV-S) had TLV MIC_{50}, MIC_{90} and MIC_{100} results of 0.03, 0.06 and 0.12 µg/ml, respectively. Equivalent MICs ($MIC_{50/90}$, 0.03/0.06 µg/ml) were obtained for methicillin-susceptible (MSSA) and -resistant (MRSA) isolates, as well as MRSA from community and nosocomial origins. TLV ($MIC_{50/90}$, 0.03/0.06 µg/ml) had similar potency against MRSA from North America and Europe, while isolates from the Asia-Pacific (APAC) and Latin America regions had slightly higher MIC_{50} values ($MIC_{50/90}$, 0.06/0.06 µg/ml). MRSA with vancomycin MICs of 2-4 µg/ml had TLV MICs ($MIC_{50/90}$, 0.06/0.12 µg/ml) 2-fold higher than those with vancomycin MICs at ≤1 µg/ml ($MIC_{50/90}$, 0.03/0.06 µg/ml), but TLV still inhibited all isolates at the S breakpoint of ≤0.12 µg/ml. SA causing endocarditis were inhibited by TLV ($MIC_{50/90}$, 0.03/0.06 µg/ml) at ≤0.12 µg/ml (100.0% S). Overall, TLV was 8-fold more potent than daptomycin ($MIC_{50/90}$, 0.25/0.5 µg/ml) and 16- to 32-fold more potent than linezolid ($MIC_{50/90}$, 1/1 µg/ml) and vancomycin ($MIC_{50/90}$, 1/1 µg/ml) against MRSA.**Conclusions:** TLV (100.0% S) demonstrated potent activity against this global and contemporary collection of SA causing bacteremia, including endocarditis. These *in vitro* results support the investigation of TLV for the treatment of SA bacteremia.

Organism (No. tested)[a]	MIC (µg/ml)							
	Telavancin		Daptomycin		Vancomycin		Linezolid	
	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
S. aureus (4,191)	0.03	0.06	0.25	0.5	1	1	1	2
MSSA (2,701)	0.03	0.06	0.25	0.5	1	1	1	2

MRSA (1,490)	0.03	0.06	0.25	0.5	1	1	1	1
MDR (569)	0.06	0.06	0.25	0.5	1	1	1	1
VAN MIC = 2-4 μg/ml (51)	0.06	0.12	0.5	1	2	2	1	2

[a] MSSA = methicillin (oxacillin)-susceptible S. aureus; MRSA = methicillin-resistant S. aureus; MDR = multidrug-resistant (MRSA resistant to three or more drug classes); VAN = vancomycin.

Author Disclosure Block:

R.E. Mendes: H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Theravance Biopharma US, Inc. **H.S. Sader:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Theravance Biopharma US, Inc. **D.J. Farrell:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Theravance Biopharma US, Inc. **J.I. Smart:** D. Employee; Self; Theravance Biopharma US, Inc. **R.K. Flamm:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Theravance Biopharma US, Inc. **R.N. Jones:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Theravance Biopharma US, Inc.

Poster Board Number:

SATURDAY-336

Publishing Title:

***In Vitro* Activity of Piperacillin-Tazobactam and Comparators against *Pseudomonas aeruginosa* in North America and Latin America: Test 2011-2015**

Author Block:

D. Hoban[1], **D. Biedenbach**[1], H. Leister-Tebbe[2], D. Sahm[1]; [1]IHMA, Inc., Schaumburg, IL, [2]Pfizer, Inc, Collegeville, PA

Abstract Body:

Background: *P. aeruginosa* infections are common among debilitated, immunocompromised and seriously ill patients and can be difficult to treat due to the emergence of resistance mechanisms that this pathogen can produce or acquire. Regional variations in antimicrobial susceptibility to multiple classes of antimicrobials also provide additional challenges with selecting appropriate antimicrobial therapy. Piperacillin-tazobactam has remained a common treatment option for infections caused by *P. aeruginosa* for many years and this surveillance study provides current susceptibility data for this agent and comparators in both Latin and North America. **Methods:** *P. aeruginosa* isolates were identified and susceptibility tested using broth microdilution according to CLSI guidelines at each participating institution in North America (3605 isolates) and Latin America (934 isolates) during The Tigecycline Evaluation Surveillance Trial (TEST) program 2011-2015. CLSI breakpoint criteria were applied to define susceptibility rates. **Results:** Susceptibility to key anti-pseudomonal agents by region are provided in the following table.

	Region (n)/MIC_{90}/%S	
Drug	**North America (3605)**	**Latin America (934)**
Piperacillin-tazobactam	64/82.2	>128/60.2
Ceftazidime	16/84.7	32/59.7
Cefepime	16/80.1	>32/62.9
Meropenem	16/77.0	>16/54.3
Levofloxacin	>8/70.7	>8/54.3
Amikacin	8/97.3	>64/77.8

Conclusions: In both regions, based on percent susceptible, piperacillin-tazobactam was among the three most active agents. However, for all drugs studied the percent susceptibility was notably lower in Latin America relative to North America. As *P. aeruginosa* continues to be a

problematic pathogen associated with patient infections and resistance to several antimicrobial classes continued surveillance monitoring on a geographic basis is warranted.

Author Disclosure Block:

D. Hoban: M. Independent Contractor; Self; IHMA, Inc. **D. Biedenbach:** M. Independent Contractor; Self; IHMA, Inc. **H. Leister-Tebbe:** D. Employee; Self; Pfizer, Inc. **D. Sahm:** M. Independent Contractor; Self; IHMA, Inc..

Poster Board Number:

SATURDAY-337

Publishing Title:

Activity of Imipenem-Relebactam (MK-7655) against *Enterobacteriaceae* and *Pseudomonas aeruginosa* from Europe - SMART 2015

Author Block:

M. Hackel[1], K. Young[2], M. Motyl[2], D. Sahm[1]; [1]IHMA, Inc., Schaumburg, IL, [2]MRL Merck & Co., Inc., Kenilworth, NJ

Abstract Body:

Objectives: Relebactam (formerly MK7655) (REL) is a newly developed beta-lactamase inhibitor of class A and class C carbapenemases. REL restores the *in vitro* activity of imipenem (IMI) against *Enterobacteriaceae,* including those producing KPC, and *Pseudomonas aeruginosa* (PA). In this study we evaluated the ability of REL to restore IMI susceptibility to a collection of Gram-negative isolates from European countries participating in the 2015 SMART surveillance program.**Methods:** 50 hospitals in 21 countries collected up to 100 consecutive Gram-negative pathogens from intra-abdominal, 50 from urinary tract and 100 from lower respiratory infections. MICs were determined for 775 PA and 3,835 non-*Proteae Enterobacteriaceae* (NPE) using CLSI broth microdilution. *Proteae* were excluded due to intrinsic non-susceptibility to IMI. REL was tested at a fixed concentration of 4 µg/mL in combination with IMI. The percent susceptible (S) was assessed using EUCAST breakpoints. IMI S breakpoints of ≤2 µg/mL (NPE) and ≤4 µg/mL (PA) were applied to IMI/REL.**Results:** The cumulative percent of isolates at each IMI and IMI/REL MIC is shown in the table.

Organism	N	Drug	MIC (µg/mL)							
			≤0.5	1	2	4	8	16	32	>32
P. aeruginosa	775	IMI	20.1	63.5	69.5	72.8	83.5	**94.7**	97.7	100
P. aeruginosa		IMI/REL	73.8	84.5	**92.8**	95.1	96.8	97.3	98.2	100
P. aeruginosa, IMI NS	211	IMI					39.3	80.6	**91.5**	100
P. aeruginosa, IMI NS		IMI/REL	9.5	44.1	73.5	82.0	88.2	**90.0**	93.4	100
NPE	3378	IMI	89.3	**96.2**	98.4	98.9	99.2	99.5	99.5	100
NPE		IMI/REL	**95.1**	98.7	99.1	99.3	99.5	99.6		100
NPE, IMI NS	55	IMI				30.9	49.1	67.3	70.9	**100**
NPE, IMI NS		IMI/REL	36.4	43.6	47.3	56.4	69.1	74.5		**100**

Shaded area indicates susceptible by EUCAST 2015 imipenem breakpoint; MIC$_{50}$ bolded; NPE, non-*Proteae Enterobacteriaceae*; IMI, imipenem; REL, relebactam; NS, non-susceptible

Among 775 PA, 73% (564) were S to IMI; of the 210 non-susceptible (NS) isolates, 173 (82%) were rendered S by the addition of REL, for a final 95% S. Among 3,835 NPE, 98% (3,758) were S to IMI; of the 77 NS isolates, 43 (56%) were rendered S by the addition of REL, for a final 99% S.**Conclusions**: Relebactam exhibited strong potential for restoring the *in vitro* activity of IMI against many pathogens otherwise NS to carbapenems. Further development of this compound could provide a valuable therapeutic option for treating infections caused by resistant Gram-negative bacilli.

Author Disclosure Block:

M. Hackel: M. Independent Contractor; Self; IHMA, Inc. **K. Young:** D. Employee; Self; Merck & Co., Inc. **M. Motyl:** D. Employee; Self; Merck & Co., Inc. **D. Sahm:** M. Independent Contractor; Self; IHMA, Inc..

Poster Board Number:

SATURDAY-338

Publishing Title:

Activity of Ceftaroline against *Staphylococcus aureus* from Pediatric, Adult, and Elderly Patients

Author Block:

D. Biedenbach[1], J. Iaconis[2], D. Sahm[1]; [1]IHMA, Inc., Schaumburg, IL, [2]AstraZeneca Pharmaceuticals, Waltham, MA

Abstract Body:

Background: Ceftaroline (CPT), the active metabolite of CPT-fosamil, is a cephalosporin with in vitro activity against *S. aureus*, including methicillin resistant *S. aureus* (MRSA). This analysis evaluated the in vitro activity of CPT against *S. aureus* isolates from four geographic regions, stratified by patient age. **Methods:** *S. aureus* isolates were collected from all sources of infections in Europe, Latin America, Africa/Middle East and Asia-Pacific as part of the AWARE Surveillance Program during 2012-2014. Species were identified by a reference laboratory (IHMA, Inc., Schaumburg, IL) and susceptibility tested by broth microdilution according to CLSI guidelines. CLSI breakpoint criteria were applied to define susceptibility to CPT as follows: susceptible (S) ≤1 µg/mL, intermediate (I) 2 µg/mL, resistant (R) ≥4 µg/mL**.** Age was defined as pediatric ≤1-17, adult 18-65, and elderly >65 years old. **Results:** The table provides CPT activity by patient age and geographic region.

Region (n)[a]	MIC_{90}, µg/mL/%S					
	Pediatric (n)		**Adult (n)**		**Elderly (n)**	
	MSSA (1,790)	**MRSA (1,499)**	**MSSA (6,395)**	**MRSA (7,639)**	**MSSA (3,448)**	**MRSA (6,360)**
Europe (14,558)	0.25/100	1/96.1	0.25/>99.9	1/91.0	0.25/>99.9	1/93.6
Latin America (4,047)	0.25/100	1/91.3	0.25/100	2/83.1	0.25/100	2/68.2
Asia-Pacific (6,206)	0.25/100	1/92.2	0.25/100	2/79.1	0.25/100	2/68.4
Africa/Middle East (2,320)	0.25/100	1/91.3	0.25/100	1/91.4	0.25/100	2/89.3

[a] Includes all age groups and MSSA and MRSA combined.MSSA=methicillin-susceptible *S. aureus*; n=number of isolatesCPT non-susceptible isolates (MIC ≥ 2 µg/mL) were primarily only observed among MRSA, regardless of region or age group. CPT susceptibility among MRSA was highest among isolates from pediatric populations. Overall, CPT non-susceptibility

was predominantly due to isolates with I MICs (7.3%) compared to those with R MICs (0.4%).**Conclusions:** Regardless of patient age or geographic region CPT maintained potent and consistent *in vitro* activity against MSSA. CPT activity against MRSA did vary by region and age group, but in the majority of instances non-susceptibility was associated with the intermediate category and not frank resistance. Continued monitoring of CPT activity by regional and age parameters is needed to monitor any changes in the drug's *in vitro* activity.

Author Disclosure Block:

D. Biedenbach: M. Independent Contractor; Self; IHMA, Inc. **J. Iaconis:** D. Employee; Self; AstraZeneca Pharmaceuticals. **D. Sahm:** M. Independent Contractor; Self; IHMA, Inc..

Poster Board Number:

SATURDAY-339

Publishing Title:

Activity of Ceftazidime-Avibactam against *Pseudomonas aeruginosa* Isolated from Patients in Latin America, Asia/Pacific and Middle-East/Africa 2012-2014

Author Block:

M. Hackel[1], G. Stone[2], B. de Jonge[2], D. Sahm[1]; [1]IHMA, Inc., Schaumburg, IL, [2]AstraZeneca Pharmaceuticals, Waltham, MA

Abstract Body:

Background: Avibactam (AVI) inhibits Ambler class A β-lactamases, including extended-spectrum enzymes and KPCs, class C and some class D enzymes, but has no activity against metallo-β-lactamases (MBLs). AVI is able to restore the activity of ceftazidime (CAZ) against many CAZ-resistant *Pseudomonas aeruginosa* (PA). This study reports CAZ-AVI susceptibility data for recent clinical PA isolates from Latin America (LA), Asia/Pacific (AP), and Middle East/Africa (MEA) collected through the INFORM Surveillance initiative. **Methods:** 3,169 PA isolates were collected through 2012-2014 in LA (1,088), AP (1,392) and MEA (689). MICs were determined by CLSI broth microdilution and interpreted following CLSI 2015 or FDA (CAZ-AVI ≤8 μg/mL susceptible) guidelines.**Results**: CAZ-AVI exhibited potent *in vitro* activity against PA with 91.3% of all isolates susceptible, compared to 76.4% for CAZ alone. In the MBL-negative subset, 95.7% were susceptible to CAZ-AVI. Minor differences in activity were observed in different regions (88.7-93.2% susceptible), with higher activity against isolates with no MBLs (93.2-97.4% susceptible).

		MIC_{90}/%Susceptible					
Region	N	CAZ-AVI	CAZ	CEP	MEM	TZP	COL
All	3,169	8/91.3	64/76.4	>16/77.6	>8/72.6	>128/67.7	4/89.2
All, no MBL	3,016	8/95.7	64/80.1	16/81.3	>8/76.1	>128/70.8	4/89.0
LA	1088	16/88.7	64/71.5	>16/73.2	>8/64.9	>128/62.3	2/90.4
LA, no MBL	1031	8/93.2	64/75.4	>16/76.6	>8/68.1	>128/65.4	2/90.5
AP	1392	8/93.2	64/78.1	16/80.2	>8/77.4	>128/71.3	4/87.7
AP, no MBL	1340	8/96.7	32/81.0	16/83.1	8/80.4	>12/73.9	4/87.5
MEA	689	8/91.7	32/80.8	16/79.5	>8/74.8	>12/68.8	4/89.9
MEA, no MBL	645	8/97.4	32/85.9	16/85.0	8/79.9	128/73.2	4/89.1

CAZ-AVI, ceftazidime-avibactam; CAZ, ceftazidime; CEP, cefepime; MEM, meropenem; TZP, piperacillin-tazobactam; COL, colistin; LA, Latin America; AP, Asia/Pacific; MEA, Middle East/Africa; MBL, metallo-β-lactamase.**Conclusions**:CAZ-AVI was the most potent antimicrobial tested against PA isolates collected in LA, AP, and MEA, with 91.3% of all PA isolates susceptible. Activity was in part compromised by MBLs, although additional resistance mechanisms may also be responsible.

Author Disclosure Block:

M. Hackel: M. Independent Contractor; Self; IHMA, Inc. **G. Stone:** D. Employee; Self; AstraZeneca Pharmaceuticals. **B. de Jonge:** D. Employee; Self; AstraZeneca Pharmaceuticals. **D. Sahm:** M. Independent Contractor; Self; IHMA, Inc..

Poster Board Number:

SATURDAY-340

Publishing Title:

***In Vitro* Activity of Imipenem-Relebactam (MK-7655) against *Enterobacteriaceae* and *Pseudomonas aeruginosa* from the United States - SMART 2015**

Author Block:

M. Hackel[1], K. Young[2], M. Motyl[2], D. Sahm[1]; [1]IHMA, Inc., Schaumburg, IL, [2]MRL Merck & Co., Inc., Kenilworth, NJ

Abstract Body:

Objectives: Relebactam (formerly MK7655) (REL) is a newly developed beta-lactamase inhibitor of class A and class C carbapenemases, including KPCs. REL restores the *in vitro* activity of imipenem (IMI) against *Enterobacteriaceae* and *Pseudomonas aeruginosa* (PA). In this study we evaluated the *in vitro* activity of IMI/REL against a collection of Gram-negative isolates from the 2015 SMART surveillance program in United States.**Methods:** 21 hospitals in the US each collected up to 100 consecutive Gram-negative pathogens from intra-abdominal, 50 from urinary tract and 100 from lower respiratory infections. MICs were determined for 351 PA and 1,373 non-*Proteae Enterobacteriaceae* (NPE) using CLSI broth microdilution. *Proteae* were excluded due to intrinsic non-susceptibility to IMI. REL was tested at a fixed concentration of 4 µg/mL in combination with IMI. The percent susceptible (S) was assessed using CLSI breakpoints. IMI S breakpoints of 1 µg/mL (NPE) and 2 µg/mL (PA) were applied to IMI/REL.**Results:** The cumulative percent of isolates at each IMI and IMI/REL MIC is shown in the table.

Organism	N	Drug	MIC (µg/mL)							
			≤0.5	1	2	4	8	16	32	>32
P. aeruginosa	351	IMI	17.7	62.7	70.7	75.8	86.6	**95.7**	99.4	100
		IMI/REL	71.5	82.9	**93.4**	96.3	99.1	99.7		100
P. aeruginosa, IMI NS	103	IMI				17.5	54.4	85.4	**98.1**	100
		IMI/REL	8.7	42.7	77.7	87.4	**97.1**	99.0		100
NPE	1,373	IMI	89.4	**96.0**	98.3	98.7	99.3	99.6	99.9	100
		IMI/REL	**96.1**	99.1	99.9	100				
NPE, IMI NS	43	IMI			46.5	58.1	76.7	86.0	**95.3**	100
		IMI/REL	37.2	69.8	**95.3**	100				

Shaded area indicates susceptible by CLSI 2015 imipenem breakpoint; MIC_{90} bolded; NPE, non-*Proteae* Enterobacteriaceae; IMI, imipenem; REL, relebactam; NS, non-susceptible

Among 351 PA, 71% (248) were S to IMI; of the 103 non-susceptible (NS) isolates, 80 (78%) were rendered S by the addition of REL, for a final 93% S. Among 1,373 NPE, 97% (1,330) were S to IMI; of the 43 NS isolates, 30 (70%) were rendered S by the addition of REL, for a final 96% S.**Conclusions:** Relebactam exhibited strong potential for restoring the *in vitro* activity of IMI against many pathogens otherwise NS to carbapenems. Further development of this compound could provide a valuable therapeutic option for treating infections caused by resistant Gram-negative bacilli.

Author Disclosure Block:

M. Hackel: M. Independent Contractor; Self; IHMA, Inc. **K. Young:** D. Employee; Self; Merck & Co., Inc. **M. Motyl:** D. Employee; Self; Merck & Co., Inc. **D. Sahm:** M. Independent Contractor; Self; IHMA, Inc..

Poster Board Number:

SATURDAY-341

Publishing Title:

***In Vitro* Activity of Tigecycline and Comparators against Genotypically Characterized β-Lactam-Resistant *Enterobacteriaceae* Isolates from the United States and Canada**

Author Block:

K. Kazmierczak[1], B. Johnson[1], H. Leister-Tebbe[2], D. Sahm[1]; [1]IHMA, Inc., Schaumburg, IL, [2]Pfizer, Inc., Collegeville, PA

Abstract Body:

Background: The Tigecycline Evaluation Surveillance Trial (TEST) monitors the *in vitro* activity of the glycylcycline tigecycline (TGC) against clinical isolates collected globally. This study reports the *in vitro* activity of TGC and comparators against *Enterobacteriaceae* (*Ebac*) collected in the United States (US) and Canada (CAN) in 2012-2014. **Methods:** Non-duplicate clinical isolates were collected from 60 participating sites. Susceptibility testing was performed by broth microdilution by the local laboratory using supplied panels and interpreted using CLSI breakpoints. Confirmation of extended-spectrum β-lactamase (ESBL) activity and meropenem (MEM) non-susceptibility (NS) was performed at a central laboratory.A subset of *Ebac* that were MEM-NS and/ or phenotypically ESBL positive (ESBLp+) were screened for β-lactamase (BL) genes by PCR, followed by sequencing.**Results:** TGC demonstrated good *in vitro* activity against *Ebac* collected in the US and CAN, with 97% of overall isolates inhibited by ≤2 µg/mL TGC. A total of 605 isolates (293 *Escherichia coli*, 256 *Klebsiella pneumoniae*, and 56 other *Ebac*) were molecularly characterized for BL genes. MIC_{90}s of TGC against ESBLp+, MEM-susceptible (S) subsets from both countries were 1 µg/mL, and were 1-2 µg/mL against MEM-NS subsets, including isolates carrying carbapenemases (KPC, OXA-48-like, NDM) and those producing ESBLs or AmpC BL that are assumed to also harbor changes in permeability.

	Drug (MIC_{90} [µg/ml]/ % S)				
Country/ phenotype (n)	**TGC**	**CAZ**	**MEM**	**LVX**	**AMK**
CAN					
Ebac All (1975)	1/ 97.6	16/ 87.0	0.12/ 99.2	8/ 85.3	4/ 99.8
ESBLp+, MEM-S (111)	1/ 96.4	>16/ 41.4	0.12/ 100	>8/ 27.0	8/ 98.2
MEM-NS (13)	1/ 100	>16/ 69.2	>16/ 0.0	8/ 84.6	8/ 92.3
US					
Ebac All (8781)	1/ 97.0	16/ 86.7	0.12/ 98.0	8/ 85.1	4/ 99.3
ESBLp+, MEM-S (340)	1/ 95.9	>16/ 25.6	0.12/ 100	>8/ 19.7	16/ 96.8

MEM-NS (141)	2/ 92.2	>16/ 5.0	>16/ 0.0	>8/ 14.9	32/ 88.7

TGC, tigecycline; CAZ, ceftazidime; MEM, meropenem; LVX, levofloxacin; AMK, amikacin; S, susceptible; NS, non-susceptible.**Conclusions:** Amikacin was the only tested agent that displayed similar activity to that of TGC against subsets of ESBLp+ and MEM-NS isolates carrying BL. TGC continues to display good *in vitro* activity against β-lactam-resistant pathogenic *Ebac*.

Author Disclosure Block:

K. Kazmierczak: M. Independent Contractor; Self; IHMA, Inc. **B. Johnson:** M. Independent Contractor; Self; IHMA, Inc. **H. Leister-Tebbe:** D. Employee; Self; Pfizer, Inc. **D. Sahm:** M. Independent Contractor; Self; IHMA, Inc..

Poster Board Number:

SATURDAY-342

Publishing Title:

Activity of Ceftaroline and Comparators against *Staphylococcus* spp. and *Streptococcus* spp. from Patients with Blood Stream Infections (BSI): Aware 2014 Surveillance Program

Author Block:

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Abstract Body:

Background: Ceftaroline (CPT), the active metabolite of CPT-fosamil, is a cephalosporin developed for treating infections caused by *S. aureus* (MSSA and MRSA), *S. pneumoniae* (SPN), β-hemolytic streptococci (BHS), and some Gram-negative pathogens. The analysis evaluated the *in vitro* activity of CPT against Gram-positive isolates collected from BSI's in four geographic regions. **Methods:** Isolates (2018 total) were collected from patients in 37 countries in Europe, Latin America, Africa/Middle East and Asia-Pacific as part of the AWARE Surveillance Program during 2014. 13.4% of the isolates were collected from pediatric patients. Species were identified using MALDI-TOF by a reference laboratory and tested by broth microdilution according to CLSI guidelines. CLSI and EUCAST breakpoint criteria were applied to define susceptibility where applicable**. Results:** The table provides the activity of CPT and comparators against MSSA, MRSA, coagulase-negative staphylococci (CoNS), SPN, viridans group streptococci (VGS), and BHS.

	MIC_{90}, µg/mL [%S CLSI/EUCAST] [a]						
Drug[b]	**MSSA (350)**	**MRSA (427)**	**CoNS (499)**	**Drug[b]**	**SPN (471)**	**VGS (59)**	**BHS (214)**
CPT	0.25 [100/100]	1 [90.6/90.6]	1 [91.4/91.4][c]	**CPT**	0.06 [99.8/99.4]	0.03 [96.6 /94.9][c]	0.015 [100/100]
VAN	2 [100/100]	2 [100/100]	2 [99.0/99.0]	**CRO**	1 [95.1/89.8]	0.5 [93.2/91.5]	0.12 [100/NA][d]
TEC	1 [100/99.7]	2 [100/97.2]	8 [98.8/80.0]	**PEN**	1 [96.4/73.3]	0.25 [88.1/93.2]	0.06 [100/100]
DAP	1 [100/100]	1 [99.8/99.8]	1 [99.4/99.4]	**ERY**	>1 [79.2/79.2]	>1 [66.1/NA][d]	>1 [87.4/87.4]
LZD	2 [100/100]	2 [100/100]	2 [99.4/99.4]	**LZD**	2 [100/100]	2 [100/NA]	2 [100/100]

[a] Susceptibility was determined using CLSI M100-S25 (2015) and EUCAST version 5.0 (2015) breakpoint criteria.[b] Drug abbreviations: CPT (ceftaroline), VAN (vancomycin), TEC (teicoplanin), DAP (daptomycin), LZD (linezolid), CRO (ceftriaxone), PEN (penicillin), ERY (erythromycin).[c] *S. aureus* breakpoints were applied for CoNS and SPN breakpoints were applied for VGS and BHS for nterpretations of susceptibility.[d] NA=no applicable breakpoints recommended **Conclusions:** The findings demonstrate that CPT has potent *in vitro* activity against staphylococcal (including MRSA) and streptococcal isolates collected from BSIs. Given the propensity of these organisms, especially staphylococci, to develop resistance ongoing monitoring of CPT activity as the drug's use increases is warranted.

Author Disclosure Block:

D. Biedenbach: M. Independent Contractor; Self; IHMA, Inc. **J. Iaconis:** D. Employee; Self; AstraZeneca Pharmaceuticals. **D. Sahm:** M. Independent Contractor; Self; IHMA, Inc..

Poster Board Number:

SATURDAY-343

Publishing Title:

***In Vitro* Activity of Aztreonam-Avibactam (ATM-AVI) against Gram-Negative Pathogens from Latin America (LA) Collected in 2014**

Author Block:

K. Kazmierczak[1], M. Hackel[1], B. de Jonge[2], P. A. Bradford[2], D. Sahm[1]; [1]IHMA, Inc., Schaumburg, IL, [2]AstraZeneca Pharmaceuticals, Waltham, MA

Abstract Body:

Background: AVI inhibits serine β-lactamases (BL), including class A ESBLs and KPC, class C, and some class D BL. The combination of ATM, which is refractory to hydrolysis by metallo-β-lactamases (MBL), and AVI is active against carbapenem-resistant *Enterobacteriaceae* (CRE) producing MBL in combination with serine BL. This study evaluated the *in vitro* activity of ATM-AVI and comparators against *Enterobacteriaceae* (*Ebac*) and *P. aeruginosa* (*Paer*) collected in LA in 2014. **Methods:** Non-duplicate clinical isolates were collected from 23 centers in Argentina, Brazil, Chile, Colombia, Mexico, and Venezuela. Susceptibility testing was performed using CLSI broth microdilution. ATM-AVI was tested at a fixed concentration of 4 µg/mL AVI. Isolates that were non-susceptible (NS) to carbapenems, resistant to ceftazidime, or phenotypically positive for ESBL were characterized for the presence of BL genes by PCR and sequencing.**Results:**MICs of ATM-AVI were ≤8 µg/mL against 2167 of 2168 (99.9%) collected *Ebac*, and were ≤4 µg/mL against meropenem-NS isolates. The addition of AVI to ATM reduced MIC_{90} values ≥128-fold, to 0.12-1 µg/mL, against all groups of *Ebac*, including those that produced MBLs co-carried with one or more plasmid- or chromosomally-mediated serine BL (Table). Only VIM- and NDM-type MBLs were found in *Ebac* from LA (Mexico, n=3; Venezuela, n=1). ATM-AVI showed only modest activity against *Paer*.

	Drug (MIC_{90} [µg/mL], % Susceptible)									
Species/ phenotype[a] (n)	**ATM-AVI**		**ATM**		**MEM**		**COL**		**TGC**	
Ebac All (2168)	0.25	NA	128	66.1%	0.25	93.5%	>4	82.2%	2	95.1%
ESBL-positive (560)	0.25	NA	>128	4.1%	4	84.8%	2	91.4%	2	96.4%
Meropenem-S (2028)	0.12	NA	64	70.3%	0.12	100%	>4	82.7%	2	95.1%
Meropenem-NS (140)	0.5	NA	>128	5.0%	>8	0%	>4	75.0%	2	95.7%
MBL-negative (2164)	0.25	NA	128	66.1%	0.25	93.7%	>4	82.2%	2	95.1%
MBL-positive (4)	0.03-1	NA	0.06-128	50.0%	2->8	0%	1-1	100%	0.5-2	100%
Paer All (595)	32	NA	64	58.2%	>8	62.2%	2	90.4%	>8	NA

ATM-AVI, aztreonam-avibactam; ATM, aztreonam; MEM, meropenem; COL, colistin; TGC, tigecycline; NA, no breakpoints available; S, susceptible; NS, non-susceptible. Range is shown for n<10.**Conclusions:** ATM-AVI shows potent activity against β-lactam-resistant *Ebac*, especially isolates producing MBLs, that are becoming globally disseminated.

Author Disclosure Block:

K. Kazmierczak: M. Independent Contractor; Self; IHMA, Inc. **M. Hackel:** M. Independent Contractor; Self; IHMA, Inc. **B. de Jonge:** D. Employee; Self; AstraZeneca Pharmaceuticals. **P.A. Bradford:** D. Employee; Self; AstraZeneca Pharmaceuticals. **D. Sahm:** M. Independent Contractor; Self; IHMA, Inc..

Poster Board Number:

SATURDAY-344

Publishing Title:

Mechanisms and Molecular Epidemiology of Ampicillin-Sulbactam-Nonsusceptible *Escherichia coli*

Author Block:

T. Noguchi, Y. Matsumura, M. Yamamoto, M. Nagao, S. Takakura, S. Ichiyama; Kyoto Univ. Graduate Sch. of Med., Kyoto, Japan

Abstract Body:

Background: A recent increase in the prevalence of ampicillin-sulbactam-nonsusceptible *Escherichia coli* has challenged an appropriate therapy for various infections such as bacteremia. However, data on resistance mechanisms, especially about outer membrane proteins (OMPs), and molecular epidemiology are limited. We performed a detailed analysis on clinical ampicillin-sulbactam-nonsusceptible *E. coli* isolates that caused bloodstream infections. **Methods:** We included 41 non-duplicate ampicillin-sulbactam-nonsusceptible *E. coli* isolates that were collected at a Japanese university hospital between 2010 and 2013. We performed PCR and sesquecing of β-lactamases genes, real-time PCR expression assay of $bla_{\text{TEM-1}}$, and phenotypic AmpC detection using cefoxitin-cloxacillin disk. OMPs were characterized by gene sequencing, SDS-PAGE, and matrix-assisted laser desorption ionization time-of-flight/time-of-flight mass spectrometry. Multilocus sequence typing was performed to determine prevalent clones. **Results:** Phenotypic and genotypic characterization of the 41 ampicillin-sulbactam-nonsusceptible *E. coli* isolates showed that β-lactamases were mainly responsible for the resistance (TEM-1 hyperproduction, n=14 [34%]; chromosomal AmpC hyperproduction [cAmpC], n=9 [22%]; plasmid-mediated AmpC [pAmpC], n=7 [17%]; OXA, n=2). All of the other 11 isolates had $bla_{\text{TEM-1}}$; 5 of them had deficient/decreased OmpF and wild-type OmpC and 2 had $bla_{\text{CTX-M}}$ in addition to $bla_{\text{TEM-1}}$. A total of 13 different sequence types (STs) were found; only ST131 was the dominant ST (32%, n=13). High-level expression of $bla_{\text{TEM-1}}$ was the most frequent mechanism in ST131 isolates (n=8) followed by cAmpC hyperproduction (n=3). Isolates with altered OMPs in ST131 were rare (n=1) while 10 non-ST131 isolates had altered OMPs. **Conclusion:** Hyperproduction of TEM-1 was the most common cause that was associated with ampicillin-sulbactam-nonsusceptible *E. coli*. Mutations in OmpF or cAmpC and acquisition of pAmpC were also associated with the nonsusceptibility. Clonal expansion of the ST131 clone hyperproducing TEM-1 might contribute to the increase of ampicillin-sulbactam-nonsuscpetible *E. coli*.

Author Disclosure Block:

T. Noguchi: None. **Y. Matsumura:** None. **M. Yamamoto:** None. **M. Nagao:** None. **S. Takakura:** None. **S. Ichiyama:** None.

Poster Board Number:

SATURDAY-345

Publishing Title:

Oritavancin Longitudinal *In Vitro* Activity against Gram-Positive Organisms from USA Medical Centers: Results from the Sentry Antimicrobial Surveillance Program for 2010 - 2014

Author Block:

R. E. Mendes, R. K. Flamm, H. S. Sader, M. Castanheira, D. J. Farrell, R. N. Jones; JMI Lab., North Liberty, IA

Abstract Body:

Background: Oritavancin (ORI) is approved in the USA and European Union for the treatment of acute bacterial skin and skin structure infections caused by gram-positive (GP) pathogens. This study evaluated ORI activity over time against GP isolates collected from USA hospitals in 2010-2014.**Methods:** A total of 16,340 *S. aureus* (SA), 1,313 coagulase-negative staphylococci (CoNS), 1,861 *E. faecalis* (EF), 1,037 *E. faecium* (EFM), 2,505 beta-hemolytic streptococci (BHS) and 1,067 viridans group streptococci (VGS) were included. Bacteria were identified by standard algorithms and/or MALDI-TOF. Susceptibility (S) testing was performed by CLSI methods; interpretation of MICs used FDA (ORI), CLSI (2015) and/or EUCAST (2015) criteria.**Results:** ORI had MIC_{50} and MIC_{90} values of 0.03 and 0.06 µg/ml, respectively, against SA (99.5 - 100.0% S), the methicillin-resistant (MR) subset (99.8 - 100.0% S) and CoNS during the study period (Table). The only exception was noted in the 2011 sampling year that showed a slightly higher MIC_{50} and MIC_{90} values ($MIC_{50/90}$, 0.06/0.12 µg/ml) against SA and MRSA. Daptomycin (DAP; $MIC_{50/90}$, 0.25/0.5 µg/ml), linezolid (LZD; $MIC_{50/90}$, 1/1 µg/ml) and vancomycin (VAN; $MIC_{50/90}$, 1/1 µg/ml) also had consistent MICs against SA or MRSA over the study period. Similar (±1 doubling dilution) ORI MICs were obtained against EF and EFM over time. Ampicillin, DAP, LZD and VAN showed consistent MIC_{50} values ($MIC_{50/90}$, 1/1-2 µg/ml) against EF, while DAP ($MIC_{50/90}$, 2/2-4 µg/ml) and LZD ($MIC_{50/90}$, 1/1-2 µg/ml) had consistent MICs against EFM (80.1% VAN-resistant) over the period. VGS were highly S to ORI (100.0% S) with consistent MICs between 2010 and 2014. Similar MICs were obtained for ORI against BHS (99.1 - 99.8% S) over the study period.**Conclusions**: ORI was highly active against an extensive longitudinal USA collection of clinically important GP pathogens. No significant year-to-year variations were noted in ORI activity against these clinical isolates.

	Oritavancin MIC_{50} and MIC_{90} (µg/ml) per year:				
Organism[a] (no. tested)	**2010**	**2011**	**2012**	**2013**	**2014**
S. aureus (16,340)	0.03 / 0.06	0.06 / 0.12	0.03 / 0.06	0.03 / 0.06	0.03 / 0.06
CoNS (1,313)	0.03 / 0.06	0.03 / 0.06	0.03 / 0.06	0.03 / 0.06	0.03 / 0.06
E. faecalis (1,861)	0.015 / 0.06	0.03 / 0.12	0.015 / 0.03	0.015 / 0.03	0.015 / 0.03

E. faecium (1,037)	0.03 / 0.06	0.03 / 0.12	0.03 / 0.12	0.03 / 0.06	0.03 / 0.12
VGS (1,067)	≤0.008 / 0.06	≤0.008 / 0.06	0.008 / 0.06	0.008 / 0.06	0.08 / 0.06
BHS (2,505)	0.06 / 0.12	0.03 / 0.12	0.03 / 0.12	0.03 / 0.12	0.03 / 0.12

[a] CoNS = coagulase-negative staphylococci; BHS = beta-hemolytic streptococci; VGS = viridans group streptococci; *E. faecalis* species, 3.5% of isolates were vancomycin-resistant; *E. faecium* species, 80.1% of isolates were vancomycin-resistant.

Author Disclosure Block:

R.E. Mendes: H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from The Medicines Co. **R.K. Flamm:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from The Medicines Co. **H.S. Sader:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from The Medicines Co. **M. Castanheira:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from The Medicines Co. **D.J. Farrell:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from The Medicines Co. **R.N. Jones:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from The Medicines Co..

Poster Board Number:

SATURDAY-346

Publishing Title:

Characterization of Fosfomycin Resistance Gene, *fosB,* in Methicillin-Resistant *Staphylococcus aureus* Isolates

Author Block:

Z. Fu, Y. Guo, F. Hu, **X. Xu**, M. Wang; Inst. of Antibiotics, Huashan Hosp., Fudan Univ., Shanghai, China

Abstract Body:

Background: In China, fosfomycin alone or in combination with other antibiotics is commonly used in the treatment of infections caused by Gram-positive bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA). Although fosfomycin-resistant *S. aureus* strains have continued to emerge and increase, the research on them is rare. In order to determine prevalence, location and flanking sequences of fosfomycin-resistance (*fos*) genes in MRSA clinical isolates, this study was carried out. **Methods:** A total of 67 non-duplicate fosfomycin resistant MRSA isolates were collected from blood and cerebrospinal fluid samples at Huashan Hospital in Shanghai between 2004 and 2014. By PCR and sequencing, 9 *fosB* gene positive strains was found. The locations of *fosB* genes were determined by Southern blotting and genetic environments were analyzed by primer walking sequencing. Multiple locus sequence typing (MLST) was used to characterize genetic diversity. Conjugation of *S. aureus* ATCC25923 with *fosB* genes was performed to evaluate the genes' transferability.**Results:** Among 67 fosfomycin-resistant MRSA strains, nine high level fosfomycin resistant strains (≥128 μg/ml) were *fosB*-positive. Three new subtypes of *fosB*, designated as *fosB4*(KR870311), *fosB5*(KT032253), and *fosB6*(KR870314), were identified. Seven out of nine strains that contained the *fosB1*, *fosB4* or *fosB6* genes, which were located on small plasmids (ca. 2.5 kb) and flanked by an analogous replication gene (*rep*). The *fosB5* gene located in a unique genetic environment and was surrounded by a shorter *rep* gene and two copies of a transposon gene (*tnp*) that shared high identity with the IS*257*-like transposon. Four MLST types were found among the nine *fosB*-positive strains. Conjugants with the *fosB* genes were resistant to fosfomycin with MIC 64 or 128 μg/ml.**Conclusions:** All 3 new subtypes of *fosB* gene found in MRSA can mediate fosfomycin resistance. These different subtypes and genetic environments of *fosB* indicate that *fosB* genes have different sources.

Author Disclosure Block:

Z. Fu: None. **Y. Guo:** None. **F. Hu:** None. **X. Xu:** None. **M. Wang:** None.

Poster Board Number:

SATURDAY-347

Publishing Title:

Activity of ceftazidime-avibactam (CAZ-AVI) against OXA-48- carrying *Enterobacteriaceae* isolated from a global surveillance program, 2012-2014

Author Block:

K. Kazmierczak[1], M. Hackel[1], G. Stone[2], B. de Jonge[2], P. A. Bradford[2], D. Sahm[1]; [1]IHMA, Inc., Schaumburg, IL, [2]AstraZeneca Pharmaceuticals, Waltham, MA

Abstract Body:

Background: CAZ-AVI was developed for use against *Enterobacteriaceae* (*Ebac*) producing serine β-lactamases (BL), including carbapenemases. The AVI component of the combination is a novel non-β-lactam BL inhibitor that protects CAZ from inactivation by class A, class C, and some class D BL, including OXA-48. This study evaluated the *in vitro* activity of CAZ-AVI and comparators against OXA-48-producing isolates collected in 2012-2014 through the INFORM surveillance program. **Methods:** Isolates were collected globally in 176 sites from 39 countries. Susceptibility testing was performed by broth microdilution and interpreted using CLSI (CAZ, meropenem) or FDA guidelines (CAZ-AVI, ≤8 μg/mL susceptible). CAZ-AVI was tested at a fixed concentration of 4 μg/mL AVI. Isolates were screened for the presence of BL genes using PCR, microarray and sequencing. **Results:** Genes encoding OXA-48 enzymes were detected in 163 isolates across 10 species of *Ebac* (*K. pneumoniae*, *Enterobacter* spp., *E. coli*, *M. morganii*, *S. marcescens*, *Citrobacter* spp., *P. mirabilis*) collected in 15 countries, with 83% of isolates collected in Russia, Turkey, Romania, and Kuwait. The majority of isolates co-carried additional BL. CAZ and MEM showed poor activity against the OXA-48-containing isolates, but 95.1% of *Ebac* carrying OXA-48 were inhibited by ≤8 μg/mL of CAZ-AVI (Table). CAZ-AVI showed good *in vitro* activity against *Ebac* carrying OXA-48, including isolates that co-produce an ESBL or AmpC enzyme.

	Drug (MIC$_{90}$ [μg/mL], % Susceptible)					
Organism grouping[a] (n)	**CAZ-AVI[b]**		**CAZ**		**MEM**	
Ebac All (34062)	0.5	99.5%	64	75.6%	0.12	97.2%
OXA-48-containing *Ebac* (163)	2	95.1%	>128	24.5%	>8	25.2%
+ ESBL (118)	2	100%	>128	8.5%	>8	23.7%
+ AmpC (10)	1	100%	8	80.0%	4	40.0%

[a]Includes isolates that co-carry original spectrum β-lactamases unless noted.[b]CAZ-AVI, ceftazidime-avibactam; CAZ, ceftazidime; MEM, meropenem.**Conclusions:** CAZ-AVI showed good *in vitro* activity against complex isolates of *Ebac* carrying OXA-48 in combination with other CAZ-hydrolyzing serine BL.

Author Disclosure Block:

K. Kazmierczak: M. Independent Contractor; Self; IHMA, Inc. **M. Hackel:** M. Independent Contractor; Self; IHMA, Inc. **G. Stone:** D. Employee; Self; AstraZeneca Pharmaceuticals. **B. de Jonge:** D. Employee; Self; AstraZeneca Pharmaceuticals. **P.A. Bradford:** D. Employee; Self; AstraZeneca Pharmaceuticals. **D. Sahm:** M. Independent Contractor; Self; IHMA, Inc..

Poster Board Number:

SATURDAY-348

Publishing Title:

***In Vitro* Activity of Aztreonam-Avibactam (ATM-AVI) qgainst Gram-Negative Pathogens from Asia/Pacific (AP) Collected in 2014**

Author Block:

K. Kazmierczak[1], M. Hackel[1], B. de Jonge[2], P. A. Bradford[2], D. Sahm[1]; [1]IHMA, Inc., Schaumburg, IL, [2]AstraZeneca Pharmaceuticals, Waltham, MA

Abstract Body:

Background: ATM-AVI is a β-lactam/ β-lactamase inhibitor combination being developed for use against carbapenem-resistant *Enterobacteriaceae* (CRE), especially those carrying metallo-β-lactamases (MBL) in combination with serine β-lactamases (BL) such as KPC, OXA-48, extended-spectrum enzymes (ESBL), and AmpC. This study evaluated the *in vitro* activity of ATM-AVI and comparators against *Enterobacteriaceae* (*Ebac*) and *P. aeruginosa* (*Paer*) collected in AP in 2014.**Methods:** Non-duplicate isolates were collected from 22 centers in 8 AP countries. Susceptibility testing was performed by broth microdilution. ATM-AVI was tested at a fixed concentration of 4 µg/mL AVI. PCR and sequencing of *bla* genes was performed on isolates non-susceptible to carbapenems, phenotypically positive for ESBL, and those with ceftazidime MICs ≥16 µg/mL.**Results:**ATM-AVI was very active against *Ebac*, testing with an MIC_{90} value of 0.12 µg/mL, with 99.9% inhibited by ≤8 µg/mL. Activity was retained against resistant subsets of *Ebac,* including MBL-producing isolates. MBLs were found in 10 *K. pneumoniae*, 3 *E. cloacae*, 2 *C. freundii*, and 1 *E. asburiae* collected in the Philippines (n=14), Japan and Thailand (n=1 each), and included five variants of IMP- and NDM-type MBLs. No VIM-type MBLs were found in *Ebac* from AP. All of these MBL-producing *Ebac* co-carried serine BL, with 11 isolates carrying 1-2 SHV- and/or CTX-M-type ESBLs. ATM-AVI showed moderate activity against *Paer*.

	Drug (MIC_{90} [µg/mL], % Susceptible)									
Species/ phenotype (n)	**ATM-AVI**		**ATM**		**MEM**		**COL**		**TGC**	
Ebac All (2078)	0.12	NA	64	74.3%	0.12	98.8%	>4	83.5%	2	94.5%
ESBL-positive (427)	0.25	NA	>128	9.8%	0.12	95.8%	1	96.5%	2	96.5%
Meropenem-S (2052)	0.12	NA	64	74.9%	0.12	100%	>4	83.6%	2	94.7%
Meropenem-NS (26)	1	NA	>128	26.9%	>8	0%	>4	80.8%	4	76.9%
MBL-negative (2062)	0.12	NA	64	74.6%	0.12	99.5%	>4	83.5	2	94.6%
MBL-positive (16)	0.25	NA	128	31.3%	>8	0%	>4	87.5%	4	81.3%

Paer All (632)	32	NA	32	66.9%	>8	77.4%	4	87.7%	>8	NA

ATM-AVI, aztreonam-avibactam; ATM, aztreonam; MEM, meropenem; COL, colistin; TGC, tigecycline; NA, no breakpoints available; NS, non-susceptible.**Conclusions:** ATM-AVI was highly potent *in vitro* against CRE collected in AP, including those that produce MBLs in combination with ESBLs or other serine carbapenemases.

Author Disclosure Block:

K. Kazmierczak: M. Independent Contractor; Self; IHMA, Inc. **M. Hackel:** M. Independent Contractor; Self; IHMA, Inc. **B. de Jonge:** D. Employee; Self; AstraZeneca Pharmaceuticals. **P.A. Bradford:** D. Employee; Self; AstraZeneca Pharmaceuticals. **D. Sahm:** M. Independent Contractor; Self; IHMA, Inc..

Poster Board Number:

SATURDAY-349

Publishing Title:

Activity of Ceftazidime-Avibactam against Carbapenem-Non-Susceptible *Enterobacteriaceae* from Latin America, Asia/Pacific and the Middle East/Africa, 2012-2014

Author Block:

M. Hackel[1], K. Kazmierczak[1], G. Stone[2], B. de Jonge[2], D. Sahm[1]; [1]IHMA, Inc., Schaumburg, IL, [2]AstraZeneca Pharmaceuticals, Waltham, MA

Abstract Body:

Objectives: Avibactam (AVI) restores the *in vitro* activity of ceftazidime (CAZ) against *Enterobacteriaceae* that produce class A (including KPC), class C, and some class D β-lactamases (BLs). In this study we evaluated the *in vitro* activity of CAZ-AVI against carbapenem-non-susceptible *Enterobacteriaceae* isolated from Latin America (LA), Asia/Pacific (AP) and the Middle East/Africa (MEA) from the INFORM 2012-2014 surveillance program.**Methods:** MICs were determined using CLSI guidelines. Susceptibility (S) was assessed using FDA (CAZ-AVI, tigecycline), CLSI (CAZ, meropenem [MEM]) or EUCAST (colistin) breakpoints. Carbapenem-non-susceptible *Enterobacteriaceae* were defined as non-susceptible (NS) to MEM using CLSI breakpoints.**Results**: The %S for CAZ-AVI and comparators are shown in the table. 448 of 15,865 *Enterobacteriaceae* (2.8%) were MEM-NS. CAZ-AVI was active against 99.3% of all *Enterobacteriaceae*. Regional differences were apparent in activity against MEM-NS isolates, with lower activity in regions where carbapenem resistance was mainly due to the presence of isolates with metallo-β-lactamases (MBLs).

	% Susceptible			
Organism (N)	All	LA	AP	MEA
Enterobacteriaceae	N=15,685	N=5,317	N=7,087	N=3,281
Ceftazidime-avibactam	99.3	99.8	99.1	99.2
Ceftazidime	72.7	69.2	75.3	72.7
Colistin	83.0	82.2	83.5	83.5
Meropenem	97.1	94.9	98.5	98.0
Tigecycline	93.6	93.2	93.7	94.0
Enterobacteriaceae, MEM-NS	N=448	N=273	N=108	N=67
Ceftazidime-avibactam	80.1	97.8	48.2	59.7
Ceftazidime	5.8	5.5	3.7	10.5

Colistin	77.9	75.0	80.8	87.9
Tigecycline	93.1	95.2	87.0	94.0
Enterobacteriaceae, MEM-NS, MBL negative	N=366	N=269	N=61	N=36
Ceftazidime-avibactam	97.0	99.3	85.3	100
Ceftazidime	6.3	5.6	6.6	11.1
Colistin	75.6	74.3	70.0	88.9
Tigecycline	93.7	95.2	85.3	97.2

Conclusions: CAZ-AVI demonstrated good *in vitro* potency against *Enterobacteriaceae* isolates from LA, AP and MEA, including MEM-NS isolates, unless MEM-NS was caused by the presence of MBLs. The incidence of MBL-producing isolates amongst different regions explains to a large degree the different %S observed against MEM-NS *Enterobacteriaceae*.

Author Disclosure Block:

M. Hackel: M. Independent Contractor; Self; IHMA, Inc. **K. Kazmierczak:** M. Independent Contractor; Self; IHMA, Inc. **G. Stone:** D. Employee; Self; AstraZeneca Pharmaceuticals. **B. de Jonge:** D. Employee; Self; AstraZeneca Pharmaceuticals. **D. Sahm:** M. Independent Contractor; Self; IHMA, Inc..

Poster Board Number:

SATURDAY-350

Publishing Title:

***In Vitro* Activity of Fosfomycin against Bacterial Pathogens Isolated from Urine Specimens of Outpatients Attending Emergency Departments in Canada from 2007 to 2014**

Author Block:

J. A. Karlowsky[1], H. Adam[1], M. Baxter[2], N. Laing[2], B. Weshnoweski[1], R. Vashisht[2], G. G. ZHANEL[2]; [1]Diagnostic Services Manitoba, Winnipeg, MB, Canada, [2]Univ. of Manitoba, Winnipeg, MB, Canada

Abstract Body:

Background: In North America, fosfomycin (FOS) tromethamine is indicated for the treatment of uncomplicated urinary tract infections in women caused by *Escherichia coli* and *Enterococcus faecalis*. FOS has been shown to inactivate the enzyme UDP-*N*-acetylglucosamine-3-enolpyruvyltransferase (MurA) which ligates phosphoenolpyruvate (PEP) to the 3'-hydroxyl group of UDP-*N*-acetylglucosamine in peptidoglycan synthesis. FOS reference MIC antimicrobial susceptibility testing (AST) is rarely performed in clinical laboratories because the CLSI agar dilution method must be used. MIC data documenting the activity of FOS against outpatient urinary pathogens other than *E. coli* and *E. faecalis* are limited.**Methods:** FOS AST was performed using CLSI agar dilution testing (MHA supplemented with 25 µg/ml of glucose-6-phosphate; M100-S24 [2014]); all other antibacterial agents were tested using CLSI broth microdilution panels. MICs were interpreted using M100-S24 (2014) criteria. FOS susceptible, intermediate, and resistant breakpoints are ≤64, 128, and ≥256 µg/ml, respectively. The isolates tested were cultured from urine specimens of outpatients attending emergency departments (EDs) and submitted to the annual CANWARD surveillance study from 2007 to 2014.**Results:** The table shows MIC_{90} (µg/ml) and % susceptible data for oral antimicrobial agents.

	MIC_{90} (µg/ml) / % Susceptible				
Isolate Phenotype (n)	FOS	SXT	NIT	CIP	AMC
E. coli (877)	4 / 99.7	>8 / 78.2	32 / 97.9	>16 / 85.4	16 / 88.8
K. pneumoniae (106)	128 / 89.6	0.25 / 96.2	128 / 33.7	≤0.06 / 98.1	8 / 96.7
E. faecalis (68)	128 / 83.8	NA	8 / 100	>16 / 72.1	1 / 100*
Proteus mirabilis (40)	128 / 85.0	>8 / 87.5	128 / 0	2 / 87.5	16 / 90.0
Staphylococcus aureus (26)	32 / NA	≤0.12 / 100	16 / 100	>16 / 50.0	NA / 66.7**
Pseudomonas aeruginosa (27)	256 / NA	NA	NA	>16 / 66.7	NA
Klebsiella oxytoca (22)	64 / 90.9	≤0.12 / 100	32 / 95.5	≤0.06 / 100	16 / 89.5

Enterobacter cloacae (14)	128 / 85.7	≤0.12 / 92.9	128 / 35.7	2 / 85.7	>32 / 21.4
Abbreviations: SXT, trimethoprim-sulfamethoxazole; NIT, nitrofurantoin; CIP, ciprofloxacin; AMC, amoxicillin-clavulanate; NA, not applicable. *AMC activity predicted by testing ampicillin for *E. faecalis*. **AMC activity predicted by testing cefoxitin for *S. aureus*.					

Conclusion: The in vitro activities of SXT (78%) and CIP (85%), two frequently prescribed empiric agents for urinary tract infections, were compromised against recent urinary isolates of *E. coli* when compared to FOS (>99%). 84% of *E. faecalis* isolates were susceptible to FOS. FOS demonstrated broad spectrum activity against facultative gram-negative (Enterobacteriaceae) and gram-positive (enterococci, staphylococci) pathogens frequently isolated from urinary tract infections of Canadian outpatients attending EDs.

Author Disclosure Block:

J.A. Karlowsky: None. **H. Adam:** None. **M. Baxter:** None. **N. Laing:** None. **B. Weshnoweski:** None. **R. Vashisht:** None. **G.G. Zhanel:** I. Research Relationship; Self; Abbott, Astellas, Cubist, Galderma, Merck, Pharmascience, Sunovion, The Medicines Co, Tetraphase, Zoetis.

Poster Board Number:

SATURDAY-351

Publishing Title:

Comparison of Tigecycline Susceptibilities among Gram-Positive Bacteria Isolated in Canada, the United States, and Mexico - Results from the Test Program 2013-2015

Author Block:

M. Hackel[1], D. Sahm[1], H. Leister-Tebbe[2]; [1]IHMA, Inc., Schaumburg, IL, [2]Pfizer, Inc, Collegeville, PA

Abstract Body:

Background: The Tigecycline Evaluation Surveillance Trial (TEST) monitors the activity of tigecycline and comparators against multiple pathogens from multiple infection sources collected worldwide. This report describes the activity of tigecycline against Gram-positive isolates from Canada, Mexico and the United States during 2013-2015.**Methods:** A total of 4,198 isolates were collected including 2,675 *Staphylococcus aureus*, 1,051 *Enterococcus faecalis* and 469 *E. faecium*. Isolates were collected from Canada (n=725), Mexico (n=292) and the United States (n=3,181). MICs were determined at each participating laboratory using supplied broth microdilution panels following CLSI guidelines. MIC interpretive criteria for tigecycline followed FDA guidelines.**Results:** Susceptibility of isolates from different countries to tigecycline is shown in the table.***In Vitro*** **Activity of Tigecycline against Gram-positive Clinical Isolates**

Organism	**Canada**	**Mexico**	**USA**
	%S (n)	**%S (n)**	**%S (n)**
Enterococcus faecalis	100 (133)	100 (84)	99.5 (834)
Enterococcus faecium	100 (115)	100 (31)	98.1 (323)
Enterococcus faecium, VRE	100 (65)	100 (22)	97.3 (225)
Staphylococcus aureus, MRSA	100 (116)	100 (62)	99.8 (1040)
Staphylococcus aureus, MSSA	100 (361)	100 (115)	100 (984)

%S, percent susceptibility; (n), number of isolates; VRE, vancomycin-resistant; MRSA, methicillin-resistant *S. aureus*; MSSA, methicillin-susceptible *S. aureus***Conclusions:** Tigecycline provided excellent *in vitro* activity against >97% of the isolates in this study, including methicillin- and vancomycin-resistant phenotypes. There were no important differences in susceptibility between countries. These data confirm the continued utility of tigecycline against drug-resistant Gram-positive bacteria.

Author Disclosure Block:

M. Hackel: M. Independent Contractor; Self; IHMA, Inc. **D. Sahm:** M. Independent Contractor; Self; IHMA, Inc. **H. Leister-Tebbe:** D. Employee; Self; Pfizer, Inc..

Poster Board Number:

SATURDAY-352

Publishing Title:

Proteomic Profiles of Antibiotic Resistance Plasmid and Chromosome in *Escherichia coli*

Author Block:

N. Nolan, **F. Walsh**; Maynooth Univ., Maynooth, Ireland

Abstract Body:

This study investigated how different antibiotics treatments affect the protein abundances produced from a multi-drug resistant plasmid (pEK499) and *Escherichia coli* host chromosome. The proteomic profiles of a multi-drug resistance plasmid (pEK499) and chromosome of *Escherichia coli* under antibiotic stress were investigated using the Q-Exactive mass spectrometer. The bacteria were exposed to antibiotics, for which a resistance gene was present on the plasmid (ampicillin and cefotaxime) at high concentrations or sub-MIC concentrations for those, which the bacteria were susceptible (imipenem and ciprofloxacin). The plasmid contains the *aac(6')Ib-cr* quinolone helper resistance gene and had a ciprofloxacin MIC ≤0.125 m/L. The plasmid proteomes and chromosome proteomes of these bacteria under stress were compared to the same *E. coli* grown under the same conditions with no antibiotic stress.The results indicated that the presence of antibiotic resistance proteins from the plasmid was not dependent on the presence of the corresponding antibiotic. The quantities of bla_{TEM} and $bla_{CTX\text{-}M\text{-}15}$ proteins were similar under antibiotic stress or no antibiotic stress. The relative abundances of chromosomal proteins varied in comparison to the control from increases in 27 proteins (ampicillin) to increases in 98 proteins (ciprofloxacin) and decreases in protein abundances of 28 proteins (imipenem) to 80 proteins (ciprofloxacin). There were no common proteins to all antibiotic exposed or control bacteria. Proteins involved in gene expression were the most frequently identified metabolic pathway or process. In conclusion the variations between the relative abundances of chromosomal proteins in different samples, appears to be antibiotic specific but not antibiotic class specific and thus could indicate specific antibiotic-induction pathways or metabolic processes.

Author Disclosure Block:

N. Nolan: None. **F. Walsh:** B. Collaborator; Self; Alltech.

Poster Board Number:

SATURDAY-353

Publishing Title:

Epidemiology Of Transmissible 16s Rrna Methyltransferases In *enterobacteriaceae* Using Whole Genome Sequencing

Author Block:

L. Appalla, E. Snesrud, R. Maybank, K. Ong, R. Clifford, M. Hinkle, E. Lesho, P. Mc Gann; Walter Reed Army Inst. of Res., silver spring, MD

Abstract Body:

In the past decade, transmissible 16S rRNA methyltransferases (16S RMTase) have emerged as a new and worrisome source of aminoglycoside resistance. Increasingly, strains carrying these genes are co-producing carbapenemases, and understanding their epidemiology is essential for effective surveillance. 27 clinical isolates, representing 7 species of *Enterobacteriaceae*, had phenotypes indicative of 16S rRNA methyltransferase activity by arbekacin disk diffusion. All isolates were sequenced using a combination of long and short read sequencing using the Illumina Miseq and PacBio RSII platforms, respectively. Six genes encoding 16S RMTases were identified, with *armA* and *rmtH* being the most common (6 isolates each), followed by *rmtB* (N=5), *rmtC* (N=4), *rmtF* (N=3), and *rmtE* (N=1). 16S rRNA methyltransferases were most commonly found in *Klebsiella pneumoniae*, accounting for 19 of the 27 isolates tested (70.4%). Notably, all *K. pneumoniae* isolates carrying *armA* belonged to multi-locus sequence type (MLST) 231, whereas the six isolates carrying *rmtH* were assigned to five different ST types, including one novel ST. Three different 16S RMTases were identified among the 3 *Escherichia coli* isolates, including *rmtC* in an ST-131 strain, a globally distributed pathogenic lineage. 16S RMTase genes were also found in a single isolate of *Enterobacter cloacae* (*rmtC*), *Morganella morganii* (*rmtB*), *Proteus mirabilis* (*armA*), *Providencia sturatii* (*armA*), and *Serratia marcesens* (*armA*). All 16S RMTase genes were carried on plasmids, with those belonging to incompatibility group (Inc) FII being the most common. However, 16S RMTase genes were also carried on Inc A/C2, F1A, F1B, H1B, and L/M plasmids. Of particular concern was the observation that eight isolates also carried the carbapenemases bla_{NDM} (N=5) and bla_{KPC} (N=3). We demonstrate that transmissible 16S RMTases are carried on a wide variety of plasmids and are distributed among diverse members of the *Enterobacteriaceae*. The increasing association between carbapenemases and 16S RMTases is particularly ominous, as just two enzymes can now render the two most widely used antibiotic classes ineffective.

Author Disclosure Block:

L. Appalla: None. **E. Snesrud:** None. **R. Maybank:** None. **K. Ong:** None. **R. Clifford:** None. **M. Hinkle:** None. **E. Lesho:** None. **P. Mc Gann:** None.

Poster Board Number:

SATURDAY-354

Publishing Title:

Involvement of Two Efflux Pumps in Tigecycline Resistant *S. aureus* Mutants

Author Block:

M. Herrera[1], **S. Di Gregorio**[2], S. Fernandez[2], G. Posse[1], M. Mollerach[2], J. Di Conza[2]; [1]UAP, ER, Argentina, [2]UBA, BA, Argentina

Abstract Body:

Background: *Staphylococcus aureus* has an extraordinary ability to develop antimicrobial resistance. Previously, we reported the in vitro selection of tigecycline resistant mutants (TRM) which showed an increase in tigecycline (TIG) MICs of 128-fold. The efflux phenotype was predicted by ethidium bromide MIC determination (64-128 μg/ml). Additionally, we demonstrated that MIC for both compounds showed a decrease greater or equal than 4 dilutions in the presence of 20 μg /ml reserpine (an efflux pump inhibitor). The aim of this study was to analyze the involvement of two efflux pumps implied in glycylcicline and tetracyclines extrusion (*mep*A and *nor*B) in two TRM strains analyzed previously (94159m and 2028m). **Methods:** Efflux pump genes were amplified by PCR and sequencing. Reverse transcription-Quantitative real-time-PCR (RT-qPCR) was performed to evaluate the expression of *mep*A and *nor*B genes. Data was expressed as normalized relative quantities (NRQ) using *gyr*B and *pta* as reference genes. All experiments were performed by triplicate. Statistical analysis of the data was performed using Student t test (*alfa*=0.05).**Results:** The nucleotide sequence of *mep*A gene (1700 bp) in the parental strains 94159p and 2028p showed 100 % of identity with the corresponding reference sequences (*S. aureus* Accession number HE579073.1 and CPO12119.1). By contrast, for both mutant strains (94159m and 2028m) a transition (C to T) was observed in the position 211 of the sequence, displaying the Thr29Ile mutation in the translated amino acid sequence. In addition 2028m harbored the transition A to G in the position 985 generating the Glu287Gly mutation.NRQ values of *mep*A gene were significantly higher in both mutant strains (94159m and 2028m) when compared to parental strains ($p<0.0001$ and $p=0.0009$ respectively). On the other hand, NRQ values of *nor*B gene were significantly decreased in mutant strain 2028m when compared to parental strain 2028p ($p=0.0007$), and no difference was detected for the pair 94159p-94159m ($p=0.1093$).**Conclusions:** This study demonstrates the involvement of efflux pumps in TRM. We identified a derived, charge-changing amino acid mutation in MepA and an increase in *mep*A gene expression which would be related to the development of TIG resistance in these mutants. The decrease in *nor*B expression observed might alter the extrusion of other compounds.

Author Disclosure Block:

M. Herrera: None. **S. Di Gregorio:** None. **S. Fernandez:** None. **G. Posse:** None. **M. Mollerach:** None. **J. Di Conza:** None.

Poster Board Number:

SATURDAY-355

Publishing Title:

Resistance Mechanism and Clinical Characteristic of Linezolid-Resistant *Staphylococcus capitis* Isolated from Blood Cultures

Author Block:

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Abstract Body:

Background: Linezolid resistant *Staphylococcus* spp. have caused nosocomial infection in several countries, including China. Recent years, Linezolid resistant *Staphylococcus capitis* have continued to emerge in a teaching hospital, Shanghai, China. This study was carried out to understand the resistance mechanism and clinical characteristic of linezolid-resistant *S. capitis* isolates. **Methods:** Five linezolid resistant *S. capitis* strains were isolated from blood samples in 2012. Antimicrobial susceptibility tests were carried out to determine the antimicrobial resistance in clinical strain. By PCR and sequencing, the *cfr* genes and 23S rRNA mutations related to linezolid resistance were detected. Analysis of Pulsed-field gel electrophoresis (PFGE) and clinical data were performed to understand the characteristics of the clinical isolates. **Results:** Five strains were not only resistant to linezolid, but also resistant to most common antimicrobial agents, except vancomycin, rifampin, tigecycline and SMZco. The G2576T mutation in the domain V region of 23S rRNA was identified in all 5 strains. Four of them carried *cfr* gene. All 5 isolates were belong to one PFGE type (Table 1). The deep vein indwelling catheters were applied in 3 patients, and 2 of them had treated with linezolid. **Conclusions:** Linezolid-resistant *S. capitis* isolates have multiple-drug resistant phenotype. The linezolid resistance of *S. capitis* is mediated by *cfr* gene and 23S rRNA mutations. The application of deep vein indwelling catheter and linezolid could increase the risk of linezolid resistant *S. capitis* infection.

Table 1. Characteristics of five clinical isolates of *S. capitis*

Strains	*cfr* gene	23S rRNA mutation	PFGE type	MICs (mg/L)								
				LZD	GEN	LEV	FOS	OXA	VAN	RIF	TGC	SMZco
12-53	+	G2576T	A	256	64	8	>512	>128	1	≤0.06	0.5	0.125
12-86	+	G2576T	A	256	32	8	>512	>128	1	≤0.06	0.5	0.125
12-400	+	G2576T	A	256	32	8	>512	>128	1	≤0.06	0.5	0.125
12-498	+	G2576T	A	512	32	8	>512	>128	1	≤0.06	0.5	0.125
12-535	−	G2576T	A	32	32	8	>512	>128	1	≤0.06	0.5	0.125

*VAN, Vancomycin; TGC, Tigecycline; RIF, Rifampin; FOS, Fosfomycin; OXA, Oxacillin; GEN, Gentamicin; LEV, Levofloxacin; SMZco, Trimethoprim-sulfamethoxazole.

Author Disclosure Block:

Y. Yang: None. **D. Lin:** None. **X. Xu:** None.

Poster Board Number:

SATURDAY-356

Publishing Title:

Clarithromycin Resistance in *Helicobacter pylori* Strains from Antrum and Body from Symptomatic Colombian Adults

Author Block:

B. V. Arévalo Jaimes[1], B. Mendoza[2], C. Jaramillo[1], J. F. Vera-Chamoro[2], D. F. Rojas[1], L. F. Jiménez-Soto[3], P. Rodriguez[2], R. Haas[3], J. Álvarez[2], M. P. Delgado[1]; [1]Univ. de los Andes, Bogotá, Colombia, [2]The Univ. Hosp. Fundación Santa Fe de Bogotá, Bogotá, Colombia, [3]Max von Pettenkofer-Inst. für Hygiene und Medizinische Mikrobiologie, München, Germany

Abstract Body:

Background: *Helicobacter pylori* is a bacterium associated with gastric pathologies. Unfortunately, the effectiveness of first line of treatment decreases drastically with the raise of *H. pylori* strains resistant to clarithromycin due to point mutations within the 23S rDNA gene . Infections by *H. pylori* strains with dissimilar antimicrobial susceptibilities in diverse parts of the stomach could affect the therapy success making necessary biopsies from different stomach locations to assess clarithromycin resistance. The present study aimed to know the prevalence of *H. pylori* clarithromycin-resistant in samples from two stomach sites. **Methods:** Therefore, 42 cultures from the bank of strains of Molecular Diagnostics and Bioinformatics Laboratory, Universidad de los Andes, were isolated from biopsies taken by gastroenterologists in the Fundación Santa Fe de Bogotá, Colombia from body and antrum of 21 adult symptomatic Colombian patients. DNA was extracted and used in conventional PCR according to Álvarez et al. (2009) and followed by electrophoresis. PCR products were purified, sequenced, and compared with the *H. pylori* susceptible strain (GenBank U27270). **Results:** Twenty-seven samples had a susceptible genotype (64.3%), in the remaining fifteen (35.7%), ten strains (66.7%) showed the A2143G and five (33.3%) the A2142G mutation. In total, seven patients (33.3%) had a single resistant infection (same mutation in body and antrum strains) of which five patients had the A2143G and two the A2142G mutation; one with mixed infection where a resistant strain (A2142G mutation) was isolated from antrum and a susceptible strain from body (4.8%) and, thirteen with susceptible infection (61.9%). **Conclusions:** The resistance prevalence obtained suggest the quadruple therapy as a better eradication regimen for *H. pylori* in Bogotá according to the IV Maastricht Florence Consensus Conference (2010). Due to the heteroresistance presence, prevalence studies should be completed with at least two biopsies from different stomach locations for a more reliable result.

Author Disclosure Block:

B.V. Arévalo Jaimes: None. **B. Mendoza:** None. **C. Jaramillo:** None. **J.F. Vera-Chamoro:** None. **D.F. Rojas:** None. **L.F. Jiménez-Soto:** None. **P. Rodriguez:** None. **R. Haas:** None. **J. Álvarez:** None. **M.P. Delgado:** None.

Poster Board Number:

SATURDAY-357

Publishing Title:

Track the Cefoperazone-Sulbactam Resistance Development by Whole-Genome Comparison of Two *A. baumannii* Isolates from a Single Patient

Author Block:

X. Liu[1], W. Zhang[2], M. Zhao[1], L. Sun[1], Y. Li[3], J. Shi[3], P-y. Qian[2], J. Zhang[1]; [1]Huashan Hosp. affiliated to Fudan Univ., Shanghai, China, [2]The Hong Kong Univ. of Sci. and Technology, Hong Kong, China, [3]Roche R&D Ctr. (China) Ltd., Shanghai, China

Abstract Body:

Background: The whole genomes of two *A. baumannii* isolates were sequenced to reveal the cefoperazone-sulbactam resistance development. The two isolates were isolated from sputum of a hospital-acquired pneumonia patient who received therapy of cefoperazone-sulbactam (3g with a 45 min infusion, q8h) for 20 days and the therapy was failed. AB1845 and AB2092 were isolated before and after the therapy, and the MIC of cefoperazone-sulbactam was 16 and 128 μg/mL, respectively.**Methods:** The Molecular typing of both isolates was conducted by PFGE of *Apa*I-digested genomic and antibiotic resistance was tested by MIC following CLSI. The Genomic DNA was extracted and sequenced by Illumina Hi-seq 2000 platform. Assembly was also performed by SPAdes Genome Assembler 3.6.1. Genome annotation was searching against Kyoto Encyclopedia of Genes and Genomes (KEGG) databases. Phylogenetic analysis was performed by Mega6.06.**Results:** The PFGE patterns showed the isolates were from the same clone. MIC tests revealed both isolates were resistant to meropenem, imipenem, cefepime, amikacin, ciprofloxacin etc, and susceptible to colistin. Automated gene prediction detected 3872 and 3857 Open Reading Frames (ORFs) for AB1845 and AB2092, respectively. The G+C content was 38.9% for both strains. Comparative genomics revealed 99% similarity between the two isolates, whereas certain genes, such as a phage related protein was only present in AB1845. *A. baumannii Ab04-mff* was phylogenetically close to both isolates, which is a clinical isolate collected in Edmonton, Canada. This strain will be used as a reference to compare the resistance and virulence profiles.**Conclusions:** The whole genome shows differences between the two isolates. Further analysis will focus on comparing the resistant genes and SNPs with these two isolates to elucidate the resistant development mechanism which could lead to the treatment failure.

Author Disclosure Block:

X. Liu: None. **W. Zhang:** None. **M. Zhao:** None. **L. Sun:** None. **Y. Li:** None. **J. Shi:** None. **P. Qian:** None. **J. Zhang:** None.

Poster Board Number:

SATURDAY-358

Publishing Title:

Identification of a Novel Plasmid-Borne Multidrug-Resistance Gene, *cfr(C)*, in *Campylobacter coli*

Author Block:

Y. Tang, L. Dai, O. Sahin, Q. Zhang; Iowa State Univ., Ames, IA

Abstract Body:

The *cfr* gene, encoding an rRNA methyltransferase, confers resistance to five chemically unrelated antimicrobial classes including phenicols, lincosamides, oxazolidinones, pleuromutilins, and streptograminA (known as the $PhLOPS_A$ phenotype). Although *cfr* has been identified in both Gram-positive and Gram-negative organisms, it has not been reported in *Campylobacter*, a major foodborne pathogen. In this study, we report the identification of a novel *cfr* variant in a multidrug resistant *C. coli* isolate from cattle feces. This *C. coli* isolate was highly resistant to florfenicol (MIC= 32 ug/ml), but PCR amplification did not detect any known genes mediating resistance to phenicols. Whole genome sequence analysis revealed a gene encoding a protein that share 55.1% and 54.9% amino acid homologies to Cfr and Cfr(B), respectively. This *cfr*-like gene is named *cfr(C*) in this study and was located on a conjugative plasmid of ~48kb. Cloning of *cfr(C*) into a susceptible *C. jejuni* isolate and transfer of the *cfr(C*)-containing plasmid by conjugation confirmed its role in antimicrobial resistance. The results indicates the *cfr(C*) conferred resistance to multiple antibiotics including phenicols, lincosamides, pleuromutilins, and oxazolidinones, and resulted in 8- to 256-fold increase in their MICs in both *C. jejuni* and *C. coli*. This finding identifies a new *cfr* variant and represents the first report of *cfr*-like gene in *Campylobacter*. The localization of *cfr(C)* on a conjugative and multi-resistant plasmid will facilitate its dissemination, warranting enhanced efforts to monitor its spread in *Campylobacter* and other foodborne pathogens.

Author Disclosure Block:

Y. Tang: None. **L. Dai:** None. **O. Sahin:** None. **Q. Zhang:** None.

Poster Board Number:

SATURDAY-359

Publishing Title:

Management of Vancomycin (VAN) Mic Creep in Adult MRSA Infections

Author Block:

N. Banniettis[1], S. E. Beekmann[2], P. Polgreen[2], S. Kaushik[1], S. Kohlhoff[1], D. N. Gilbert[3], J. E. Bennett[4], M. R. Hammerschlag[1]; [1]SUNY Downstate Med. Ctr., Brooklyn, NY, [2]Univ. of Iowa, Iowa City, IA, [3]Providence Portland Med. Ctr., Portland, OR, [4]NIAID, Bethesda, MD

Abstract Body:

Background: MRSA is a significant cause of both health-care associated and community-associated infections. VAN has been the mainstay of parenteral therapy for MRSA infections. However, its efficacy has come into question, with concerns over its poor tissue penetration, slow bactericidal activity, and possible "MIC creep" among susceptible strains. We sought to assess national trends in management of adult infections due to MRSA and reports of both MRSA VAN MIC creep and clinical failures with VAN use. **Methods:** Electronic 12 question survey of adult ID physician members of the IDSA Emerging Infections Network was conducted from 11/18/2015 to 12/18/2015.**Results:** 652 (53%) physicians responded, of which 617 treat *S. aureus* infections. Of these, 95% reported their clinical micro lab used a breakpoint of ≤ 2 g/mL as indicative of MRSA susceptibility to VAN; 91% reported routine inclusion of the MIC in the susceptibility report. VAN MICs were determined via E-test by 18%, broth microdilution 3%, Vitek 38%, Microscan 25%, BD Phoenix 6%; 14% were unsure of method used. 21% reported vancomycin treatment failure with MRSA bacteremia despite adequate troughs and source-control at least once over the last year; 50% reported ≥2 times. 37% of these reported initial MIC <2 g/mL and 22% MIC=2 in their most recent treatment failure. VAN was the empiric treatment of choice for persons who inject drugs and right-side IE in 89% (509/572) followed by daptomycin, 5% (29/572); 53% would switch from VAN to alternate therapy if MIC=2 g/mL; 6% would continue treatment with VAN at same dose despite 4 d of persistent bacteremia.**Conclusions:** 59% of respondents reported initial VAN MICs ≤ 2 g/mL with persistent MRSA bacteremia, while VISA and VRSA were rarely encountered (MIC>2 g/mL, 2%), reflecting poor response to therapy at the higher end of the CSLI susceptibility-range. This may partially be due to resistance-undercall of certain testing methods. Nonetheless, VAN's poor therapeutic efficacy at MIC ≤ 2 g/mL across a wide geographic distribution renders "MIC creep" more probable than clonal spread or testing artifact. Elevated MRSA VAN MICs have been associated with elevated daptomycin MICs, rendering the latter potentially problematic as alternate therapy.

Author Disclosure Block:

N. Banniettis: None. **S.E. Beekmann:** None. **P. Polgreen:** None. **S. Kaushik:** None. **S. Kohlhoff:** None. **D.N. Gilbert:** None. **J.E. Bennett:** None. **M.R. Hammerschlag:** None.

Poster Board Number:

SATURDAY-360

Publishing Title:

***Enterobacter cloacae* Producing Ges-6 in a University Hospital in Northern Spain**

Author Block:

J. Calvo, M. E. Cano, I. Angulo, L. Martínez-Martínez; Hosp. Marqués de Valdecilla, Santander, Spain

Abstract Body:

Background: Detection of carbapenemase-producing bacteria (especially when resistance level is low) is critical. In our center, there is an ongoing outbreak of ESBL-producing *E. cloacae* complex (Eclo), with a clonal group producing CTX-M-15 coexisting with a polyclonal distribution of CTX-M-9, and other ESBL. The aim of this study was to detect the presence of carbapenemase in a collection of Eclo isolates recovered during Jan.2005-Sep.2011.**Methods:** A total of 118 (one per patient) ESBL(+)-Eclo isolates were evaluated: 72 isolates of the main group produce CTX-M-15 (n=67), CTX-M-9 (1), CTX-M-15+CTX-M-9 (1), or undefined ESBL (3); 46 isolates from other 28 clonal groups carry: CTX-M-9 (n=28), SHV-12 (11), CTX-M-3 (4), CTX-M-15 (1), SHV-12+CTX-M-9 (1) or TEM-12 (1).MICs of ertapenem (ETP), imipenem (IMP), meropenem (MPM), and other beta-lactams were determined by standardized broth microdilution. PCR multiplex to detect genes encoding IMP, VIM, NDM, SPM, AIM, DIM, GIM, SIM, KPC, BIC, OXA-48 and GES was carried out. The performance of AST-243 Vitek-2 cards [with ETP (0.5-8 mg/l) and IPM (0.25-16 mg/l)] for detection of carbapenemase-producing organisms was evaluated, considering EUCAST screening criteria (ETP or MPM >=0.12, IPM >=1).**Results:** GES-6 was detected in 28 isolates of the main clonal group. MICs of ETP and MPM above EUCAST screening criteria were observed in 28 and 4 Eclo producing GES-6, respectively. MICs (mg/l) of cefotaxime and cefepime for 3 isolates with GES-6 and lacking CTX-M-15 ranged 2-4 and 0.5-2, respectively, while these values for 44 isolates from the same clonal group with only CTX-M-15 were 64->128 and 4->16. The main clonal group was present since 2007, and isolates with GES-6 appeared in June 2010. With AST-243 cards, only 8 out of the 28 (28,6%) GES-6 (+) isolates showed ETP or IPM MICs above EUCAST screening criteria.**Conclusions:** Strains producing GES-6 were detected within the main clonal group producing CTX-M-15 using a PCR approach. Vitek2 cards should be designed in order to improve their reliability for low-level carbapenem resistance detection.

		main clonal group producing GES-6 (n=28)	main clonal group not producing GES-6 (n=44)	others clones not producing GES-6 (n=46)
ETP	**Range**	0.25-4	0.03-1	0.015-4

	MIC 50/90	0.25/2	0.06/0.25	0.25/2
IMP	**Range**	0.5-1	0.03-1	0.12-2
	MIC 50/90	0.5/1	0.5/1	0.5/2
MPM	**Range**	0.06-0.25	0.015-0.12	0.015-1
	MIC 50/90	0.12/0.25	0.03/0.06	0.03/0.12

Author Disclosure Block:

J. Calvo: None. **M.E. Cano:** None. **I. Angulo:** None. **L. Martínez-Martínez:** None.

Poster Board Number:

SATURDAY-361

Publishing Title:

Genotypic Diversity and Pathogenic Potential of *Campylobacter jejuni* Strains Isolated from Animal, Human and Food in Brazil

Author Block:

M. R. Frazao[1], S. S. Duque[2], M. I. C. Medeiros[3], J. P. Falcão[1]; [1]Sch. of Pharmaceutical Sci. of Ribeirão Preto - Univ. of São Paulo, Ribeirao Preto, Brazil, [2]Oswaldo Cruz Inst. (Fiocruz-RJ), Rio de Janeiro, Brazil, [3]Adolfo Lutz Inst. of Ribeirão Preto (IAL-RP), Ribeirao Preto, Brazil

Abstract Body:

Background: *Campylobacter jejuni* is an important causative agent of human diarrheal diseases worldwide. Specifically, in Brazil *C. jejuni* has been underdiagnosed and understudied. Therefore, studies that molecularly characterize strains of this species isolated in the country are of great importance. The aims of this study were to genotype and investigate the presence of some virulence-associated genes of *C. jejuni* strains isolated in Brazil. **Methods:** A total of 90 *C. jejuni* strains isolated from humans (36), animals (28) and food (26) between 1996 and 2012 in Brazil were genotyped by PFGE and *flaA*-SVR sequencing. Additionally, the presence of 16 virulence-associated genes was searched by PCR. **Results:** The dendrogram of genetic similarity of PFGE grouped the strains in three clusters with more than 43.7% of similarity. Cluster PFGE-A comprised 18 strains isolated from animals, humans and food. Cluster PFGE-B comprised 35 strains isolated from animals, humans and food. Cluster PFGE-C comprised 36 strains isolated from animals, humans and food. In the three PFGE clusters, 76 (84.4%) strains of clinical and non-clinical sources were grouped in subclusters with a similarity of ≥ 80%. The dendrogram of genetic similarity of *flaA*-SVR sequencing grouped the strains in two clusters with more than 81.5% of similarity. Cluster SVR-A comprised 8 strains isolated from animals and humans. Cluster SVR-B comprised 82 strains isolated from animals, humans and food. All the 90 strains studied presented the genes *flaA*, *flhA*, *cadF*, *docA*, *cdtA*, *cdtB*, *cdtC*, *iamA*, *ciaB*, *racR*, *crsA*, *pldA*, *dnaJ* and *sodB*. The gene *wlaN* was found in 9 (10.0%) strains and the gene *virB11* was found in only one (1.11%) strain. **Conclusions:** The results obtained by PFGE and *flaA*-SVR sequencing showed a high genomic similarity among some *C. jejuni* strains which may suggest that a possible contamination may have occurred among clinical and non-clinical sources during 16 years in Brazil. Furthermore, the high frequency of the majority of the virulence-associated genes searched highlighted the pathogenic potential of those strains.

Author Disclosure Block:

M.R. Frazao: None. **S.S. Duque:** None. **M.I.C. Medeiros:** None. **J.P. Falcão:** None.

Poster Board Number:

SATURDAY-362

Publishing Title:

Comparison of Ribotyping and MLST for Genotyping *Bordetella bronchiseptica*

Author Block:

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Abstract Body:

Bordetella bronchiseptica is a widespread bacterial pathogen that infects a variety of domesticated and wild animals. Multilocus sequence typing (MLST) and *PvuII* ribotyping have proven useful to distinguish among strains of *B. bronchiseptica*. Both are highly discriminatory and have been used to infer relationships among isolates and identify likely sources of exposure. It is unclear which of these methods is most discriminatory since they have not been directly compared using a single set of isolates and relatively few isolates have been typed by both methods. The purpose of this study was to compare the discriminatory power of these methods using a single set of geographically and genetically diverse strains. A total of 122 isolates was analyzed, including representatives of the 22 *PvuII* ribotypes so far recognized and 25 of the 61 MLST sequence types currently associated with *B. bronchiseptica* isolates in the *Bordetella* PubMLST database. Isolates were sourced from the United States, Europe, Israel and Australia and include 24 from humans, 20 from seals, 17 from pigs, 13 from turkeys, 12 from rabbits, 10 from dogs, 8 from guinea pigs, 7 from horses, 6 from cats, 3 from koalas, 1 from a leopard and 1 of unknown origin. A total of 32 ribotype patterns was identified based on various combinations of 35 restriction fragments ranging in size from ~1.8 Kb to 5.9 Kb. DNA from one isolate could not be digested completely with *PvuII* such that a ribotype could not be assigned. In comparison, all isolates were typeable by MLST and a total of 30 sequence types was identified. The discrimination indices for ribotyping and MLST are nearly identical, 0.920 and 0.919, respectively. Nonetheless, for 10 ribotypes and 10 MLST sequence types, the alternative method discriminates among isolates that otherwise type identically. These data indicate *Pvu*II ribotyping and MLST are complementary but equally discriminatory methods. Because MLST offers practical advantages over ribotyping, including ease of standardization, scalability, portability and the ability to type all isolates, it is the preferred primary typing tool for *B. bronchiseptica*. *PvuII* ribotyping provides a secondary method that may be applied to further discriminate isolates not distinguishable by MLST.

Author Disclosure Block:

K.B. Register: None. **T.L. Nicholson:** None. **B.W. Brunelle:** None.

Poster Board Number:

SATURDAY-363

Publishing Title:

Molecular Epidemiological Analysis of *Burkholderia* Isolates from Cystic Fibrosis Patients Attended at Two Reference Centers in Brazil

Author Block:

C. P. C. Capizzani[1], N. C. Caçador[1], L. A. G. M. Torres[2], C. E. Levy[3], P. Vandamme[4], A. C. Darini[1]; [1]Sch. of Pharmaceutical Sci. of Ribeirão Preto - Univ. of São Paulo, Ribeirão Preto - SP, Brazil, [2]Clinic Hosp. of the Ribeirão Preto Med. Sch. - Univ. of Sao Paulo, Ribeirão Preto - SP, Brazil, [3]Sch. of Med. Sci. - State Univ. of Campinas, Campinas - SP, Brazil, [4]Lab. of Microbiol. - Ghent Univ., Ghent, Belgium

Abstract Body:

Many species of *Burkholderia cepacia* complex (Bcc) from cystic fibrosis (CF) patients are highly resistant to antibiotics and transmissible. MLST has been recently used as a powerful tool for species identification and genetic typing in this group of bacteria. The aim of this study was to perform molecular epidemiological analysis of Bcc species of CF patients from two Centers in Brazil by PFGE and MLST and analyze the antibiotics sensitivity. About 66 Bcc isolates were obtained from 48 CF patients. Species were identified by *recA* gene sequencing analysis. Isolates were typed by (i) MLST according to Bcc MLST Databases and (ii) *Spe*I-PFGE, and the results were analyzed by BioNumerics. Antibiotics sensitivity was performed according to CLSI. *B. vietnamiensis* was the most prevalent specie in both centers, which is very unusual since normally the most common species in CF patients are *B. multivorans* and *B. cenocepacia.* PFGE analysis showed a slightly higher molecular diversity (6%) among the isolates than MLST. In total, 17 different STs were typed by MLST. No new species of Bcc was identified; however, 14 novel STs were obtained and 3 STs (ST911, ST17 and ST369) have presented intercontinental distribution. *B. cenocepacia* seems to be more commonly spread among CF patients and associated with outbreaks. Nevertheless, *B. vietnamiensis* reached the largest number of patients with the same ST (27 patients). In most cases, the first and last isolates from each patient belonged to the same sequence type. It could explain the strong ability of Bcc strains to persist in CF patients lungs. Different results in relation to antibiotics sensitivity were obtained between the centers, which were also showed by other studies. It demonstrates the importance in perform susceptibility test looking for the best therapeutic option and to avoid treatment failure.This study contributed to update the Bcc MLST database providing more data for global epidemiological investigations, besides correlating these results with bacteria resistance.

Author Disclosure Block:

C.P.C. Capizzani: None. **N.C. Caçador:** None. **L.A.G.M. Torres:** None. **C.E. Levy:** None. **P. Vandamme:** None. **A.C. Darini:** None.

Poster Board Number:

SATURDAY-364

Publishing Title:

***Aacinetobacter baumannii* and the Conflict in Iraq: New Insights from Whole Genome Sequencing**

Author Block:

E. C. Snesrud, P. Mc Gann, **A. C. Ong**, R. Maybank, L. Appalla, F. Onmus-Leone, R. Clifford, M. K. Hinkle, E. Lesho; Walter Reed Army Inst. of Res., Silver Spring, MD

Abstract Body:

Background: Acinetobacter baumannii emerged as a significant source of infection during the conflict in Iraq. Over the course of the war, carbapenem resistant A. baumannii (CRAB) increased exponentially, primarily via the introduction of Class D carbapenemases. Chronicling the emergence CRAB using conventional typing methods, such as PFGE and MLST, is difficult due to resolution limitations, but whole genome sequencing (WGS) offers the prospect of changing this paradigm. **Methods:** In this study, WGS was employed to sequence every de-duplicated A. baumannii clinical isolate archived at the Walter Reed Army Medical Center (WRAMC) from 2003-2011 (n= 1,024). WGS was performed using a combination of short-read and long-read sequencing on the NextSeq 500 and PacBio RSII, respectively. A comprehensive analysis was subsequently performed to unravel the epidemiology of these isolates.**Results:** 970 (94.7%) of isolates were identified as A. baumannii, with Acinetobacter non-baumannii accounted for the remaining 54 isolates. In silico multi-locus sequence typing (MLST) revealed 73 ST's, including nine novel ST's. Despite the large number of ST's, >85% of isolates were assigned to just 6 ST's: ST-1, 2, 20, 25, 81 and 94. Single Nucleotide polymorphism (SNP)-based analysis revealed that ST-1 was composed of 4 different clonal clusters that were temporally separated. In contrast, isolates of ST-2, 20, 25, 81, and 94 were all caused by a single clone that persisted and spread throughout the healthcare facility over 1 to 6 years. 387 (38.5%) isolates carried blaOXA, with blaOXA-23 identified in 312 (80.6%). WGS indicated that OXA-negative strains were gradually replaced by new strains carrying Class D carbapenemases.**Conclusions:** Despite being responsible for hundreds of infections during the course of the conflict, WGS revealed that the majority of infections by CRAB were caused by just 9 different strains. Overall, the majority of CRAB represented the emergence of new strains that supplanted previously carbapenem-sensitive populations.

Author Disclosure Block:

E.C. Snesrud: None. **P. Mc Gann:** None. **A.C. Ong:** None. **R. Maybank:** None. **L. Appalla:** None. **F. Onmus-Leone:** None. **R. Clifford:** None. **M.K. Hinkle:** None. **E. Lesho:** None.

Poster Board Number:

SATURDAY-365

Publishing Title:

Characterisation Of Multi Resistant *pseudomonas Aeruginosa* From Clinical Isolates From Animals

Author Block:

J. Hordijk, E. M. Broens, V. Scheele, J. A. Wagenaar, B. Duim; Utrecht Univ., Utrecht, Netherlands

Abstract Body:

Background: *Pseudomonas aeruginosa* can cause opportunistic infections in both humans and animals. It is intrinsically resistant to multiple classes of antibiotics. Resistant *P. aeruginosa* is often isolated from clinical animal samples. Little is known about both phenotypic and genotypic characteristics of veterinary isolates. The purpose of this study was to determine susceptibility profiles and genetic diversity of *P. aeruginosa* isolates from animals. **Methods:** 113 presumptive *P. aeruginosa* isolates, obtained from clinical samples from the Veterinary Microbiological Diagnostics Center (VMDC), were included. These were isolated from dogs (99), cats (6), horses (6) and rabbits (2) between January and September 2015. Species determination was confirmed by MALDI-TOF, susceptibility testing was performed by broth microdilution. A ChromID agar was used for detection of carbapenemase activity. Presence of common beta-lactamase genes was screened by PCR. Multi Locus Sequence Typing was performed to determine the sequence types (ST).**Results:** In total, 109 isolates were confirmed to be *P. aeruginosa* by MALDI-TOF. The remaining four isolates were excluded for further analysis. Based on Minimal Inhibitory Concentration data, all isolates were resistant to more than three antimicrobial classes and therefore multi resistant. 33 unique resistance profiles and five recurring profiles were identified. The recurring profiles consisted of 5, 7, 10, 14 and 16 isolates respectively. 58.7% of the isolates were phenotypically positive for carbapenemase activity, but all tested negative for the most common beta-lactamase genes by PCR. In total 36 known sequence types (ST) and 41 novel STs were identified by MLST. Comparison with the PubMLST isolate database, showed that several previously described high risk clones in human, were also identified (ST244, ST274 and ST277) among the veterinary isolates. Interestingly, 6 isolates from cats were screened, of which 4 belonged to ST1320 and all 4 showed the same resistance pattern, whereas the strains were epidemiologically unrelated.**Conclusions:** 1: The diversity in STs of animal isolates of *P. aeruginosa* correlated with the diversity observed among STs of human isolates.2: No common beta-lactamase genes were identified.

Author Disclosure Block:

J. Hordijk: None. **E.M. Broens:** None. **V. Scheele:** None. **J.A. Wagenaar:** None. **B. Duim:** None.

Poster Board Number:

SATURDAY-366

Publishing Title:

Molecular Epidemiology of Betalactamase Resistance in *Neisseria gonorrhoeae* Isolates in France

Author Block:

H. Jacquier[1], M. Micaelo[1], A. Goubard[2], G. La Ruche[3], E. Denamur[4], O. Tenaillon[4], E. Cambau[1], B. Bercot[1]; [1]Hôpital Lariboisière, IAME, Univ Paris Diderot, PARIS, France, [2]Inst. Fournier, PARIS, France, [3]French Inst. for Publ. Heath Surveillance, Depart. Infect. Dis.,, PARIS, France, [4]Univ. Paris Diderot, IAME, UMR 1137, Sorbonne Paris Cité,, PARIS, France

Abstract Body:

Background: In France, the resistance of *Neisseria gonorrhoeae* to penicillin by beta-lactamase production (PPNG) is increasing (18% in 2014). This resistance is related to the presence of the $bla_{\text{TEM-1}}$ gene which is carried by small plasmids pJD4, pJD5 and pJD7. We recently reported the emergence of the betalactamase TEM-135 and new TEM-1 variants. The aim of this new study was to investigate the distribution of these strains by looking at their genetic materials and their clonality.**Methods:** Our collection included 176 isolates collected between 2010 and 2012 in various French regions producing TEM-1 (n=155), TEM-135 (n=14) and TEM variants defined by mutations P14S/P14L (n=8), G228S (n=2) and Q269K (n=1). Plasmids were characterized by PCR or complete sequence. Genotyping of the strains was carried out by the NG-MAST technique.**Results:** The 176 PPNG isolates were divided into 106 Sequence Type. We highlighted four main clusters of PPNG circulating in France: cluster A, related to the ST1479 (10.2%), cluster B, related to the ST1582 (8.5%), ST8922 (5.7%) and ST1285 (5.1%). The pJD5 plasmid was predominant (89.2%) whereas pJD7 and pJD4 were found in respectively 7.4% and 3.4% of the isolates. All the pJD7 and 61% of pJD4 harbored the $bla_{\text{TEM-135}}$ gene, while 94% of pDJ5 harbored $bla_{\text{TEM-1}}$ and 6% the other bla_{TEM} variants.All the PPNG isolates included in the cluster B, ST1285 or ST8922 harbored the pDJ5 plasmid and produced the TEM-1 protein. Conversely, the two plasmids pDJ4 and pDJ5 were highlighted among the isolates comprised in the cluster A. Isolates producing the TEM variants G228S and Q269K were identified in the cluster A, suggesting an emergence *de novo* of these new mutations.**Conclusion:** This work highlights the wide dissemination of $bla_{\text{TEM-1}}$ gene in France in different isolates and different genetic materials and circulation of clonal isolates producing variants of ß lactamase TEM-1.

Author Disclosure Block:

H. Jacquier: None. **M. Micaelo:** None. **A. Goubard:** None. **G. La Ruche:** None. **E. Denamur:** None. **O. Tenaillon:** None. **E. Cambau:** None. **B. Bercot:** None.

Poster Board Number:

SATURDAY-367

Publishing Title:

Genotype Diversity of Cefixime-resistant *Neisseria gonorrhoeae* Isolated in France 2014-2015

Author Block:

P. Gaspar[1], S. Kumanski[2], A. Goubard[3], N. Ndeikoundam[4], H. Jacquier[2], F. Meunier[2], E. Cambau[2], **B. Bercot**[2]; [1]Brazilian Ministry of Hlth., Brasilia, Brazil, [2]GH St Louis-Lariboisière–FW, APHP, Assoc. Lab. for the Natl. Reference Ctr. for gonococci,, PARIS, France, [3]Inst. Fournier, PARIS, France, [4]IFrench Inst. for Publ. Heath Surveillance, Depart. Infect. Dis, Saint-Maurice, France

Abstract Body:

Background: Sexually transmitted infections due to *Neisseria gonorrhoeae* (NG) are a recurrent public health problem, with worldwide increasing incidence. The recent emergence of NG isolates resistant to cephalosporin critically complicates the treatment guidelines and requires resistance surveillance. This study investigates NG isolates with decrease ssusceptibility or resistance to cefixime.**Methods:** Twenty-six NG isolated in France during 2014-2015 showing decrease susceptibility (Etest MIC= 0.125 µg/ml, EUCAST breakpoint) or resistance (MIC>0.125 µg/ml) to cefixime were submitted to molecular epidemiology typing by the reference method NG-MAST. Sequence types were assigned *via* the on line NG-MAST data base. A maximum-likelihood phylogenetic tree was constructed with PHYML program using concatenated *porB* and *tbpB* alleles.**Results:** Among the 26 strains, there were 13 with MIC of 0.125, 11 with MIC of 0.19 and 2 with MIC of 0.25 µg/ml. All isolates were susceptible to ceftriaxone. The NG isolates distributed into 14 different Sequence Type (ST) with 3 major clusters: ST6778 (n=6; 23%), one related to ST11018 (n=6; 23%), and the Genogroup G1407 (n=5; 19%). Among the resistant isolates, there were 5 ST11018, 3 G1407, 2 ST 6778 and 3 others. The phylogenetic study showed that ST11018 and ST6778 have probably a common ancestor although they are not closely related. Among the 6 isolates of ST 6778, 4 were diagnosed in the Paris area in 2015 and may be epidemiologically related.**Conclusions:** Although the well-known Genogroup 1407 is still observed among the strains with decreased susceptibility or resistance to cefixime resistance, we described two new STs, which were prevalent among the resistant strains and which require surveillance.

Author Disclosure Block:

P. Gaspar: None. **S. Kumanski:** None. **A. Goubard:** None. **N. Ndeikoundam:** None. **H. Jacquier:** None. **F. Meunier:** None. **E. Cambau:** None. **B. Bercot:** None.

Poster Board Number:

SATURDAY-368

Publishing Title:

St-11 Clonal Complex Causing Invasive Meningococcal Infection in the Basque Country, Northern Spain, in the Last 28 Years

Author Block:

J. M. Marimón[1], C. Mojica[2], D. Vicente[1], M. Alkorta[2], G. Cilla[1], E. Perez-Trallero[3]; [1]CIBERES & H.U.Donostia-IIS Biodonostia, San Sebastián-Donostia, Spain, [2]H.U.Donostia-IIS Biodonostia, San Sebastián-Donostia, Spain, [3]EHU/UPV, CIBERES & H.U.Donostia-IIS Biodonostia, San Sebastián-Donostia, Spain

Abstract Body:

Background: Meningococcal disease is related to some serogroups but also to some genotypes, as the hypervirulent ST-11 clone, responsible of epidemic outbreaks in the past decades. The main objective of this work was to analyze the epidemiology of ST-11 clonal complex (cc11) in our area in the last 3 decades. **Methods:** From June 1987 to June 2015, 887 non-duplicated invasive meningococcal isolates from the Basque Country, Northern Spain, were analyzed. Capsular serogroup were identified by latex agglutination (Murex Biotech Ltd., Dartford, England) and/or by PCR of the *siaD* and *orfA* genes. The genosubtype was determined after amplification and sequencing of variable regions (VR1/VR2) of the *PorA* gene. Genotyping was performed by MLST and PFGE after *Spe*I restriction. Antibiotic susceptibility was determined by the E-test. Meningococcal C conjugated vaccine was introduced in our region for infants in 2000 and for adolescents in 2005. **Results:** Of 887 invasive episodes, 165 (18.6%) were caused by cc11 isolates: 63 (38.2%) serogroup B and 102 (61.8%) serogroup C. MLST: 155 (93.4%) isolates were ST11 (99 serogroup C, 56 serogroup B), being the others ST3419 (n=3), ST6648 (n=2), ST67 (n=2), ST761, ST4091 and ST4706. PFGE: Two main cc11 clones: SPE-1 was prevalent between 1987-1994 representing 94.4% (34/36) of cc11 isolates obtained before 1995. SPE-2 clone was mainly isolated during 2000-2010: 121 among total 124 cc11 isolates. Genosubtype: the most frequent porA type (VR1/VR2) within SPE-1 and SPE 2 isolates were: 5/2-1 and 5-1/10-8, respectively. The *fHbp* B1 gene was present in all 17 SPE-2 isolates studied but in none 19 SPE-1. However, 12/19 SPE-1 isolates studied had the *NadA* variant 2/3. All isolates were susceptible to cefotaxime but 58 (35.2%) were penicillin non-susceptible. The mortality rate related to ST-11 clonal complex was 13.6%. **Conclusions:** Most ST-11 clonal complex strains belonged to the hypervirulent ST11 ET-37 complex, 62.5% of them being serogroup C. The 4CMenB vaccine would have covered most cc11 isolates.

Author Disclosure Block:

J.M. Marimón: None. **C. Mojica:** None. **D. Vicente:** None. **M. Alkorta:** None. **G. Cilla:** None. **E. Perez-Trallero:** None.

Poster Board Number:

SATURDAY-369

Publishing Title:

Molecular Epidemiological Analysis of *Bordetella pertussis* Isolates from Nuevo LeóN, MéXico

Author Block:

J. Gutiérrez Ferman[1], J. Ramírez-Aranda[1], A. Camacho Ortíz[1], R. Ballesteros-Elizondo[2], M. Moreno-Juárez[3], E. Garza-González[1]; [1]Univ. Autónoma de Nuevo León, San Nicolás, Mexico, [2]Secretaría de salud of Nuevo Leon, Guadalupe, Mexico, [3]Secretaría de salud of Nuevo Leon, San Nicolás, Mexico

Abstract Body:

Background: *Bordetella pertussis* is the major agent of whooping cough, a highly contagious disease of the respiratory tract. Despite the vaccination, the disease remains as a public health problem in México. The aim of this study was to determine the genetic diversity and temporal distribution of *B. pertussis* isolates in Nuevo León, México.**Methods:** Isolates were recovered from patients with clinical presentation of pertussis, from 2006 to 2014. The isolates were identified by phenotypic and genotypic tests. A total of 119 *B. pertussis* isolates (α=0.005, CI=10) were selected to determine the clonal diversity by Pulsed-Field Gel Eelectrophoresis (PFGE). Epidemiological and demographic data were collected included.**Results:** Of the 356 isolates collected, 98% were identified as *B. pertussis*, 1.7% as *Bordetella parapertussis* and 0.3% as *Bordetella bronchiseptica*. A total of 25 different genotypes with profiles comprising 11 to 16 restriction fragments and a similarity of 75 to 100% were obtained by PFGE. Three predominant clones were detected, clone A (52.9%), A1 (6.7%) and A2 (5%). The years with the highest number of *B. pertussis* isolates were 2009, 2012 and 2014 with 72, 74 and 64 isolates collected, respectively. The age group most affected was the younger than 2 months with 156 cases reported. From the infant population the 85.9% who should have received at least one dose of acellular vaccine, did not have any doses.**Conclusion:** The results suggest that the clone A is an endemic strain of Nuevo Leon and persisted for seven consecutive years, from 2008 to 2014. Further analysis is necessary to determinate if the persistence and spread of the endemic clone are a result a particularly virulent strain rather than the immune status of the study population.

Author Disclosure Block:

J. Gutiérrez Ferman: None. **J. Ramírez-Aranda:** None. **A. Camacho Ortíz:** None. **R. Ballesteros-Elizondo:** None. **M. Moreno-Juárez:** None. **E. Garza-González:** None.

Poster Board Number:

SATURDAY-370

Publishing Title:

Risk Factors for Mortality in Patients with Extended-spectrum Beta-Lactamase-Producing Enterobacteriaceae Bloodstream Infections: A Multi-Centre, Cohort Study

Author Block:

N. F. Dewhurst[1], S. M. Poutanen[2], B. Hamandi[3], L. W. Goneau[4], L. Dresser[3], M. Pitre[3], A. Y. C. Lin[5], M. D. Mistry[6], L. Graham[3], D. O. Lowe[3]; [1]St. Michael's Hosp., Toronto, ON, Canada, [2]Mount Sinai Hosp., Toronto, Ontario, Canada, Toronto, ON, Canada, [3]Univ. Hlth.Network, Toronto, ON, Canada, [4]Univ. of Toronto, Toronto, ON, Canada, [5]London Hlth.Sci. Ctr., London, ON, Canada, [6]Kingston Gen. Hosp., Kingston, ON, Canada

Abstract Body:

Background: While controversy exists whether extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae increase mortality among hospitalized patients with bloodstream infections (BSIs), limited data exist regarding predictors of mortality. We examined predictors of mortality and 7- and 30-day mortality rates among patients with ESBL-producing Enterobacteriaceae BSIs.**Methods:** Retrospective cohort study of 4 tertiary care hospitals in Toronto, Canada from Nov. 2005 to Nov. 2012. All adult inpatients with ESBL-producing Enterobacteriaceae BSIs during the study period were included (n=220). The primary outcome measures were 7- and 30-day mortality rates. Cox regression analyses were conducted to determine associations between survival and potential risk factors.**Results:** Mortality rate at 30-days among patients with ESBL-producing Enterobacteriaceae BSIs was 50/209 (23.9%). Univariate analysis revealed that 30-day mortality rates for adequately and inadequately treated patients were 37/184 (20.1%) and 13/25 (52.0%), respectively; for patients with non-nosocomial and nosocomial-associated ESBL BSIs were 20/112 (17.9%) and 30/97 (30.9%), respectively; and for patients with Charlson co-morbidity indexes of 0-4 (reference), 5-6 and ≥ 7 were 33/162 (20.4%), 9/28 (32.1%), and 8/19 (42.1%), respectively. A Cox proportional hazards model revealed that inadequate therapy [hazard ratio (HR)=4.0; p<0.001], nosocomial-associated infections (HR=2.6; p=0.002) and a Charlson co-morbidity index ≥7 (HR=3.1; p=0.007) demonstrated a significant mortality risk at 30-days.**Conclusions:** Among patients with ESBL-producing Enterobacteriaceae BSIs, inadequate therapy, nosocomial-associated infection and Charlson co-morbidity index ≥7 are associated with an increased risk of death. These clinical factors should be taken into consideration when assessing mortality risk in patients with ESBL-producing Enterobacteriaceae BSIs.

Author Disclosure Block:

N.F. Dewhurst: None. **S.M. Poutanen:** J. Scientific Advisor (Review Panel or Advisory Committee); Self; Optimer Pharmaceuticals, Inc./Merck & Co., Inc./Cubist Pharmaceuticals Inc,,

Paladin Labs Inc.,. L. Speaker's Bureau; Self; Merck & Co., Inc., Pathogenica, Inc.,. N. Other; Self; Expert forum honorarium from Accelerate Diagnostics, Inc.. **B. Hamandi:** None. **L.W. Goneau:** None. **L. Dresser:** E. Grant Investigator; Self; Sunovion Pharmaceuticals Inc, Merck & Co., Inc, Astellas Pharma Inc., and grants from Cubist Pharmaceuticals Inc, (Optimer Pharmaceuticals, Inc.). N. Other; Self; Personal fees from Sunovion Pharmaceuticals Inc.. **M. Pitre:** None. **A.Y.C. Lin:** None. **M.D. Mistry:** None. **L. Graham:** None. **D.O. Lowe:** None.

Poster Board Number:

SATURDAY-371

Publishing Title:

Extended-spectrum Beta-Lactamase-Producing *Enterobacteriaceae* are Not Associated with Decreased Survival in Patients with Bloodstream Infections

Author Block:

N. F. Dewhurst[1], S. M. Poutanen[2], B. Hamandi[3], L. W. Goneau[4], L. Dresser[3], M. Pitre[3], A. Y. C. Lin[5], M. D. Mistry[6], E. Chung[7], L. Graham[3], D. O. Lowe[3]; [1]St. Michael's Hosp., Toronto, ON, Canada, [2]Mount Sinai Hosp., Toronto, Ontario, Canada, Toronto, ON, Canada, [3]Univ. Hlth.Network, Toronto, ON, Canada, [4]Univ. of Toronto, Toronto, ON, Canada, [5]London Hlth.Sci. Ctr., London, ON, Canada, [6]Kingston Gen. Hosp., Toronto, ON, Canada, [7]The Hosp. for Sick Children, Toronto, ON, Canada

Abstract Body:

Background: Factors impacting mortality among patients with Enterobacteriaceae bloodstream infections (BSIs) are not well defined. Controversy exists whether extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae are independently associated with increased mortality compared to non-ESBL-producing Enterobacteriaceae among patients with BSIs. We examined 7- and 30-day mortality rates along with mortality risk in patients with ESBL compared to non-ESBL-producing Enterobacteriaceae BSIs.**Methods:** Retrospective matched cohort study of 4 tertiary care hospitals in Toronto, Canada from Nov. 2005 to Nov. 2012. All adult inpatients with ESBL-producing Enterobacteriaceae BSIs during the study period (n=162) were matched (1:1) to those with non-ESBL-producing Enterobacteriaceae BSIs (n=162). Seven and 30-day mortality rates were determined. Mortality risk was assessed using time to death, censored at 30 days, for the Cox regression analysis.**Results:** Seven-day mortality rates for the ESBL and non-ESBL groups were 21/161 (13.0%) and 19/160 (11.9%), respectively [odds ratio (OR), 1.1, 95% confidence interval (CI)= 0.6-2.2, p=0.73]; 30-day mortality rates were 35/152 (23.0%) and 32/153 (20.9%), respectively (OR=1.3, 95% CI: 0.7-2.3, p=0.38). Using Cox regression analysis, controlling for inadequate therapy, nosocomial-association and Charlson comorbidity index, 30-day mortality was not statistically different between patients with ESBL or non-ESBL-producing Enterobacteriaceae BSIs (HR=1.1; p=0.61).**Conclusions:** Patients with ESBL-producing Enterobacteriaceae BSIs do not have an independent increased mortality risk compared to those with non-ESBL-producing Enterobacteriaceae BSIs. When assessing mortality risk in patients with Enterobacteriaceae BSIs, emphasis should be placed on clinical factors such as adequate therapy, nosocomial-association and comorbidities.

Author Disclosure Block:

N.F. Dewhurst: None. **S.M. Poutanen:** J. Scientific Advisor (Review Panel or Advisory Committee); Self; Optimer Pharmaceuticals, Inc./Merck & Co., Inc./Cubist Pharmaceuticals Inc.,

Paladin Labs Inc.. L. Speaker's Bureau; Self; Merck & Co., Inc., Pathogenica, Inc.. N. Other; Self; Expert forum honorarium from Accelerate Diagnostics, Inc.. **B. Hamandi:** None. **L.W. Goneau:** None. **L. Dresser:** E. Grant Investigator; Self; Sunovion Pharmaceuticals Inc, Merck & Co., Inc, Astellas Pharma Inc., and grants from Cubist Pharmaceuticals Inc, (Optimer Pharmaceuticals, Inc.). N. Other; Self; Personal fees from Sunovion Pharmaceuticals Inc.. **M. Pitre:** None. **A.Y.C. Lin:** None. **M.D. Mistry:** None. **E. Chung:** None. **L. Graham:** None. **D.O. Lowe:** None.

Poster Board Number:

SATURDAY-372

Publishing Title:

***Acinetobacter* Infections and Outcomes in Critically Ill Patients at an Academic Medical Center**

Author Block:

N. Law, C. Emery, K. Raible, S. Joshi, T. Bias; Hahnemann Hosp., Philadelphia, PA

Abstract Body:

Background: *Acinetobacter baumanni* has emerged as a major cause of healthcare-associated infections, posing an important challenge to clinicians as conventional therapy often fails. This study aims to describe the epidemiological characteristics, microbial resistance patterns and clinical outcomes associated with acquisition of *Acinetobacter* infections in critically ill patients. **Methods**: A retrospective, observational study was conducted to evaluate adult patients (age ≥ 18 years) admitted to the intensive care unit who had a documented *Acinetobacter* infection between June 2013 and October 2015. Demographics and disease specific information were collected. The following clinical outcomes were assessed: 30-day mortality, infection-related mortality, microbiological cure, length of stay, and intensive care unit (ICU) length of stay.**Results:** Fifty-three patients met criteria for inclusion with a total of 111 clinical isolates of *Acinetobacter* identified. Patients with *Acinetobacter* infections were frequently admitted from healthcare facilities (38%), required mechanical ventilation (83%), had 2 or more invasive devices (66%) and antibiotic exposure within 30 days of acquisition (49%). The most common source of infection was lung (66%), followed by blood (21%), skin (9%) and urine (4%). Carbapenem resistance was identified in 62% of isolates, with colistin being most susceptible (100%) and subsequently tobramycin (65%). Despite primary source control, the overall infection related mortality and all cause 30 day mortality rates were 36% and 38% respectively. Patients who received inappropriate therapy based on Vitek 2 susceptibilities had higher rates of infection related mortality (42% vs. 25%, p=0.2). Overall, hospital length of stay (33 days [interquartile range (IQR), 13-59]) and ICU related length of stay (19 days [8-39]) was prolonged in our cohort. **Conclusions:** *Acinetobacter* infections have a high attributable mortality and remain a serious public health concern. New strategies aimed at promptly recognizing infections, selecting effective antimicrobial treatment options, preventing transmission through infection control measures and implementing stewardship initiatives for high risk patients.

Author Disclosure Block:

N. Law: None. **C. Emery:** None. **K. Raible:** None. **S. Joshi:** None. **T. Bias:** None.

Poster Board Number:

SATURDAY-373

Publishing Title:

Multidrug-Resistant Clones of *Acinetobacter baumannii* from a Tertiary Care Hospital in MéXico Are Highly Biofilm Producers, Resistant to Human Serum and Potent Inflammatory Immune Response Inducers

Author Block:

R. Rosales-Reyes[1], D. Calderón-Campos[1], C. Rivera-Benítez[2], J. I. Santos-Preciado[1], D. Alcántar-Curiel[1]; [1]UNAM, Mexico, Mexico, [2]Hosp. Gen. de México, Mexico, Mexico

Abstract Body:

Background: During the period from January to December of 2014, we identified 113 isolates of *A. baumannii* causing nosocomial infections in the Hospital General de Mexico. The great majority (97%) was resistant to carbapenem associated with the production of metallo-beta-lactamases (89%) and 84.6% carried the $bla_{\text{OXA-type}}$ gene. The aim of this study was to determine the ability of these 113 clinical isolates to form biofilm over abiotic surfaces, resistance to human serum and their ability to induce an inflammatory immune response. **Methods:** The biofilm production was assessed over polystyrene surfaces. The resistance to serum was assessed on *in vitro* assays using normal human serum (NHS). The TNFa released into supernatants from infected macrophages with *A. baumannii* were quantified by ELISA. Cell death was determined by quantification of the Lactate Dehydrogenase (LDH) activity.**Results:** Virtually all (98.41%) of clinical isolates of *A. baumannii* were biofilm producers. The 82.5% of were resistant to 40% of normal human serum (NHS) and only 12.5 % of all the clinical isolates were highly susceptible to NHS (>98% of susceptibility). All isolates tested were high inducers of TNFα release by infected macrophages with a variable cell death induction.**Conclusions:** The high prevalence of *A. baumannii* in the Hospital General de México could be due to their high ability to form biofilm over abiotic surfaces. Based on these results, we propose that the ability of these *A baumannii* isolates to form biofilm coupled to their high resistance to human serum (82.5%) and their ability to induce TNFa may explain in part their pathogenicity and their persistence in the hospital environment.

Author Disclosure Block:

R. Rosales-Reyes: None. **D. Calderón-Campos:** None. **C. Rivera-Benítez:** None. **J.I. Santos-Preciado:** None. **D. Alcántar-Curiel:** None.

Poster Board Number:

SATURDAY-374

Publishing Title:

Evaluation of *in vitro* Combination Effects of Trimethoprim-Sulfamethoxazole Combined with Levofloxacin, Ticarcillin/Clavulanate, or Minocycline Against *Stenotrophomonas maltophilia*

Author Block:

H. Araoka, M. Baba, C. Okada, M. Abe, M. Kimura, A. Yoneyama; Toranomon Hosp., Tokyo, Japan

Abstract Body:

Background: *Stenotrophomonas maltophilia* is an important cause of hospital-acquired infection including bacteremia and pneumonia, particularly in severely immunocompromised patients. The aim of this study was to evaluate the *in vitro* combination effects of trimethoprim-sulfamethoxazole (TMP-SMX) with other antimicrobial agents against *S. maltophilia* strains.**Methods:** Eighty-nine *S. maltophilia* strains isolated from blood (29) and the respiratory tract (60) between June 2012 and October 2014 were investigated. Levofloxacin (LVFX), ticarcillin/clavulanate (T/C), and minocycline (MINO) were selected to examine their effects, when combined with TMP-SMX, by the checkerboard method. Synergy is defined in checkerboard tests when the fractional inhibitory concentration (FIC) index is ≤0.5. Antagonism is defined by an FIC of >4.0.**Results:** The results of TMP-SMX + LVFX showed synergy in 21, no interaction in 61, and antagonism in 7. The results of TMP-SMX + T/C showed synergy in 71, no interaction in 18, and antagonism in 0. The results of TMP-SMX + MINO showed synergy in 10, no interaction in 79, and antagonism in 0. There were no significant differences between the combination effects of strains from blood and those from the respiratory tract.**Conclusions:** These *in vitro* data suggest that TMP-SMX + T/C was microbiologically beneficial and not antagonistic. Antagonism was observed only with the TMP-SMX + LVFX combination.

Author Disclosure Block:

H. Araoka: None. **M. Baba:** None. **C. Okada:** None. **M. Abe:** None. **M. Kimura:** None. **A. Yoneyama:** None.

Poster Board Number:

SATURDAY-375

Publishing Title:

Relationship between Daptomycin (DAP) Minimum Inhibitory Concentration (MIC) and Patient Outcomes in Vancomycin-Resistant Enterococci (VRE) Bacteremia

Author Block:

J. T. Babic[1], K. W. Garey[2], H. R. Russo[1]; [1]CHI St. Luke's Hlth.Baylor St. Luke's Med. Ctr., Houston, TX, [2]Univ. of Houston, Houston, TX

Abstract Body:

Background: Patients with increased DAP MICs may have poorer outcomes compared to those with lower MICs. To optimize therapy, a dosing policy of daptomycin ≥ 8 mg/kg was implemented for non-urinary VRE infections. The purpose of this study was to assess the effectiveness of this policy on outcomes of patients stratified by low versus high DAP MICs.**Methods:** A retrospective cohort study was performed on hospitalized patients with VRE bacteremia (2010-2014). Adult patients who received at least 72 hours of DAP were included. Area under the curve (AUC)/MIC ratios were calculated for each patient based on mean population pharmacokinetics (AAC, 2004). Clinical and microbiological outcomes were compared based on MIC (≤ 2 and >2 mcg/mL) and AUC/MIC ratios.**Results:** Sixty-nine patients (59% male, 38% Caucasian) aged 61 ± 15 years were identified. Average DAP dose was 8.9 mg/kg ± 1.31 (range 4.6 – 13.2) with 81% of patients receiving ≥ 8 mg/kg. Forty-three of 69 (62%) patients had DAP MICs > 2 mcg/mL. No difference was found in clinical cure rate between groups with low MIC [18/26 (69%)] vs high MIC [27/43 (63%); p=0.612]. Microbiological cure occurred more frequently in patients with low MICs [26/26 (100%) vs high MICs [37/43 (86%);p=0.046]. Average AUC/MIC was 491 ± 365 (range 143-1736). No significant difference in clinical or microbiological outcomes was seen in patients with low versus high AUC/MIC ratios.**Conclusions:** In this retrospective study, there was no significant difference in clinical cure rates of VRE bacteremia between patients with low and high DAP MICs, however patients with low DAP MICs had a higher rate of microbiological cure. Our institution’s dosing strategy appears to be effective clinically in treating VRE bacteremia with DAP MICs up to 4 mcg/mL, but higher doses or alternative antibiotics may be needed for microbiologic cure.

Author Disclosure Block:

J.T. Babic: None. **K.W. Garey:** I. Research Relationship; Self; Merck & Co.. **H.R. Russo:** None.

Poster Board Number:

SATURDAY-376

Publishing Title:

Five-Year Decreased Incidence of Surgical Site Infections Following Gastrectomy and Prosthetic Joint Replacement Surgery Through Active Surveillance by Konis

Author Block:

H. Choi[1], L. Adiyani[2], J. Sung[2], J. Choi[3], H. Kim[4], S. Han[5]; [1]Ewha Womans Univ. Sch. of Med., Seoul, Korea, Republic of, [2]Seoul Natl. Univ., Seoul, Korea, Republic of, [3]Yonsei Univ. Coll. of Med., Seoul, Korea, Republic of, [4]Seoul Natl. Univ. Coll. of Med., Seoul, Korea, Republic of, [5]Soonchunhyang Univ. Bucheon Hosp., Seoul, Korea, Republic of

Abstract Body:

Background: Surveillance of health care-associated infection has been associated with a reduction in surgical site infection (SSI). The Korean Nosocomial Infection Surveillance system (KONIS) was evaluated to assess its effects on SSI since it was introduced. **Methods:** SSI data after gastrectomy, total hip arthroplasty (THA), and total knee arthroplasty (TKA) between 2008 and 2012 were analysed in an evaluation of the KONIS. The pooled incidence of SSI was calculated for each year; the same analyses were also conducted from hospitals that had participated in KONIS for at least three consecutive years. Standardized SSI rates for each year were calculated by adjusting for SSI risk factors. SSI trends were analysed using the Cochran-Armitage test. **Results:** The SSI rate following gastrectomy was 3.12% (522/16,918). There was a significant trend of decreased crude SSI rates over five years. This trend was also evident in analysis of hospitals that had participated for more than three years. The SSI rate of THA was 2.05% (157/7,656) and it significantly decreased from 2008 to 2012. The risk factors for SSI after THA included the National Nosocomial Infections Surveillance risk index, trauma, re-operation, and age (60-69 years). The SSI rate of TKA was 1.67% (165/9,862), and it also decreased significantly over five years. However, the risk-adjusted analysis of SSI did not significantly show a decrease in all surgeries. **Conclusions:** The SSI incidence of gastrectomy and prosthetic joint replacement declined over five years as a result of active surveillance by the KONIS.

Author Disclosure Block:

H. Choi: None. **L. Adiyani:** None. **J. Sung:** None. **J. Choi:** None. **H. Kim:** None. **S. Han:** None.

Poster Board Number:

SATURDAY-377

Publishing Title:

Clinical and Economic Impact of Delaying Catheter Removal before Preliminary Microbiological Reports in Catheter-Related Bloodstream Infections

Author Block:

K-H. Park, M. Jung, S-y. Moon, J. Son, M. Lee; Kyung Hee Univ. Sch. of Med., Seoul, Korea, Republic of

Abstract Body:

Background: Central venous catheter (CVC) removal is recommended in most patients with catheter-related bloodstream infection (CRBSI). However, it remains unclear whether delaying of CVC removal before preliminary microbiological reports influence length of hospital stay and hospital charge, compared with early CVC removal. The objective of this study was to quantify the impact of delayed catheter removal on outcomes in these patients.**Methods:** This study was performed at two tertiary care hospitals, Seoul, Republic of Korea. Patients with early CVC removal were defined as those who had the CVC removed within 2 days after onset of CRBSI. Outcomes were evaluated using the following measures: in-hospital mortality, length of hospital stay after onset of CRBSI, and hospital charge after onset of CRBSI**Results:** During the study period, 187 episodes of CRBSI were identified. Of the 187 episodes of CRBSI, 118 (63.1%) were early removal group and 69 (36.9%) were delayed removal group. Delayed removal group was more likely to be associated with absence of fever, long-term catheter, and community-onset infection. After multivariate analysis, compared with early catheter removal, delayed catheter removal was independently associated with increased length of hospital stay (hazard ratio 1.79, 95% CI, 1.23-2.61; $P = 0.03$) and hospital charges (1.74 odds ratio, 95% CI, 1.25-2.41; $P = 0.001$). There was a trend towards an increased in-hospital mortality among delayed catheter removal group, this was not statistically significant (2.02 odds ratio, 95% CI, 0.91-4.45; $P = 0.08$).**Conclusions:** Our data suggest that an initial delay of 1-2 days in CVC removal before the preliminary microbiological reports was not associated with in-hospital mortality, but it was associated with significant increases in the length of hospital stay and hospital charges.

Author Disclosure Block:

K. Park: None. **M. Jung:** None. **S. Moon:** None. **J. Son:** None. **M. Lee:** None.

Poster Board Number:

SATURDAY-378

Publishing Title:

An Under-Diagnosed Cause Of Morbidity And Mortality In Hospitalized Patients: Healthcare-Acquired Viral Infections

Author Block:

A. K. Skrzynski, V. Russo, B. Boyanton, M. D. Sims, J. D. Band; Beaumont Hlth.- Royal Oak, Royal Oak, MI

Abstract Body:

Background: Healthcare-associated infections are a major issue in healthcare today and lead to significant morbidity and mortality. Though respiratory viral infections are among the most common infections in the general population, very little research has been conducted on the epidemiology and clinical implications of healthcare-acquired respiratory viral infections (HA-RVIs).**Methods:** We conducted a retrospective analysis of all patients with a positive respiratory virus panel (RVP) PCR from September 2011 through May 2015, aged ≥ 18 years and admitted to the hospital >5 days with symptoms of respiratory illness (utilizing the CDC concept of "influenza-like illness" as our case definition). Medical records of patients meeting criteria were reviewed for relevant clinical data and mortality.**Results:** A total of 4065 positive RVPs were noted during our study period with 44 patients (1.1%) developing a viral-associated respiratory illness after >5 days of hospitalization including *influenza* (13), *parainfluenza* (10), *rhinovirus* (9), *respiratory syncytial virus* (6), *human metapneumovirus* (4) and *coronovirus* (3) (one patient was co-infected with both *coronavirus and respiratory syncytial virus*). The average age of infected patients was 73.8 years. Cases peaked during two seasons with *parainfluenza* predominating in spring and *influenza* predominating in winter. Our cohort had significant respiratory symptoms: cough, sputum production and hypoxic respiratory failure being the most common and significant. The mortality rate for our cohort was 42%.**Conclusions:** Though under-appreciated, HA-RVIs appear to target the sickest patients in the hospital and lead to significant morbidity and mortality. Careful attention to infection control measures is paramount. Encouraging awareness among healthcare workers of the dangers transmissible respiratory viral illnesses pose for their patients and enforcement of policies to prevent acutely ill healthcare workers from caring for patients should be implemented. Likewise, ill family members should be restricted from visiting during their illness. Immunization of healthcare personnel against *influenza* should be made mandatory.

Author Disclosure Block:

A.K. Skrzynski: None. **V. Russo:** None. **B. Boyanton:** None. **M.D. Sims:** None. **J.D. Band:** None.

Poster Board Number:

SATURDAY-379

Publishing Title:

Patterns of Antimicrobial Resistance among Nosocomial Infections in Peru from 2011-2015

Author Block:

M. P. Simons[1], M. Lopez[1], E. Canal[1], M. Bernal[1], B. Pizango[1], R. Abadie[1], W. Prudencio[2], V. Changano[2], J. Celis[3], E. Zamora[3], C. Coral[4], H. Rodriguez[4], E. Linares[5], M. Calisto[5], J. Guevara[6], N. Reynolds[1], D. Tilley[7], C. Rocha[1]; [1]Naval Med. Res. Unit No. 6 Peru, Washington, DC, [2]Hosp. de la Fuerza Aerea del Peru, Lima, Peru, [3]Hosp. Regional de Loreto, Punchana, Peru, [4]Hosp. Apoyo Iquitos, Iquitos, Peru, [5]Hosp. Edgardo Rebagliati Martins, Lima, Peru, [6]Hosp. Daniel Alcides Carrion, Callao, Peru, [7]Naval Med. Ctr., San Diego, San Diego, CA

Abstract Body:

Enhanced surveillance is critical to control the spread of antimicrobial-resistant (AMR) strains, especially in underreported regions. From 2011 to 2015, we established an AMR surveillance network with 3 hospitals in Lima and 2 hospitals in Iquitos, Peru we collected 1,690 isolates from 2,665 patients and paired environmental samples classified as nosocomial infections. These isolates were retested in our laboratory for identification and susceptibility versus 37 antibiotics by disk diffusion. Epidemiological data was paired with the laboratory results on all isolates (220 variables in total). Of the 1,143 isolates collected from Lima, the most common isolates were coagulase-negative *Staphylococcus* (CONS) (26.7%), *Pseudomonas aeruginosa* (15.8%), *Klebsiella pneumoniae* (13.7%), *S. aureus* (10.4%), *E. coli* (8.7%), *Acinetobacter baumanii/calcoaceticus* complex (8.3%), and *Stenotrophomonas maltophila* (3.9%). From the 547 isolates collected in Iquitos, the most prevalent were *E.coli* (21.8%), *P.aeruginosa* (14.8%), *K.pneumoniae* (13.5%), CONS (13.4%), *Acinetobacter* spp. (12.1%), *S. aureus* (7.9%), and *Klebsiella* spp. (4.8%). Of the *S. aureus* isolates, 124/162 (76.5%) were resistant to oxacillin. Among Gram-negatives, 555/733 (75.7%) resistant to ceftriaxone and 277/1036 (26.7%) resistant to imipenem. Imipenem resistance (IMP-R) was most commonly found among *A. baumanii* (72/92, 78.3%) and *P. aeruginosa* (125/258, 48.5%). Among the IMP-R isolates, 200/277 (72.2%) were resistant to ciprofloxacin, 182/277 (65.7%) were resistant to aztreonam, and 166/277 (59.9%) resistant to amikacin, indicating high rates of MDR. Infection with an IMP-R isolate was associated with an increased hospital stay of 7.6 days (p=0.0746). Collectively, these data provide insight into causes of HAI and antibiotic resistance trends in an under-reported region of the world.

Author Disclosure Block:

M.P. Simons: None. **M. Lopez:** None. **E. Canal:** None. **M. Bernal:** None. **B. Pizango:** None. **R. Abadie:** None. **W. Prudencio:** None. **V. Changano:** None. **J. Celis:** None. **E.**

Zamora: None. **C. Coral:** None. **H. Rodriguez:** None. **E. Linares:** None. **M. Calisto:** None. **J. Guevara:** None. **N. Reynolds:** None. **D. Tilley:** None. **C. Rocha:** None.

Poster Board Number:

SATURDAY-380

Publishing Title:

***Legionella indianapolisensis sp.* Nov., Isolated from a Patient with Pulmonary Disease**

Author Block:

R. F. Relich[1], B. H. Schmitt[1], H. Raposo[2], L. Barker[2], S. J. Blosser[3], M. May[2]; [1]IU Hlth.Pathology Lab., Indianapolis, IN, [2]Univ. of New England, Biddeford, ME, [3]Indiana State Dept. of Hlth.Lab., Indianapolis, IN

Abstract Body:

Background: To date, at least 50 species of *Legionella* have been described. These organisms are ubiquitous in nature and have been isolated from diverse ecological environments, including man-made structures such as cooling towers and spas. Legionellae have also been isolated from human and veterinary clinical specimens, and their roles in disease are well-established. In this report, we describe the isolation of a novel *Legionella* species from a respiratory specimen from a patient with influenza and suspected pulmonary embolus.**Case:** A 68-year-old male presented to an Indianapolis-area hospital with pulmonary disease and, upon workup, was found to have influenza. However, the bronchoalveolar lavage fluid was also submitted for conventional bacterial culture and *Legionella* culture. The patient was prescribed broad-spectrum and recovered.**Results:** A *Legionella*-like microorganism was isolated on buffered charcoal yeast extract agar, and it was determined not to be *Legionella pneumophila* by immunofluorescence. Subsequently, the isolate was referred to the IU Health Pathology Laboratory and the state health department for identification. Mass spectrometry and comparative 16S rRNA gene sequencing inconclusively identified the isolate as *Legionella* sp. Further analysis of the 16S rRNA gene and multilocus sequence typing confirmed the strain to be a new species, related to *Legionella jamestownensis.* Physiochemical and morphologic testing were used to confirm the discovery of a novel species, *Legionella indianapolisensis* sp. nov.; type strain KT361191.

Author Disclosure Block:

R.F. Relich: None. **B.H. Schmitt:** None. **H. Raposo:** None. **L. Barker:** None. **S.J. Blosser:** None. **M. May:** None.

Poster Board Number:

SATURDAY-381

Publishing Title:

Predictors Of Multidrug Resistant (Mdr) *Pseudomonas Aeruginosa* Nosocomial Pneumonia (Pa-Np) In The Intensive Care Unit (Icu)

Author Block:

T. D. Trinh, E. J. Zasowski, K. C. Claeys, A. Lagnf, S. L. Davis, M. J. Rybak; Wayne State Univ., Detroit, MI

Abstract Body:

Background: PA-NP in the ICU has high morbidity/mortality. MDR PA makes appropriate empiric antibiotic selection difficult, warranting clinical decision tools to better predict MDR PA-NP in this population. Risks associated with MDR PA-NP in critically ill patients require further exploration. We sought to determine independent predictors of MDR PA-NP in ICU patients. **Methods:** Retrospective cohort study at two centers from 2013 - 2015. Inclusion: age ≥18 years, PA-NP culture obtained in ICU. Exclusions: PA colonization, cystic fibrosis, death ≤24 hours of empiric antibiotic administration for NP. Primary objective: to determine risk factors for MDR PA-NP in ICU patients. MDR PA isolates defined as non-susceptible to ≥3 antipseudomonal classes. Clinical characteristics of patients with and without MDR PA-NP were compared using χ^2/Fisher's exact test and Student's t-test/Mann-Whitney U test. Variables associated with MDR PA-NP at a p-value <0.20 were entered into a multivariable logistic regression model to determine predictors of MDR PA-NP.**Results:** Included 178 PA-NP episodes; 22.5% MDR. Mean (SD) age 64.3 (15.2) years; 65.2% male; 60.7% African-American; median (IQR) sequential organ failure assessment score 9 (7,12); 61.2% medical ICU; 24.7% surgical/trauma ICU; 54.5% clinically-defined NP; 30.3% ventilator-associated pneumonia (VAP); 51 (28.7%) experienced 30-day mortality. In bivariate analysis, diabetes mellitus (DM), solid organ transplant, prior hospitalization ≤60 days of PA-NP episode, admission from nursing home/skilled nursing facility (SNF), and VAP were candidates for entry into the multivariable model. The final model results are shown below.

Variable	MDR (N=40) n (%)	Non-MDR (N=138) n (%)	OR (95% CI)	aOR (95% CI)
DM	27 (67.5)	66 (47.8)	2.27 (1.08 - 4.75)	2.28 (1.05 - 4.96)
Prior hospitalization ≤60 days of PA-NP episode	17 (42.5)	33 (23.9)	2.35 (1.12 - 4.92)	2.25 (1.01 - 5.04)

Admission from SNF	13 (32.5)	17 (12.3)	3.43 (1.49 - 7.89)	2.80 (1.15 - 6.81)

Conclusions: History of DM, prior hospitalization ≤60 days of PA-NP episode, and admission from SNF were independently associated with MDR PA-NP. Patients transferred to the ICU with clinical signs of NP should be assessed for MDR PA risk via history of DM, recent hospitalizations, and nursing home residency.

Author Disclosure Block:

T.D. Trinh: None. **E.J. Zasowski:** None. **K.C. Claeys:** None. **A. Lagnf:** None. **S.L. Davis:** C. Consultant; Self; Pfizer Pharmaceuticals. E. Grant Investigator; Self; Allergan, Merck & Co. J. Scientific Advisor (Review Panel or Advisory Committee); Self; Allergan, Merck & Co, Melinta. **M.J. Rybak:** C. Consultant; Self; Allergan, Bayer, Cempra, The Medicines Company, Merck & Co, Sunovian, Theravance. E. Grant Investigator; Self; National Institutes of Health, Allergan, Bayer, Cempra, The Medicines Company, Merck & Co, Sunovian, Theravance. J. Scientific Advisor (Review Panel or Advisory Committee); Self; Allergan, Cempra, Bayer, The Medicines Company, Merck & Co, Sunovian, Theravance. L. Speaker's Bureau; Self; Allergan, Bayer, Cempra, The Medicines Company, Merck & Co, Sunovian, Theravance.

Poster Board Number:

SATURDAY-382

Publishing Title:

Impact of Clinical Underestimated in Phenotypic ESBLs by Co-Harboring ESBL and pAmpC

Author Block:

F. Nishimura, Y. Morinaga, N. Kaku, K. Takeda, N. Uno, K. Kosai, H. Hasegawa, K. Yanagihara; Nagasaki Univ., Nagasaki, Japan

Abstract Body:

Background: ESBL and plasmid-mediated AmpC (pAmpC) have been known as plasmid-mediated cephalosporinases, but the AmpCs are not routinely identified. When isolates have both ESBLs and AmpCs, there is some possibility of misidentifying ESBLs for AmpCs in the clinical laboratory. In this study, we studied the prevalence of pAmpCs and the impact of pAmpCs on laboratory detection of ESBLs in potential ESBL-producers with cefepime (FEP) sensitive. **Methods:** Clinical isolates of *Escherichia coli*, *Klebsiella* spp., and *Proteus* spp. were analyzed. We defined the potential ESBL-producers with FEP sensitive as the isolate with MIC for ceftazidime ≥2μg/mL and FEP ≤8μg/mL. The phenotypic ESBLs were detected by automated microbiology system (AMS), and the isolates were analyzed for ESBL genotyping and pAmpC types by in-house PCR. **Results:** Potential ESBL-producers with FEP sensitive were observed in 528 of all 8299 isolates. Of the 528 isolates, the prevalence of pAmpCs, ph-ESBLs and genotypic ESBLs (g-ESBLs) were 27.5%, 43.6% and 57.6%, respectively. Of the ph-ESBL, 19 were in pAmpC group, and 211 were in non-pAmpC group (Table). The isolates, which were not detected ESBL by AMS but detected ESBL genes by in-house PCR (non-ph-ESBL/g-ESBL), were more frequently observed in pAmpC isolates, compared with non-pAmpC isolates (42.8% vs. 16.7%, p< 0.01) (Table).**Conclusions:** Since non-ph-ESBL/g-ESBL was observed frequently observed in pAmpC in comparison with non-pAmpC, the co-harbouring ESBL and pAmpC might mask the ESBL-phenotype in the laboratory testing.

Characteristic of ESBL between pAmpC and non-pAmpC group

ESBL phenotype/genotype	pAmpC group, n, (%)	non-pAmpC group, n, (%)	P values
ph-ESBL/g-ESBL	12 (8.3)	166 (43.3)	< 0.01
non-ph-ESBL/g-ESBL	62 (42.8)	64 (16.7)	< 0.01
ph-ESBL/non-g-ESBL	7 (4.8)	45 (11.7)	< 0.01
non-ph-ESBL/non-g-ESBL	64 (44.1)	108 (28.1)	< 0.01
Total	145 (100.0)	383 (100.0)	

Author Disclosure Block:

F. Nishimura: None. **Y. Morinaga:** None. **N. Kaku:** None. **K. Takeda:** None. **N. Uno:** None. **K. Kosai:** None. **H. Hasegawa:** None. **K. Yanagihara:** None.

Poster Board Number:

SATURDAY-383

Publishing Title:

Rapid and Efficient Approach to Urine Culture Screening Using CHROMagar Orientation/ESBL Medium

Author Block:

A. Nakayama[1], A. Niwa[1], H. Ohta[1], N. Furuta[1], H. Ito[2], N. Murakami[1], K. Ohkusu[3], M. Seishima[2]; [1]Gifu Univ. Hosp., Gifu, Japan, [2]Gifu Univ. Graduate Sch. of Med., Gifu, Japan, [3]Tokyo Med. Univ., Tokyo, Japan

Abstract Body:

Background: CHROMagar Orientation(CHO)/ESBL (Kanto Chemical, Japan) is a bi-plate consisting of CHO medium and CHROMagar ESBL for the differentiation and presumptive identification of clinically important gram-negative bacteria and detection of ESBL-producers. We report a rapid and efficient approach to urine cultures using CHO/ESBL medium. **Methods:** A total of 1995 urine culture results generated during the months of February, April, July and October of 2012 (1073 reports) and 2015 (922 reports), were selected at random for comparison of time to result between conventional urine culture and improved method. In the conventional method, 10 μl of urine was streaked on a sheep blood agar plate. A sample of the urine was also prepared for gram-staining. In the improved method, in addition to plating, the urine sample was centrifuged at 1,500 g for 10 min after which the supernatant was discarded and the pellet was smeared onto a glass slide for gram staining. If gram-negative organisms were observed, a CHO/ESBL plate was added.**Results:** The mean time to reporting was 4.1 days for the conventional method compared to 3.9 days with the improved method. When ESBL-producing organisms were present in the urine specimen, the mean time to reporting was 4.6 days compared to 3.5 days with the improved method. With respect to negative urine cultures, the mean time to reporting was 3.2 days compared to 2.4 days with the improved method.**Conclusions:** The use of CHO/ESBL bi-plates allow differentiation of bacteria the day after plating and providing information on the presence or absence of ESBL-producing organisms. As this medium allows confirmatory testing of resistant organisms to be setup at the same time as antimicrobial susceptibility testing, CHO/ESBL allows reporting of resistant organisms 1 day earlier compared to conventional methodology. Furthermore, the staining of centrifuged urine sediments has reduced false negative microscopies, allowing release of final negative culture reports on the next day as well as workup of cultures with a cutoff of 10^3cfu/ml for specimens showing >5 WBC/hpf. The improved efficiency contributes to rapid reporting of clinically useful information.

Author Disclosure Block:

A. Nakayama: None. **A. Niwa:** None. **H. Ohta:** None. **N. Furuta:** None. **H. Ito:** None. **N. Murakami:** None. **K. Ohkusu:** None. **M. Seishima:** None.

Poster Board Number:

SATURDAY-384

Publishing Title:

Development of Simplified Assay for Detection of *van*-Gene Harbored Enterococci Using Automated BD MAX Platform

Author Block:

Y. Yamagishi, D. Sakanashi, H. Suematsu, H. Mikamo; Aichi Med. Univ., Aichi, Japan

Abstract Body:

Backgound: Molecular technologies have largely replaced culture in the clinical laboratory. The BD Max system (BD Diagnostics, Sparks, MD) is an automated molecular platform that combines specimen extraction and real-time PCR with a number of FDA-cleared assays. In addition, their generic extraction and PCR reagents for the "open platform" allow users to design their own assays using their own primers and probes. Since nosocomial spread of vancomycin-resistant enterococci (VRE) is serious problem in each hospital, rapid detection of VRE would be very important. **Methods:** We developed and evaluated of multiplex real-time PCR assay for detection of vancomycin-resistant genes (*vanA, vanB, vanC1* and *vanC2/C3*) using the new, fully automated BD MAX platform.**Results:** Cycle threshold value analyses of real-time PCR simultaneous repeatability test have showed the usefulness; coefficient of variation: CV (%) were determined 2.09%, 1.72%, 1.41% and 1.52% with *vanA*, *vanB, vanC1* and *vanC2/C3*, respectively. We also evaluated with 43 strains of enterococci were characterized by conventional PCR method; 4/4 for *vanA*-positive, 14/14 for *vanB*-positive, 1/1 for *vanB* plus *vanC1*-positive, 6/6 for *vanC1*-positive, 4/4 for *vanC2/C3*-positive and 14/14 for all-van gene-negative strains were identified correctly. This assay was automatically performing before and after PCR operations previously done manually by operator, such as DNA extraction, sample dispensing and gel electrophoresis or the ethidium bromide dyeing. As a result, work burden and the risk of the contamination were largely reduced and were shortened to about half for measurement time.**Conclusions:** We conclude that our newly developed assay might be able to greatly contribute to efficient and rapid detection of vancomycin-resistant genes.

Author Disclosure Block:

Y. Yamagishi: None. **D. Sakanashi:** None. **H. Suematsu:** None. **H. Mikamo:** None.

Poster Board Number:

SATURDAY-385

Publishing Title:

Improved EPI-CRE Test for Rapid Carbapenemase Detection

Author Block:

A. J. Slesar, P. C. Schreckenberger; Loyola Univ. Med. Ctr., Maywood, IL

Abstract Body:

Background: The Modified Hodge Test (MHT) is used to confirm the presence of KPCs but requires 24 h and has limited ability to detect other non-KPC CREs. The Carba-NP test is rapid and has been endorsed by CLSI, but is tedious to make. As an alternative, we evaluated the improved EPI-CRE® test (Pilots Point LLC, Sarasota FL) to detect carbapenemases directly from bacterial colonies. EPI-CRE is a colorimetric test that measures fermentation of a proprietary substrate present in a solution containing meropenem and bromocresol purple. This causes the solution to change from purple to yellow, indicating the presence of a CRE.**Methods**: EPI-CRE was prepared by adding 40 ml dH_2O to the powdered test reagent. The pH was adjusted to 7.5 (±0.1) using 1 M NaOH. 1 ml aliquots of the solution were added to test tubes. The tubes were inoculated with a 1 µl loopful of an overnight bacterial subculture from a blood agar plate. The tubes were incubated at 37°C and monitored for color change from purple to yellow at 1 h intervals. KPC isolates tested included: *K.pneumoniae* (2), *E.coli* (1), *E.cloacae* (1). OXA-48 isolates tested included: *E.coli* (1), *E. cloacae* (1), *K.pneumoniae* (1). MBL isolates tested included: *E.coli* (4), *K.pneumoniae* (1). ESBL isolates tested included: *E.coli* (1), *E.cloacae* (1), *K.oxytoca* (1), *K.pneumoniae* (1). AmpC isolates tested included: *E.aerogenes* (2), *S.marcesens* (2), *E.cloacae* (1). The Xpert CARBA-R RUO cartridge (Cepheid, Sunnyvale, CA), the MHT, and the EDTA Etest (BioMeriéux) were tested on all isolates. ESBL isolates were confirmed using the combination disk diffusion method. AmpC isolates were identified as bacteria belonging to the MYSPACE group of chromosomal AmpC producers.**Results:** The EPI-CRE test detected 12 out of 12 CREs in 6 h or less, including 6 isolates that were negative by the MHT. See Table.**Conclusion**: EPI-CRE is a simple, inexpensive assay that requires no sophisticated equipment or lengthy setup. Due to its ease of use, EPI-CRE has the potential to be an effective tool in screening for CREs at the bench, especially as confirmation for a questionable MHT, or as an alternative to the Carba-NP test, or molecular assays.

	MHT	MBL Etest	EPI-CRE	Xpert CARBA-R (RUO)
KPC	4 / 4	0 / 4	4 / 4	4 / 4
OXA-48	2 / 3	0 / 3	3 / 3	3 / 3
MBL	0 / 5	5 / 5	5 / 5	5 / 5

ESBL	0 / 4	0 / 4	3 / 4	0 / 4
AmpC	0 / 5	0 / 5	0 / 5	0 / 5
Sensitivity	6 / 12 = 50.0%	5 / 12 = 41.7%	12 / 12 = 100%	12 / 12 = 100%
Specificity	9 / 9 = 100%	9 / 9 = 100%	6 / 9 = 66.7%	9 / 9 = 100%

Author Disclosure Block:

A.J. Slesar: None. **P.C. Schreckenberger:** None.

Poster Board Number:

SATURDAY-386

Publishing Title:

A Real Time Multiplex PCR Assay Using Rapid Amplification Technology for the Simultaneous Detection of 6 OXA Carbapenemases

Author Block:

S. Morrow[1], R. Fowler[2], N. D. Hanson[1]; [1]Creighton Univ., Omaha, NE, [2]Univ. of NE Med Ctr, Omaha, NE

Abstract Body:

Background: The emergence of OXA carbapenemases in *Acinetobacter spp* and members of *Enterobacteriaceae* threatens the clinical efficacy of carbapenems. The purpose of this study was to design a real time multiplex PCR assay that detects six important OXA carbapenemases in two real time PCR reactions. **Methods:** Purified DNA was prepared using the DNeasy Blood and Tissue Kit®. Primers and hydrolysis probes were designed for *bla*$_{\text{OXA-48}}$, *bla*$_{\text{OXA-143}}$, *bla*$_{\text{OXA-58}}$, *bla*$_{\text{OXA-23}}$, *bla*$_{\text{OXA-24/40}}$, and *bla*$_{\text{OXA-51}}$. Degenerate primers specific for the 16S-rRNA gene were used as an internal control (IC). Primer set sensitivity was evaluated by singleplex endpoint PCR. PhilisaFAST® DNA polymerase was used to optimize the real time assays, which were validated using the 7500 Fast Real-Time PCR System. Individual positive controls were synthesized for each target. 122 clinical isolates representing 32 negative and 90 positive isolates for the target genes were used to validate the multiplex assay and included *E. coli, Klebsiella spp, Enterobacter spp, Morganella spp, S. marcescens, P. aeruginosa,* and *Acinetobacter spp*. Two master mixes were optimized to identify the six OXA carbapenemases with the IC included in both mixes. PCR reactions for both master mixes consisted of primer and probe concentrations optimized to simultaneously amplify all targets using the same PCR conditions. **Results:** This assay detected multiple OXA targets within characterized clinical isolates. The sensitivity and specificity of this assay was 97% (115/119) and 100% (32/32), respectively. The IC product was detected in all 122 isolates. The total time required for DNA extraction, PCR set-up, amplification, and analysis was ~2.5 hrs. **Conclusions:** This real time OXA-multiplex assay is a rapid molecular diagnostic tool for the detection of important OXA carbapenemase genes. With the increasing prevalence of microorganisms carrying one or more OXA carbapenemases, this assay offers fast and reliable detection of six clinically important OXA carbapenemases. Implementation of such a rapid assay can have a positive impact for infection control and patient care.

Author Disclosure Block:

S. Morrow: E. Grant Investigator; Self; STRECK. **R. Fowler:** F. Investigator; Self; Streck. **N.D. Hanson:** E. Grant Investigator; Self; Streck.

Poster Board Number:

SATURDAY-387

Publishing Title:

Commercial Multiplex PCR Kit for the Detection of Gram Negative β-Lactamase Genes: Adapting Procedures for Improved Workflow

Author Block:

K. M. Riederer, J. T. Fishbain, S. M. Szpunar; St. John Hosp. and Med. Ctr., Detroit, MI

Abstract Body:

Background: Multiplex PCR detection of resistance genes among *Enterobacteriaceae* can provide useful epidemiologic information and confirmation of phenotypic results. Commercial kits are available for use but are validated using specific assay conditions and equipment. We investigated the utility of the ARM-D™ for Beta-Lactamase ID kit (Streck) using a rapid DNA extraction method and standard thermocycler. **Methods:** DNA was extracted in parallel for 50 organisms using the validated QIAGEN® DNeasy® Blood and Tissue kit (Q) and a rapid boil method (R). Briefly, a 10µl loop of organism was suspended in 200µl water, heated at 99ºC for 15 minutes then centrifuged at 16,000 x g for 2 minutes. DNA yield and purity of supernatant (R) and eluate (Q) was assessed by spectrophotometry. DNA template from each method was amplified in parallel with internal kit controls on the Eppendorf Mastercycler® Nexus thermocycler, following kit manufacturer conditions. Gels were stained in EtBr and molecular weight of resulting bands compared. ATCC strains #25922, 700603 and BAA-1705 were included.**Results:** Selected isolates included *E. coli* (11), *Klebsiella* (16), *Enterobacter* (13), *Acinetobacter* (4), *Serratia* (2), *Proteus* (2), *Pseudomonas* (1) and *Citrobacter* (1). DNA yield was significantly higher ($p<0.0001$) with the R method (mean 57.2 µg/ml ± 52.30) compared to Q extraction (mean 13.9 µg/ml ± 8.86). Purity (A_{260}/A_{280} ratio) was also better ($p=0.013$): mean 1.72 ± 0.10 and mean 1.67 ± 0.12 for R and Q extraction, respectively. Extraction time was faster for R (20 minutes) compared to Q (1.5 hours). Positive bands for CTX-M (10), KPC (19) and NDM (1) genes were concordant using both extraction methods; 2 *K. pneumoniae* had CTX-M and KPC genes. Negative samples (22) were also concordant. Presence of non-specific bands was observed in both R (50%) and Q (34%) samples, most often with *Klebsiella* and *Enterobacter*. Non-specific bands did not correlate with DNA yield or purity.**Conclusions:** Rapid DNA extraction is a suitable option for detection of Gram negative resistance genes with the ARM-D™ for Beta-Lactamase ID kit. Optimization of amplification conditions for alternate thermocyclers may reduce or eliminate non-specific bands. The ability to use rapid DNA extraction with existing thermocyclers may facilitate use of this assay and improve workflow.

Author Disclosure Block:

K.M. Riederer: None. **J.T. Fishbain:** None. **S.M. Szpunar:** None.

Poster Board Number:

SATURDAY-388

Publishing Title:

High-Plex UTI Panel for the Detection of Bacterial Resistance Genes in Infected Urines

Author Block:

K. Schmidt[1], K. Stanley[2], D. M. Livermore[1]; [1]Univ. of East Anglia, Norwich, United Kingdom, [2]AusDiagnostics, Sydney, Australia

Abstract Body:

Introduction: Increasing resistance drives wider empirical use of previously-reserved antibiotics, including in complicated urinary tract infections (UTIs). Rapid resistance profiling, without culture, might better guide early therapy in deteriorating UTI patients and we explored the potential of the AusDiagnostics High-Plex PCR UTI Panel to deliver this.***Methods***: The High-Plex UTI panel was designed to identify 16 resistance genes by multiplexed tandem PCR; amplification is tracked in real time, with the product melting temperature determined to confirm identity. Assays were performed on 74 infected urines and 35 pure cultures. Human cells were removed from urines at 300*g*; bacterial pellets were then resuspended in water and denaturated by heat, with the lysate used as template for the High-Plex PCR. Depending on the resistance, results were compared with sequencing, real-time SybrGreen PCR, or phenotypic susceptibility.***Results*:** Performance was similar regardless of whether urines or cultured bacteria were used; time from specimen to results was *c.* 3.5h. The assay detected extended-spectrum β-lactamase (41 bla$_{CTX\text{-}M\ Gp1}$, 17 bla$_{CTX\text{-}M\ Gp9}$), *ampC* (16 bla$_{CMY}$), penicillinase (55 bla$_{TEM}$, 22 bla$_{SHV}$, 27 bla$_{OXA\text{-}1}$) and carbapenemase(7 bla$_{OXA\text{-}48}$, 5 bla$_{KPC}$, 10 bla$_{NDM}$, 1 bla$_{VIM}$) genes with 100% sensitivity and 96-100% specificity compared with reference molecular methods. *dfrA1/A5/A7/A12* genes were found in 77 of the 82 urines or isolates with phenotypic trimethoprim resistance, with sensitivity 93.9%, and specificity 100%. *aac(6')-1b* was reliably identified and consistently associated with tobramycin resistance (sensitivity 100%, and specificity 93.3%); other tobramycin and gentamicin modifying enzymes were not sought. Fluoroquinolone -susceptible and -resistant *E. coli* (only) were distinguished by the melting temperatures of their *gyrA* products.***Conclusion***: The assay rapidly identified resistance genes from Gram-negative bacteria in infected urines, potentially guiding early therapy. Limitations were (i) a narrow range of aminoglycoside targets;(ii) detection of fluoroquinolone resistance only in *E. coli*, (iii) inability to detect rarer resistance determinants e.g. to trimethoprim.

Author Disclosure Block:

K. Schmidt: None. **K. Stanley:** D. Employee; Self; Australia. **D.M. Livermore:** C. Consultant; Self; Accelerate, Achaogen, Adenium, Alere, Allecra, Altermune, Astellas, AstraZeneca, Auspherix, Basilea, Bayer, BioVersys, Cubist, Cycle, Discuva, GSK, Meiji, Nordic, Pfizer, Roche, Shionogi, Tet. H. Research Contractor; Self; Melinta, Tetraphase, AstraZeneca, Meiji,

Roche, Wockhardt. K. Shareholder (excluding diversitied mutual funds); Self; GSK, Dechra, Pfizer, Perkin Elmer. L. Speaker's Bureau; Self; AstraZeneca, Merck, Pfizer, AOP Orphan, Nordic.

Poster Board Number:

SATURDAY-389

Publishing Title:

Standard Detection and Reporting of pAmpC Helps Limiting Its Spread in the Hospital Setting

Author Block:

Z. Daoud, E. Najem, E. Ragheb, R. Taoutel, M. Bousaab, I. Dandachi; Univ. of Balamand, Beirut, Lebanon

Abstract Body:

Background: Chromosomal and plasmidic AmpC enzymes (pAmpC) have received relatively little attention in comparison to Extended-spectrum beta lactamases (ESBLs). An increasing prevalence of pAmpCs has been reported recently in different areas of the world. While many infection control activities addressed ESBLs in the hospitals, pAmpCs remain inappropriately detected in most of the clinical labs what contributes to its spread. Our aim is to evaluate the importance of detection and reporting of pAmpC on its prevalence in the hospital setting. **Methods:** Between 31/12/2010 and 31/12/2015, *E.coli* and *K. pneumonia* isolates were screened for AmpC using a combination of reduced susceptibility to 3rd generation cephalosporins and cefoxitin as a screening strategy. As a confirmation test, we used the combination disk consisting of cefoxitin and ceftazidime combined with boronic acid or cloxacillin as inhibitors. ESBL was also detected using the Double Disc Synergy test. Prior to 1/1/2015, no infection control measures were taken concerning pAmpC patients. Between 1/1/2015 and 31/12/2015, isolates that gave positive confirmatory test were reported to the infection control department and isolation of the patient was performed accordingly and percentage of pAmpC was determined yearly for comparison. Statistical analyses were performed using SPSS V 20.0**Results:** While *E.coli* isolates showed an ESBL rate increase from 2011- 2012 from 25% to 29% and then reached 30% and stabilized between 2012 -2015; a progressive increase at the level of AmpC production from 2011 -2014 (5 to 9%) was noted when isolation of the patient was not performed. In 2015, an abrupt decrease from 9 to 4% (p<0.05) was noted in parallel to the implementation of patient isolation. A similar but less significant pattern was observed with *Klebsiella* most probably due to the lower incidence of pAmpC in this genus.**Conclusions:** Our data show that the appropriate detection of pAmpC and its subsequent standard reporting as a Multi-Drug-Resistant organism that can spread in the hospital, decreases the incidence of this type of resistance. Therefore, clinical microbiology labs should adopt a phenotypic technique to detect pAmpC and differentiate it from ESBL and report it to the infection control department for follow up. This will help containing the spread of this enzyme with high potential of transmission.

Author Disclosure Block:

Z. Daoud: None. **E. Najem:** None. **E. Ragheb:** None. **R. Taoutel:** None. **M. Bousaab:** None. **I. Dandachi:** None.

Poster Board Number:

SATURDAY-390

Publishing Title:

Rapid Identification of *bla*$_{IMP-1}$ and *bla*$_{IMP-6}$ by Multiplex Arms PCR

Author Block:

A. Nakano[1], R. Nakano[1], F. Mizuno[1], S. Endo[2], K. Kasahara[1], H. Yano[1]; [1]Nara Med. Univ., Nara, Japan, [2]Tohoku Univ. Graduate Sch. of Med., Miyagi, Japan

Abstract Body:

Background: Carbapenem-resistant Enterobacteriaceae have been globally reported because of the acquisition of carbapenemase genes. Transferable carbapenem resistance genes, IMP (*bla*$_{IMP-1}$ and *bla*$_{IMP-6}$), are most commonly identified in clinical settings in Japan. They have different substrate specificity; IMP-1 producers were more resistant to imipenem than to meropenem, while IMP-6 (IMP-1 variant differs only a single nucleotide) producers were more resistant to meropenem than to imipenem. In this study, we developed a rapid multiplex amplification refractory mutation system (ARMS) PCR assay to discriminate between *bla*$_{IMP-1}$ and *bla*$_{IMP-6}$ by using allele-specific PCR primers. **Methods:** Multiplex ARMS PCR primers, including two primers sets, were designed based on the nucleotide sequence of *bla*$_{IMP-1}$ and *bla*$_{IMP-6}$. Two primers (forward and reverse) were designed to recognize conserved sequences of *bla*$_{IMP-1}$ and *bla*$_{IMP-6}$, and two other primers were designed to recognize specific nucleotide sequences of *bla*$_{IMP-1}$ (forward) and *bla*$_{IMP-6}$ (reverse), respectively. PCR products of *bla*$_{IMP-1}$ and *bla*$_{IMP-6}$ were confirmed by electrophoretic analysis. The specificity of the assay was evaluated using 40 IMP (10 IMP-1 or 30 IMP-6)-producing clinical isolates of Enterobacteriaceae (*Escherichia coli*, *Klebsiella pneumoniae*, and *Enterobacter cloacae*). **Results:** The multiplex ARMS PCR assay conducted amplification within 60 min. *bla*$_{IMP-1}$ and *bla*$_{IMP-6}$ PCR products had the expected size of *ca*. 400 and 150 bp and *ca*. 400 and 300 bp, respectively. Moreover, PCR amplification for 40 IMP-producing Enterobacteriaceae strains was successful with expected product sizes. Cross-reactivity was not observed for other types of carbapenemase producers (NDM, VIM, KPC, and OXA-48), and false positive or false negative results were not observed. **Conclusions:** The developed multiplex ARMS PCR assay successfully discriminates between *bla*$_{IMP-1}$ and *bla*$_{IMP-6}$. This method could serve as a specific, rapid, and easier alternative for the detection of IMP-1 or IMP-6 producers in clinical settings. Therefore, this method might guide the appropriate choice of antimicrobial therapy and facilitate epidemiological studies.

Author Disclosure Block:

A. Nakano: None. **R. Nakano:** None. **F. Mizuno:** None. **S. Endo:** None. **K. Kasahara:** None. **H. Yano:** None.

Poster Board Number:

SATURDAY-391

Publishing Title:

Volatile Metabolites for Rapid Diagnosis of Carbapenem Resistance in *Klebsiella pneumoniae*

Author Block:

C. A. Rees[1], A. Smolinska[2], M. Nasir[1], P. C. Zucchi[3], E. B. Hirsch[3], J. E. Hill[1]; [1]Dartmouth Coll., Hanover, NH, [2]Maastricht Univ., Maastricht, Netherlands, [3]Northeastern Univ., Boston, MA

Abstract Body:

Background: Infections caused by carbapenem-resistant *Enterobacteriaceae* are a growing global public health threat, resulting in high mortality. More rapid diagnosis could reduce mortality and length of hospitalization by expediting appropriate antimicrobial therapy. Here, we show that the volatile metabolites produced by *Klebsiella pneumoniae* can be used to reliably predict carbapenem resistance. **Methods:** Sixty-two clinical isolates of *K. pneumoniae* (30 carbapenem-resistant [C-R] and 32 carbapenem-susceptible [C-S], 3 replicates per strain) were grown in Mueller-Hinton broth to early stationary phase. C-R isolates were positive for bla_{KPC} by PCR and sequencing and resistant to meropenem (MEM), while C-S isolates were neither. Culture supernatants were collected in air-tight vials, and volatile metabolites were analyzed using GCxGC-TOFMS. The Mann-Whitney U test with Benjamini-Hochberg correction was used to identify volatiles that differed in abundance between the two groups. Random Forest (RF) analysis was used to identify volatiles that most accurately predicted MEM susceptibility (one-third of the data was used as an independent validation set). The receiver operating characteristic (ROC) curve was used to assess accuracy of the classification model.**Results:** 212 volatile metabolites differed significantly ($p < 0.05$) in relative abundance between the C-R and C-S groups. RF identified a set of 25 volatiles predicting the MEM susceptibility with accuracy of 94.4% in our training set and 95.1% in the validation set. ROC analysis showed a sensitivity of 1, a specificity of 0.91, and an area under the curve of 0.96 (95% C.I. of 0.91-1.0) for the detection of C-R isolates in the validation set. Sixty of 64 C-S and 57/60 C-R isolates in the training set, and 29/32 C-S and 30/30 C-R isolates in the validation set classified correctly.**Conclusion:** Amongst the 62 strains analyzed, volatile metabolite suites could distinguish C-R from C-S isolates with approximately 95% accuracy. The identification of predictive volatile biomarkers linked to carbapenem susceptibility could inform the development of a rapid, minimally-invasive breath-based diagnostic for lung infections, and could also serve in the analysis of patient blood and/or urine samples.

Author Disclosure Block:

C.A. Rees: None. **A. Smolinska:** None. **M. Nasir:** None. **P.C. Zucchi:** None. **E.B. Hirsch:** None. **J.E. Hill:** None.

Poster Board Number:

SATURDAY-392

Publishing Title:

Evaluation of Alere^{tm} Pbp2a Assay for Detection of Methicillin-resistance in *Staphylococcus* Isolates

Author Block:

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Abstract Body:

Objective: Penicillin binding protein 2a (PBP2a), encoded by mecA gene, is responsible for beta-lactam resistance in *Staphylococcus spp*. The aim of this study was to evaluate the performance of Alere^{TM} PBP2a assay, a rapid immunochromatographic assay, for the detection of PBP2a in *Staphylococcus* isolates. **Methods:** Retrospectively collected 150 *S. aureus* and 377 coagulase-negative staphylococcus (CoNS) isolates as identified by Staphaurex® latex agglutination assay were utilized for this study. CoNS isolates were speciated with Microscan Automated ID system. Isolates were grown on columbia agar with 5% sheep blood (CA), tryptic soy agar with 5% sheep blood (TSA) or Mueller-Hinton agar (MHA). Isolates from different plate types were tested by the Alere^{TM} PBP2a assay and results compared to the cefoxitin disk diffusion test.**Results:** Methicillin-resistance was detected in 63/150 *S. aureus* isolates and 140/377 CoNS isolates by cefoxitin disk diffusion testing. The most common CoNS species detected in the study was *S. epidermidis* (n=238). See table 1 for Alere^{TM} PBP2a assay performance on different media types.**Conclusion:** The Alere^{TM} PBP2a assay had high sensitivity and high specificity for rapid detection of methicillin-resistance in *S. aureus* isolates. For CoNS isolates, the sensitivity and specificity was 95% or above for all plate types. Alere^{TM} PBP2a assay was found to be quick, easy to use, highly sensitive and specific method to detect methicllin-resistance in Staphylococcus isolates.Table 1: Performance characteristics of Alere^{TM} PBP2 assay

***S. aureus* (n=150)-Alere^{TM} PBP2a results**						
Cefoxitin-KB	TSA		CA		MHA	
	Neg	Pos	Neg	Pos	Neg	Pos
Resistant	1	62	1	62	1	62
Susceptible	87	0	87	0	87	0

Sensitivity (95% CI)	100.0% (92.7-100.0%)		100.0% (92.7-100.0%)		100.0% (92.7-100.0%)	
Specificity (95% CI)	98.9% (92.9-99.9%)		98.9% (92.9-99.9%)		98.9% (92.9-99.9%)	
***CoNS* (n=377)-Alere™ PBP2a results**						
Cefoxitin-KB	TSA		CA		MHA	
	Neg	Pos	Neg	Pos	Neg	Pos
Resistant	9	131	7	133	8	132
Susceptible	230	7	230	7	231	6
Sensitivity (95% CI)	94.9% (89.4-97.7%)		95.0% (89.6-97.8%)		95.7% (90.4-98.2%)	
Specificity (95% CI)	96.2% (92.7-98.2%)		97.1% (93.8-98.7%)		96.7% (93.3-98.4%)	

Author Disclosure Block:

N. Kanwar: None. **A. Nguyen:** None. **R. Selvarangan:** None.

Poster Board Number:

SATURDAY-393

Publishing Title:

Evaluation of a Rapid Immunochromatographic Test for Detection of Distinct Variants of Kpc Carbapenemase Among *Enterobacteriaceae*

Author Block:

A. Ramos[1], A. Gales[1], J. Monteiro[1], S. Suzane[2], A. Machado[1], C. Carvalhaes[1]; [1]Univ.e Federal de São Paulo - UNIFESP, São Paulo, Brazil, [2]Tampa Gen. Hosp., Tampa, FL

Abstract Body:

Background: The prevalence of carbapenemase-producing *Enterobacteriaceae* isolates is raising worldwide. Therefore, rapid and feasible tests for their detection are highly desirable. Media used to bacteria growth is known as a potentially confounder for carbapenemase detection by CARBA-NP and MALDI-TOF, mainly MacConkey agar. KPC is one of the most prevalent carbapenemase described among *Enterobacteriaceae*. To date 23 KPC variants were reported. This study evaluated the performance of a rapid immunochromatographic test for detection of different variants of KPC carbapenemase directly from a single bacterial colony. The influence of distinct growth media was also studied. **Methods:** A total of 26 Gram-negative isolates were studied, including 17 KPC-producing enterobacteria (7 distinct variants of KPC: KPC-2; -3; -4; -6; -7; -8; -11), 4 carbapenemase-producing isolates other than KPC (BKC-1-; NDM-1-; OXA-48-producing *K. pneumoniae* and OXA-23-producing *A. baumannii*) and 4 non-carbapenemase-producing *K. pneumoniae* (ESBL-producing isolates possessing porin losses). The isolates were subcultured onto Müeller-Hinton (MHA), MacConkey (MAC) and ChromID CPS® agar (CPS). Briefly, a bacterial colony was resuspended and homogenized in 10 drops of LY-LA buffer (TRIS, NAN_3, detergent; pH 7.5) from KPC *K*-SeT® kit (CORIS BioConcept). Three drops of this solution were applied into the cassette sample spot and read up to 15 minutes. A positive result was observed when two visible red lines were detected. **Results:** None of the non-KPC-producing isolates recorded positive results by KPC *K*-SeT (100% specificity). However, the isolates harboring the variants KPC-6 and KPC-8 could not be detected (sensitivity 88%). All the positive results were observed within 1-3 minutes. The distinct growth medium did not influence the results, independently of the KPC variant tested. **Conclusions:** The KPC *K*-SeT immunochromatographic kit showed to be a feasible and rapid test for detection of the main variants of KPC. The test was able to detect all tested KPC variants but KPC-6- and KPC-8, without influence of distinct culture media.

Author Disclosure Block:

A. Ramos: None. **A. Gales:** None. **J. Monteiro:** None. **S. Suzane:** None. **A. Machado:** None. **C. Carvalhaes:** None.

Poster Board Number:

SATURDAY-394

Publishing Title:

Rapid Identification of Oxa-48, Oxa-163 Subfamily and Kpc in Carbapenemase-producing *Enterobacteriaceae*

Author Block:

L. Denorme[1], I. Ote[1], F. Pasteran[2], S. Gomez[2], A. Corso[2], **P. Y. M. G. Mertens**[1];
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Abstract Body:

Background: CPE are a leading cause of antibiotic resistance worldwide. OXA-48 and KPC are two carbapenemases expressed in CPE and their identification present a serious challenge for laboratories. Noteworthy, some allelic variants of OXA-48, such as OXA-163 which is highly prevalent in Argentina, show weak carbapenemase activity while it hydrolyses broad-spectrum cephalosporins. Definitive confirmation of OXA-48 variants currently relies on molecular assays and gene sequencing. We developed the OXA-163/48 Duo *K*-SeT test, a new lateral flow assay that identifies OXA-48 and OXA-163-like carbapenemases, and we combined this test with the KPC identification in the OXA-163/48 and KPC Trio *K*-SeT test.**Methods:** Immunochromatographic sandwich tests were developed by using monoclonal antibodies in both capture (coating on membrane) and detection (coupling to colloidal gold). Tests are performed on bacterial colonies grown on solid medium after suspension in a specific buffer.**Results:**Two anti-OXA-48 antibodies were selected as specific capture reagents on two lines for the Duo test: (1) a first antibody directed against OXA-48 (but not OXA-163) and (2) another antibody directed against another epitope of OXA-48 and other variants (including OXA-163). A third antibody directed against all OXA-48 variants (including the OXA-163 subfamily) was chosen as a detection reagent. If the sample contains OXA-48, it will remain bound to the first capture antibody (anti-OXA-48). If the sample contains the OXA-163, it will not react with the first antibody and will bind to the second capture antibody (anti-OXA-163+48). This OXA-163/48 Duo *K*-SeT test allowed to accurately and rapidly identify (i) OXA-163 and other related variants (OXA-247 and -438) and (ii) OXA-48 and -181 from a panel of 40 positive clinical strains with 100% sensitivity. All 30 non-OXA-48 strains remained negative. The OXA-163/48 and KPC Trio *K*-SeT test allowed to detect KPC in all tested KPC positive strains. **Conclusions:**We developed the OXA-163/48 Duo and OXA-163/48 and KPC Trio *K*-SeT tests for the rapid identification of carbapenemases from the OXA-48 family, OXA-163 subfamily and KPC. The 100% performance of the tests will be further assessed on a larger panel of clinical bacterial isolates

Author Disclosure Block:

L. Denorme: D. Employee; Self; Coris BioConcept. **I. Ote:** D. Employee; Self; Coris BioConcept. **F. Pasteran:** None. **S. Gomez:** None. **A. Corso:** None. **P.Y.M.G. Mertens:** D. Employee; Self; Coris BioConcept.

Poster Board Number:

SATURDAY-395

Publishing Title:

Effectiveness of the Immunochromatographic Method for the Rapid Identification of Oxa-48 Carbapenemase

Author Block:

S. Aksaray[1], E. Sanmak[2], I. Davarci[2], S. Akcay[2], A. Kuskucu[2]; [1]Haydarpasa Numune Hosp., istanbul, Turkey, [2]haydarpasa numune Hosp., istanbul, Turkey

Abstract Body:

Hospital infections due to carbapenemase producing enteric gram negative bacterias cause high morbidity and mortality as a result of multiple drug resistance. This is why it is vital to detect carbapenemase existence correctly and rapidly. While some part of carbapenemase can be detected using phenotyping methods, D group carbapenemase can be detected with molecular methods. In this study, we aimed to determine the efficacy of immunochromatographic method to show OXA-48 carbapenemase existence at Klebsiella pneumoniae strains. We have included 39 *Klebsiella pneumoniae* strains, isolated from several clinical samples from inpatients during one year, that are detected as carbapenem resistant or lower sensitivity using standard disc diffusion method and Vitek-2 system (BioMerieux-France). We have tested OXA-48 by Real-time PCR (Corbett research,Australia) and OXA-48 K SeT (Coris Bio, Belgium) at all strains. OXA-48 was found positive at 27 *Klebsiella pneumoniae* strains with both methods. The sensitivity and specificity of immunochromatographic method was found as 100%. Enteric bacteria infections that cause carbapenemase, present a serious problem in our country as worldwide. However, OXA-48 has been reported from Turkey for the first time in world. Molecular methods are time consuming and expensive and this causes difficulties in diagnosis. According to our preliminary results; altough the number of strains in our study is low (we are planning to continue our study with more samples); we have come to a conclusion that the OXA-48 K SeT will be a vary good alternative at rapid diagnosis, giving results in a very short time, having high sensitivity and specificity along with its easy procedure.

Author Disclosure Block:

S. Aksaray: None. **E. Sanmak:** None. **I. Davarci:** None. **S. Akcay:** None. **A. Kuskucu:** None.

Poster Board Number:

SATURDAY-396

Publishing Title:

A Molecular Multidrug Resistance Gene Panel as an Innovative and Accurate Alternative to Antibiotic Susceptibility Testing

Author Block:

P. Dawson, M. Stonebraker, D. Stalons, L. Malone, E. Grigorenko; Diatherix Lab., LLC, Huntsville, AL

Abstract Body:

Background: Antibiotic susceptibility testing (AST) is one of the most important tests in a clinical microbiology laboratory. Accurate and rapid detection of antibiotic resistance patterns is crucial for all aspects of antimicrobial stewardship, including resistance surveillance and effective patient treatment. Existing traditional culture-based ASTs are time-consuming and often lack specificity for new generations of antimicrobials. In this study, we compare the performance of a rapid molecular multidrug resistance gene (MDRG) panel to gold-standard Kirby-Bauer AST methodology. **Methods:** Twenty-six isolates were obtained from IHMA, Inc., with all information about them withheld. Isolates were cultured on a non-selective medium, followed by nucleic acid extraction and testing on custom OpenArray® qPCR plates with seventeen MDRG targets. Simultaneously, an antibiotic resistance profile (ARP) was determined using the Kirby-Bauer method of AST. Corresponding resistance phenotypes of detected genes were assigned from published literature and compared with the Kirby-Bauer method's ARPs.**Results:** The MDRG Panel was 100% accurate in detecting the correct resistance gene(s) in the blinded isolates. There was excellent agreement between the ARPs generated through the Kirby-Bauer method and those generated from the detected antibiotic resistance genes. There was a 92% correlation in detection for resistance to carbapenems; 100% to cephalosporins, fluoroquinolones, and macrolides; and 53% to the monobactam, aztreonam. Expected carbapenem resistance was observed in all but one strain which had a zone of inhibition just beyond that of the resistant phenotype, indicating intermediate susceptibility and a possible evolution into the resistant phenotype. The low agreement of monobactam resistance between the two methods seemed largely due to the presence of CTX-M genes in the strains. This can be explained from the variability in antibiotic resistance expression among the different CTX-M gene classes reported in published literature.**Conclusions:** Our findings indicate that the accuracy of the MDRG Panel is comparable to the Kirby-Bauer method in predicting antibiotic class resistance phenotypes and is an innovative and rapid alternative to conventional AST methods.

Author Disclosure Block:

P. Dawson: D. Employee; Self; Diatherix Laboratories. **M. Stonebraker:** D. Employee; Self; Diatherix Laboratories. **D. Stalons:** D. Employee; Self; Diatherix Laboratories. **L. Malone:** D. Employee; Self; Diatherix Laboratories. **E. Grigorenko:** D. Employee; Self; Diatherix Laboratories.

Poster Board Number:

SATURDAY-397

Publishing Title:

Detection of Esbls, Mbls, Kpcs, and Plasmid-Mediated *amp*c β-Lactamase Genes by Multiplex Real-Time Pcr

Author Block:

M. P. Torres[1], S. Morrow[2], J. Lechner[1], L. Porter[1], C. Connelly[1], N. D. Hanson[2]; [1]Streck, Omaha, NE, [2]Creighton Univ., Omaha, NE

Abstract Body:

Background: The production of extended-spectrum β-lactamases (ESBLs) by Gram-negative bacteria has emerged as a major resistance mechanism that threatens healthcare facilities. Current phenotypic tests do not differentiate clinically important β-lactamases and may not identify several co-existing resistance mechanisms. Novel diagnostic tests that lead to the accurate identification of these resistance mechanisms can improve antibiotic stewardship and infection control. This study describes two multiplex Real-Time PCR assays for the detection of 9 different β-Lactamase gene families and 6 different plasmid-mediated *amp*C β-Lactamase genes.**Methods:**Streck's ARM-D® for β-Lactamase ID and *amp*C ID Real-Time PCR Kits (Streck, Omaha, NE) were used to detect the following β-Lactamases: IMP-1, NDM, OXA-48, CTX-M-14, CTX-M-15, CMY-2, DHA, VIM, and KPC; and plasmid-mediated *amp*Cs: MOX, DHA, ACC, EBC, FOX, and CMY-2. An internal control (IC) that targets a conserved region in Gram-negative bacteria was included in each mix to discriminate false negative results. PCR amplification was monitored using hydrolysis probe chemistries. DNA samples extracted from characterized clinical isolates were used to validate gene identification. Data for both Multiplex assays was generated using the ABI QuantStudio™7 Flex and Bio-Rad CFX96 Touch™ real-time PCR instruments. However, each kit has been developed as an endpoint real-time detection assay compatible with multiple real-time PCR instrument platforms using Philisa*FAST*® DNA polymerase.**Results:**The ARM-D® for β-Lactamase ID and the Philisa® *amp*C ID Real-Time PCR kits have been validated with previously characterized clinical isolates. Specificity and sensitivity for both assays was 100%. β-Lactamase positive isolates amplified within the first 20 cycles of the PCR.**Conclusions:**These assays provide a rapid detection strategy for monitoring β-Lactamase-based antibiotic resistance in Gram-negative bacteria. Given the broad range of β-Lactamase families identified by these kits, these assays represent a valuable tool to improve antibiotic stewardship practices and active surveillance of resistance mechanisms.

Author Disclosure Block:

M.P. Torres: None. **S. Morrow:** None. **J. Lechner:** None. **L. Porter:** None. **C. Connelly:** None. **N.D. Hanson:** None.

Poster Board Number:

SATURDAY-398

Publishing Title:

Screening and Detection of Antimicrobial Agents Production Bioprospects in Metagenomic Libraries from Aquatic Bodies in Puerto Rico

Author Block:

B. M. Soriano, L. M. Del Valle Perez, C. Rios Velazquez; Univ. of Puerto Rico at Mayaguez, Mayaguez, PR

Abstract Body:

Background: Antimicrobials have revolutionized human health care, but in recent years, their effectiveness has been diminishing. Antimicrobial resistance is a global public health concern causing nearly 19,000 deaths annually in United States and around $20 billion in care related costs each year. Being aware of the enormous repercussions of this problem, it is imperative to do more research in order to reduce the impact of this issue. Knowing the scarce discovery of novel antibiotics by culture-dependent methods, it is necessary to implement culture-independent methods such as Metagenomics as an alternative solution for the increasing antimicrobial resistance issue. **Methods:** For this, an antimicrobial agent production functional analysis methods were performed to four high molecular weight aquatic bodies metagenomic libraries (AML) generated from samples of Guajataca water reservoir (GWR- G1 and G2), Rio Grande de Añasco (RGA), Playuela Beach (PB). The functional screening was done using two assays: (1) overlay inhibition test and (2) Kirby Bauer assay (KBA). In the first test, 500 clones of the respective AML were spreaded on Petri plates with Luria Bertani (LB) and after 24 hrs of incubation, an overlay of *Klebsiella pneumoniae* as target was done on top of the AML clones. A total of 1% of the clones per AML was screened for antimicrobial agents production. In the KBA, supernatants of the AML, one after centrifuging the un-lysed AML culture (S1) and the other one after lysing the AML culture (S2). After spreading *K. pneumoniae* on LB plates, 5mm individual disks impregnated with S1 and S2 were placed on top and incubated for 24 hrs.**Results:** While no antibiosis halos were detected for S1, halos with variable inhibition zones were found in the S2 tested. For PB no inhibition halos were found, however for G1 43% of the disk demonstrated inhibition halos, for G2 thebinhibition ratio was 21% and 36% inhinition ratio for RGA.**Conclusions:** These results demonstrated the reliability of Metagenomics in the discovery of antimicrobial products in these environments, representing an opportunity and a new alternative in solving the antibiotic resistant issue.

Author Disclosure Block:

B.M. Soriano: None. **L.M. Del Valle Perez:** None. **C. Rios Velazquez:** None.

Poster Board Number:

SATURDAY-399

Publishing Title:

The Isolation of Ocean Microbes That Produce Antibiotics Against *Pseudomonas aeruginosa* Strains from Cystic Fibrosis Patients

Author Block:

D. M. Walsh; MassBay Community Coll., Wellesley Hills, MA

Abstract Body:

Microbes are the source of antibiotics. It is necessary to find new antibiotics against the bacterium *Pseudomonas aeruginosa*. This is the causative agent of infection in cystic fibrosis patients and it can develop antibiotic resistance.Water samples were taken from various marine environments. With aseptic technique, 100 µl of marine water was added to each of the following microbial media; *Streptomyces* agar, R2A agar, Sabourauds Dextrose agar (SAB), and R2A and SAB both supplemented with sea water. The marine water was streaked for isolation, and incubated at 25ºC-35°C for 3-5 days.The cross streak method was used with all of the isolated colonies against *Pseudomonas aeruginosa* ATCC 39324 (PAcf), a strain isolated from a cystic fibrosis patient. If a potential antibiotic is produced by the isolated water microbe, there would be growth inhibition of PAcf near the streaked area.Of the many tests performed only 1 isolate proved to inhibit PAcf. The microbe was identified by Gas Chromatography (GC) as *Pseudoalteromonas nigrifaciens*. The potential antibiotics produced were acetone extracted, and identified by GC-Mass Spectrometry (GC-MS) as benzophenone, 1, 2-butanediol, and cetene. These molecules could perhaps be used in clinical phase I trials in cystic fibrosis patients. This could lead to a possible cure for this terrible disorder.

Author Disclosure Block:

D.M. Walsh: None.

Poster Board Number:

SATURDAY-400

Publishing Title:

Isolation and Identification of Antibiotic Producing Bacteria from Soil

Author Block:

M. Bailey, J. Pifer, E. Hardin; Lewis Univ., Romeoville, IL

Abstract Body:

Most antibiotics currently prescribed to treat infections are derivatives of natural compounds produced by bacteria and fungi. Unfortunately, most antibiotics will not remain clinically effective long-term due to the increasing frequency of antibiotic resistance. Increasing instances of multidrug resistance coupled with a decrease in the discovery and development of novel antibiotics is setting the stage for a global healthcare crisis. To combat this increasing public health threat, the idea of "crowdsourcing" to isolate and identify new antibiotic producers and new drugs is growing in prominence. This work represents the results of implementation, in our undergraduate microbiology laboratory, of the Small World Initiative crowdsourcing for antimicrobial discovery. Suspensions of soil samples from thirty-two locations were plated to yield individual colonies. Diverse colonies and any colonies exhibiting clear zones of growth inhibition of neighboring cell growth were selected. Isolates were tested for antimicrobial activity against a panel of ESKAPE pathogen safe relatives. Isolates exhibiting greatest antimicrobial activity against the ESKAPE panel were identified using 16S rRNA sequencing followed by nucleotide BLAST sequence alignment. Additionally, isolates exhibiting the most significant antimicrobial activity against the ESKAPE tester strains were subjected to organic extraction with ethyl acetate. The organic extracts were dried, resuspended and spotted onto LB agar plates inoculated with the same ESKAPE strains to test for organic antimicrobial activity. Over 400 diverse colonies were tested against a panel of Gram positive and Gram negative ESKAPE pathogen safe relative strains. Sixteen examples that exhibited the most substantial growth inhibition of tester panel strains were sequenced and subjected to the organic extraction process. Sequencing identified species of the genera *Bacillus, Pseudomonas, Microbacterium, Staphylococcus, Streptomyces* and *Enterobacter*. One particular isolate, ELHA18b, identified as a strain of *Bacillus pumilus,* was found to actively grow and produce an active organic antimicrobial across a range of temperatures from -80C to 42C. The data support the concept that soil yields a diverse population of bacteria that produce active antimicrobial compounds and may harbor as yet unidentified antibiotic compounds.

Author Disclosure Block:

M. Bailey: None. **J. Pifer:** None. **E. Hardin:** None.

Poster Board Number:

SATURDAY-402

Publishing Title:

Controlling Bacterial Weapons: Biosynthesis and Regulation of Antifungal, Antibacterial and Nematicidal Metabolites in *Serratia*

Author Block:

M. A. Matilla[1], J. E. E. U. Hellberg[2], G. P. C. Salmond[3], T. Krell[1]; [1]EEZ-CSIC, Granada, Spain, [2]Univ. of Cambridge, Cambridfe, United Kingdom, [3]Univ. of Cambridge, Cambridge, United Kingdom

Abstract Body:

The extended use of antibiotics and chemical pesticides has driven the emergency of multidrug resistance microorganisms. As a consequence, great efforts are being made to isolate and develop new broad-spectrum antibiotics. Soil bacteria are a promising source of secondary metabolites, which are mainly synthesized by large multifunctional enzymes known as polyketide synthases (PKSs) and non-ribosomal peptide synthetases (NRPSs). During the screening of a collection of soil and plant-associated bacteria, we found that one of them, the rhizobacterium, *Serratia plymuthica* A153, showed a broad spectrum of biological activities - including antifungal, anti-nematode, and antibacterial. Using genome sequencing, mutagenesis, chemical analyses and *in vivo* antagonistic and virulence assays, we identified three secondary metabolites as responsible for the observed bioactivities. Firstly, we isolated and characterized for the first time the PKS gene cluster responsible for the biosynthesis of the antifungal and anti-oomycete haterumalide, oocydin A. Secondly, we found that A153 produces the hybrid NRPS/PKS antibacterial acetyl-CoA carboxylase inhibitor, andrimid. Finally, we demonstrated that the observed nematicide properties are due to the production of the PKS/NRPS antibiotic, zeamine. Using the nematode worm, *Caenorhabditis elegans* as a model organism, we showed that early larvae are more sensitive to zeamine. We also investigated the genetic regulation of these three secondary metabolites. Our results showed that the expression of their respective gene clusters is highly controlled at both transcriptional and posttranscriptional levels - highlighting the complexity of the regulatory mechanisms controlling secondary metabolism in this enterobacterium.

Author Disclosure Block:

M.A. Matilla: None. **J.E.E.U. Hellberg:** None. **G.P.C. Salmond:** None. **T. Krell:** None.

Poster Board Number:

SATURDAY-403

Publishing Title:

Urinary *Lactobacillus crispatus* Produces a Molecule Which Kills Uropathogenic *E. coli*

Author Block:

K. A. Diebel, T. Price, A. J. Wolfe; Loyola Univ. Chicago, Chicago, IL

Abstract Body:

Background: As many as 1 in 2 women will have at least one urinary tract infection (UTI) in their lifetime. UTIs can cause complications in pregnancy and decrease quality of life, and their treatment and prevention are expensive. Uropathogenic *E. coli* (UPEC) is the primary cause of UTI. A vaginal suppository of the bacterium *Lactobacillus crispatus* has been shown to protect against recurrent UTIs caused by UPEC, and vaginal *Lactobacillus* isolates have been shown to produce by-products that inhibit the growth of UPEC. The probiotic and bactericidal capacities of gut and vaginal *Lactobacillus* isolates have been studied, but the same attention has not been paid to urinary strains. *L. crispatus* strains isolated from the bladders of women without lower urinary tract symptoms are significantly genetically different from vaginal and gut strains. These urinary isolates of *L. crispatus* appear to have a greater killing capacity against UPEC and this bactericidal activity does not depend on the cells themselves, consistent with the hypothesis that they secrete a molecule with anti-UPEC activity. In the future, this bacterium could be useful as a probiotic and molecules it produces could be used as antibacterial compounds.**Methods:** *L. crispatus* isolates were grown in de Man, Rogosa and Sharpe broth (MRS), at 37°C in CO_2, for 48 hours. The spent culture supernatant (SCS) was removed and filter-sterilized. NU14 was aerated in tryptic soy broth (TSB) at 37°C in O_2 for an average of 19 hours. Two mL each of NU14 and SCS were combined and aerated at 37°C in O_2. Aliquots of 100 µl were harvested at chosen time points, serially diluted and spread onto tryptic soy agar (TSA) plates, which are placed at 37°C in O_2 for 24 hours. Colonies were counted and the CFU/mL of NU14 were calculated.**Results:** The SCS of one urinary isolate of L. crispatus killed several logs of UPEC within 2 hours of exposure. This isolate creates a more acidic environment than isolates of other Lactobacillus species, but the killing of UPEC was not due to low pH alone, as buffered of the SCS delayed but did not eliminate the bactericidal effect. This effect became stronger after the SCS was left to sit for 24 hours. The molecule was not heat sensitive.**Conclusions:** A urinary L. crispatus isolate produces a unique soluble molecule that can kill up to 9 logs of UPEC within 24 hours. The molecule may be an antimicrobial peptide or bacteriocin. Further experiments are required.

Author Disclosure Block:

K.A. Diebel: None. **T. Price:** None. **A.J. Wolfe:** None.

Poster Board Number:

SATURDAY-404

Publishing Title:

Designing a Chronic Wound Anti-biofilm Strategy Using Probiotics

Author Block:

J. G. Thomas, L. listico, R. Kreft; Allegheny Hlth.Network, Pittsburgh, PA

Abstract Body:

Background: Since 2011, we have reported on the efficacy of silver dressings against a broad spectrum of wound isolates in planktonic (P/P) and biofilm phenotypes (P/BF), unmasking developing biofilm resistance and gauze colonization. Hence, we wanted to investigate a bi-phasic intervention, "Disruption: Reconstruction" with silver and probiotics, here, focusing on the selection of probiotics. **Methods:** Initial studies targeted Methicillin Resistant *Staph aureus* and gauze colonization using 3 probiotic challenges containing different combinations *of Lactobacillus sp., Bifidobacterium sp.,* and/or *Saccharomyces boulardi*i MRSA P/BF were grown in BHI for 24-72 hrs. in 25 mm petri dishes with 1 cm square gauzes evaluated using 1) FISH with species specific *Staph aureus* 16 S rRNA probe and 2) Live /Dead stain background quantifying 1) MRSA P/BF mass, 2) average thickness and 3) average roughness.Secondary studies targeted 7 clinical isolates measuring anti-planktonic activity *of Lactobacillus rhueteri* (LR), ATCC 53608, and *Bifidobacterium pseudocatenulatum (BP)*, ATCC 27919, using a disc diffusion/zone of inhibition(ZOI) assay at 24, 48 and 7 days. **Results:** Two probiotic pools containing 4 species, LB and SLB, showed inconsistent results via both CLSM and Live/Dead stains; studies with LR, *Lactobacillus rheuteri*, showed promising results highlighting a 30% reduction in biomass and 80% reduction in MRSA gauze biofilm thickness, stabilizing gauze integrity. Secondary studies showed varying anti-planktonic ZOI (7-10mm) of both LR and BP against 10^3 CFUs/ml of clinical isolates. **Conclusions:** CLSM data suggested that 1) *Lactobacillus rheuteri*, can disrupt/ reduce the 3-D biofilm architecture of MRSA while maintaining gauze integrity and 2) a combination of anti-planktonic probiotics may be necessary.

Author Disclosure Block:

J.G. Thomas: None. **L. listico:** None. **R. Kreft:** None.

Poster Board Number:

SATURDAY-405

Publishing Title:

Ahl-Lactonases of *Bacillus cereus*, Characterization of Activities and Sequencing Encoding Genes

Author Block:

M. Sakr, M. Aboulwafa, K. Aboshanab, N. Hassouna; Faculty of Pharmacy, Ain Shams Univ., Cairo, Egypt

Abstract Body:

Background: N-Acyl homoserine-lactone-dependent quorum sensing plays a major role in the virulence of a number of pathogenic bacteria, therefore is considered a useful target for antimicrobial therapy. In this study, activities of crude AHL-lactonase enzymes from five *B. cereus* isolates were characterized followed by sequence analysis of the genes/enzymes involved. **Methods:** Total protein concentration was determined by Lowry's method. Thermal and pH stabilities were determined. Effect of some divalent metals (Ca++, Fe++, Mg++ Cu++, Zn++) and EDTA on activity was tested. Catalytic rates, effect of temperature (5-70°C) and pH (6-9) on activity were studied. Kinetic constants were determined and enzyme substrate specificity was measured using standard synthetic signals (C4, C6, C7 and C8-HSL). Lactonase genes were sequenced, analyzed, assembled, ORFs detected and the full nucleotide sequences were submitted to GenBank database. Domain analysis and prediction of putative tertiary structures were performed.**Results:** The enzymes displayed high thermal and pH stabilities. Maximum enzymes activities were obtained within the temperature range of 28-50°C and pH 6-9. Their activities were neither enhanced by any of the tested metals nor inhibited by EDTA. However, a concentration of 10 mM Fe^{+2} either completely or partially inhibited their activities. Enzymes proved to be constitutively produced. AHL lactonase activity ranged between 17.7-32.97 U/mg. Vmax values ranged between 59.9-113.6 $nM.min^{-1}$ per mg protein, and Km between 14.27-27.2 nM. All tested enzymes have a broad activity spectrum. DNA sequences were submitted to GenBank database (accession codes: KF254906, KF254908, KF254909, KF254907, KF254910). The protein sequences revealed conserved multi-histidine motif and position 194-tyrosine residue. Predicated structures showed them to be metalloenzymes with binding sites for 2 zinc atoms. **Conclusion:** Displaying high physical stability and relatively strong catalytic activity, the five studied lactonase enzymes proved to be potential candidates for development into antipathogenic drugs.

Author Disclosure Block:

M. Sakr: None. **M. Aboulwafa:** None. **K. Aboshanab:** None. **N. Hassouna:** None.

Poster Board Number:

SATURDAY-406

Publishing Title:

A Novel Natural Ramoplanin Analog Produced by an Actinomycete Strain

Author Block:

F. VICENTE, M. DE LA CRUZ; FUNDACION MEDINA, GRANADA, Spain

Abstract Body:

Ramoplanin is a glycolipodepsipeptide obtained of *Actinoplanes* sp. with activity against clinically multi-drug-resistant pathogens including vancomycin-resistant *Enterococcus*, methicillin-resistant *Staphylococcus aureus* and vancomycin-intermediate resistant *Clostridium difficile*. It disrupts bacterial cell wall through a novel and a unique mechanism of action by sequestering the peptidoglycan intermediate Lipid II and does not show cross-resistance. However, while demonstrating excellent antimicrobial activity in systemic use in infection animal models, ramoplanin presents low tolerability intravenously. Therefore, new derivatives are desirable to overcome this issue.A natural product program developed to discover compounds that inhibit peptidoglycan transglycosylation binding the intermediate Lipid II permitted the identification of a novel ramoplanin analog produced by an actinomycete fermentation. Minimum inhibitory concentration of the purified compound was measured against a panel of isolates. Cytotoxicity, cytochrome inhibition and kinetic aqueous solubility at different pH were also measured by MTT, LC-MS and turbidimetric assays.This new ramoplanin (2572 Da), exhibited specific activities against Gram-positive bacteria (methicillin-resistant *S. aureus*, *E. faecalis*, *E. faecium* and *C. difficile*). No relevant activity was observed when the compound was tested against Gram-negative bacteria. The compound displayed no significant toxicity (IC_{50} above 23.6 µM) against human hepatocyte cell line, and weak CYP450 inhibition with IC_{50} values above 36 µM. According to the kinetic solubility classification, the compound can be categorized into acceptable (>50 µM).We present here a novel natural ramoplanin analog whose favorable pharmacological profile and excellent antibacterial activities support further investigation. Data obtained in this study reinforce the fact that natural products are a source of potential drugs, in particular of antibiotics.

Author Disclosure Block:

F. Vicente: None. **M. De la cruz:** None.

Poster Board Number:

SATURDAY-407

Publishing Title:

Bioinspired Antimicrobial Therapeutics to Treat Drug-Resistant Infections

Author Block:

C. de la Fuente[1], R. Citorik[1], M. Mimee[1], F. Reffuveille[2], K. Fairfull-Smith[3], T. Coenye[4], R. Hancock[5], T. Lu[1]; [1]Massachusetts Inst. of Technology; Broad Inst. of MIT and Harvard Univ., Cambridge, MA, [2]Univ. de Reims, Reims, France, [3]Queensland Univ. of Technology, Queensland, Australia, [4]Lab. of Pharmaceutical Microbiol., Ghent Univ., Ghent, Belgium, Ghent, Belgium, [5]Univ. of British Columbia, Vancouver, BC, Canada

Abstract Body:

Background: Antibiotic-resistant infections are predicted to kill 10 million people worldwide per year by 2050. The majority of these infections are caused by biofilms, which demonstrate increased adaptive resistance to conventional antibiotics. Host defense systems have evolved to fight off infections, including the human innate immune system that produces small molecules such as cationic peptides and nitric oxide (NO), and the adaptive immune system CRISPR-Cas of bacteria that operates by cleaving invading phages and plasmids. Here, we leveraged these systems for the design and implementation of next-generation antimicrobials. **Methods:** For peptide work, microplate, flow cell assays, NMR and TLC were used. CRISPR phages were generated by programming the *S. pyogenes* Type II CRISPR-Cas9 system to recognize specific DNA targets and packaging this system using the M13 filamentous bacteriophage. The resultant phage particles were purified and used to deliver the CRISPR construct into recipient bacteria containing or lacking the target sequences.**Results:** We identified synthetic peptides that:i) are broad-spectrumii) interfere with the signalling nucleotide ppGppiii) synergize with antibioticsiv) protect *C. elegans* and *G. mellonella* from otherwise lethal *Pseudomonas aeruginosa* biofilm infectionsNO mimetics eradicated biofilms and synergized with fluoroquinolones.Finally, when CRISPR phages were used to target carbapenem-resistant *E. coli*, a highly discriminatory lethal activity was noted that depended on the presence of the pre-defined sequence within the recipient cells. Programmed phages were also used to selectively remove single *E. coli* strains from a three-strain consortium by targeting the different resistance genes contained within the strains. A CRISPR phage therapeutic targeting a virulence factor of enterohemorrhagic *E. coli* O157:H7 significantly improved *G. mellonella* survival.**Conclusions:** We have generated bioinspired therapeutics that effectively target drug-resistant infections both *in vitro* and *in vivo*.

Author Disclosure Block:

C. de la Fuente: None. **R. Citorik:** None. **M. Mimee:** None. **F. Reffuveille:** None. **K. Fairfull-Smith:** None. **T. Coenye:** None. **R. Hancock:** None. **T. Lu:** None.

Poster Board Number:

SATURDAY-408

Publishing Title:

The Antibiotic Actinorhodin: From Phenotypic Marker in Streptomycete Research to Potential Antibacterial Drug Candidate

Author Block:

N. M. Nass, C. P. Randall, R. F. Seipke, A. J. O'Neill; Univ. of Leeds, Leeds, United Kingdom

Abstract Body:

Background: New antibacterial drugs are urgently required to replace those whose therapeutic utility has become compromised by antibiotic resistance. One approach to identifying antibacterial drug candidates is to revisit known antibiotics that have not been clinically exploited. Actinorhodin (ACT) is a pigmented antibiotic produced by *Streptomyces coelicolor*, first discovered in the 1940's, and often employed as a phenotypic marker in streptomycete research. Here we report on the antibacterial properties of ACT, concluding that this compound may have potential as an antistaphylococcal agent.**Methods**: ACT was extracted from *S. coelicolor* L646 with ethyl acetate, and further purified by HPLC. Broth microdilution MICs and time-kill studies were performed according to CLSI guidelines. Cytotoxicity was evaluated by measuring lactate dehydrogenase release and ATP levels in human proximal tubular HK-2 cells. Antibacterial mode of action (MOA) was investigated by macromolecular synthesis (MMS) studies using radiolabeled precursors, and effects upon membrane potential/ integrity were assessed using $DiSC_3(5)$ and potassium (K^+) leakage assays, respectively.**Results**: ACT demonstrated bacteriostatic antibacterial activity (MIC of 8 µg/ml) against multi-drug resistant (MDR) *S. aureus*, but lacked activity against Gram-negatives. ACT exhibited an LC_{50} >100 µg/ml in mammalian cytotoxicity tests. ACT caused rapid, non-specific inhibition of MMS (≥ 87% inhibition at 10 min), a result typically observed for membrane perturbing compounds. However, assays detecting changes in membrane potential [$DiSC_3(5)$] and membrane integrity (K^+ leakage), revealed limited effects (<30% reduction) after 60 min, suggesting that the membrane may not be the primary target of ACT. No reduction in ACT susceptibility was selected upon serial passage of *S. aureus* in the presence of ACT for 5 days.**Conclusion**: ACT exhibits selective antibacterial activity against MDR staphylococci at concentrations in range of established antibacterial drugs, and demonstrates low resistance potential. Although further studies will be required to precisely elucidate the MOA of ACT, we consider that ACT represents a promising candidate for the treatment of staphylococcal infection.

Author Disclosure Block:

N.M. Nass: None. **C.P. Randall:** None. **R.F. Seipke:** None. **A.J. O'Neill:** None.

Poster Board Number:

SATURDAY-409

Publishing Title:

Antibiofilm Activity of Bioactive Compound from *Pseudomonas stutzeri* AF3 Against Pathogenic Bacteria

Author Block:

D. E. Waturangi, L. Suteja, M. T. Suhatono; Atma Jaya Catholic Univ., Jakarta, Indonesia

Abstract Body:

Background: Biofilm is an assemblage of surface-attached microorganism embedded in exopolysaccharide (EPS) matrix. It is one of the key factor for microbial survival in adverse environment. In the recent years, various means have been explored in order to control biofilm formation and accumulation. Many recent studies suggest that antibiofilm compounds extracted from diverse microorganism have the ability to control biofilm without exhibiting biocidal activity (Kostakioti*et al.* 2015).From previous studies, endophytic bacteria AF3 (*Pseudomonas stutzeri*) had been isolated from *Anrederacordifolia* and had been shown antibiofilm activity against wide range of human pathogenic bacteria (Jessica 2011; Febri 2013).**Methods:** In this study we purified the compound from *Pseudomonas stutzeri* AF3 using ion exchange chromatography as well as desalting to concentrated the sample followed by antibiofilm assay using static biofilm assay to inhibit and destruct biofilm formation of *Pseudomonas aeruginosa* and *Staphylococcus aureus.* **Results:** The compound was purified using ion exchange chromatography showed single peak when eluted using linear gradient of NaCl 2 M. While desalting was done to obtain concentrated sample.. Significant increase in antibiofilm activity of purified samples was observed. The highest increase observed in inhibition and destruction antibiofilm of *P. aeruginosa* at 10% concentration of sample. Concentrated sample exhibited relatively high antibiofilm activity when compared with purified sample even after 10-fold dilution. There was an exception for biofilm inhibition against *S. aureus* where the antibiofilm activity of concentrated sample diluted 10-fold was much lower than purified sample. The highest increase in biofilm inhibition activity was showed against *P. aeruginosa* when 5% of compound was added whereas in destruction activity 10% of compound added to *P. aeruginosa* showed highest increase.**Conclusions:** Bioactive compound from *Pseudomonas stutzeri* showed antibiofilm activity agains *P. aeruginosa* and *S. aureus,* the activity increased after purification. This suggested that polysaccharide might have an important role in biofilm destruction activity of this compound.

Author Disclosure Block:

D.E. Waturangi: None. **L. Suteja:** None. **M.T. Suhatono:** None.

Poster Board Number:

SATURDAY-410

Publishing Title:

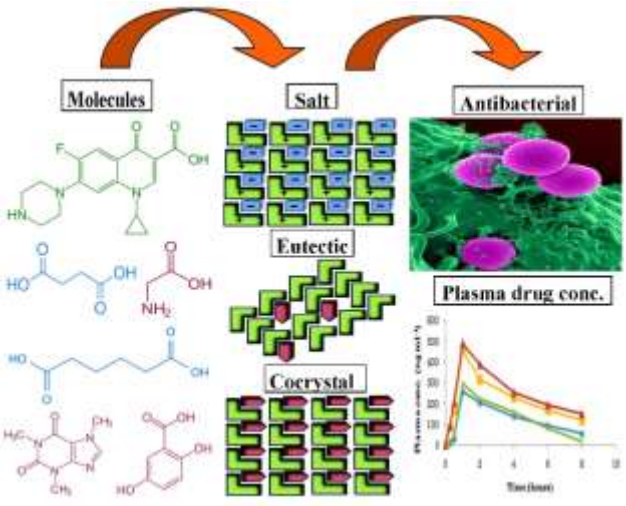
Synergistic Antibacterial & Pharmaceutical Advantage of the Ciprofloxacin Multi-Component Forms

Author Block:

P. Singh, R. Chadha; Panjab Univ., Chandigarh, India

Abstract Body:

Background: Ciprofloxacin, the active ingredient of the antibacterial drug Cipro (in USA), is potent and active against both Gram-positive and Gram-negative bacteria; however, it is known to have poor solubility and absorption. Thus, to improve solubility and bioavailability characteristics, multi-components forms of the drug have been prepared and also been tested for synergistic antibacterial activity.**Methods:** Multi-components are formed with succinic acid (salt), adipic acid (salt), caffeine (eutectic), glycine (eutectic) and gentisic acid (cocrystal) using solvent assisted grinding technique.Antibacterial activity (MIC) was tested on *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli* and *Klebsiella pneumoniae* using broth dilution method.**Results:** The MIC value for these multi-component forms ranged between 0.05µg/mL and 0.8µg/mL. Moreover, the cocrystal and eutectics as multi-components exhibits improved solubility and dissolution compared to the salt forms in water. Pharmacokinetic studies on rats showed that the cocrystal and eutectic forms exhibits doubled plasma AUC values in a single dose.**Conclusions:** To conclude, the design, synthesis and characterization of these kinds of multi-components are essential in order to further expand the scope of the available pre-formulation options beyond pure API forms.



Author Disclosure Block:

P. Singh: None. **R. Chadha:** None.

Poster Board Number:

SATURDAY-411

Publishing Title:

The Isolation and Characterization of Marine Antibiotic Producing Bacteria from Sarasota Bay, Florida

Author Block:

D. Christou-ader, E. Warrick; State Coll. of Florida, Bradenton, FL

Abstract Body:

The current emergence of a plethora of multi-drug resistance bacteria has left us with a dwindling variety of effective antibiotics to fight infections. The once elementary infection may now be of great concern as the cause of the infection may have developed the ability to combat one of our only outside defenses, antibiotics. This emerging crisis has stimulated the need for researchers to discover and develop new antibiotics to target these multi-drug resistance bacteria. In order to discover new antibiotics, samples should be taken from more unique environmental locations. To accomplish this, sediment and water samples taken from offshore and inshore sites along the Gulf of Mexico and Sarasota bay were collected and cultured on various media looking for antibiotic producing bacteria. Media recipes included potato dextrose agar, lysogeny broth, and actinomycetes isolation agar. All of the media were supplemented with 0.5M NaCl and 100µg/ml cycloheximide. Incubation conditions were designed to be optimal for the enrichment and isolation for marine Actinomycetes. All samples were plated at dilutions yielding plates that contained 30-300 colonies and then transferred to a gridded master plate for individual testing. Each colony was tested against several ESKAPE safe relatives; *Staphylococcus epidermidis, Bacillus subtilis, Enterobacter aerogenes, and Citrobacter freundii* as well as the non-pathogenic marine organism *Chromohalobacter salexigens* for the ability to produce a zone of inhibition. Colonies that showed inhibition of an ESKAPE safe relative were preliminarily identified using 16s rDNA gene. Initial testing resulted in *Streptomyces aurues, Bacillus amyloliqefaciens, Chromobacterium aquaticum* and an unknown species of *Microbacterium*. Many samples came back as known antibiotic producing bacterium. The unknowns will be used for continued research. Chemical analysis of the antibiotic compound(s) is ongoing and will be discussed.

Author Disclosure Block:

D. Christou-ader: None. **E. Warrick:** None.

Poster Board Number:

SATURDAY-412

Publishing Title:

Accurate Quantification of D-Ala-D-Lac Terminated Peptidoglycan Stem Structures in Cell Wall of Vancomycin-Resistant *Enterococcus faecalis*

Author Block:

J. Chang, E. Foster, S. Kim; Baylor Univ., Waco, TX

Abstract Body:

Background: Vancomycin-resistant *Enterococcus faecalis* (ATCC 51299) is VRE of *VanB* type which replaces D-Ala-D-Ala peptidoglycan (PG) stem with D-Ala-D-Lac in the presence of vancomycin. This substitution reduces the binding affinity of PG-stem to vancomycin by thousand fold and confers VRE vancomycin resistance. Although D-Ala-D-Lac terminated cytoplasmic PG precursors have been previously observed, evidence for the existence of D-Ala-D-Lac terminated PG resulting from these precursors has remained elusive. This has led to suggestions that D,D-carboxypeptidase completely cleaves all D-Lac away from PG in the cell wall. In this study, we investigate using LC-MS and solid-state NMR vancomycin induced changes to the chemical composition of the cell wall in VRE. **Methods:** VRE was grown with or without the presence of vancomycin (4 μg/ml) in defined media containing [2-^{13}C]pyruvate, or L-[1-^{13}C]Ala and L-[ε-^{15}N]Lys. Solid-state NMR was used to determine the extent of accumulated Park's Nucleotide in intact whole cells by monitoring the amount of D-[2-^{13}C]Lac and D,L-[2-^{13}C]Ala converted from [2-^{13}C]pyruvate. The effect of D-Ala-D-Lac substitution on PG bridge-linking efficiency was determined by ^{13}C{^{15}N} REDOR NMR. LC-MS was used to quantify the chemical composition of PG fragments resulting from mutanolysin digestion of the isolated cell wall. **Results:** Induction of vancomycin resistance shows excess accumulation of cytoplasmic D-Ala-D-Lac terminated Park's Nucleotide in VRE. Approximately 20% of PG pentapeptide in the cell wall during the exponential growth phase were composed of D-Ala-D-Lac, and this proportion increased to 60% once the stationary growth phase was reached. **Conclusion:** REDOR NMR confirmed that the bridge-linking efficiency was not affected by D-Ala-D-Lac substitution. D-Ala-D-Lac substituted PG fragments from the cell wall were observed and differentiated from D-Ala-D-Ala terminated PG fragments via LC-MS through m/z values and chromatographic retention time. D-Ala-D-Lac substituted fragments were only observed upon the induction of vancomycin resistance.

Author Disclosure Block:

J. Chang: None. **E. Foster:** None. **S. Kim:** None.

Poster Board Number:

SATURDAY-413

Publishing Title:

Murine Lung Infection Model Demonstrates Reduced *In Vivo* Resistance Emergence to Wall Teichoic Acid Inhibitors Compared to Observed *In Vitro* Resistance

Author Block:

L. Liang, C. Gill, S. Lee, J. Liu, P. Mann, H. Wang, J. Xiao, C. Tan, T. Roemer, A. Flattery; MERCK RESEARCH LABORATORIES, Kenilworth, NJ

Abstract Body:

Increasing multiple drug resistance to marketed antibacterials is limiting the effective options for treatment of infections by Methicillin Resistant *Staphylococcus aureus* (MRSA). There is emerging evidence that wall teichoic acid (WTA) synthesis plays an important role in the expression of ß-lactam resistance in MRSA. We have previously demonstrated that inhibition of WTA synthesis in MRSA via mutations or with WTA inhibitor compounds leads to reduced virulence and restored susceptibility to the β-lactam antibiotic imipenem in mouse infection models (Wang *et al* 2013). These in vivo studies demonstrated that inhibition of WTA synthesis is an attractive target for combination therapy with ß lactams to combat MRSA infections. In vitro studies however revealed a higher frequency of resistance (FoR) than acceptable over an extended 48 hr time period and had the potential to derail this program. In order to examine the significance of this drug resistance observed in vitro, we developed a novel in vivo infection model. Herein we describe studies in a murine MRSA lung infection model utilizing both MRSA COL and an isogenic strain deficient in WTA as the first gene involved in WTA synthesis, tarO, was deleted. The ΔTarO mutant serves as a genetic surrogate for WTA inhibition by a small molecule and simplifies FoR analysis as only the high frequency bypass mutants observed in vitro can be obtained. We compared the efficacy of 2 β-lactam antibiotics imipenem and dicloxacillin against both MRSA and its ΔTarO mutant in immune competent and neutropenic mice. Our results demonstrate that the MRSA ΔTarO mutant strain is more sensitive to the immune response and more susceptible to both imipenem and dicloxacillin treatment compared to MRSA COL in this lung infection model. Recovery of resistant mutants in our lung model was dramatically reduced compared to the in vitro FoR, suggesting that the likelihood of resistance emergence in the clinic would be lower than anticipated based on the in vitro data. Moreover, this innovative in vivo FoR model may be broadly applicable to other antibacterial programs.

Author Disclosure Block:

L. Liang: D. Employee; Self; Merck. **C. Gill:** D. Employee; Self; Merck. **S. Lee:** D. Employee; Self; Merck. **J. Liu:** D. Employee; Self; Merck. **P. Mann:** D. Employee; Self; Merck. **H. Wang:** D. Employee; Self; Merck. **J. Xiao:** D. Employee; Self; Merck. **C. Tan:** D.

Employee; Self; Merck. **T. Roemer:** D. Employee; Self; Merck. **A. Flattery:** D. Employee; Self; Merck.

Poster Board Number:

SATURDAY-414

Publishing Title:

Molecular Analysis of *Streptococcus pyogenes*, Macrolide Resistance, *emm* Types and Virulence Genes of Pediatric Isolates During a 7-Year Period

Author Block:

A. Michos[1], F. Koutouzi[1], A. Tsakris[1], P. Chatzichristou[1], E. Koutouzis[1], G. Daikos[1], A. Stathi[2], V. Syriopoulou[1]; [1]Natl. and Kapodistrian Univ. of Athens, Med. Sch., Athens, Greece, Greece, [2]Aghia Sophia Children's Hosp., Athens, Greece, Greece

Abstract Body:

Background: Increased macrolide resistant of Group *A streptococcus* (GAS) has been reported in many countries posing a significant problem in cases of β- lactam allergy. The aim of the study was the molecular characterization of pediatric GAS isolates regarding macrolide resistance, virulence genes and relevant *emm* types in Greece. **Methods:** Pharyngeal and non-pharyngeal GAS isolates were collected during a 7-year period (2007-2013) and examined for antibiotic susceptibility, macrolide-resistant genes (*mef*(A), *erm*(A), and *erm*(B)), *emm* typing and streptococcal pyrogenic exotoxins genes (*speA, speB, speC* and *ssa*).**Results:** The overall GAS macrolide resistance was 20.4% (270/1324); Macrolide resistance varied during the study period with maximum in 2008 (29.57%) and minimum in 2013 (10.95%) (*P*-value for trend=0.007). At the same period consumption of pediatric macrolide packages was gradually reduced by 55%. Regarding macrolide resistance genotypes, *mef*(A) was detected in 87(32.2%), *erm*(A) in 136 (50.37%), *erm*(B) in 44(16.29%) and both *mef*(A) and *erm*(A) in 3(1.1%) isolates. The most prevalent *emm* types among erythromycin-resistant isolates were *emm*77 (31.48%), *emm*4 (18.15%) and *emm*12 (10.74%). Ten *emm* types accounted for 90.3% of macrolide-resistant isolates. Among 415 randomly selected GAS isolates *spe*A was detected in 61(14.7%), *speB* in 280(67.5%), *speC* in 252(60.7%) and *ssa* in 82(19.7%) isolates. Increased prevalence of *speC* was found among macrolide-resistant isolates (72.6% vs 56.6%, respectively; *P*=0.01) and of *speB* among invasive isolates (79.48% vs 62.41%; *P*=0.0009). Distribution of virulence genes differed among GAS *emm* types with all virulence genes detected only in *emm* types 1, 3, 4, 12.**Conclusions:** GAS macrolide resistance remained significant in our area during the study period with a reduction in the last year of the study, that followed the reduced consumption of macrolides.

Author Disclosure Block:

A. Michos: None. **F. Koutouzi:** None. **A. Tsakris:** None. **P. Chatzichristou:** None. **E. Koutouzis:** None. **G. Daikos:** None. **A. Stathi:** None. **V. Syriopoulou:** None.

Poster Board Number:

SATURDAY-415

Publishing Title:

Combination Antibiotic Exposure Selectively Alters the Development of Vancomycin Intermediate Resistance in *Staphylococcus aureus*

Author Block:

X. Zheng[1], A. Berti[2], W. Rose[2]; [1]China Med. Univ., Shenyang, China, [2]Univ. of Wisconsin-Madison, Madison, WI

Abstract Body:

Background: Heterogeneous vancomycin-intermediate *Staphylococcus aureus* (hVISA) is associated with high rates of vancomycin (VAN) and secondary treatment failure. Combination therapy with VAN is one strategy to prevent resistance, but comprehensive assessments of potential combinations are limited. This study identifies optimal combinations to prevent the emergence of vancomycin-intermediate *Staphylococcus aureus* (VISA). **Methods:** hVISA Mu3 (VAN MIC=1 mg/L) was exposed for 28 d *in vitro* to VAN alone, VAN with beta-lactams (BLs) (cefazolin (CFZ), piperacillin-tazobactam (TZP), meropenem (MEM)) and VAN with other antibiotics (gentamicin (GEN), trimethoprim-sulfamethoxazole (SXT), rifampin (RIF), and fosfomycin (FOS)). VAN was increased from 1-6 mg/L according to growth, while the secondary antibiotic was maintained at the serum free average concentration. VAN MIC was determined every 7 d. Antibiotic susceptibility, cell wall thickness (CWT), pigment, and membrane fluidity were determined prior to and following exposure. **Results:** Exposure to VAN alone or VAN+GEN, SXT, RIF, or FOS increased the Mu3 VAN MIC to 8-16 mg/L at day 28. Combinations with GEN, SXT, RIF or FOS prevented VAN resistance at day 7 but were not effective thereafter, while FOS combination was no different than VAN alone at any time point. VAN plus any BL limited the VAN MIC increase to 2-4 mg/L at day 28, with CFZ and TZP as the most effective agents (VAN MIC=2 mg/L). The combination of VAN and BLs exposure resulted in no significant change to DAP or TLV MIC, while other combinations increased the MIC of DAP and TLV. LZD MIC was unchanged with all treatments. No changes to the MIC of secondary antibiotic were observed in any combination except VAN and RIF which increased the RIF MIC 512-fold. No correlation was observed between pigment or fluidity and VAN MIC. VAN and BLs exposure decreased CWT significantly compared with VAN alone (mean 52.0-58.3 vs 60.1±6.7 nm, $p<0.05$) whereas VAN plus other antibiotics increased the CWT (mean 63.5-68.5 vs 60.1±6.7 nm, $p<0.05$). **Conclusions:** Only the combination exposures of VAN and BLs suppresses the development of VAN resistance and the transition from hVISA to VISA. This combination should be further explored as a long-term treatment of serious MRSA infections due to ability to suppress VAN resistance.

Author Disclosure Block:

X. Zheng: None. **A. Berti:** None. **W. Rose:** C. Consultant; Self; Visante, Inc. E. Grant Investigator; Self; Merck. J. Scientific Advisor (Review Panel or Advisory Committee); Self; Theravance. L. Speaker's Bureau; Self; The Medicines Company, Merck.

Poster Board Number:

SATURDAY-416

Publishing Title:

Molecular and Cellular Changes That Accompany the Development of Low-Level of Vancomycin Resistance in Methicillin-Resistant *Staphylococcus aureus* during *In Vitro* Therapy

Author Block:

C. VIDAILLAC[1], G. SAKOULAS[2], R. TEWHEY[3], T. KAOMA[4], F. BERNARDIN[4], A. MULLER[4], G. KAATZ[5], W. ROSE[6], L. VALLAR[4], M. J. RYBAK[1]; [1]Wayne State Univ., Detroit, MI, [2]Univ. of California, San Diego, CA, [3]Harvard Univ., Cambridge, MD, [4]Luxembourg Inst. of Hlth., Luxembourg, Luxembourg, [5]John D. Dingell VA Med. Ctr., Detroit, MI, [6]Univ. of Wisconsin, Madison, WI

Abstract Body:

Background: Sequential molecular and cellular alterations accompanying the development of low-level VAN resistance (LVR) in MRSA during therapy remain unclear. **Methods:** We used an *in-vitro* pharmacokinetic/pharmacodynamic model with simulated endocardial vegetations (SEV) to expose the VAN susceptible MRSA JH-1 to a standard VAN regimen (1g q12h) over 60 days. SEV samples were collected daily to further characterize the bacterial population and selected LVR mutants. Over the treatment course, 6 isolates (JH3R to JH8R) exhibiting increased VAN MICs (> 2 µg/mL) were recovered and evaluated for phenotypic and genotypic changes, using JH1 and its *in-vivo* derivative JH2 as comparators. Techniques used included imaging, genome sequencing and microarray data analysis. **Results:** Similar to the *in-vivo* derivative JH2, JH3R-8R isolates exhibited increased VAN and daptomycin MIC values (≥3 and 1 µg/mL, respectively). Consistent with VISA characteristics, JH3R-8R demonstrated reduced autolysis and hemolysis phenotypes, progressive increase in cell wall thickness (up to 68%), and reduced cell separation (increased cellular clumping). Altered virulence phenotypes included decreased staphyloxanthin pigment production (up to 89%) and increased biofilm formation. Consistent mutations supporting the cellular changes were identified in *yycF*, *yycG* and *rpoA*. Microarray data analysis revealed a significant downregulation of *pur* genes involved in the purine/pyrimidine biosynthesis in selected LVR mutants exposed to VAN (up to -3.02 $\log_2$ FC with a FDR ≤ 0.001). **Conclusions:** Our results lend additional support towards a significant role of *yycFG* and *rpo* associated genes in the initial steps of the emergence of LVR during VAN therapy, and highlight the complexity of this inducible resistance mechanism, with multiple and significant changes in the expression of genes associated with cell envelop biosynthesis, regulation, virulence and metabolism.

Author Disclosure Block:

C. Vidaillac: None. **G. Sakoulas:** L. Speaker's Bureau; Self; Cubist, Forest, and Pfizer. **R. Tewhey:** None. **T. Kaoma:** None. **F. Bernardin:** None. **A. Muller:** None. **G. Kaatz:** None. **W. Rose:** C. Consultant; Self; The Medicines Company, Cubist, Astellas, and Visante. E. Grant Investigator; Self; The Medicines Company, Cubist, Astellas, and Visante. L. Speaker's Bureau; Self; The Medicines Company, Cubist, Astellas, and Visante. **L. Vallar:** None. **M.J. Rybak:** C. Consultant; Self; Cubist, Forest, Cepheid, Cerexa, Novartis, and Theravance. E. Grant Investigator; Self; Cubist, Forest, Cepheid, Cerexa, Novartis, and Theravance. L. Speaker's Bureau; Self; Cubist, Forest, Cepheid, Cerexa, Novartis, and Theravance.

Poster Board Number:

SATURDAY-417

Publishing Title:

Emergence of a Mrsa Clone Harbouring a New Composite Scc*cad/Ars*-Scc*mec* Cassette in France

Author Block:

O. Barraud[1], P. Martins Simões[2], N. Hidri[1], M. Bes[2], C. Martin[1], M-C. Ploy[1], F. Garnier[1], F. Laurent[2]; [1]INSERM, Université de Limoges, CHU Limoges, Limoges, France, [2]CNR Staphylocoques, CIRI INSERM U1111, Hospices Civils de Lyon, Université de Lyon, Lyon, France

Abstract Body:

Background: MRSA are responsible for a wide range of infections, notably bacteraemia. In France, MRSA have been decreasing for several years with prevalence close to 20% in 2015. At the Limoges University Hospital Centre, we observed that, among the MRSA collected from blood cultures, the proportion of isolates susceptible to kanamycin (Kana-S) increased from 15% to 78% in the last decade (2005-2015). **Methods:** In 2014, a Kana-S MRSA isolate (LIM88), not detected as MRSA by the Xpert MRSA/SA BC kit (Cepheid) was characterized by *spa*-typing and WGS (IonProton™). WGS data allowed i) *in silico* MLST, ii) characterization of SCCmec cassette, iii) design of a multiplex PCR (*mecA*, *ccrA4B4*, *ccrC*) specific to the SCCmec of this isolate. All 2015 Kana-S MRSA isolates from bacteraemia in the Limoges Hospital were tested using the multiplex PCR.**Results:** LIM88 belonged to ST5 and harboured a t777 *spa*-type. WGS analysis revealed the presence of a novel composite 40 kb-long SCC*cad/ars*-SCC*mec* element including i) a SCC*cad/ars* domain harbouring genes related to detoxification of heavy metals, *cadA (cadmium) and arsCBAD (arsenic), associated to a ccr*C1 recombinase, ii) a SCC*mec* domain harbouring a *mec*A classD complex (IS*431-mec*A-delta*mec*R1), a *ccr*A4B4 complex, and a *cop*A gene coding for resistance to copper. Comparison with other SCC elements reveals a mosaic structure composed of i) the SCC*mec* domain related to the SCC*mec* element of the "new" Paediatric MRSA clone (ST5-MRSA-VI-t777), ii) the operon *ars*CBAD similar to the one found in *S. haemolyticus* JCSC1435, iii) *ccrC* domain almost identical to the one of the ZH43 MRSA isolate. Phylogenetic analysis using other published ST5 whole genomes confirmed that LIM88 represents a new ST5 MRSA lineage.Screening with the multiplex PCR revealed that 20% of the Kana-S bloodstream MRSA isolated in 2015 were positive for the 3 targeted genes. Further investigations are underway concerning the other Kana-S MRSA isolates.**Conclusion:** Here, we report the emergence of a new ST5-t777 MRSA clone with a novel, composite SCC*cad/ars-SCCmec* element in Limoges University Hospital Centre. Interestingly, this clone represents in 2015 20% of all bloodstream Kana-S MRSA in this hospital.

Author Disclosure Block:

O. Barraud: None. **P. Martins Simões:** None. **N. Hidri:** None. **M. Bes:** None. **C. Martin:** None. **M. Ploy:** None. **F. Garnier:** None. **F. Laurent:** None.

Poster Board Number:

SATURDAY-418

Publishing Title:

Characterization of Gene Expression Profile Associated with Daptomycin Nonsusceptibility in *Staphylococcus aureus*

Author Block:

G. L. Oliva[1], R. Shah[1], J. Lu[1], S. V. Catlett[1], M. P. Pai[1], W. E. Rose[2], G. Sakoulas[3], M. Malik[1]; [1]Albany Coll. of Pharmacy, Albany, NY, [2]Univ. of Wisconsin-Madison Sch. of Pharmacy, Madison, WI, [3]Univ. of California, San Diego, La Jolla, CA

Abstract Body:

Background: Extensive use of daptomycin for treating complex methicillin-resistant *Staphylococcus aureus* (MRSA) infections has led to the emergence of daptomycin-non-susceptible *Staphylococcus aureus* (DNSA) strains. An understanding of the molecular mechanisms underlying daptomycin non-susceptibility is required for early diagnosis and intervention with alternate combination therapies. While phenotypic changes associated with DNSA strains have been well established, the genotypic changes, especially, alteration in the kinetics of expression of genes responsible for daptomycin nonsusceptibilty are not well understood. In this study, we used a phenotypically well characterized DNSA strain along with its daptomycin-susceptible (DSSA) isogenic pair to study gene expression profile and genetic mutations associated with daptomycin nonsusceptibility.**Methods:** We determined expression profiles of genes involved in cell membrane charge (*mprF, dltABCD*), autolysis (*atl, lytM*), cell wall synthesis (*femA, femB, vraSR, walkR*), and penicillin binding proteins (*pbp2*, *pbp2a*, *pbp4, pbp1*) in D592 (DSSA) and D712 (DNSA) strains.**Results:** A characteristic signature of genes involved in cell surface charge (*mprF, dltABCD*), cell wall synthesis (*vraS, femB*) and penicillin binding protein gene *pbp2a* that were observed to be significantly upregulated in D712 strain. In contrast, the gene profile signature of D592 strain was an enhanced expression of the master controller of peptidoglycan metabolism gene *walkR*, the autolysis genes *atl* and *lyt*M; and *pbp1* and *4*. Together, these alterations in gene expression profiles provide a mechanistic explanation for the resultant phenotypic changes such as low membrane binding affinity for daptomycin and increased cell wall thickness of these strains. The *mprF* gene is the major target for genetic mutations and these mutations most likely contribute to daptomycin non-susceptibility.**Conclusions:** Differences in gene expression profiles in the DNSA strain result in phenotypic changes associated with daptomycin nonsusceptibility and that these expression profiles may serve as excellent surrogate for differentiation of DNSA and DSSA strains.

Author Disclosure Block:

G.L. Oliva: None. **R. Shah:** None. **J. Lu:** None. **S.V. Catlett:** None. **M.P. Pai:** None. **W.E. Rose:** None. **G. Sakoulas:** None. **M. Malik:** None.

Poster Board Number:

SATURDAY-419

Publishing Title:

Exploring the Role of Two Abc Transporters Essential for Acquired Nisin Resistance in *Staphylococcus aureu*s

Author Block:

C. P. Randall, B. Utley, A. J. O'Neill; Univ. of Leeds, Leeds, United Kingdom

Abstract Body:

Background: Nisin (NIS) is a lantibiotic routinely used as a food preservative, and may have a role in the chemotherapy of bacterial infection. We demonstrated recently that spontaneous resistance to NIS can arise in *Staphylococcus aureus* through mutation in the sensor kinase NsaS, leading to constitutive phosphorylation of the response regulator NsaR and subsequent upregulation of BraDE and VraDE, two putative ABC transporters. However, the role of these transporters in NIS resistance is unclear. Here we report on studies to examine this issue. **Methods:** Markerless deletions of *braDE* and *vraDE* were created in NIS-resistant S. aureus (SH1000 [$NsaS_{A208E}$]) using plasmid pIMAY. Complementation analysis was achieved using the regulable expression vector pRMC2, and the effect of deletions/complementation on NIS susceptibility was assessed by MIC determination according to CLSI methods. The impact of *braDE* deletion on expression of *vraDE* was evaluated by qRT-PCR, whilst interaction between BraDE and NsaRS/VraDE was assessed using the BACTH two hybrid (2H) system.**Results:** Deletion of *braDE* or *vraDE* in SH1000 ($NsaS_{A208E}$)(NIS MIC 64 mg/L) resulted in strains displaying NIS susceptibility equivalent to that of NIS-susceptible, wild-type SH1000 (NIS MIC 4 mg/L), and complementation of the deletion *in trans* restored NIS resistance. Overexpression in SH1000 of *vraDE* alone, but not *braDE*, resulted in an increase in NIS resistance to a level comparable to that seen for SH1000 ($NsaS_{A208E}$). Whilst expression of *vraDE* in SH1000 ($NsaS_{A208E}$) was ~65-fold greater than that observed in SH1000, deletion of *braDE* in SH1000 ($NsaS_{A208E}$) returned expression of *vraDE* to wild-type levels. 2H analysis revealed that BraDE interacts directly with NsaRS *in vivo*, but not VraDE.**Conclusions:** The ability of VraDE alone, but not BraDE alone, to confer high-level NIS resistance when overexpressed *in trans* in SH1000 indicates that VraDE is solely responsible for NIS detoxification in acquired NIS resistance. The loss of VraDE expression in NIS-resistant strains deleted for *braDE*, and the direct interaction observed between BraDE and NsaRS *in vivo* implies that BraDE participates in transduction of the signal through NsaS that leads to VraDE overexpression.

Author Disclosure Block:

C.P. Randall: None. **B. Utley:** None. **A.J. O'Neill:** None.

Poster Board Number:

SATURDAY-420

Publishing Title:

Iron/Manganese Homeostasis Contribute to Mutagenesis during Acquisition of Hetero/Homo β-Lactam Resistance in Mrsa

Author Block:

M. Fatouuraei, L. Paz, R. R. Rosato, **A. E. ROSATO**; Houston Methodist Res. Inst., houston, TX

Abstract Body:

Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) remains a major public health problem and a therapeutic challenge. The majority of MRSA strains display a Hetero-resistant (HeR) phenotype (mix of cells expressing low (HeR) or high (HoR) levels of resistance). We previously demonstrated that exposure of HeR-MRSA to β-lactams leads to important perturbations in cell wall/division machinery that require of metabolic adaptations that, as a by-product, stimulate intracellular production of reactive oxygen species (ROS). Furthermore, we found that oxidative damage and iron/manganese homeostasis are associated with ROS production which constitute major drivers of mutagenesis that, together with metabolic signal pathways and cell wall adaptations, favor the survival of HeR-MRSA in presence of β-lactams.**Methods:** The mechanistic role of Fe(II) and Mn(II) during oxidative stress induced by exposure to β-lactams were analyzed in cells grown in Fe-repleted conditions ± OXA. In the case of Mn, cells were supplemented with Mn(II). The role of Fur regulator in sensing iron during β-lactam-mediated HeR/HoR selection was determined using a *fur* mutant. Gene expression analysis was performed by RNA-Seq; ROS production was measured by fluorescence (dihydrorhodamine 123, DHR123). Mutation frequencies for resistance to rifampicin and β-lactams were determined by E-test. ROS-induced DNA damage was investigated by Comet assay. **Results:** Blockade of ROS with iron chelators determined important effects during acquisition of β-lactam-mediated HoR phenotype. Growth and phenotypic analysis of MRSA-*Δfur::tet* strain ± OXA (0.5 μg/ml) showed a significant delay in growth, persistent levels of ROS production and increased mutation rate due to continuous intake of Fe and reduced expression of detoxifying KatA, SodA and SodM enzymes, while GraSR, a two component regulator of CAMP in *S. aureus*, showed a marked upregulation in cells repleted with Mn +OXA, increased expression of enzymes and reduced formation of ROS. **Conclusions:** Oxidative damage regulated by GraSR and iron/manganese homeostasis associated with ROS production are major drivers of mutagenesis that, together with both metabolic signal pathways and cell wall adaptations, favor the survival of MRSA.

Author Disclosure Block:

M. Fatouuraei: None. **L. Paz:** None. **R.R. Rosato:** None. **A.E. Rosato:** None.

Poster Board Number:

SATURDAY-421

Publishing Title:

Beta-Lactams (Bl) Plus Vancomycin (Van) Prevent Emergence of Van Resistance in *Staphylococcus aureus* (*S. aureus*) *In Vitro*

Author Block:

N. B. Singh[1], J. Yim[1], G. Sakoulas[2], M. J. Rybak[1]; [1]Wayne State Univ., Detroit, MI, [2]Univ. of California San Diego Sch. of Med., San Diego, CA

Abstract Body:

Background: Vancomycin (VAN) is a widely used antibiotic for treatment of Methicillin-resistant *S. aureus* (MRSA) infections and Methicillin-susceptible *S. aureus* (MSSA) infections in patients allergic to penicillin. Development of heterogeneous VAN-intermediate *S. aureus* (*h*VISA) and VAN-intermediate *S. aureus* (VISA) during VAN therapy has been reported, especially at subtherapeutic(STh) VAN concentrations. Although synergy between β-lactams (BL) and lipopeptides /glycopeptides has been demonstrated, there is little to no data on whether BL, combinations with VAN could prevent the emergence of VISA. Our objective is to determine if BL+VAN could prevent the emergence of VAN resistance in *S. aureus* under STh VAN exposure. **Methods:** RN 9120, a MSSA isolate with dysfunctional accessory gene regulator group II (*agr- II)* that is susceptible to VAN (MIC = 1 mg/L), was evaluated in vitro. The organism was exposed to STh VAN concentrations in a 1-compartment pharmacokinetic/pharmacodynamic (PK/PD) model, simulating human PK of VAN 200 mg q 12h over 72 h to induce resistance. At 72h, organisms recovered from the model were re-exposed to the same VAN regimen for an additional 72-144 h exposure to generate VISA. The same experiment was repeated with continuous infusion of cefazolin (CFZ) at 0.5x MIC (MIC= 0.25 mg/L). Changes in MIC were evaluated at the end of each 72 h exposure. A population analysis profile (PAP) was performed to evaluate for shifts in population susceptibility.**Results:** VAN MIC of RN 9120 increased to 4 mg/L as soon as 144h under STh VAN exposure. When CFZ was concomitantly infused at 0.5x MIC, VAN MIC increased to 2 mg/L at 72h. However, no further increase in MIC was noted up to 216h of STh VAN administration. PAP revealed a shift in the overall population towards non-susceptibility.**Conclusions:** The addition of low concentration of CFZ appears to prevent emergence of VISA under STh exposure to VAN. Further investigations with MRSA isolates are warranted using various BL combinations.

Author Disclosure Block:

N.B. Singh: None. **J. Yim:** None. **G. Sakoulas:** C. Consultant; Self; The Medicines Company. E. Grant Investigator; Self; Allergan Pharmaceuticals. L. Speaker's Bureau; Self; Merck, Allergan, Sunovion, and The Medicines Company. **M.J. Rybak:** C. Consultant; Self; Allergan, Bayer, Cempra, Merck, Sunovian, The Medicine Company , Theravance. E. Grant Investigator;

Self; Allergan, Bayer, Cempra, Merck, Sunovian, The Medicine Company , Theravance, NIAID R01AI12400-01 and R21 AI109266-01. L. Speaker's Bureau; Self; Allergan, Bayer, Cempra, Merck, Sunovian, The Medicine Company , Theravance.

Poster Board Number:

SATURDAY-422

Publishing Title:

A Novel *erm*(44) Gene Variant From a Human *Staphylococcus saprophyticus* Isolate Confers Resistance to Macrolides, Lincosamides but not Streptogramins

Author Block:

V. Perreten, C. Strauss; Univ. of Berne, Berne, Switzerland

Abstract Body:

Staphylococcus saprophyticus, widespread in the environment, in animals and in humans, is a major cause of urinary tract infection and cystitis. Although macrolides and lincosamides are not used for the treatment of urinary tract infections, their general use may contribute to the selection of resistant bacteria of the normal human flora, including staphylococci from the skin. Genes conferring resistance to macrolide, lincosamide and streptogramin B (MLS_B) antibiotics in staphylococci have been associated with erythromycin ribosome methylase (*erm*) genes.One *S. saprophyticus* isolated from the skin of a healthy person was found to exhibit resistance to erythromycin (MIC 64µg/ml) and clindamycin (MIC >256µg/ml). Whole genome sequence (Ion Torrent technology) analysis of the isolate revealed the presence of a 23S rRNA methylase gene which belonged to the Erm(44) determinant based on the nomenclature of the MLS_B resistance genes. It showed 81% amino acid identity to Erm(44) of *S. xylosus* from bovine milk and 77% amino acid identity with Erm(44) of *S. saprophyticus* from environmental origin. The novel *erm*(44) variant differed for the two other *erm*(44) genes by the absence of a streptogramin B phenotype as demonstrated by cloning in vector pTSSCm-Pcap and expression in *S. aureus* RN4220 and after measurement of the MIC of pristinamycin Ia and virginiamycin S1. The *erm* gene was located on a putative 19’400-bp genetic island, which is absent in the MLS_B-susceptible strain *S. saprophyticus* KACC16562. It was flanked by two unrelated integrases and integrated at a specific 19-bp integration site (*attC*).This study revealed the presence of a novel *erm*(44) variant which does not confer resistance to streptogramin B antibiotics thus differing from all the other *erm* determinants described to date. The discovery of this novel *erm* gene emphasizes once again the role of staphylococci as a large reservoir of MLS_B resistance genes and their particular ability to acquire new genes through multifaceted mechanisms.

Author Disclosure Block:

V. Perreten: None. **C. Strauss:** None.

Poster Board Number:

SATURDAY-423

Publishing Title:

Detection and Characterization of a Novel Integron Gene Cassette in the Human Oral Metagenomic DNA That Confers Resistance to D-Cycloserine

Author Block:

M. Rahman, F. Kaiser, P. Mullany, A. P. Roberts; Univ. Coll. London, London, United Kingdom

Abstract Body:

Background: A PCR-based metagenomic approach was used to investigate the presence of integrons carrying antibiotic resistance genes (ARGs) in the oral cavity of healthy human individuals from the UK and Bangladesh. Integrons are gene capture and expression system which are known for their role in dissemination of ARGs in clinically important pathogens. Although the oral cavity is a reservoir of ARGs, no study has been carried out using metagenomic DNA to detect if these genes are part of integrons.**Methods:** Saliva samples were collected from healthy volunteers and metagenomic DNA was prepared. PCR primers were used to target the integrons and associated gene cassettes and a library of amplicons was constructed. These libraries were analysed using bioinformatic tools to detect the presence of putative antibiotic resistance genes. The genes of interest were sub-cloned into pHCMC05 (*Bacillus subtilis- E coli* shuttle vector) and pET28a (expression vector) to determine the minimum inhibitory concentration (MIC) of antibiotics in *B. subtilis* and to express and purify proteins for *in vitro* analysis of biological activities, respectively. MICs of antibiotics were determined using agar dilution method.**Results:** We identified two novel variants of an integron located D-alanine-D-alanine ligase gene (*ddl*) in the oral metagenomic library among other putative ARGs such as virginiamycin acetyl transferase (*vat*) and bleomycin binding protein. We observed that both constitutive and induced expression of the *ddl* variants confer high level resistance to D-cycloserine (DCS; MIC=256 µg/mL) in *Escherichia coli* and *Bacillus subtilis*. Over-expression of these genes significantly increases the cell-wall thickness of *B. subtilis* which confirms their role in cell-wall synthesis. Using paper chromatography and an inorganic phosphate detection assay we further confirmed the formation of D-ala-D-ala dipeptide in the reactions catalyzed by purified 6xHis-tagged Ddl and showed this was D-alanine specific. The reactions catalyzed by Ddl were also found to be inhibited by DCS in a dose-dependent manner.**Conclusions:** These results represent the first time that *ddl* has been found on an integron. Enzymatic characterization of Ddl showed that it is biologically active, inhibited by DCS and plays an important role in cell-wall synthesis.

Author Disclosure Block:

M. Rahman: None. **F. Kaiser:** None. **P. Mullany:** None. **A.P. Roberts:** None.

Poster Board Number:

SATURDAY-424

Publishing Title:

Strain Dependent Genetic Networks for Antibiotic-Sensitivity in a Bacterial Pathogen with a Pan-Genome

Author Block:

T. van Opijnen, S. Dedrick; Boston Coll., Chestnut Hill, MA

Abstract Body:

The interaction between an antibiotic and bacterium is not merely restricted to the drug and its direct-target, rather antibiotic induced stress resonates through the bacterium, creating selective pressures that drive the emergence of adaptive mutations not only in the direct-target, but in genes involved in many different (fundamental) processes as well. Surprisingly, it has been shown that adaptive mutations do not necessarily have the same effect in all species, indicating that the genetic background influences how phenotypes are manifested. However, to what extent the genetic background affects the manner in which a bacterium experiences antibiotic stress, and how this stress is processed is unclear. Here we employ the genome-wide tool Tn-seq to construct antibiotic-sensitivity profiles for different strains of the bacterial pathogen *Streptococcus pneumoniae*. Remarkably, over half of the genes that are important for dealing with antibiotic-induced stress in one strain are dispensable in another. By confirming hundreds of genotype-phenotype relationships, probing potassium-loss, employing genetic interaction mapping as well as temporal gene-expression experiments we reveal genome-wide conditionally important/essential genes (antibiotic-induced weakest-links), we discover roles for genes with unknown function, and uncover antibiotic mode-of-action. Moreover, by mapping the underlying genomic network we encounter little conservation in network connectivity between strains as well as profound differences in regulatory relationships. Our approach uniquely enables genome-wide fitness comparisons across strains, enabling the discovery that antibiotic responses are complex events that can vary widely between strains, which suggests that the emergence of resistance may be strain specific and at least for species with a pan-genome less predictable.

Author Disclosure Block:

T. van Opijnen: None. **S. Dedrick:** None.

Poster Board Number:

SATURDAY-425

Publishing Title:

Vancomycin Intermediate *Staphylococcus aureus*- Visa: It's Everywhere You Don't Want to Be

Author Block:

A. R. McMullen, M. Wallace, A. Shupe, C-A. Burnham; Washinton Univ. Sch. of Med., St Louis, MO

Abstract Body:

Background: At Barnes-Jewish Hospital, approximately 5% of the *Staphylococcus aureus* strains isolated from clinical specimens have a vancomycin-intermediate phenotype (VISA). Few studies that have examined the antimicrobial susceptibility profile of a large collection of VISA isolates.**Methods:** We evaluated the minimum inhibitory concentration (MIC) of a panel of antibiotics against 100 VISA and 50 vancomycin susceptible *S. aureus* (VSSA) isolates using the TREK microbroth dilution panel GPALL3F and gradient diffusion (Etest) assays. Isolates were characterized using a multiplex PCR to detect and differentiate SCC*mec* types I-IV. Additionally, PCR assays to detect high level mupirocin resistance (*mupA*) and chlorhexidine tolerance (*qacA/B*) were performed. Chart reviews were performed for patient demographics and source of each isolate.**Results:** The demographics of the patients and specimen source from which the VSSA and VISA isolates were not significantly different. Antimicrobial susceptibility profiling of the isolates is shown in Table 1. For vancomycin (VAN), VISA isolates had a mean MIC of 2.35 µg/ml with a range of 1-4 µg/ml. The VAN MIC50 and MIC90 for VISAs was also elevated at 2 µg/ml and 4 µg/ml, respectively. VISA isolates demonstrated increased MICs to daptomycin (DAP) but similar MICs against other drugs including ceftaroline (CPT) and linezolid (LZD). About 60% of the VSSA and VISA isolates were susceptible to methicillin (MSSA). Few isolates were *mupA* positive, but nearly 1/3 carried *qacA/B*, the gene associated with chlorhexidine tolerance.**Conclusions:** Using *SCCmec* typing, we are able to demonstrate that the isolates in our study are not clonal. There were relatively few differences in the patient demographics, specimen source and antimicrobial profile between VSSA and VISA isolates with the exception that VISA isolates had elevated MICs to cell-wall active agents including VAN and DAP.

	VSSA (n=50)	**VISA (n=100)**
VAN Mean/MIC$_{50}$/ MIC$_{90}$ (Range) (µg/ml)	1.0/1.0/1.0 (0.5-1.0)	2.3/2.0/4.0 (1.0-4.0)
DAP Mean/MIC$_{50}$/ MIC$_{90}$ (Range) (µg/ml)	<0.5/<0.5/<0.5 (<0.5)	0.8/<0.5/1.0 (<0.5-4.0)

LZD Mean/MIC$_{50}$/ MIC$_{90}$ (Range) (µg/ml)	2.7/4.0/4.0 (2.0-4.0)	3.1/4.0/4.0 (<1.0-4.0)
CPT Mean/MIC$_{50}$/ MIC$_{90}$ (Range) (µg/ml)	0.5/0.25/0.5 (<0.12-4.0)	0.6/0.5/1.0 (<0.12-2.0)
% *SCCmec* II/ IV	9.6/35.5	40.0/27.0
% *mupA* positive	2	9
% *qacA/B* positive	34	35

Author Disclosure Block:

A.R. McMullen: None. **M. Wallace:** None. **A. Shupe:** None. **C. Burnham:** None.

Poster Board Number:

SATURDAY-426

Publishing Title:

Antibiotic Resistance Profiling and Panton-Valentine Leukocidin Gene Detection among *Staphylococcus aureus* from Nasal Passage of Hospital Staff in Haripur and Abbottabad Cities of Pakistan

Author Block:

M. A. Syed, Sr.[1], M. Rukan[1], H. Jamil[1], S. Ali[1], M. Gul[1], A. Khan[2], H. A. Bokhari[2]; [1]Univ. of Haripur, Haripur, Pakistan, [2]COMSATS Inst. of Information Technology, Islamabad, Pakistan

Abstract Body:

Background: *Staphylococcus aureus* is one of the most common causes of nosocomial infections. Health care professionals may also be a source of infection for those receiving treatment in hospitals or health care settings. It was a cross-sectional study carried out on health care staff of different hospitals of Hazara division during the period January 2015 to June 2015 to determine the nasal carriage of Methicillin resistant *Staphylococcus aureus* by Operation Theater and ward staff of different hospitals in Haripur and Abbottabad cities of Pakistan. Panton Valentine Leukocidin gene was also detection in the isolated strains using PCR.**Methods:** The samples were collected from the participants who were working in hospital for one or more than one year of time duration and cultured in microbiology lab at University of Haripur. Microbiological and biochemical tests were performed for *Staphylococcus aureus* confirmation. In order to determine antibiotic resistance of *S. aureus* by disc diffusion test, eight antibiotics were used including methicillin. Furthermore, multiplex PCR was performed to detect *mecA* and *pvl* genes.**Results:** A total of 206 samples were collected, out of that 167 (81%) samples were found positive for *S. aureus.* Majority of the strains (54%) were methicillin resistant, whereas large number of isolates showed resistance against all tested antibiotics (Ofloxacin= 34% (n= 57), Erythromycin=77% (n= 128), Doxycycline=21% (n=36), Lincomycin=70% (n= 117), Augmentin = 59% (n= 99), Ciprofloxacin= 37% (n= 62), Ciprofloxacin = 53% (n=88)). *Pvl* gene was present in 29% of the strains.**Conclusions:**The results of this study show high level of resistance against antibiotics. This study concludes that due to exposure of different antibiotics the resistant strains are emerging. It might be possible that the staff members are transferring MRSA to the patients and causing nosocomial infections.

Author Disclosure Block:

M.A. Syed: None. **M. Rukan:** None. **H. Jamil:** None. **S. Ali:** None. **M. Gul:** None. **A. Khan:** None. **H.A. Bokhari:** None.

Poster Board Number:

SATURDAY-427

Publishing Title:

Design And Evaluation Of SER-262: A Fermentation-Derived Microbiome Therapeutic For The Prevention Of Relapse In Patients With Primary C*lostridium difficile* Infection

Author Block:

J. Lachey, J. R. Wortman, M. J. Lombardo, K. D. Litcofsky, J. E. Button, C. L. Morse, M. Vulic, S. Sykes, K. Halley, C. McChalicher, J. Winkler, J. G. Aunins, M. R. Henn, D. N. Cook, J. M. Otero; Seres Therapeutics, Cambridge, MA

Abstract Body:

Background: Approximately 25% of patients relapse after a primary episode of *Clostridium difficile* infection (CDI) in part because antibiotic treatments do not repair the underlying dysbiosis of the gut microbiome. SER-109, an ecology of spore-forming bacteria from healthy screened stool donors, was effective in preventing CDI recurrence in a Phase 1b/2 study of subjects with a history of multiply recurrent CDI. Here, we present development of a second-generation microbiome therapeutic, SER-262, produced by *in vitro* fermentation of bacterial strains for the prevention of CDI recurrence in subjects with a primary episode. **Methods:** Microbes were selected based on relatedness to clades identified in SER-109, prevalence in healthy humans, spore-forming potential, and safety. Compositions of up to 15 strains were formulated to mimic the phylogenetic and functional diversity of SER-109. Compositions were evaluated in the CDI mouse model for efficacy in preventing body weight loss, clinical symptoms and death. **Results:** The 100+ compositions tested exhibited variable efficacy; however, the composition selected for clinical development as SER-262 exhibited robust and reproducible protection from CDI across experiments.

Table 1. Select data demonstrating SER-262 efficacy in the mouse CDI model

Treatment	**Endpoint**		
	Mortality (%)	**Body weight loss (mean ± SEM)**	**Maximum Clinical Score (Range 0-4; mean ± SEM)**
PBS control	40%	18.7% ± 2.6	2.8 ± 0.3
SER-262	0%***	1.3% ± 0.4**	1.0 ± 0.0**
Naïve (no CDI)	0%***	0.6% ± 0.2**	0.0 ± 0.0**

Study details: C57Bl/6 mice, n=10/group.
Body weight loss is calculated as the percent change relative to Day -1.
Maximum clinical score is a composite score of lethargy, posture, grooming, wet tail/abdomen

(indicative of diarrhea) and hypothermia, with a higher score denoting worsening clinical symptoms.
***$p \leq 0.001$ using a Fisher's exact test.
**$p \leq 0.001$ compared to PBS group using 1-way ANOVA with Fisher's LSD.

Conclusions: SER-262 is a novel, defined composition of spores designed as an oral Ecobiotic™ drug to prevent recurrence in patients with primary CDI. The composition is fermentation-based making it more amenable to very large-scale manufacturing. SER-262 was highly efficacious in the mouse CDI model and has been extensively characterized and the preclinical data support continued development into a Phase 1b clinical study.

Author Disclosure Block:

J. Lachey: D. Employee; Self; Seres Therapeutics. **J.R. Wortman:** D. Employee; Self; Seres Therapeutics. **M.J. Lombardo:** D. Employee; Self; Seres Therapeutics. **K.D. Litcofsky:** D. Employee; Self; Seres Therapeutics. **J.E. Button:** D. Employee; Self; Seres Therapeutics. **C.L. Morse:** D. Employee; Self; Seres Therapeutics. **M. Vulic:** D. Employee; Self; Seres Therapeutics. **S. Sykes:** D. Employee; Self; Seres Therapeutics. **K. Halley:** D. Employee; Self; Seres Therapeutics. **C. McChalicher:** D. Employee; Self; Seres Therapeutics. **J. Winkler:** D. Employee; Self; Seres Therapeutics. **J.G. Aunins:** D. Employee; Self; Seres Therapeutics. **M.R. Henn:** D. Employee; Self; Seres Therapeutics. **D.N. Cook:** D. Employee; Self; Seres Therapeutics. **J.M. Otero:** D. Employee; Self; Seres Therapeutics.

Poster Board Number:

SATURDAY-428

Publishing Title:

Characterization Of Aurone X As A Potential Drug Candidate Against *cryptococcus Neoformans*

Author Block:

Y. Mohammed, D. Araujo, E. McClelland; Middle Tennessee State Univ., Murfreesboro, TN

Abstract Body:

Background: *Cryptococcus neoformans* is an opportunistic, fungal pathogen that can spread from the lungs to the central nervous system and cause life-threatening meningitis, most commonly in immune depressed individuals. Amphotericin B and fluconazole are the standard treatment; however, this has resulted in renal toxicity and resistant strains. The lack of safe, effective medication for treating cryptococcal meningitis is the motivation for identifying if Aurone X is a potential drug candidate by characterizing the compound's inhibition.**Methods:** A previous screen of an aurone library using the A27-M2 CLSI standard micro-dilution method identified aurone X, which showed >90% inhibition to *C. neoformans*. Toxicity assays conducted on rat L6 fibroblasts and human THP1 macrophages showed minimal toxicity. Additional tests were performed to determine aurone x's potential as a drug candidate. We characterized the minimum inhibitory concentrations (MIC) of aurone X in different medias, at different cell concentrations, with different serotypes and strains, and tested for synergy with Fluconazole, Flucytosine, and Amphotericin B.**Results:** Thirty-six extracts showed >90% inhibition of *C. neoformans* at 100 µM. Aurone X was selected for further characterization based on its low MIC and its low toxicity to THP1 macrophages and fibroblasts (>100 µM). Aurone X was found to inhibit *C. neoformans* in RPMI+MOPS, asparagine media, and YPD at 25,100, and >200 µg/ml respectively. The MIC's at cell concentrations of 10^3, 10^4, and 10^5 cells/ml were 25, 50, and 100 µg/ml, respectively. Clinical serotype A strains B18 and B45 had an MIC of 25 µg/ml each, and B58 had an MIC of 50 µg/ml. Serotype D strains 24067 and JEC21 both had an MIC of 25 µg/ml, and B3501 had an MIC of 50 µg/ml. Both strains of *Cryptococcus gattii*, R265 and R272, had MIC's of 50 µg/ml. Preliminary results suggest that aurone X is synergistic with all three drugs tested.**Conclusions:** Preliminary data on the inhibition of *C. neoformans* by aurone X suggests that it may be a potential drug candidate. This cannot be confirmed unless further tests are done including more experiments on synergy and a mouse challenge study. Based on aurone x's low toxicity and potential effectiveness at low dosages, aurone X could be a possible drug candidate against *C. neoformans* infections.

Author Disclosure Block:

Y. Mohammed: None. **D. Araujo:** None. **E. McClelland:** None.

Poster Board Number:

SATURDAY-429

Publishing Title:

Novel Broad-Spectrum Antibacterial Agents with Activity Against Mdr Gram-Negative Pathogens

Author Block:

P. Picconi[1], C. Hind[2], M. E. Wand[2], M. J. Sutton[2], K. M. Rahman[1]; [1]King's Coll. London, Univ. of London, London, United Kingdom, [2]Publ. Hlth. England, Salisbury, United Kingdom

Abstract Body:

Background: Bacterial resistance to antibiotics is becoming a major global issue and the development of novel antimicrobial agents, especially active against Gram-negative pathogens, is an urgent priority. We developed a new class of DNA minor groove binder with good efficacy against multi-drug resistant (MDR) Gram-negative pathogens through modification of an existing scaffold that showed excellent activity against Gram-positive pathogens.**Methods:** The newly designed chemical entities were synthesized, using solution phase chemistry through a consolidated synthetic route. The activity of various compounds was assessed against several Gram-negative pathogens to determine the MIC (minimum inhibitory concentration). Lead compounds were further evaluated against several MDR isolates to determine their bactericidal/bacteriostatic mode of action through time-kill assays and the effect of efflux on activity was analysed using efflux pump inhibitors in an adapted MIC method. Compounds were also examined for their potential synergy with existing antibiotics using standard chequerboard assays.**Results:** To date, 30 compounds presenting three distinct structural modifications have been synthesized, showing good efficacy against several MDR Gram-negative pathogens with MICs in the range of 0.25-4 µg/ml. Tested strains included isolates of MDR *Klebsiella pneumoniae*, *Acinetobacter baumannii* and strains from the *Burkholderia cepecia* complex. Existing drug-resistance mechanisms appeared to have no influence over the compounds efficacy with MDR strains showing similar MIC levels to more antibiotic susceptible isolates. The MIC of some of the compound was decreased by the addition of efflux pump inhibitor PaβN and by the addition of the membrane permeabilizing agent PMBN. The compounds did not show significant toxicity against eukaryotic cell lines at the highest concentration tested.**Conclusions:** A new class of broad spectrum antibacterial agents belonging to the class of DNA minor groove binder has been developed and presents a good activity against a range of MDR Gram-negative pathogens. The microbiological data set provides the basis for further med-chem modification and lead optimisation to develop a broad spectrum antibiotic as a pre-clinical candidate.

Author Disclosure Block:

P. Picconi: None. **C. Hind:** None. **M.E. Wand:** None. **M.J. Sutton:** None. **K.M. Rahman:** None.

Poster Board Number:

SATURDAY-430

Publishing Title:

Newly Synthesized Quinolinium Derivatives and Their Antimicrobial Effect

Author Block:

E. Kim, S-H. Lee, O-P. O-Pil Kwon, H. Yoon; Ajou Univ., Suwon, Korea, Republic of

Abstract Body:

Antibiotic resistant pathogens become a serious and urgent concern in public health due to an increase incidence of failure with existing antibiotics and the abuse of antibiotics facilitates the occurrence of a variety of Multi Drug Resistant (MDR) pathogens. Quaternary Ammonium Compounds (QACs) have been widely used as antimicrobial agents against a broad range of microorganisms. In this study, we tested the antibacterial activities of rationally designed quinolinium derivatives to compete against resistance toward current QACs. Minimum Inhibitory Concentration (MIC) of quinolinium derivatives was determined on several species of Gram-positive and Gram-negative bacteria using Alamar Blue redox indicator that exhibits a colorimetric change in response to cellular metabolic activity. Among fifteen quinoliniums synthesized, six compounds had antibacterial effects against Gram-positive bacteria showing MICs of 4.7 ~ 37.5 μg/ml and two of these six showed inhibitory activities to Gram-negative bacteria as well. Bacterial susceptibility to the quinolinium compounds was further verified with Kirby-Bauer disc diffusion method. The synergistic effect was tested and analyzed using a Fractional Inhibitory Concentration Index (FICI) with ampicillin. Among four tested quinoliniums, one compound showed an additive effect against Gram-positive bacteria. For the case of Gram-negative bacteria, three compounds showed an additive effect against *Salmonella-enterica* subsp. and one compound against Enterohaemorrhagic *E. coli*. These results would provide a possible aspect for designing future quinolone derivatives having antimicrobial effect against pathogenic bacteria.

Author Disclosure Block:

E. Kim: None. **S. Lee:** None. **O. O-Pil Kwon:** None. **H. Yoon:** None.

Poster Board Number:

SATURDAY-431

Publishing Title:

Discovery of Three New Compounds Series Targeting Bacteria Peptidoglycan Glycosyltransferases (Pgt)

Author Block:

P. Warn[1], D. Kahne[2], S. East[3], A. Parkes[3], D. Corbett[1], M. Whittaker[3], F. Krieger[4], S. Walker[5]; [1]Evotec (UK) Ltd, Manchester, United Kingdom, [2]Harvard Univ., Cambridge, MA, [3]Evotec (UK) Ltd, Abingdon, United Kingdom, [4]Evotec Ltd, Hamburg, Germany, [5]Harvard Univ., Boston, MA

Abstract Body:

Background: There is an urgent need for new antimicrobials to treat MDR infections. Multiple antimicrobials are in the pipeline but most have targets that have been extensively exploited. Moenomycin (Moe) is a potent inhibitor of Gram positive (Gm pos) and negative (Gm neg) bacterial PGTs. We used a Moe-based probe to identify inhibitors of PGTs, apply hit expansion, structure-based and biology techniques to progress novel inhibitors**Methods:** A Moe-based probe was used in a displacement fluorescence polarization (FP) HTS of Evotec and Harvard chemical libraries in 2 campaigns to identify inhibitors that bind to the invariant amino acid residues in the PGT active site. Following hit confirmation, secondary screens were performed incl. nephelometry, secondary FP and SPR binding assays. Hit series were pursued using medicinal chemistry and structural biology approaches. Optimisation was driven by toxicity, solubility and *in vitro* potency. MICs were performed against Gm pos and Gm neg isolates (incl. *E. coli* BW25113, JW5503-1 (ΔtolC::kan) and JW0452-3 (ΔacrA::kan) to determine the contribution of the major multi-drug efflux pumps and *E. coli* MC4100 and MC4100*imp4213* to determine the contribution of outer membrane permeability).**Results:** HTS screens of >460K compounds resulted in ~700 hits. Following profiling and additional selection 61 were followed up, resulting in 3 series progressing to medicinal chemistry expansion. Series 1 compounds have potent activity v Gm pos bacteria (*S. aureus* MICs <0.5µg/mL) and active highly active against MC4100*imp421.* Series 2 compounds have potent activity v Gm pos bacteria (*S. aureus* MICs <0.25µg/mL) and are highly active against the efflux pump mutants. Series 3 compounds have potent activity v Gm pos bacteria (*S. aureus* MICs <0.125µg/mL) and are active against wild-type Enterobacteriaceae (MICs <0.25µg/mL) and highly active against the efflux pump and outer membrane mutants**Conclusions:** We identified 3 novel series of inhibitors of bacterial PGTs that have potent activity against Gm pos bacteria, one series has promising activity against Gm negs. These studies support continued development of PGT for the treatment of MDR infections

Author Disclosure Block:

P. Warn: D. Employee; Self; Evotec. **D. Kahne:** None. **S. East:** D. Employee; Self; Evotec. **A. Parkes:** D. Employee; Self; Evotec. **D. Corbett:** D. Employee; Self; Evotec. **M. Whittaker:** D. Employee; Self; Evotec. **F. Krieger:** D. Employee; Self; Evotec. **S. Walker:** None.

Poster Board Number:

SATURDAY-432

Publishing Title:

Mouse Tolerability, Pharmacokinetics (Pk) and *In Vivo* Efficacy of Novel Compounds Targeting Bacteria Peptidoglycan Glycosyltransferases (Pgt)

Author Block:

P. Warn[1], D. Kahne[2], A. Parkes[3], M. Whittaker[3], F. Nutter Howard[1], J. Smith[1], S. Walker[4]; [1]Evotec (UK) Ltd, Manchester, United Kingdom, [2]Harvard Univ., Cambridge, MA, [3]Evotec (UK) Ltd, Abingdon, United Kingdom, [4]Harvard Univ., Boston, MA

Abstract Body:

Background: Peptidoglycan (PG) is an essential structural macromolecule in the bacterial cell wall, assembled by membrane-bound glycosyltransferases and transpeptidases. Inhibition of synthesis of PG leads to bacterial lysis and death making it an attractive antimicrobial target. We have developed 3 series of PGT inhibitors with potent *in vitro* activity against Gm posbacteria (one series effective against Gm neg). Examples from all series were assessed in murine models for tolerability and PK; an example of one series was assessed in a mouse model of *S. aureus* sepsis**Methods:** Naïve male ICR mice were used. Compounds were formulated in 5% DMSO/10% cremophor/85% saline and dosed at 5-20mg/kg IV, SC or IP at 10mL/kg. For tolerability studies mice were observed for 24h compared to vehicle controls. For PK studies mice were treated IV and blood sampled by tail vein stab (~40µL/sample) at 10, 30, 60 and 240 min post dose. Bioanalysis was by LSMS-MS & data analysed in a non-compartmental model. For the efficacy study, mice were infected IP using *S. aureus* EMRSA 16. Mice were treated 1 & 5h post infection (PI) with 7.5mg/kg IP and the burden determined in IP wash & blood 9h PI, vancomycin (VAN) 10mg/kg IP was administered at the same times as a control**Results:** All compounds were well tolerated at the max deliverable dose with no adverse effects. Series 1 compounds dosed at 5 or 20mg/kg IV had T½ and Vdss of 0.7-1.5h and 2.4-11L/kg respectively. Series 2 compounds dosed at 10mg/kg IV had T½ and Vdss of 0.4h and 0.3L/kg. Series 3 compounds were dosed at 5 - 20mg/kg IV, SC or IP had T½ and Vdss of 0.7-0.9h and 0.6-1.6L/kg. Infected mice developed a robust infection with vehicle burdens of 6.0 and 6.1$\log_{10}$cfu/mL in wash and blood respectively. Treatment with a series 3 compound resulted in a reduction of burden in wash and blood of 0.45 & 2.14$\log_{10}$cfu/mL respectively, similar to VAN (1.2 & 2.0$\log_{10}$cfu/mL)**Conclusions:** All 3 series of PGT inhibitors were well tolerated following IV, IP or SC dosing. All series demonstrated acceptable PK parameters. A series 3 compound was highly effective in a murine model of *S. aureus* sepsis. These studies support continued development of PGT for the treatment of MDR infections

Author Disclosure Block:

P. Warn: D. Employee; Self; Evotec. **D. Kahne:** None. **A. Parkes:** D. Employee; Self; Evotec. **M. Whittaker:** D. Employee; Self; Evotec. **F. Nutter Howard:** D. Employee; Self; Evotec. **J. Smith:** D. Employee; Self; Evotec. **S. Walker:** None.

Poster Board Number:

SATURDAY-433

Publishing Title:

Antiviral Activity of Novel Di-Dabco Molecules on Non-enveloped Viruses

Author Block:

K. A. MELKONIAN, L. Chrastecka, E. Stirling; LONG ISLAND Univ., Brookville, NY

Abstract Body:

We have previously engineered novel DABCO (1,4-diazabicyclo[2.2.2]octane)-hydrocarbon molecules that display numerous antimicrobial properties when covalently bound to carbohydrate- or protein-based surfaces. Using a prokaryotic system, we have shown that specific novel DABCO-hydrocarbon cloths have the ability to reduce (>90%) the number of T4 bacteriophage virus particles available for infection after incubation. Using a plaque assay, we have shown that the cloth removes virus from the solution, rendering it incapable of infecting bacteria.DABCO-hydrocarbon surfaces have also been shown to prolong the life of MDCK (Madin-Darby canine kidney) cells that have been exposed to the influenza H3N2 virus. However, DABCO-hydrocarbons have shown little to no anti-viral activity against certain non-enveloped viruses, such as adenovirus. Therefore, di-DABCO variants of the surface modifications were prepared. Using fluorescence microscopy, one specific di-DABCO molecule (when covalently bound to cotton cloth) was shown to inhibit GFP-adenovirus infection of HeLa cells. We have shown that the spacing between the DABCO molecules is critical to the surface's antiviral activity. In addition, by neutralizing the charges on the DABCO molecule, we have shown that a charge interaction is responsible for the inhibition of viral infection. Therefore, a combination of DABCO-hydrocarbon and di-DABCO modifications applied to a surface will provide anti-bacterial, anti-fungal and anti-viral activity against a wide range of microorganisms. These surfaces may prove to be an invaluable first line of defense for our medical workers (hospital linens, scrubs, etc.) and for our armed forces (protective uniforms, wound dressings, etc.) in the future.

Author Disclosure Block:

K.A. Melkonian: None. **L. Chrastecka:** None. **E. Stirling:** None.

Poster Board Number:

SATURDAY-434

Publishing Title:

Developing Safer Polymyxins (Pms): Structure-Activity (Sar) and Structure-Toxicity (Str) Relationships of Modifications to Positions 6 and 7

Author Block:

K. D. Roberts[1], J. Wang[1], H. Yu[1], L. Wang[1], O. Lomovskaya[2], D. Griffith[2], S. Hecker[2], M. Dudley[2], P. E. Thompson[1], R. L. Nation[1], T. Velkov[1], J. Li[1]; [1]Monash Univ., Melbourne, Australia, [2]The Med.s Company, San Diego, CA

Abstract Body:

Background: PM B and colistin (COL) are last-line antibiotics for the treatment of multidrug-resistant (MDR) Gram-negative pathogens; however, their use is hampered by their potential for nephrotoxicity. The *N*-terminal fatty acyl chain and Dab residues have been implicated in nephrotoxicity. Unfortunately, modification of these structural regions has yet to deliver safer PMs with comparable efficacy. Using a novel medicinal chemistry approach, we substituted the residues at positions 6 (D-Phe^6/Leu^6) and 7 (Leu^7) with less hydrophobic amino acids (Thr, Ala, Val, Ser, Abu) and examined the SAR and STR of these modifications using animal models.**Methods:** Peptides were synthesized using solid-phase peptide synthesis. MICs were determined against a panel of 17 PM-susceptible ATCC and clinical MDR Gram-negative isolates (*Pseudomonas aeruginosa* (Pa), *Acinetobacter baumannii* (Ab), *Klebsiella pneumoniae* (Kp), and *Enterobacter cloacae*) using broth microdilution. Nephrotoxicity was investigated (accumulated dose 60 mg/kg for 24 h) in a mouse model and kidney histological examination was conducted. *In vivo* efficacy was evaluated against *Pa* ATCC 27853, Ab ATCC 19606, Kp M320455 at 4 h following a single intravenous bolus dose (4 mg base/kg) in a neutropenic mouse blood infection model.**Results:** Substitution of either or both the D-Phe^6/Leu^6 and Leu^7 with Thr, Ala, Val, Ser or Abu resulted in no observable kidney damage, compared to PM B and COL (mild to severe nephrotoxicity) in mice. While changes at position 7 retained antimicrobial activity, a minimum level of hydrophobicity (D-Leu^6) is required at position 6 to maintain comparable *in vitro* (MIC <0.125-2 μg/mL) and *in vivo* ($\Delta\log_{10}$ >-1 to -3) antibacterial activity to PM B and COL. Further modification, changing the stereochemistry of position 3 to the D-configuration nullified the decrease in the nephrotoxicity achieved by modifications at positions 7.**Conclusions:** PM nephrotoxicity can be modulated through substitution of position 7 with less hydrophobic residues without compromising antibacterial potency. Our lead peptides show potential for obtaining safer PMs, and are undergoing further preclinical evaluation.

Author Disclosure Block:

K.D. Roberts: I. Research Relationship; Self; Involved in a NIH funded research project with the Medicines Company. **J. Wang:** None. **H. Yu:** None. **L. Wang:** None. **O. Lomovskaya:** D.

Employee; Self; Employee at The Medicines Company. **D. Griffith:** D. Employee; Self; Employee at the The Medicines Company. **S. Hecker:** D. Employee; Self; Employee at The Medicines Company. **M. Dudley:** D. Employee; Self; Employeee at The Medicines Company. **P.E. Thompson:** I. Research Relationship; Self; Involved in NIH finded research project with the The Medicines Company. **R.L. Nation:** I. Research Relationship; Self; Involved in a NIH funded research project with The Medicines Company. **T. Velkov:** I. Research Relationship; Self; Involved in a NIH funded research project with the Medicines Company. **J. Li:** I. Research Relationship; Self; Involved in a NIH funded research project with The Medicines Company.

Poster Board Number:

SATURDAY-435

Publishing Title:

Novel Isolate, Novel Compound - Lipopeptide from *Paenibacillus tyrfis* Mst1, a Malaysian Tropical Peat Swamp Soil Isolate

Author Block:

Y. Aw, C. Yule, Y. Cheow, S. Lee; Monash Univ. Malaysia, Selangor, Malaysia

Abstract Body:

Rapid emergence of antimicrobial resistance limits therapeutic options for infections by antimicrobial resistant bacteria (ARB). New antimicrobial compounds are needed to combat ARB related infections and scientists have shifted their attention towards exploration of unique, understudied ecosystem for novel compounds. Tropical peat swamps are examples of such ecosystems, with unique characteristics such as acidic pH, phenolic rich blackwater and constantly waterlogged substrate. Screening peat for antimicrobial producing bacteria yielded a novel species of *Paenibacillus* known as *Paenibacillus tyrfis* that exhibits broad spectrum antimicrobial activity against bacteria, yeasts and fungi. This study focused on characterization of the antimicrobial compounds and prediction of the structure of the compounds based on genome sequence analysis of *P. tyrfis* MSt1. The antimicrobial compounds were subjected to partial purification using reverse phase column chromatography and active fraction exerted strong antimicrobial activity against *Escherichia coli* ATCC 25922 (MIC = 1.5 µg/mL), MRSA ATCC 700699 (MIC = 25 µg/mL) and *Candida albicans* IMR (Institute Medical Research, Malaysia) (MIC = 12.5 µg/mL). Active fraction caused membrane rupture on *E. coli* ATCC 25922 when viewed with SEM. LC-MS analysis showed mass fragments with *m/z* of 1091, 1105 and 1119 together with base peak of *m/z* 678 which highly suggested the presence of lipopeptide compounds in the active fraction. Analysis of the genome of *P. tyrfis* MSt1 also revealed a lipopeptide biosynthetic gene cluster that is closely related to the biosynthesis of pelgipeptin, a lipopeptide produced by *Paenibacillus elgii* B69. Using the adenylation domain in the non-ribosomal peptide synthetase (NRPS) modules, the nonapeptide sequence of the lipopeptide was predicted to possess the following amino acid sequence; DAB-Val-DAB-Phe-Phe-DAB-Val-Phe-Ser where DAB stands for 2,4-diaminobutyric acid. This is the first lipopeptide compound with this amino acid sequence and strongly highlights the novelty of the lipopeptide compound produced by *P. tyrfis* MSt1. Further purification and identification of the lipopeptide compound will be performed in the future to accurately identify and characterize the lipopeptide compound.

Author Disclosure Block:

Y. Aw: None. **C. Yule:** None. **Y. Cheow:** None. **S. Lee:** None.

Poster Board Number:

SATURDAY-437

Publishing Title:

A Novel Approach for Identifying Molecules with Activity Against New Targets in Bacteria

Author Block:

C. E. PETERS, A. Lamsa, J. Durrant, J. L. Garrido, R. Amaro, J. Pogliano, K. Pogliano; Univ. of California San Diego, La Jolla, CA

Abstract Body:

The emergence of multi-drug resistant bacteria and the decline in the number of new antibiotics coming to market poses a major global health threat. There is an urgent need to discover antibiotics that act by novel mechanisms of action (MOA). We have developed a rapid and versatile platform for identifying drugs that act by novel MOAs called Bacterial Cytological Profiling (BCP). BCP utilizes fluorescence microscopy to observe changes in cytological parameters of bacteria exposed to lethal concentrations of antibiotics. Antibiotics that hit targets in different pathways generate different cytological profiles. For novel drug targets, those for which there are currently no antibiotics, we have developed Rapid Inhibition Profiling (RIP), in which we generate cytological profiles by degrading essential proteins, providing a visual screen for antibiotics hitting novel targets. We have utilized RIP to obtain profiles of targets within a number of essential pathways in *Bacillus subtilis* and selected several proteins with novel profiles as targets for virtual screens. Here we combine BCP with a novel virtual screening approach that utilizes snapshots of target proteins extracted from state-of-the-art molecular dynamics simulations. We used simulation-based virtual screening to identify candidate molecules active against our selected novel drug targets and then determined the MOA of these compounds using BCP. We show that combining these approaches provides a rapid and efficient platform identifying molecules with activity against new targets in bacteria.

Author Disclosure Block:

C.E. Peters: None. **A. Lamsa:** None. **J. Durrant:** None. **J.L. Garrido:** None. **R. Amaro:** None. **J. Pogliano:** C. Consultant; Self; Linnaeus Bioscience Inc.. K. Shareholder (excluding diversitied mutual funds); Self; Linnaeus Bioscience Inc. **K. Pogliano:** C. Consultant; Self; Linnaeus Bioscience Inc.. K. Shareholder (excluding diversitied mutual funds); Self; Linnaeus Bioscience Inc..

Poster Board Number:

SATURDAY-438

Publishing Title:

Adjunctive Use of Clavulanic Acid Abolishes the Cefazolin Inoculum Effect of Methicillin-Sensitive *Staphylococcus aureus* (MSSA) *in vitro*

Author Block:

W. R. Miller, K. V. Singh, C. A. Arias, B. E. Murray; Univ. of Texas McGovern Med. Sch., Houston, TX

Abstract Body:

Background: The inoculum effect (InE) refers to an increase in minimum inhibitory concentration (MIC) of an antibiotic when large numbers of organisms are present. In MSSA, this effect has been linked to the production of a staphylococcal type A β-lactamase (β-lac) and is associated with clinical failures of cefazolin (CFZ), which appears to be efficiently hydrolyzed by the enzyme. We investigated the ability of clavulanate, a β-lac inhibitor, to reverse the InE *in vitro*.**Methods:** Three *S. aureus* strains were used in this study ***i)*** ATCC 25923, a β-lac negative strain, ***ii)*** ATCC 29213, previously shown to produce small amounts of β-lac and ***iii)*** TX0117, a β-lac producing clinical isolate known to show the CFZ InE. MICs by broth microdilution at both standard inoculum (SI, 5 x 10^5 CFU/mL) and high inoculum (HI, 5 x 10^7 CFU/mL) were performed using Mueller-Hinton II media. CFZ alone and combined with amoxicillin/ clavulanate (AM/CL) at 0.5/0.125 µg/mL (1/16 MIC) and 1/0.25 µg/mL (1/8 MIC) were tested in triplicate for all three strains. We tested AM/CL together as it is a common co-formulation. Time-kill curves (TKC) for strain TX0117 were performed with CFZ (64 µg/mL) alone at SI and HI or in combination with AM/CL (1/8 MIC) at HI. Samples were taken at 0, 4 and 24 hours, centrifuged and washed to remove antibiotics and dilutions were plated for colony counts.**Results:** At SI, both β-lac producing stains had CFZ MICs in the susceptible range. At HI, CFZ MICs for both ATCC 29213 and TX0117 increased from 0.5 to 4 µg/mL and 1 to 128 µg/mL, respectively. Using HI, the addition of AM/CL at 1/16 MIC to CFZ reverted the CFZ MIC to 1 µg/mL. This effect was not seen with CFZ plus ampicillin (to control for possible synergism between β-lactams). In TKCs, CZF alone at HI resulted in a 0.7 log10 reduction at 4 hours before regrowth to a level similar to the antibiotic free control at 24 hours. In contrast, CFZ plus sub-MIC AM/CL at HI achieved killing similar to CZF at SI (2.86 log10 decrease from T=0) and a 4.5 log10 decrease at 24 hr compared to CFZ alone at HI; growth in sub-MIC AM/CL alone did not differ from the control.**Conclusions:** The use of sub-MIC AM/CL with CFZ abolished the InE of MSSA observed *in vitro*. In select MSSA infections where a high inoculum may be present, the addition of oral AM/CL to CFZ may theoretically offer protection against the InE.

Author Disclosure Block:

W.R. Miller: None. **K.V. Singh:** I. Research Relationship; Self; Merck. **C.A. Arias:** C. Consultant; Self; Theravance, Bayer. I. Research Relationship; Self; Forest, Theravance. J. Scientific Advisor (Review Panel or Advisory Committee); Self; Bayer, Pfizer. L. Speaker's Bureau; Self; Forest, Theravance, Pfizer, Astra-Zeneca, The Medicines Company, Novartis. **B.E. Murray:** C. Consultant; Self; Theravance, Targanta Therapeutics, Pfizer, AstraZeneca. I. Research Relationship; Self; Johnson & Johnson, Theravance.

Poster Board Number:

SATURDAY-439

Publishing Title:

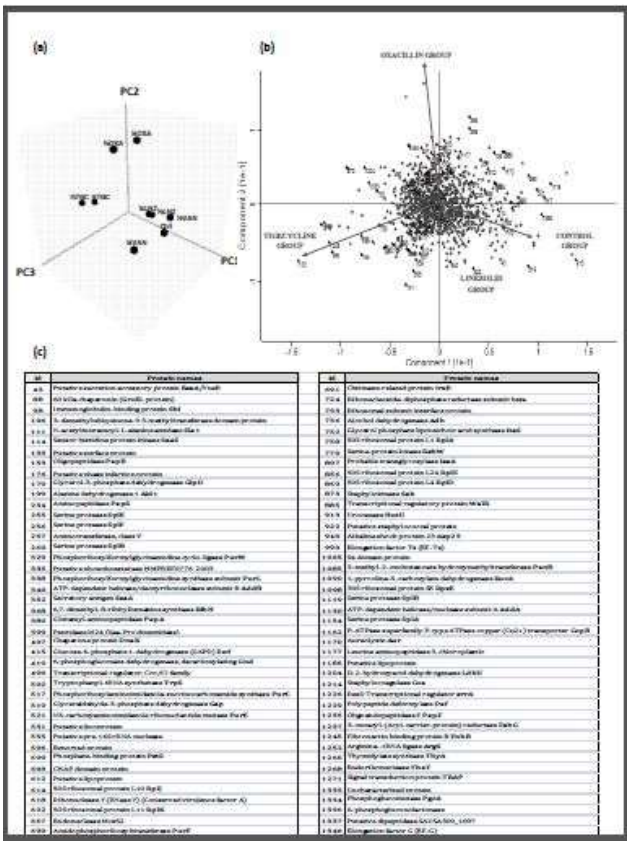
Response of USA300 to Sub-Concentrations of Antibiotics

Author Block:

E. Torres-Sangiao[1], B. Cillero-Pastor[2], **M. Cartelle-Gestal**[3], H. G. Wiker[4], C. G. Riestra[1]; [1]USC, LCG, Spain, [2]Maastricht Univ., MST, Netherlands, [3]Univ. of Georgia, Athens, GA, [4]UiB, BGO, Norway

Abstract Body:

Staphylococcus aureus is a magnificent example of evolving pathogen and one of the most common causes of infections. Over the past decades CA-MRSA has emerge as an important pathogen. USA300 clone has the ability to infect healthy individuals due to its highly virulence. The aim was clarify the response against antibiotic pressure of the USA300 strain**Method:**USA300 cells were grown under ¼ & ½ MIC of LNZ, TGC, OXA & VAN (TSB, 37ºC, 250 rpm). The peptide mix from SDS-PAGE followed by in-gel trypsin digestion, were analysed by nLC-MS/MS Orbitrap. MaxQuant (*v*1.5.3.28) was used for protein identifications & LFQ intensities calculation. Multivariate analysis was performed by Perseus (*v*1.5.2.6). Missing values were imputed before normalization. *Z*-scoring was performed on columns and substract components on the first 3 principal components (PC). PCA was performed by singular value decomposition**Results:**The PC1, PC2 & PC3 represented the 52.2, 35.2 & 12.6 % (100 %) of variance, resp. (**Figure a**). The bi-plot (**Figure b**) shows the first 2 PC and 86 significant proteins by ANOVA test (**Figure c**)Control cells showed an up-regulation of purine and serineproteases biosynthesis pathway, as well as SaeS two-component System & Esa System, both involve in the establishment of infection and dispensable for laboratory growth.TGC & OXA showed a response to stress by up-regulation of proteins involved in pathogenesis (Coa & Sak, resp.) or biofilm formation (FnbB & PsmA1, resp.)**Conclusion:**PCA identified 4 patterns. LNZ did not lead relevant changes. VAN showed similar patterns to control. The major variability was observed with TGC & OXA.The results show that sub-MICs can lead to a strong mechanism of protection by USA300 against the used antibiotics.



Author Disclosure Block:

E. Torres-Sangiao: None. **B. Cillero-Pastor:** None. **M. Cartelle-Gestal:** None. **H.G. Wiker:** None. **C.G. Riestra:** None.

Poster Board Number:

SATURDAY-440

Publishing Title:

Protective Efficacies of Tedizolid Phosphate, Linezolid, and Vancomycin in Rabbit Model of Mrsa Necrotizing Pneumonia

Author Block:

H. Le, V. Le, M. Pinheiro, K. Hahn, M. Dinh, B. Diep; Univ. of California, San Francisco, San Francisco, CA

Abstract Body:

Background: Linezolid has previously been shown to be superior to vancomycin in a rabbit model of necrotizing pneumonia because it inhibits *Staphylococcus aureus* from producing two key lung-damaging toxins, alpha-toxin and Panton-Valentine leucocidin. Herein we compared the protective efficacy of tedizolid phosphate, a second-generation oxazolidinone that potently inhibits *S. aureus* protein synthesis, to those of linezolid, vancomycin, or saline in the same model. **Methods:** Using a well-characterized rabbit model of necrotizing pneumonia with the USA300/SF8300 strain, 48 infected rabbits were randomized into 1 of 4 groups that were administered the following treatment regimens at 1.5 h post infection: 1) 6 mg/kg tedizolid phosphate [TR-701 FA] intravenously twice daily, 2) 50 mg/kg linezolid subcutaneously three times daily; 3) 30 mg/kg vancomycin intravenously twice daily; and 4) 5 ml saline intravenously twice daily. Animals were monitored every 2 h for the first 30 h postinfection, and every 6 h thereafter until 48 hours for mortality. Animals with profound pulmonary dysfunction (respiration rate >75, cyanosis, and cough) were euthanized for humane reasons.**Results:** The rabbit dosing regimen of 6 mg/kg tedizolid phosphate IV BID, which yielded $AUC_{0\text{-}24h}$ values approximating those in humans, was used. Survival of rabbits treated with 6 mg/kg tedizolid phosphate BID was similar to those treated with 50 mg/kg linezolid TID (10/12 vs. 10/12, P=0.98), but was significantly improved compared to those treated with 30 mg/kg vancomycin BID (10/12 vs. 2/12, P=0.002) and saline (10/12 vs. 2/12, P=0.001) using two-sided log-rank (Mantel-Cox) test with Bonferroni correction for multiple comparisons. Bacterial count in lungs of rabbits treated with tedizolid phosphate was significantly decreased compared to those treated with saline (log_{10}CFU ± std dev of 6.3±1.3 vs. 8.1±1.1, P=0.010), although this was not significantly different from those treated with vancomycin (vs. 7.5±1.9, P=0.11) or linezolid (vs. 6.4±1.4, P=1.00) by nonparametric one-way ANOVA with Kruskal-Wallis test followed by Dunn's multiple comparisons test.**Conclusions:** Tedizolid phosphate, like linezolid, improves survival outcomes in a rabbit model of staphylococcal necrotizing pneumonia.

Author Disclosure Block:

H. Le: None. **V. Le:** None. **M. Pinheiro:** None. **K. Hahn:** None. **M. Dinh:** None. **B. Diep:** None.

Poster Board Number:

SATURDAY-441

Publishing Title:

Evaluation of Vitamin D3 and Vitamin C in Cooperation with Doxycycline against *Borrelia burgdorferi* and *Borrelia garinii*

Author Block:

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Abstract Body:

Background: Little is known about the anti-borrelieae activity of vitamins with commercially available agents against spirochetes of *Borrelia sp.* that cause Lyme disease. To better understand such cooperation, we studied the anti-bacterial efficacy of vitamin D3 and vitamin C (known for their antimicrobial activity) with doxycycline (one of the most frequently prescribed drugs for Lyme patients) against *Borrelia sp*. **Methods:** Doxycycline was tested in combination with vitamin D3, and with vitamin C for their *in vitro* effectiveness against vegetative (spirochetes) and dormant (rounded bodies, biofilm) forms of *Borrelia burgdorferi* and *Borrelia garinii* using dark field and fluorescence microscope. Synergistic antibacterial effect of these agents was evaluated at their MIC values and three dilutions above (2-6 X MIC) as well as three dilutions below their MICs (1/2-1/8 X MIC), according to checkerboard assays, and defined by fractional inhibitory concentration index. Additionally, a Student t-test statistical analysis was performed for further validation. All experiments were performed in triplicates. **Results:** The results showed that doxycycline in combination with vitamin D3 exhibits additive anti-spirochetal and amended anti-biofilm effects, whereas with vitamin C additive anti-spirochetal and amended anti-rounded bodies effect was observed. Although complete elimination of rounded forms and mature biofilm was not noted, tested combinations of agents greatly reduced viable organisms in term of qualitative effect. **Conclusions:** The data obtained from this *in vitro* study revealed that doxycycline in combination with vitamin D3 and vitamin C expresses compelling antibacterial action against examined species of *Borrelia*. This indicates that a commercially and widely used drug doxycycline may act with vitamins D3 and C and that these vitamins may serve as an important addition to treatment of Lyme disease bacteria.

Author Disclosure Block:

A. Goc: None.

Poster Board Number:

SATURDAY-442

Publishing Title:

Evaluation of the Antimicrobial Activity of Cetylpyridinium Chloride against Oral and Throat Flora

Author Block:

A. M. García[1], A. M. Aristizabal[2], I. P. Torres[2], O. M. Gómez[2], D. H. Cáceres[2], C. de Bedout[2], G. I. Mejía[2]; [1]Univ. de Antioquia, Medellín, Colombia, [2]Corporación para investigaciones Biologicas - CIB, Medellín, Colombia

Abstract Body:

Background: Quaternary ammonium salts such as cetylpyridinium chloride, act penetrating the cell membrane altering bacterial metabolism and inhibiting cell growth (Ozdemir 2013; Liu 2013). This compound has shown a beneficial effect in reducing dental plaque as it has a broad spectrum of antimicrobial activity against bacteria and fungi found in the oral cavity that produce odor. The objective of this study was to assess the minimum inhibitory concentration (MIC) of cetylpyridinium chloride as an antimicrobial agent against some pathogens from oral and throat, and some microorganism common skin flora, such as Candida albicans (Ca), Streptococcus mutans (Sm), Streptococcus pyogenes (Sp), Haemophilus influenzae (Hi), Porphyromonas gingivalis (Pg), Fusobacterium nucleatum (Fn), *Actinomyces* israelii (Ai) and Prevotella loescheii (Pi). All microorganism tested were ATCC strains. **Methods:** Agar disk diffusion technique was employed, preparing cetylpyridinium chloride sensidiscs at different concentrations (0.125, 0.5, 2, 8 and 32 ug). As inhibition control different antibiotics sensidiscs specific for each microorganism were used (25 ug fluconazole - Ca, 10 ug ampicillin - Sm, Sp, and Hi, 5 ug ampicillin - Pg, 2 ug ampicillin - Fn, ampicillin 15 ug - Ai and 5 ug ampicillin - Pi). The incubation time and the inhibition zone read were performed according to the recommendations of the CLSI. The data were analyzed with the program GraphPad Prism version 4. t Student test was performed to determine statistically significant differences, with $p < 0.05$.**Results:** The inhibitory activity of cetylpyridinium chloride activity was evidenced from 0.5 ug in Sm; for Ca, Sp, and Pi from 8 ug; for Hi and Pg from 2 ug. In all organisms the inhibition zone diameter rise with increasing concentration tested, except for Ai which showed inhibition only in the highest concentration (32 ug) and in Fn where the inhibition was not observed. **Conclusions:** In conclusion, cetylpyridinium chloride exerted its antimicrobial power at low levels in almost all organisms tested except for A. israelii who needs higher concentrations and in F. nucleatum where not effect was observed.

Author Disclosure Block:

A.M. García: I. Research Relationship; Spouse/Life Partner; Tecnoquímicas S.A, Cali, COLOMBIA. **A.M. Aristizabal:** H. Research Contractor; Spouse/Life Partner; Técnoquimicas

S.A. **I.P. Torres:** H. Research Contractor; Self; Tecnoquímicas S.A, Cali, COLOMBIA. **O.M. Gómez:** H. Research Contractor; Self; Tecnoquímicas S.A, Cali, COLOMBIA. **D.H. Cáceres:** None. **C. de Bedout:** None. **G.I. Mejía:** None.

Poster Board Number:

SATURDAY-443

Publishing Title:

Aminoglycosides and Triclosan for the Kill: Drug Synergism That Eradicates *Pseudomonas aeruginosa* Biofilms

Author Block:

A. M. A. Hunt, J. A. Gibson, C. M. Waters; Michigan State Univ., East Lansing, MI

Abstract Body:

Chronic infections caused by *Pseudomonas aeruginosa* are difficult to eradicate. As biofilms form, microbial cells protected by an exopolysaccharide matrix, and cells within the biofilm in a reduced metabolic state become tolerant to conventional antimicrobials. As a result, patients suffering from diseases like cystic fibrosis and diabetic foot ulcers have recurrent infections without the prospect of biofilm elimination. The search for new drugs is a long and costly process. Therefore, the identification of known biologically active compounds that exhibit synergistic effects with clinically used antibiotics represents an attractive approach to treat biofilms. A screen of over 6000 biologically active molecules identified 26 hits that exhibited synergistic activity when combined with tobramycin to kill *P. aeruginosa* biofilms while exhibiting no activity themselves. From these compounds, triclosan, known to have little to no effect against *P. aeruginosa* was selected for further investigation. High throughput assays combined with luminescence cell viability were used to test the killing effect of different concentrations of the compounds. Associations with other aminoglycosides and classes of antibiotics were evaluated, as well as their activity against different microorganisms. *In vitro* results demonstrated that in combination the antimicrobials killed nearly 100% of all strains of *P. aeruginosa*, including 19 clinical isolates, at the highest concentrations of 250 µg/mL tobramycin and 100 µM triclosan. Moreover, even at a 5-fold dilution 87% of the biofilm was eradicated with one dose. Synergistic effects were confirmed with other aminoglycosides and treated biofilms of *Burkholderia* spp also exhibited reduced cell viability. *In vivo* testing with a *P. aeruginosa* alginate-producing isolate showed a 3 log killing improvement with the dual treatment. The combination eradicated *P. aeruginosa fabI* mutant biofilms, the known target of triclosan, demonstrating that the killing effect is not a consequence of triclosan's inhibition of fatty acid production. The striking antimicrobial properties of aminoglycosides-triclosan both *in vitro* and *in vivo* is a promising approach to manage recalcitrant biofilms.

Author Disclosure Block:

A.M.A. Hunt: None. **J.A. Gibson:** None. **C.M. Waters:** None.

Poster Board Number:

SATURDAY-444

Publishing Title:

Antimicrobial Activity and Synergistic Combinations of Methyl Gallate with Antibiotics against Multidrug-Resistant Bacteria

Author Block:

F. K. Zeeshan[1], M. Yousuf[2], S. Faizi[2], S. u. Kazmi[3]; [1]Dow Univ. of Hlth.Sci., karachi, Pakistan, [2]HEJ Res. Inst. of Chemistry, Intl. Ctr. for Chemical and Biological Sci. (ICCBS), Univ. of Karachi, Karachi, karachi, Pakistan, [3]Dada Bhoy Inst. of Higher education, karachi, Pakistan

Abstract Body:

Objective: The main purpose of study is to check antimicrobial activity of Methyl gallate and its synergistic combinations with antibiotics against MDR (multi drug resistant) Bacteria.**Background:** Antibiotic resistance has now become serious health problem. Alternative treatment regime are needed to fight against MDR pathogens. Plants are used in herbal medicine since ages. Methyl gallate is a phenolic compound isolated from plant *Caesalpinia pulcherrima*, known to have antimicrobial activities. Therefore, Methyl gallate was choosen in this study. **Methods:** Methanol extract of fresh pods of *Caesalpinia pulcherrima* was subjected to bi phasic liquid separation which gave Hexane, Ethyl acetate, Butanol and Aqueous phases separately. Ethyl acetate phase was further fractionated , led to the isolation of a Methyl gallate. Minimum Inhibitory Concentration (MIC) of Methyl gallate was performed by micro broth dilution and tube dilution method. Synergistic combinations of Methyl gallate with Amoxycillin and Ciprofloxacin were determined by Fractional Inhibitory concentration index. **Results:** MIC of Methyl gallate (125-250µg/ml) against Methicillin Resistant Staphylococcus aureus, MDR *E. coli, P. aeruginosa, S. typhi, P. mirabilis, Acinetobactor spp,* ATCC *E.coli* 25922, and ATCC *S. aureus* 29213 . Synergistic combination of Ciprofloxacin and Methyl gallate showed fall in MIC from 512 µg/ml to 0.0312 µg/ml and 250 µg/ml to 15.6 µg/ml, respectively against MDR *E.coli*. Synergistic combination of Amoxycillin and Methyl gallate from 256µg/ml to 0.0625µg/ml and 250 µg/ml to 31.25µg/ml, respectively against MRSA. .**Conclusion:** Methyl gallate showed remarkable antimicrobial activity and synergistic combinations against MDR bacteria. Detailed pharmacological studies are required in order to use Methyl gallate as therapeutic agent in future.

Author Disclosure Block:

F.K. Zeeshan: None. **M. Yousuf:** None. **S. Faizi:** None. **S.U. Kazmi:** None.

Poster Board Number:

SATURDAY-445

Publishing Title:

Activity of Telavancin Tested against Gram-Positive Pathogens Isolated from Bone and Joint Infections in European (EU), Latin American (LATAM), USA and Asia-Pacific (APAC) Nations

Author Block:

R. N. Jones[1], J. I. Smart[2], R. E. Mendes[1]; [1]JMI Lab., North Liberty, IA, [2]Theravance Biopharma US, Inc, South San Francisco, CA

Abstract Body:

Background: Telavancin (TLV), a lipoglycopeptide, has broad activity against *S. aureus* (SA; MIC_{90}, 0.06 µg/ml), coag-negative staphylococci (CoNS; 0.06 µg/ml), β-hemolytic (BHS) and viridans group (VGS) streptococci (≤0.06 µg/ml) and vancomycin-susceptible (VAN-S) enterococci (0.12 µg/ml). TLV, as a candidate for osteomyelitis therapy, was tested against a worldwide collection of pathogens (940) isolated from bone-joint infections (BJI).**Methods**: A SENTRY Program collection of BJI strains (2011-2014) was tested by reference broth microdilution susceptibility (S) methods for TLV and 12 other agents including VAN, oxacillin, daptomycin (DAP) and teicoplanin (TPN). Data were analyzed by region (EU, LATAM, USA, APAC), patient age (≤17 and ≥18 years) and resistant (R) subgroups. Organism numbers tested were: SA (693), CoNS (117), BHS (93), VGS (37) and enterococci (65).**Results**: The most common pathogen was SA (73.7%; range 51.2% [APAC] to 79.1% [USA]), having a 35.7% MRSA rate and TLV $MIC_{50/90}$ of 0.03/0.06 µg/ml (100% S by USA-FDA/EUCAST breakpoint criteria). CoNS (12.4% of BJI) had TLV MIC $_{50/90}$ at 0.06/0.06 µg/ml (100.0% S), but 64.1 and 13.7% were MR CoNS and TPN-R, respectively. Monitored streptococci were 100% S to TLV (MIC_{90}, 0.03-0.06 µg/ml). Most BJI isolates were from USA (52.8%) followed by EU (24.7%), LATAM (13.3%) and APAC (9.1%). Only 12.5% of SA were observed in patients ≤17 years and 31.2% were MRSA (36.6% in adults). DAP-R strains (0.3%) were noted in USA, all MRSA in adults and TLV-S (≤0.12 µg/ml). Enterococci overall had TLV MIC $_{50/90}$ at 0.12/0.25 µg/ml, but VAN-S strains had TLV MICs at ≤0.12 µg/ml. All VRE had a VanA-phenotypes.**Conclusion**: TLV was active against all SA strains regardless of phenotype. Only VRE (all VanA) were not S to TLV (MICs, 0.25-2 µg/ml). TLV appears to be a viable candidate for BJI/osteomyelitis treatment caused by gram-positive cocci in adults and children.

	TLV MIC 50/90 by age and region:						
BJI Pathogen (no.)	**All (% S)**	≤17 yrs	≥18 yrs	USA	EU	LATAM	APAC

SA (642)	0.03/0.06 (100)	0.03/0.06	0.03/0.06	0.03/0.06	0.03/0.06	0.06/0.06	0.06/0.06
CoNS (117)	0.06/0.06 (100)[a]	--	--	0.03/0.06	0.06/0.06	0.06/0.06	0.06/0.06
BHS (106)[b]	0.03/0.06 (100)	--	--	0.03/0.06	≤0.015/0.03	≤0.015/--	0.03/0.06
VGS (37)[c]	≤0.015/0.03 (100)	--	--	≤0.015/0.03	≤0.015/0.03	0.03/--	0.03/--
Enterococci (65)[d]	0.12/0.25	--	--	0.12/1	0.06/0.12	0.12/0.12	≤0.015/--
a. SA breakpoint applied to CoNS for comparison only; b. Four species groups; c. 13 species or species groups. d. Includes 7 VAN-R strains, All VAN-S strains were TEL-S (≤0.12 μg/ml)							

Author Disclosure Block:

R.N. Jones: H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Theravance Biopharma US, Inc. **J.I. Smart:** D. Employee; Self; Employee of Theravance Biopharma US, Inc. **R.E. Mendes:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Theravance Biopharma US, Inc.

Poster Board Number:

SATURDAY-446

Publishing Title:

In Vitro* Synergistic Activity of Antimicrobial Agents in Combination against Clinical Isolates of Colistin-Resistant *Acinetobacter baumannii

Author Block:

S. Bae, T. Kim, S. Park, S-H. Kim, H. Sung, S-O. Lee, M-N. Kim, S-H. Choi, Y. Kim, J. Woo, Y. Chong; Asan Med. center, Seoul, Korea, Republic of

Abstract Body:

Background: Emerging resistance to colistin in clinical isolates of *Acinetobacter baumannii* is of growing concern. As the treatment options are extremely limited, we investigated *in vitro* activities of various antimicrobial combinations against colistin-resistant *A. baumannii*.**Methods:** 9 clinical isolates of colistin-resistant *A. baumannii* were collected in Asan Med. Ctr., Seoul, Korea between Jan 2010 and Dec 2012. To screen synergy, combinations of every double antimicrobial among 12 agents were tested using the multiple-combination bactericidal test (MCBT). Checkerboard tests were then performed to validate the existence of synergism.**Results:** On the MCBT, the most effective combinations were colistin-rifampin and colistin-teicoplanin as in both of these combinations 8 of 9 strains were inhibited. Colistin combined with aztreonam, meropenem, and vancomycin showed inhibition of growth on 7 strains. Colistin was the most common component of the antimicrobial combinations which were active against *A. baumannii*. Further checkerboard tests were conducted in colistin-based combination regimens. Notably, colistin-rifampin was fully synergistic (FICI ≤ 0.5) against all the 9 strains (100%). Glycopeptide or β-lactam containing combinations showed fairly successfully effects and the remaining regimens revealed limited synergism.**Conclusions:** Using MCBT and the checkerboard test, we found that colistin-based combination regimens, particularly with rifampin, glycopeptides or β-lactams, can offer a therapeutic benefit for infection caused by colistin-resistant *A. baumannii*.Table 1. Results of the checkerboard test for colistin-resistant A. baumannii

Agent	Synergy (FIC ≤ 0.5)	Partial synergy (0.5 < FICI <1)	Additive/Indifferent (1 ≤ FICI < 4)
CST + SAM	h	b,d,f,g,i	a,c,e
CST + RFP	a,b,c,d,e,f,g,h,i	-	-
CST + TGC	h	f	a,b,c,d,e,g,i
CST + TMP/SMX	f	a,g,h	b,c,d,e,i
CST + VAN	a,b,d,e,f,g,h	c,i	-

CST + TEC	a,e,f,i	b,c,d,g,h	-
CST + AMK	f,g,h	-	a,b,c,d,e,i
CST + AZM	f	-	a,b,c,d,e,g,h,i
CST + ATM	a,b,d,i	c,g,h	e,f
CST + CAZ	a,f,g,h	b,c,d	e,i
CST + MEM	e,g,h	a,b,d,f	c,i

Nine strains were described as alphabet letters ‘a’ to ‘i’CST, colistin; SAM, ampicillin/sulbactam; TGC, tigecycline; AMK, amikacin; AZM, azithromycin; ATM, aztreonam; CAZ, ceftazidime; MEM, meropenem; RFP, rifampin; TMP/SMX, trimethoprim/sulfamethoxazole; VAN, vancomycin; TEC, teicoplanin.

Author Disclosure Block:

S. Bae: None. **T. Kim:** None. **S. Park:** None. **S. Kim:** None. **H. Sung:** None. **S. Lee:** None. **M. Kim:** None. **S. Choi:** None. **Y. Kim:** None. **J. Woo:** None. **Y. Chong:** None.

Poster Board Number:

SATURDAY-447

Publishing Title:

Evaluation of Outcome for Empiric Treatment of *Escherichia coli* Bacteraemia with Piperacillin-Tazobactam or Cefuroxime

Author Block:

J. Knudsen, F. Jansåker, S. Thønnings; Depart of Clin Microbiol, Hvidovre Hosp., Hvidovre, Denmark

Abstract Body:

Background: In one of six hospitals in Copenhagen, the empiric antimicrobial treatment was changed due to high prevalence of ESBL-producing *Klebsiella pneumoniae*, and cephalosporins were avoid after January 2010. We wanted to study the outcome after bacteremia with *E. coli*, two years before and after 2010, in six hospitals, when using piperacillin-tazobactam (PT) and cefuroxime (XM). **Methods:** We retrospectively identified *E. coli* bacteremia patients in the Copenhagen area, 2008 to 2011. The ESBL-rate in *E. coli* in this period was 4-9%. Data on the empiric treatment, patient demographics, Charlson co-morbidity index (CCI), and 30 and 90 days all-cause mortality were retrieved, for patients treated with either PT of XM. The study was approved by the Danish authorities.**Results:** 1207 episodes were found, 228 and 979 cases, were treated with PT and XM, respectively. Before 2010, the patients treated with PT compared to XM, had higher 30 and 90 day mortality, i.e. 53% and 58% for PT, and 14% and 22% for XM ($p<0.01$). After 2010, the same figures were for PT 15% and 22%, and for XM 16% and 23%, respectively ($p's>0.8$). Among patients with ESBL-producing isolates, treated with PT 16% died, and for XM 18% died within 30 days ($p=1.0$). In the multivariate analyze of the 30 day mortality, no difference were seen between the two treatments, and mortality could only be correlated to high age, high co-morbidity, and to be hospital-associated or acquired.**Conclusions:** The higher 30 and 90 days mortality rate for PT treatment of *E. coli* bacteremia seen before 2010 (53% and 58%), where not predictive for outcome when PT was used more often (14% and 22%). We saw no differences in outcome for PT and XM for patients with ESBL-producing *E. coli*. In an area with low prevalence of resistance, no difference in outcome after *E. coli* bacteremia could be seen for PT and XM. Our results highlight the importance on the phenomenon confounding by indication.

Author Disclosure Block:

J. Knudsen: None. **F. Jansåker:** None. **S. Thønnings:** None.

Poster Board Number:

SATURDAY-448

Publishing Title:

Ceftaroline Fosamil Use in a National Health Care System

Author Block:

C. R. FREI[1], G. C. Lee[1], K. R. Reveles[1], **N. K. Boyd**[1], K. M. Sorensen[1], X. Jones[2], M. Bollinger[2]; [1]The Univ. of Texas at Austin and The Univ. of Texas Hlth.Sci. Ctr. San Antonio, SAN ANTONIO, TX, [2]The Univ. of Texas Hlth.Sci. Ctr. San Antonio and the South Texas Veterans Hlth.Care System, SAN ANTONIO, TX

Abstract Body:

Background: The United States (U.S.) has approved ceftaroline fosamil for acute bacterial skin and skin structure infections and community acquired bacterial pneumonia, but it is unknown how ceftaroline is being used in real-world settings and how adverse effects and mortality compare to clinical trials. The study objective was to describe ceftaroline use, adverse effects, and mortality in patients admitted to U.S. Veterans Health Administration (VHA) hospitals. **Methods:** This was a phase 4, population based, epidemiologic study of patients at least 18 years of age who received at least one ceftaroline dose within 14 days of admission at 69 VHA hospitals in 41 U.S. states/territories from 10/1/10 to 9/30/14. Data from VHA repositories were linked using a unique patient identifier. ICD9CM codes were used to determine diagnoses and adverse effects. Descriptive statistics were used to summarize patient demographics, adverse effects after 14 days of therapy, and patient mortality. **Results:** A total of 824 patients met study criteria. Patients were 97% male, 55% white, 26% black, 15% Hispanic, and 4% other/missing race, 61 years of age (median), with a Charlson score of 4 (median). Diagnoses included skin (39%), sepsis (34%), osteomyelitis (28%), diabetic foot (21%), pneumonia (17%), bacteremia (14%), endocarditis (8%), meningitis (3%), and device (2%) infections. Ceftaroline use was 37% first-line and 56% second-line. The typical patient received ceftaroline 3 days (median) after their hospital admission and for 5 days (median) in the hospital. Rates of eosinophilia, leukopenia, leukocytosis, fibromyalgia, myalgia and myositis, and polymyalgia were less than 1%. Rates of 30/60/90-day mortality were: overall (4/6/9%), skin (2/3/6%), sepsis (5/8/11%), osteomyelitis (4/5/7%), diabetic foot (2/2/5%), pneumonia (8/14/16%), bacteremia (6/10/12%), endocarditis (6/11/11%), meningitis (0/4/8%), and device (0/0/0%). **Conclusions:** Ceftaroline is used in VHA hospitals for many types of infections. Adverse effects and patient mortality are low, and comparable with rates from clinical trials. Additional studies are needed to compare these ceftaroline results to other drugs used in similar situations.

Author Disclosure Block:

C.R. Frei: E. Grant Investigator; Self; Bristol-Myers Squibb, Actavis/Forest, Pfizer, Pharmacyclics. **G.C. Lee:** None. **K.R. Reveles:** E. Grant Investigator; Self; Merck/Cubist. **N.K. Boyd:** None. **K.M. Sorensen:** None. **X. Jones:** None. **M. Bollinger:** None.

Poster Board Number:

SATURDAY-449

Publishing Title:

A Polymyxin Based Triple Combination Combats ST258 KPC-2 Producing *Klebsiella pneumoniae*

Author Block:

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Abstract Body:

Background: High bacterial density KPC-producing *K. pneumoniae* cause severe infections with excessively high mortality rates. Our objective was to identify an antimicrobial regimen capable of achieving bactericidal activity against a variety of clinical KPC-2 producing *K. pneumoniae* isolates.**Methods:** Four clinical KPC-2 producing *K. pneumoniae* isolates were used (Isolate, PMB/MERO/RIF MICs: 9A, 0.5/16/64; 24A, 0.5/64/>64; 27A, 1/32/>64; 5A, 64/32/64 mg/L) and each contained other β-lactamases concurrently. Static 48h time-kill experiments with polymyxin B (PMB) (6 mg/L), meropenem (MERO) (100, 200 mg/L), and rifampin (RIF) (3.5 mg/L) were conducted at a starting inoculum of 10^8 CFU/mL. A 10-day hollow fiber infection model (HFIM) was utilized to simulate human pharmacokinetic profiles at traditional dosage regimens for the triple combination at an inoculum of 10^8 CFU/mL. **Results:** In time-kill studies, PMB alone caused >3 $\log_{10}$CFU/mL killing within 6h against 9A, 24A and 27A but regrowth approaching growth controls occurred by 24h. PMB alone showed no killing activity against 5A. PMB (6 mg/L), MERO (100 mg/L), and RIF (3.5 mg/L) in combination displayed sustained bactericidal activity from 6h through 48h against 9A, 24A and 27A (Strain, 6h log reduction: 9A, -4.6; 24A, -8.3; 27A, -4.7). Against 5A, a combination of PMB 6 mg/L, MERO 100 mg/L and RIF 3.5 mg/L achieved -2.2 and -1.6 log reductions at 24 and 48h, respectively. This triple combination with MERO 200 mg/L instead of 100 mg/L achieved maximal bacterial reduction at 48h with a -5.0 log reduction against 5A. Among our 4 clinical isolates in time-kill experiments, PMB MIC predicted bacterial killing well. Regrowth in the HFIM occurred more rapidly than in time-kill studies although the PMB, MERO, and RIF triple sustained bactericidal activity longer than any double combination. **Conclusion:** Triple drug combination therapy using polymyxin B, meropenem and rifampin was bactericidal *in vitro* and may be of clinical utility in the fight against KPC-producing *K. pneumoniae*.

Author Disclosure Block:

Z.P. Bulman: None. **M.J. Satlin:** None. **B. Kreiswirth:** None. **J.B. Bulitta:** None. **C.B. Landersdorfer:** None. **J.R. Lenhard:** None. **V. Petraitis:** None. **T.J. Walsh:** None. **P.N. Holden:** None. **A. Forrest:** None. **R.L. Nation:** None. **J. Li:** None. **B.T. Tsuji:** None.

Poster Board Number:

SATURDAY-450

Publishing Title:

Empiric Meropenem versus Ceftazidime for Severe Community Acquired Pneumonia (SCAP)

Author Block:

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Abstract Body:

Background: SCAP is associated with 67% mortality in Singapore. Empiric β-lactam plus macrolide or fluoroquinolone is recommended for SCAP in many guidelines but the most optimal regimen is not established. Locally, ceftazidime (CEF) or meropenem (MER) is preferred for melioidosis cover. We compare the outcomes between these 2 regimens. **Methods:** A single center, retrospective cohort study was performed from Jan 11 to Apr 15 on patients (18 years & above) with SCAP, requiring intensive care unit (ICU) admission & mechanical ventilation within 48 hours of admission. Collected data included demographics, clinical data, microbiological data & antibiotic use data. Primary outcome was overall mortality (OM) within 30 days. Secondary outcomes were CAP-related mortality (CM) & clinical response. Chi-Square, Fisher's Exact, Mann Whitney U, log-rank tests & Kaplan-Meier survival analysis were employed. Multivariate Cox regression was used to adjust for confounders. **Results:**59 patients received CEF while 41 received MER; median age was 64 (interquartile range, IQR 56-75) & 62 years (IQR 54-74) respectively. Age, gender, Charlson comorbidity index, APACHE II score, CURB-65 score & pneumonia severity index were similar for both groups. CEF was mostly paired with antipneumococcal quinolones (88%) while MER was paired with azithromycin (73%). 30-day OM was 63% & 29% for CEF & MER groups respectively (p=0.001) while 30-day CM was 34% & 22% respectively (p=0.195). Median time to OM for CEF was 7 days vs >30 days for MER (p<0.001) while median time to CM was not significantly different (p=0.071). After adjusting for confounders, age & immunocompromised state, CEF use was associated with greater OM - hazards ratio, HR 3.0 (95% CI: 1.5-5.8). After adjusting for concurrent bacteremia, CEF use was associated with greater CM - HR 2.5 (95% CI: 1.1-5.7). More patients on MER had antibiotic de-escalation after a median of 3 days (68% vs 32%, p<0.001) & recovered at the end of therapy (59% vs 29%, p=0.003). 22% of patients on CEF required escalation to a carbapenem. Length of hospital & ICU stay was similar for survivors in both groups. **Conclusions:** Empiric MER plus macrolide may be more effective than CEF plus fluoroquinolone in reducing early mortality among patients with SCAP. Prompt antibiotic de-escalation can be considered after 3 days if patients have adequate response.

Author Disclosure Block:

N.G. Chua: None. **W. Lee:** None. **Y.X. Liew:** None. **S. Tang:** None. **Y. Zhou:** None. **K. Patel:** None. **M.P. Chlebicki:** None. **A.L. Kwa:** None.

Poster Board Number:

SATURDAY-451

Publishing Title:

Patient Outcomes with Complicated Intra-Abdominal Infections (cIAI) Caused by Gram-Negative Pathogens from IGNITE1: A Phase 3 Study to Evaluate the Efficacy and Safety of Eravacycline (ERV) versus Ertapenem (ETP)

Author Block:

J. Sutcliffe[1], A. Marsh[1], S. Izmailyan[1], L. Tsai[1], M. Castanheira[2], J. Solomkin[3], P. Horn[1]; [1]Tetraphase Pharmaceuticals, Inc., Watertown, MA, [2]JMI Lab., North Liberty, MA, [3]Univ. of Cincinnati Coll. of Med., Cincinnati, OH

Abstract Body:

Background: Eravacycline (ERV) is a novel, fully synthetic fluorocycline antibiotic of the tetracycline class with broad-spectrum activity. It is being developed for the treatment of serious infections, including those caused by MDR pathogens. ERV was evaluated in a phase 3, randomized, double-blind, multicenter study where patients with cIAI received either ERV (1.0 mg/kg IV BID) or ETP (1g IV QD). ERV demonstrated non-inferiority to ertapenem in the primary analysis (1). This analysis evaluated patient outcomes based on the resistant phenotypes and genotypes present in baseline Gram-negative pathogens.**Methods:** Baseline blood and cIAI specimens were obtained from all randomized patients. Susceptibility to study antibiotics and relevant comparators was determined by CLSI broth microdilution methods. Isolates of the same bacterial species from a single subject were evaluated by pulsed-field gel electrophoresis (PFGE) to assess genetic relatedness. Gram-negative aerobes resistant to any $3^{rd}/4^{th}$ generation cephalosporin and/or carbapenem were screened using published PCR conditions for narrow- and extended-spectrum β-lactamases (ESBL) and carbapenemases. Overexpression of chromosomal AmpC β-lactamases (↑AmpC) was evaluated.**Results:** 541 patients were randomized; baseline isolates were cultured from 446 patients. Following PFGE testing of 708 isolates, 1353 unique isolates were identified among the 446 patients. 151 Gram-negative isolates were tested for β-lactamase resistance mechanisms. CTX-M enzymes were the most common ESBLs among Enterobacteriaceae. KPC, IMP, VIM and OXA carbapenemases were detected.**Conclusions:** ERV was efficacious in patients with cIAI including those infected with difficult-to-treat Gram-negative pathogens.

IGNITE 1: Favorable Outcomes[1] (Clinical Cure at TOC in the micro-ITT Population) for Patients with Gram-Negative Pathogens		
Pathogen	**ERV**	**ETP**
All baseline pathogens	191/220 (86.8)	198/226 (87.6)
Gram-negative pathogens	155/182 (85.2)	162/186 (87.1)

CEPH-R[2]	25/27 (92.6)	24/28 (85.7)
Confirmed ESBL	22/24 (91.7)	16/19 (84.2)
Confirmed ↑AmpC	8/8 (100)	7/8 (87.5)
Carbapenem-resistant	6/6 (100)	8/9 (88.9)
Confirmed carbapenemase	4/4 (100)	4/5 (80.0)
MDR[3]	18/20 (90.0)	15/16 (93.8)
[1] Results are presented as n/N (%), where n=subjects with favorable outcomes, N=total subjects in the category, and % = n/N*100; [2] CEPH-R=3rd/4th generation cephalosporin-resistant according to 2015 CLSI M100 guidance; [3] multidrug resistance according to modified ESCMID criteria (Magiorakos, et al., 2011)		

Author Disclosure Block:

J. Sutcliffe: D. Employee; Self; Joyce Sutcliffe. **A. Marsh:** D. Employee; Self; Andrew Marsh. **S. Izmailyan:** D. Employee; Self; Sergey Izmailyan. **L. Tsai:** D. Employee; Self; Larry Tsai. **M. Castanheira:** D. Employee; Self; Mariana Castanheira. **J. Solomkin:** C. Consultant; Self; Joseph Solomkin. **P. Horn:** D. Employee; Self; Patrick Horn.

Poster Board Number:

SATURDAY-452

Publishing Title:

Understanding the Inhibition of Mexb and Adeb Efflux Pump Transporters by PaβN

Author Block:

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Abstract Body:

Background: Multi drug (MDR) efflux transporters play key roles in bacterial pathogenicity and resistance to antibiotics. Agents that can inhibit the tripartite efflux pump systems, MexB, AcrB and AdeB, can play an important role in reviving antibiotics that have become resistant in a variety of Gram-negative pathogens. Computational approaches provide useful insight into the binding of inhibitors to the MDR efflux pumps and designing new inhibitors. We studied the interaction of PAβN with MexB and AdeB transporters both from *Acinetobacter baumannii* to explore the interactions that play key roles in inhibiting these transporters.**Methods:** The interaction of PAβN with MexB and AdeB transporters was explored using computational methods. The structures of the transporters were generated by homology modelling. After molecular docking of PAβN to the transporters, 50s molecular dynamics (MD) simulations combined with MM-PBSA/MM-GBSA were performed to understand the molecular interactions of PAβN with the binding pockets of the transporters.**Results:** Our results showed that PAβN passes through the multisite binding pocket of AdeB and MexB. The Phe-loop of the binding monomer guides the ligand, by shifting through the narrow channel to pass the proximal binding pocket, towards the distal binding pocket. Ser134, Thr668, GLn42 and Glu665 of AdeB and Ser132, Ser133, Ser135, Thr44, Phe618 and Thr668 of MexB formed hydrogen bonds with PAβN during the course of the simulation. MD simulations further showed that PAβN behaves like a climber; it uses Phe residues of the Phe-cluster as hooks to go forward in the channel, then it climbs and goes upward in distal binding pockets along the intramolecular channel of transporters. The differences in amino acid sequences in the binding site of the pumps dictate in the way PAβN interacts and inhibits these transporters by generating hydrophobic microenvironments with diverse strength in the binding site of different complexes.**Conclusions:** Information obtained from this study provides detailed insight into the interaction of PAβN with AdeB and MexB transporters in *A. baumannii*. This molecular interaction with the key residues within the binding pocket will contribute to the design of new effective and selective efflux pump inhibitors that could play key roles in reversing antimicrobial resistance.

Author Disclosure Block:

S. Jamshidi: None. **J.M. Sutton:** None. **K.M. Rahman:** None.

Poster Board Number:

SATURDAY-453

Publishing Title:

A Reducing Cell-free *In Vitro* Expression System to Generate High Quantities of Antibacterial Microcin S

Author Block:

C. Auerbach[1], K. Zimmermann[2], F. Gunzer[1]; [1]TU Dresden, Med. Faculty 'Carl Gustav Carus', Dresden, Germany, [2]SymbioPharm GmbH, Herborn-Hörbach, Germany

Abstract Body:

The number of infections caused by antibiotic resistant bacteria is vastly increasing. Often, conventional antibiotics don't make an impact anymore. Therefore, detection of so far unknown antimicrobial substances is required. The probiotic *Escherichia coli* G3/10, a component of the drug Symbioflor2, is producing such an antibacterial peptide, called microcin S (MccS). To investigate its antibacterial spectrum and mode of action in more detail quantitative amounts of biologically active MccS are needed. Since recombinant intrabacterial expression delivered no sufficient concentrations - due to its assumed toxicity - a bacteria based but cell-free *in vitro* expression system was set up and optimized. This system consists of a cell extract of *E. coli* BL21 Rosetta 2(DE3), a fructose-1,6-bisphosphate based energy buffer enriched with ATP and NAD, amino acids and template DNA encoding either a carboxy- or an amino-terminally 6 x His-tagged or an untagged microcin S (*mcsS*). Linear templates were generated by a new three round PCR technique with primers containing all up- and downstream elements required for efficient protein expression. Cloning of linear amplicons into pUC19 provided circular templates. To improve protein folding, any reducing substances were excluded from the reaction mixture. The impact on antimicrobial activity was analyzed after MccS samples were treated with oxidizing glutathione. Performance of MccS synthesis was measured by dot blot followed by immune detection. Activity of MccS was determined by performing an agar-diffusion assay against sensitive strains. Best *in vitro* expression rates could be achieved by using circular DNA templates in combination with a concentrated cell extract. However, antibacterial activity could only be detected when the C-terminus of MccS is tag-free. Presence of the MccS signal peptide didn't cause any influence on expression rate and activity. Biological activity was improved by treating the samples with glutathione. The results confirm that we were able to develop a system to produce high yields of active MccS *in vitro* under reducing conditions to improve formation of disulfide bonds necessary for proper folding. MccS concentrations reached with this system are sufficient to get better insight into its structure and mode of action.

Author Disclosure Block:

C. Auerbach: None. **K. Zimmermann:** None. **F. Gunzer:** None.

Poster Board Number:

SATURDAY-454

Publishing Title:

Isolation of *Shigella* Cross-Reactive Environmental Bacteria as Potential Shigellosis Live Vaccine Candidate Strains

Author Block:

N. Azmuda[1], R. Bilkis[1], S. I. Khan[2], N-K. Birkeland[3]; [1]Jahangirnagar Univ., Dhaka, Bangladesh, [2]Univ. of Dhaka, Dhaka, Bangladesh, [3]Univ. of Bergen, Bergen, Norway

Abstract Body:

Background: Shigellosis is recognized as an endemic disease worldwide**,** including Bangladesh and one of the most common causes of morbidity and mortality among children. Approximately 125 million shigellosis cases and 14,000 related deaths occur annually in Asia. There is a strong need for a shigellosis vaccine, particularly because of the global emergence of antibiotic-resistant shigellae strains. Protective immunity against shigellosis is serotype specific and directed against the surface lipopolysaccharide antigens. At present, no effective vaccine against *Shigella* infection exists. Noninfectious bacterial genera sharing common surface antigens with *Shigella* spp. could be potential vaccine candidates for combating shigellosis. **Methods:** A number of serologically cross-reactive environmental bacteria were isolated by an immunocapturing technique using group specific polyvalent *Shigella*-antisera. The isolates were identified and characterized by cultural and biochemical properties, 16S rRNA gene sequencing and Western blot analysis.**Results:** Environmental isolates of *Aeromonas veronii*, *Enterobacter cloacae*, *Escherichia coli*, *Klebsiella pneumoniae*, *Stenotrophomonas maltophilia* and *Plesiomonas shigelloides* showed cross-reactivity with a number of group- and type-specific *Shigella* antisera due to sharing of common surface proteins and/or LPS. *Aeromonas veronii* and *Enterobacter cloacae* cross-reacted with *Shigella boydii* type 16 and 15 monovalent antisera, respectively, through reaction with O-antigens, thus revealing a type of inter-species cross-reaction not reported earlier. Many of the isolates showed cross-reactivity with more than one group of type-specific *Shigella*-antisera due to the presence of immunogenic proteins. However, *Plesiomonas shigelloides* were cross-reactive with *Shigella sonnei* phase I monovalent antisera only due to immunogenic LPS.**Conclusions:** This study revealed that important *Shigella* spp. surface antigens are shared by a number of environmental bacteria. Naturally occurring environmental bacteria expressing surface lipopolysaccharide antigens specific for certain types of *Shigella* could be a good choice for vaccine candidates against shigellosis.

Author Disclosure Block:

N. Azmuda: None. **R. Bilkis:** None. **S.I. Khan:** None. **N. Birkeland:** None.

Poster Board Number:

SATURDAY-455

Publishing Title:

***In Vitro* Activity of Wck 4873 (Nafithromycin) Against Resistant Subsets of *Streptococcus pneumoniae* from a Global Surveillance Program (2014)**

Author Block:

D. J. Farrell, H. S. Sader, P. R. Rhomberg, R. K. Flamm, R. N. Jones; JMI Lab., North Liberty, IA

Abstract Body:

Background: WCK 4873 (INN: Nafithromycin) is a novel lactone ketolide, currently in clinical development for the treatment of community acquired bacterial pneumonia (CABP). WCK 4873 has completed SAD and MAD Phase 1 studies in Europe and an intra-pulmonary pharmacokinetic study in USA. WCK 4873 was awarded QIDP status in 2015. In this study, WCK 4873 was tested against contemporary *Streptococcus pneumoniae* (SPN) clinical isolates (including resistant subsets) collected in medical centers worldwide as part of the 2014 SENTRY Antimicrobial Surveillance Program.**Methods**: A total of 1,911 SPN, were tested for susceptibility (S) against WCK 4873 and multiple comparator agents by reference broth microdilution methods and interpretive criteria. Number of isolates by geography (country or region) were as follows: USA (712), Europe (698, 23 countries), Asia Pacific (251, 8 countries) and Latin America (250, 11 countries).**Results**: Against all 1,911 SPN, WCK 4873 was very active ($MIC_{50/90}$, 0.015/0.06 µg/mL) with all strains inhibited at MIC values ≤0.25 µg/mL (Table). Telithromycin (TEL) S (CLSI/EUCAST) rates were 99.9/89.3% and WCK 4783 was up to eight-fold more potent than TEL ($MIC_{50/90}$, 0.015/0.5 µg/mL). Using the EUCAST S breakpoint for TEL of ≤0.25 µg/mL, 204 strains were non-S to TEL and all 204 strains demonstrated WCK 4873 MIC values of ≤0.25 µg/mL ($MIC_{50/90}$, 0.06/0.12 µg/mL). WCK 4873 was four-fold less active against penicillin (PEN) -intermediate and -resistant (R) strains compared to PEN-S strains with WCK 4783 $MIC_{50/90}$ values of 0.06/0.12, 0.06/0.12 and 0.015/0.03 µg/mL, respectively. Ceftriaxone (CRO) non-S was 9.3% using CLSI non-meningitis breakpoints. Erythromycin (ERY) R was 37.9%, and 19.7% were R to clindamycin (CLI). WCK 4783 retained good activity against ERY-R/CLI-S strains ($MIC_{50/90}$, 0.03/0.06 µg/mL) and ERY-R/CLI-R strains ($MIC_{50/90}$, 0.03/0.12 µg/mL).**Conclusions**: WCK 4873 demonstrated potent activity against contemporary (2014) global SPN isolates and retained good activity against strains with lower activity to TEL (including 204 strains non-S by EUCAST breakpoints), PEN, CRO, ERY, and CLI.

Author Disclosure Block:

D.J. Farrell: H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Wockhardt. **H.S. Sader:** H. Research Contractor; Self; This study and

abstract presentation were funded by a research grant from Wockhardt. **P.R. Rhomberg:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Wockhardt. **R.K. Flamm:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Wockhardt. **R.N. Jones:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Wockhardt.

Poster Board Number:

SATURDAY-456

Publishing Title:

WCK 4873 (Nafithromycin): *In Vitro* and *In Vivo* Activity of Novel Lactone-Ketolide, against Clinically Relevant *S. pneumoniae* (SPN) Resistotypes and Methicillin-Sensitive *S. aureus* (MSSA)

Author Block:

J. S. Satav, S. S. Takalkar, A. M. Kulkarni, **S. S. Bhagwat**, M. V. Patel; Wockhardt Res. Ctr., Aurangabad, India

Abstract Body:

Background: WCK 4873 is a lactone-ketolide (Phase 1 studies completed in US, Europe) with a broad spectrum activity against respiratory-tract pathogens (RTI). WCK 4873 is characterized by its potent activity against macrolide and ketolide resistant SPN. Herein, we describe *in vitro* and *in vivo* activity of WCK 4873 against key RTI pathogens. **Methods:** WCK 4873 MICs were determined for six SPN strains (resistotypes: *ermB*, *mef*, *ermB+mef*, L4+L22) and two MSSA. MICs were determined employing agar dilution methods (Medium: MHA + 5% sheep blood). *In vivo* efficacy ($ED_{50/90}$) was determined through murine peritonitis model. Mice were intraperitoneally infected ($5x10^7$-$2x10^8$ CFU/mL) and oral treatment (twice-daily) was initiated 1h post-infection. $ED_{50/90}$ values were calculated by probit analysis. PK studies were carried at the corresponding efficacy doses. Mouse serum drug concentrations were estimated by LCMS-MS method.**Results:** WCK 4873 MICs for various RTI pathogens ranged from 0.007-0.5 µg/mL, while telithromycin MICs were 0.015-8 µg/mL. *In vivo*, ED_{50} for WCK 4873 ranged from 0.59-27.2 mg/kg while ED_{90} ranged from 0.79-35 mg/kg (table). ED_{90} values of telithromycin ranged from 3.3->150 mg/kg. Mouse 24h serum exposures ($fAUC_{0-24}$) at doses 50 and 100 mg/kg were 1.0 and 2.5 µg.h/mL respectively.

Organism (Resistance mechanism)	WCK 4873 MIC (µg/mL)	ED_{90} (mg/kg)
SPN ATCC 49619 (WT)	0.015	3.1
SPN 772 (*mef*)	0.06	31.1
SPN 3591 (*ermB*)	0.007	11.8
SPN 3773 (*ermB*)	0.5	35.0
SPN 1179 (*mef+ermB*)	0.12	8.32
SPN 6683 (Mutations in L4 and L22)	0.03	15.5
MSSA ATCC 25923	0.06	6.2

MSSA 5044	0.06	11.8

Conclusion: WCK 4873 (Nafithromycin) provided promising therapeutic effect against both SPN expressing diverse clinically relevant resistance mechanisms and MSSA at significantly low doses.

Author Disclosure Block:

J.S. Satav: D. Employee; Self; Wockhardt Research Center. **S.S. Takalkar:** D. Employee; Self; Wockhardt Research Center. K. Shareholder (excluding diversitied mutual funds); Self; Wockhardt Ltd.,. **A.M. Kulkarni:** D. Employee; Self; Wockhardt Research Center. **S.S. Bhagwat:** D. Employee; Self; Wockhardt Research Center. K. Shareholder (excluding diversitied mutual funds); Self; Wockhardt Ltd.,. **M.V. Patel:** D. Employee; Self; Wockhardt Research Center. K. Shareholder (excluding diversitied mutual funds); Self; Wockhardt Ltd.,.

Poster Board Number:

SATURDAY-457

Publishing Title:

Activity of Novel Oxazolidinone Tedizolid against Clinical Gram-Positive Pathogens Collected from Canadian Hospitals: CANWARD 2013-15

Author Block:

A. R. Golden[1], H. J. Adam[2], M. Baxter[1], K. A. Nichol[2], B. Weshnoweski[2], R. Vashisht[1], S. Biju[1], J. A. Karlowsky[2], D. J. Hoban[2], G. G. Zhanel[1], The Canadian Antimicrobial Resistance Alliance (CARA); [1]Univ. of Manitoba, Winnipeg, MB, Canada, [2]Diagnostic Services Manitoba, Winnipeg, MB, Canada

Abstract Body:

Background: The novel oxazolidinone tedizolid (TZD) has been approved for the treatment of acute bacterial skin and skin structure infections. TZD demonstrates high antibacterial potency, and is active against Gram-positive pathogens including methicillin-susceptible and -resistant *Staphylococcus aureus* (MSSA/MRSA). TZD demonstrates numerous positive attributes, including 6-day short course therapy, once daily oral or intravenous dosing with no requirement for dose adjustment across a range of patient factors, a low potential for drug-drug interactions and a well-tolerated safety profile. Currently, TZD is being evaluated in Phase 3 trials for the treatment of hospital-acquired and ventilator-associated bacterial pneumonia.**Methods:** Beginning in January 2013, Canadian hospital laboratories (15 sites in 2013, 13 sites in 2014 and 2015) were asked to submit consecutive pathogens from blood, respiratory, urine and wound infections as part of the CANWARD 2013, 2014 and 2015 studies. Antimicrobial susceptibility testing was performed in accordance with CLSI guidelines.**Results:** The table below demonstrates the activity of TZD (MIC, μg/mL) and select comparators against Canadian clinical Gram-positive pathogens tested to date during CANWARD 2013, 2014 and 2015 studies:

Organism (n)	TZD Susceptibility*			TZD MIC_{50}/MIC_{90}	TZD Range	Comparators (MIC_{50}/MIC_{90})			Fold Reduction in TZD MIC_{50}/MIC_{90} vs. LZD MIC_{50}/MIC_{90}
	%S	%I	%R			LZD	VAN	DAP	
MRSA (417)	99.7	0.3	-	0.25/0.25	0.12-1	2/2	0.5/1	0.25/0.5	8/8
HA-MRSA (248)	99.4	0.6	-	0.25/0.5	0.12-1	2/2	1/1	0.25/0.5	8/4

CA-MRSA (169)	100	-	-	0.25/0.25	0.12-0.5	2/2	0.5/1	0.25/0.5	8/8
MSSA (1,857)	100	-	-	0.25/0.25	0.06-0.5	2/2	0.5/1	0.25/0.5	8/8
S. epidermidis (200)	NA	NA	NA	0.12/0.12	0.06-0.5	0.5/1	1/2	0.25/0.25	4/8
E. faecalis (309)	100	-	-	0.25/0.25	0.12-0.5	2/2	1/2	1/2	8/8
VAN-resistant *E. faecium* (25)	NA	NA	NA	0.25/0.5	0.12-2	2/4	>32/>32	2/2	8/8
VAN-susceptible *E. faecium* (107)	NA	NA	NA	0.25/0.5	0.12-0.5	2/2	0.5/1	2/2	8/4
S. agalactiae (178)	100	-	-	0.25/0.25	≤0.03-0.25	1/2	0.5/0.5	0.25/0.25	4/8
S. pneumoniae (481)	NA	NA	NA	0.12/0.25	≤0.03-0.5	1/2	0.25/0.25	0.12/0.12	8/8
S. pyogenes (137)	100	-	-	0.25/0.25	≤0.03-0.25	1/2	0.5/0.5	0.12/0.12	4/8
*, Interpretive breakpoints defined by FDA; S, susceptible; I, intermediate; R, resistant; NA, breakpoints not available; HA, healthcare-acquired; CA, community-acquired; LZD, linezolid; VAN, vancomycin; DAP, daptomycin.									

Conclusion: Based on MIC_{50} and MIC_{90} values, TZD demonstrated four- to eight-times greater activity than LZD and greater potency than VAN versus Gram-positive organisms isolated from Canadian hospitals in the surveillance period. TZD also demonstrated equal or greater potency than DAP against *Staphylococcus* and *Enterococcus* spp. The highest recorded MIC value for TZD was 2 μg/mL in a VAN-resistant *E. faecium*.

Author Disclosure Block:

A.R. Golden: None. **H.J. Adam:** None. **M. Baxter:** None. **K.A. Nichol:** None. **B. Weshnoweski:** None. **R. Vashisht:** None. **S. Biju:** None. **J.A. Karlowsky:** None. **D.J. Hoban:** I. Research Relationship; Self; Abbott Laboratories Ltd, Astellas Pharma Canada Inc, Cubist Pharmaceuticals, Galderma, Paladin Labs, Pharmascience Inc, Sunovion Pharmaceuticals, Tetraphase Pharmaceuticals, The Medicines Company, Pfizer Inc. **G.G. Zhanel:** I. Research Relationship; Self; Abbott Laboratories Ltd, Astellas Pharma Canada Inc, Cubist

Pharmaceuticals, Galderma, Paladin Labs, Pharmascience Inc, Sunovion Pharmaceuticals, Tetraphase Pharmaceuticals, The Medicines Company, Pfizer Inc.

Poster Board Number:

SATURDAY-458

Publishing Title:

Comparison of *In Vitro* Susceptibility Testing of Tedizolid (Tzd) and Linezolid (Lzd) Against Isolates of Nontuberculous Mycobacteria (Ntm)

Author Block:

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Abstract Body:

NTM are responsible for infections including respiratory, cutaneous, and disseminated disease. Most clinically relevant species of NTM are multidrug resistant, and there is a paucity of antimicrobials with efficacy against these organisms. Multiple species of NTM have been shown to be susceptible to LZD, the first commercially available oxazolidinone. TZD is a new oxazolidinone with improved *in vitro* and intracellular potency against *Mycobacterium tuberculosis,* including multidrug resistant strains, and some species of NTM compared with LZD. Using the current Clinical and Laboratory Standards Institute (CLSI) recommended method of broth microdilution, susceptibility testing of 170 isolates of rapidly growing mycobacteria (isolates of *M. fortuitum*, *M. smegmatis, M. mucogenicum, M. chelonae,* and *M. abscessus* complexes) showed equivalent or lower (1- to 8-fold MIC_{50} and/or MIC_{90} values) MIC_{90} values for TZD compared to LZD. The TZD MIC_{90} for *M. abscessus* subsp. *abscessus* (n=81 isolates) and *M. abscessus* subsp. *massiliense* (n=12) was 8 μg/mL and 4 μg/mL, respectively, compared to a LZD MIC_{90} of 32 μg/mL for both. The MIC_{90} for *M. fortuitum* (n=20) was 2 μg/mL for TZD and 4 μg/mL for LZD. *M. chelonae* (n=22) had TZD and LZD MIC_{90} values of 2 μg/mL and 16 μg/mL, respectively. Slowly growing NTM (n=148), including 7/7 *M. marinum*, 7/7 *M. kansasii*, and 128/128 of other less commonly isolated species had TZD MIC values ≤1 μg/mL and LZD MIC values ≤8 μg/mL. Less than 10% of *Mycobacterium avium* complex isolates (n=100) and *M. simiae* isolates (n=8) had TZD MIC values ≤1 μg/mL or LZD MIC values ≤8 μg/mL. *M. arupense* (n=9 isolates) had MIC_{50} values of 4 μg/mL and 16 μg/mL for TZD and LZD, respectively. These findings demonstrate greater *in vitro* potency of TZD than LZD against NTM and suggest that evaluation of TZD as a potential treatment agent for infections caused by selected NTM is warranted.

Author Disclosure Block:

B.A. Brown-Elliott: E. Grant Investigator; Self; Merck. **J.V. Philley:** N. Other; Self; Clinician. **D.E. Griffith:** N. Other; Self; Clinician. **R.J. Wallace:** F. Investigator; Self; Co-investigator.

Poster Board Number:

SATURDAY-459

Publishing Title:

***In Vitro* Activity of Tedizolid Against Gram-positive Pathogens Isolated from Patients with Skin and Skin Structure Infections in the United States and Europe (2014-2015)**

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Abstract Body:

Background: Tedizolid (TZD) is approved for treatment of acute bacterial skin and skin structure infections (ABSSSI) in the United States (USA), European Union (EU), and Canada. The*in vitro* activity of TZD was tested against Gram-positive pathogens isolated from patients with SSSIs collected in 2014 and 2015 as part of an ongoing resistance surveillance program. **Methods:** Non-duplicate, single-patient SSSI clinical isolates (USA/EU) of *Staphylococcus aureus* (SA; 3019/1778), coagulase-negative staphylococci (CoNS; 159/283), β-hemolytic streptococci (BHS; 384/231), and enterococci (ENT; 233/328) were tested for antimicrobial susceptibility (S) as per CLSI guidelines. MIC values for TZD and linezolid (LZD) were read both at (1) the first well where trailing begins (80% read; TZD80 and LZD80) as per CLSI M07-A10 and (2) at no-growth endpoint (100% growth inhibition; TZD100 and LZD100). USA-FDA and EUCAST breakpoints were used to determine %S.**Results:** TZD activity ($MIC_{50/90}$/Range/%S) was similar for isolates obtained from USA or EU (Table). For SA, 100.0% of isolates were S to both LZD and TZD, and TZD was four- to eight-fold more potent than LZD. For 442 CoNS isolates, $MIC_{50/90}$ values for TZD80 and TZD100 were 0.06/0.12 µg/mL and 0.12/0.25 µg/mL, respectively, and for LZD80 (100.0% S) and LZD100 were 0.5/1 µg/mL and 1/1 µg/mL, respectively. All 615 isolates of BHS (TZD80/100 MIC_{90}, 0.12/0.25 µg/mL; LZD80/100 MIC_{90}, 1/2 µg/mL) were S to TZD and LZD according to both EUCAST and USA-FDA-approved breakpoints. For 561 ENT isolates, the $MIC_{50/90}$ values for TZD80 and TZD100 were 0.12/0.25 µg/mL and 0.25/0.5 µg/mL, respectively, and for LZD80 and LZD100 were 1/1 µg/mL and 2/2 µg/mL, respectively. **Conclusion**: TZD had very potent activity against recent USA and European SSSI isolates of SA, CoNS, BHS, and ENT. In general, TZD80 MIC values (read as per CLSI M07-A10, 2015) were one doubling dilution lower than those read at TZD100. Based on MIC_{90} values, TZD was 4- to 8-fold more potent than LZD, consistent with previous reports.

	***S. aureus* (USA; n = 3,019)**			***S. aureus* (Europe; n = 1,778)**		
	MIC (µg/mL)			**MIC (µg/mL)**		
	50/90%	**Range**	**%S**	**50/90%**	**Range**	**%S**
TZD80	0.12/0.12	0.015-0.25	-	0.12/0.12	0.03-0.25	-
LZD80	1/1	≤0.12-2	100.0	1/1	≤0.12-2	100.0

TZD100	0.25/0.25	0.03-0.5	100.0	0.25/0.25	0.03-0.5	100.0
LZD100	1/2	0.25-4	-	1/2	≤0.12-4	-

Author Disclosure Block:

D.J. Farrell: H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Merck & Co. **R.E. Mendes:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Merck & Co. **R.K. Flamm:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Merck & Co. **R.N. Jones:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Merck & Co..

Poster Board Number:

SATURDAY-460

Publishing Title:

Wck 4873 (Nafithromycin): Safety Studies of a Novel Lactone-Ketolide in Rat and Dog

Author Block:

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Abstract Body:

Background: Nafithromycin (WCK4873) is a novel non-fluoroketolide with differentiating characteristics of the "lactone-ketolide". Unlike other ketolides,WCK 4873 is a five membered lactone ring, to which the alkyl aryl side chain is attached via chiral carbon of lactoneBased on its unique structure it overcomes macrolide, telithromycin, penicillin and quinolone resistance in *S. pneumoniae*. Safety of WCK 4873 was studied in 28-day toxicity studies, both in Rat and Dog.**Method:**All studies were designed in accordance with the ICH guidelines. WCK 4873 was administered once-daily (oral) at doses of 0, 50, 100 and 200 mg/kg in Rat and at doses of 0, 25, 50 and 75 mg/kg in Dog. In both the studies, recovery group was maintained at the highest dose. For toxicokinetic profiling, blood was sampled at multiple time points (on 1, 14 and 28 days). Clinical signs, body weight, food consumption, ophthalmoscopy, clinical pathology, ECG, organ weight, gross and histopathology evaluations were performed.**Results:**Increased macrophages in lung and vacuolated histiocytes in lymphoid organs (suggestive of phospholipidosis - a class specific effect), were observed at 200 mg/kg in rats. In both the studies, unlike other macrolides/ketolides, WCK 4873 did not show any indications of liver toxicity even at the highest dose. Liver specific enzymes (ALT, AST and bilirubin) and liver histopathology were normal at the end of 28-day treatment. The increased pulmonary macrophages and vacuolated histiocytes in lymphoid organs were reversed to normal at the end of recovery period. ECG did not reveal any alterations. Based on the minor reversible changes in lungs and lymphoid organs, No Observable Adverse Effect Level (NOAEL) was established to be 100 mg/kg in rat and 50 mg/kg in dog. The average AUC at NOAEL dose in rat and dog was 105.7 µg.h/mL and 50.96 µg.h/mL, respectively.**Conclusions:**WCK 4873 (Nafithromycin) showed a promising safety profile including hepatic safety in both rat and dog.

Author Disclosure Block:

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Poster Board Number:

SATURDAY-461

Publishing Title:

Wck 4873 (Nafithromycin): Mouse and Dog Pharmacokinetic (Pk) Profile of a Novel Lactone-Ketolide

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Abstract Body:

Background: For macrolide/ketolide class of agents, gaining good oral bioavailability is a major challenge in view of they being hydrophilic molecules and substrate of both intestinal P-gp and CYP3A. WCK 4873 is a broad spectrum novel lactone ketolide antibacterial drug (Phase I studies recently completed in Europe and US) with a promising activity against macrolide and telithromycin-NS Pneumococci. We investigated Mouse and Dog pharmacokinetic profile of WCK 4873. **Method:** WCK 4873 (15 and 30 mg/kg) or telithromycin (TEL) was administered either oral or intravenous (i.v.) route to Swiss mice. Serum, lung and liver concentration of WCK 4873 and TEL were measured over 24h by LC-MS/MS method. PK parameters were determined by non-compartmental analysis using Win-Nonlin. **Results:** Following single oral dose administration (15, 30 mg/kg), the mean serum C_{max} of WCK 4873 and TEL were 1.83, 3.82 and 3.11, 4.73 µg/mL respectively. Similarly, the AUC of WCK 4873 and TEL (15, 30 mg/kg) were 8.8, 15.01 and 13.97, 29.43 µg.h/mL respectively. However, lung exposures of WCK 4873 were 4-6 times higher compared to TEL (15 mg/kg per oral: WCK 4873: 116.7 µg.h/mL and TEL: 20.51 µg.h/mL; 30 mg/kg: WCK 4873: 272.7and TEL: 68.94 µg.h/mL). In liver, the exposure of WCK 4873 was considerably lower compared to TEL (30 mg/kg per oral: WCK 4873: 200.3 µg.h/mL and TEL: 428.7µg.h/mL).The mean serum $t_{1/2}$ of WCK 4873 and TEL following i.v. administration was 1.8h and 1.3h, respectively. Dog AUCs at i.v doses of 15 and 30 mg/kg was 18.7 and 38.9 µg.h/mL, respectively. The mean serum $t_{1/2}$ of WCK 4873 was in the range of 3.9-4.5h. In Dog, the oral bioavailability at the dose of 30 mg/kg was 100%. **Conclusion:** WCK 4873 (Nafithromycin) showed good PK profile and favourable tissue distribution including higher lung penetration in Mouse. Dog PK profile was suggestive of once a day dosing potential in clinic.

Author Disclosure Block:

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Poster Board Number:

SATURDAY-462

Publishing Title:

Outpatient Treatment of Acute Bacterial Skin and Skin Structure Infections (Absssi) with Tedizolid Phosphate and Linezolid in Patients in the United States

Author Block:

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Abstract Body:

ABSSSI are a frequent cause of hospital admissions in the United States (US). Tedizolid phosphate, the prodrug of the oxazolidinone tedizolid (TZD; 200 mg once daily for 6 d), demonstrated noninferior efficacy to linezolid (LZD; 600 mg twice daily for 10 d) for treatment of ABSSSI due to confirmed or suspected Gram-positive pathogens in two randomized, controlled, Phase 3 trials. The subpopulation of US-based outpatients from both trials combined was explored retrospectively. The pooled intent-to-treat population included 403 TZD and 410 LZD patients from the US who were not hospitalized during the study. Patient demographics and clinical characteristics were comparable between both treatment arms; however, systemic inflammatory response syndrome was about twice as common in the TZD arm (Table). Based on clinical characteristics, the outpatient population in both treatment groups was severely ill, with high rates of large lesions (>300 cm^3), lymphadenopathy, hepatitis C, and intravenous drug use. Early clinical response (≥20% reduction in lesion size at 48-72 h) and clinical success at end of therapy (EOT) and 7-14 d post-EOT were similar between TZD and LZD (Table). Overall incidence of treatment-emergent adverse events (AEs) was comparable between TZD and LZD (50.9% [204/401] vs 51.9% [210/405], respectively); however, gastrointestinal AEs were more common with LZD (21.4% [86/401] and 29.4% [119/405], respectively). These findings, observed in a study population in which comorbidities and fairly severe infections were frequent, suggest that ABSSSI due to confirmed or suspected Gram-positive pathogens can be successfully treated with short-course TZD in an outpatient setting.

Table. Patient Characteristics and Clinical Outcomes in US-Based Outpatients (ITT population)

Patient Characteristics, n (%)	**Tedizolid (N = 403)**	**Linezolid (N = 410)**
WBC count (≥10,000 or <4000 cells/mm^3)	182 (45.2)	156 (38.0)
Fever (≥38°C)	32 (7.9)	21 (5.1)
SIRS	55 (13.6)	29 (7.1)
Lymphadenopathy	366 (90.8)	375 (91.5)
Hepatitis C	152 (37.7)	173 (42.2)
IV drug use	178 (44.2)	200 (48.8)
Lesion surface area, cm^2, mean (range)	253.4 (22.5-2030.0)	259.8 (27.0-2490.0)
>300 cm^2	83 (20.6)	88 (21.5)
Early clinical response, n (%)	332 (82.4)	324 (79.0)
Clinical success, n (%)		
EOT	351 (87.1)	353 (86.1)
Post-EOT evaluation	335 (83.1)	343 (83.7)

EOT, end of therapy; ITT, intent-to-treat; IV, intravenous; SIRS, systemic inflammatory response syndrome; WBC, white blood cell.

Author Disclosure Block:

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Poster Board Number:

SATURDAY-463

Publishing Title:

Wck 4873 (Nafithromycin): Pk/Pd Analysis Against *S. pneumoniae* (Spn) Including Telithromycin-Non-Susceptible (Tel-Ns) Strains Through Murine-Lung Infection Model

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Abstract Body:

Background: WCK 4873 is a lactone-ketolide with activity against typical and atypical respiratory pathogens including potent activity against TEL-NS Pneumococci. Recent surveillance studies show that WCK 4873 MIC_{90} for global SPN (n=1911) including macrolide and TEL-NS was 0.12 µg/mL. We employed 6 SPN strains (1 *ermB*$^-$, 5 *ermB*$^+$ including 2 TEL-NS) in murine lung infection studies to establish bactericidal exposures. PK/PD analyses aimed at identifying the efficacy driving PK/PD index and its magnitude employing mouse serum and ELF PK.**Methods:** PK data was obtained by administering single oral doses in mice ranging from 2.5-600 mg/kg. Serum and BALF were collected over 24h and assayed for WCK 4873 by LC-MS/MS method. Urea measurement (colorimetric assay) in serum and BALF were used to determine WCK 4873 concentrations in ELF. Neutropenic mice were infected intra-nasally (10^7-10^9 CFU), so that the bacterial burden at the initiation of treatment (2h post-infection) was in the range of 5.82–7.71 $\log_{10}$ CFU/lung. 27h post-infection, animals were sacrificed to enumerate the bacterial lung load. To arrive at the representative PK/PD index in SPN 49619, various doses and their fractionated regimens were employed (dose range 0.6-25 mg/kg, q6h, q12h and q24h). For *ermB*$^+$ strains, various doses (2.5-600 mg/kg, q24h) were administered to arrive at the magnitude of the PK/PD index. Exposure-response analysis to arrive at PK/PD index and its magnitude was undertaken using Graph Pad prism.**Results:** With SPN 49619 (WCK 4873 MIC 0.015 µg/mL) 24h *f*AUC/MIC best correlated with efficacy (r^2=0.934) followed by Time >MIC (r^2=0.853) and *f*C_{max}/MIC (r^2=0.777). For 6 SPN strains (WCK 4873 MIC: 0.015-0.5 µg/mL) *f*serum AUC_{0-24}: MIC and ELF AUC_{0-24}: MIC associated with 1 $\log_{10}$ CFU reduction from the baseline were in the range of 1.8–17.48 and 30.6–1984.2, respectively. Similar pooled analysis involving all the 6 strains showed that for 1 $\log_{10}$ CFU reduction, *f*serum AUC_{0-24}: MIC and ELF AUC_{0-24}: MIC requirement was 3.06 and 336.4, respectively.**Conclusions:** WCK 4873 PK/PD targets based on mouse unbound serum and ELF PK were identified. This data would form the basis of probability of target attainment analysis and clinical dose justification employing population PK and Monte Carlo Simulations.

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Poster Board Number:

SATURDAY-464

Publishing Title:

WCK 4873 (Nafithromycin): Structure-Activity Relationship (SAR) Identifying a Lactone-Ketolide with Activity Against Telithromycin-Resistant (TEL^R) Pneumococci (SPN) and *S. pyogenes* (SPY)

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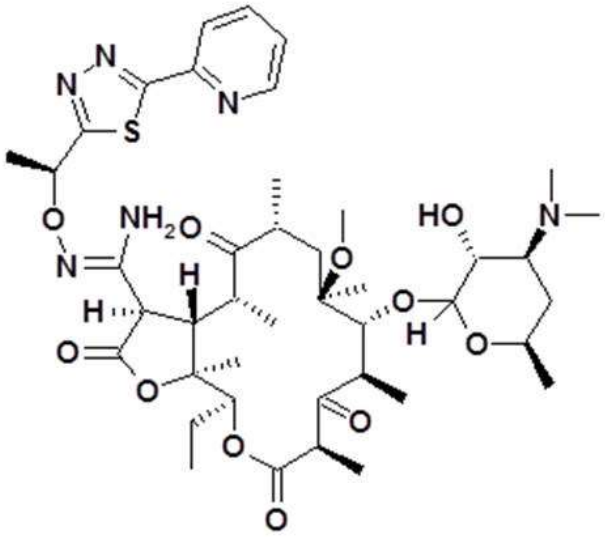
Abstract Body:

Background:SAR objectives were to identify the ketolide candidate with activity against TEL^R SPN, improved hepato-safety and good oral bioavailability with favorable tissue distribution profile.**Methods:**Various lactone-ketolides were synthesized via coupling appropriate side chains to amidoxime core followed by oxidation and deprotection. Agar dilution MICs were undertaken in MHA+5% sheep blood employing inoculum of 10^4 CFU/spot. In addition to macrolide-sensitive and *mef*-expressing pneumococci, TEL^R SPN 3773 and SPY 3530 were employed for SAR studies.**Results:**Several compounds were found to be superior in antibacterial activity over Telithromycin. This was attributed to unique amidoxime core having un-conventional chiral carbon in fused five member ring attached to macrocycle. Specific orientation and length shortening double bond geometry was critical. 1,3,4-thiadiazole and 2-pyridine moieties were found to be optimal as ring A and B, respectively. The specific orientation conferred by 'S' stereochemistry in the side chain rendered higher potency against SPN 3773 and SPY 3530. Expectedly, 2-fluoro ketolide versions were found to be 1 fold more potent than non-fluoro ketolides against SPN 3773.**Conclusion:**WCK 4873 (Nafithromycin)- a non-fluoro ketolide compound was identified as a candidate for further development.

WCK 4873



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Poster Board Number:

SATURDAY-465

Publishing Title:

Comparison of *In Vitro* Susceptibility Testing of Tedizolid (Tzd) and Linezolid (Lzd) Against Isolates of Nocardia

Author Block:

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Abstract Body:

Nocardia are responsible for various types of infections including respiratory, cutaneous and disseminated disease. Most clinically relevant species of *Nocardia* are multidrug resistant and there is a paucity of efficacious antimicrobials (especially oral) against these organisms. To date, all species of *Nocardia* have been shown to be susceptible to LZD, the first commercially available oxazolidinone. TZD is a new oxazolidinone with previously reported improved *in vitro* and *in vivo* (intracellular) potency against *Mycobacterium tuberculosis*, including multidrug resistant strains, and *Nocardia brasiliensis* compared with LZD. Using the current Clinical and Laboratory Standards Institute (CLSI) recommended method of broth microdilution, 103 isolates of *Nocardia* spp, including 29 *N. cyriacigeorgica*, 17 *N. farcinica*, 13 *N. nova* complex, 22 *N. brasiliensis*, 5 *N. pseudobrasiliensis*, and 5 *N. wallacei* were tested for susceptibility to TZD and LZD. Additional less common species (n=12 isolates) were also tested. For the most commonly encountered and clinically relevant species of *Nocardia*, TZD MIC_{50} values were 0.25 μg/mL for *N. nova* complex, *N. brasiliensis*, *N. pseudobrasiliensis*, and *N. wallacei*, with LZD MIC_{50} values of 1, 2, 0.5, and 1 μg/mL respectively. For *N. nova* complex, the TZD and LZD MIC_{90} was 2 μg/mL and for *N. brasiliensis*, the TZD MIC_{90} was 0.5 μg/mL and the LZD MIC_{90} was 2 μg/mL. TZD MIC_{50} and MIC_{90} values for both *N. cyriacigeorgica* and *N. farcinica* were 0.5 μg/mL and 1 μg/mL, respectively, compared to LZD MIC_{50} and MIC_{90} values of 2 and 4 μg/mL, respectively, for both species. TZD MIC_{90} values were 1 μg/mL for *N. cyriacigeorgica* and *N. farcinica*, compared to LZD MIC_{90} values of 4 μg/mL. This study shows that based on MIC_{90} values, TZD was 4 fold more active than LZD *in vitro* against species of *Nocardia* ($n \geq 10$), with the exception of available isolates from the *N. nova* complex (n=13) and *N. brasiliensis* (n=21) which were the same. These results may warrant evaluation of TZD as a potential treatment option for *Nocardia* infections.

Author Disclosure Block:

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Poster Board Number:

SATURDAY-466

Publishing Title:

Tedizolid or Tedizolid Combination Therapy with Rifampin Is Effective in a Rat Model of Methicillin-Resistant *Staphylococcus aureus* (Mrsa) Chronic Foreign Body Osteomyelitis

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Abstract Body:

Background: MRSA implant associated infections, such as prosthetic joint infection (PJI), are difficult to treat. Tedizolid, a novel oxazolidinone, has MRSA activity *in vitro*. Rifampin is active against MRSA; however, when used as monotherapy, resistance may emerge. We compared tedizolid and tedizolid plus rifampin with vancomycin plus rifampin in a rat model of MRSA chronic foreign body osteomyelitis. **Methods:** The study strain (PJI-associated clinical isolate) tedizolid, vancomycin, and rifampin MICs were determined using CLSI guidelines. Steady state (after 3 days) pharmacokinetic profiles for intraperitoneal administration of 30 mg/kg tedizolid once daily, 50 mg/kg vancomycin twice daily, and 25 mg/kg rifampin twice daily were determined in 3-5 healthy rats. Rat chronic foreign body osteomyelitis was established by injecting MRSA into the proximal tibia and implanting a threaded K-wire. Four weeks after infection, rats received 21 days of no treatment (n=17), tedizolid (n=14), tedizolid plus rifampin (n=11), or vancomycin plus rifampin (n=13). After treatment, quantitative cultures of bone within 5 mm of the implanted wire were performed. **Results:** The MIC was 0.5, 1.0, and ≤0.015 µg/ml for tedizolid, vancomycin, and rifampin, respectively. The mean peak (0.5 hour) plasma concentration (µg/ml) / mean $AUC_{0\text{-}24}$ (µg·h/ml) for tedizolid, vancomycin and rifampin was 12 / 60, 47 / 264, 28 / 332, respectively. Total tedizolid AUC was 2-fold that seen at steady state for human 200 mg once daily intravenous dosing. The median quantity of control, tedizolid, tedizolid plus rifampin, and vancomycin plus rifampin was 5.76, 3.86, 2.95, and 0.50 $\log_{10}$ cfu/g bone, respectively. Bacterial quantities of all treatments were significantly lower than control. Tedizolid was not significantly different than tedizolid plus rifampin. Tedizolid plus rifampin was not significantly different than vancomycin plus rifampin. No appreciable rat weight loss was seen over the treatment period. Rats receiving tedizolid had some alopecia. Those receiving rifampin showed some coarse fur, mild lethargy, and alopecia. No tedizolid resistance was detected in isolates recovered from bones. **Conclusion:** Tedizolid alone and combined with rifampin is effective against MRSA in a rat model of chronic foreign body osteomyelitis.

Author Disclosure Block:

K.E. Greenwood-Quaintance: None. **K. Park:** None. **R. Patel:** H. Research Contractor; Self; Merck Pharmaceuticals.

Poster Board Number:

SATURDAY-467

Publishing Title:

Microbiological Diagnoses from a Recent Community-Acquired Bacterial Pneumonia (CABP) Trial - Solitaire-IV

Author Block:

A. Sheets, K. Keedy, B. Jamieson, D. Oldach, P. Fernandes; Cempra, Inc., Chapel Hill, NC

Abstract Body:

Background: CABP is the most common cause of death from infectious disease in the US, and most patients do not have a microbiological diagnosis. Solithromycin (SOLI) is a 4th generation macrolide with potent activity against CABP pathogens, including macrolide-resistant strains. Solitaire-IV was a global Phase 3 non-inferiority trial of IV-to-oral SOLI versus moxifloxacin (MOXI) in the treatment of CABP.**Methods:** The trial was conducted under the new FDA CABP Guidance with clinical outcomes measured using an objective endpoint of early clinical response (ECR) at 72 hours postdose. 863 patients with confirmed CABP (PORT II to IV) were randomized between January 2014 and July 2015 to receive IV SOLI or MOXI on Day 1 and were permitted to switch to oral dosing on subsequent days.A variety of techniques were used to enhance the detection of pathogens including cultures of blood and sputum, detection of *Streptococcus pneumoniae* and *Legionella pneumophila* antigen in urine, *L. pneumophila* and *Mycoplasma pneumoniae* serologies (4-fold diagnostic rise in titer between baseline and 4 week sera), culture and PCR of oropharyngeal swabs for *M. pneumoniae*, and quantitative PCR of nasopharyngeal swabs for *S. pneumoniae*.**Results:** SOLI was non-inferior to MOXI in ECR in the microbiological intent-to-treat population (80.3% vs 79.1%; 95% Confidence Interval: -8.1, 10.6). The most frequently identified pathogens were *S. pneumoniae* (18%), *M. pneumoniae* (8%), and *H. influenzae, S. aureus,* and *L. pneumophila* (all 4%). The rate of macrolide resistance in *S. pneumoniae* (defined as resistance to azithromycin [AZI] or erythromycin) was 27% and SOLI maintained activity against these isolates with MICs ≤1 mg/L. SOLI MICs were generally lower than MOXI and AZI for Gram-positive and atypical pathogens, while MOXI MICs were lower for Gram-negative pathogens.

Pathogen (N)	**MIC90**		
	AZI	**SOLI**	**MOXI**
S. pneumoniae (98)	>32	0.06	0.12
Macrolide-resistant *S. pneumoniae* (26)	>32	0.5	0.12
S. aureus (37)	>32	0.12	2
H. influenzae (35)	2	2	0.06
M. pneumoniae (26)	0.0005	≤0.000032	0.125

Conclusions: SOLI is a new macrolide with potent activity against typical and atypical CABP pathogens, including macrolide-resistant ones, and shows promise as a new macrolide for inpatient and outpatient treatment of CABP.

Author Disclosure Block:

A. Sheets: D. Employee; Self; Cempra. **K. Keedy:** D. Employee; Self; Cempra. **B. Jamieson:** D. Employee; Self; Cempra. **D. Oldach:** D. Employee; Self; Cempra. K. Shareholder (excluding diversitied mutual funds); Self; Cempra. **P. Fernandes:** A. Board Member; Self; Cempra. D. Employee; Self; Cempra. K. Shareholder (excluding diversitied mutual funds); Self; Cempra.

Poster Board Number:

SATURDAY-468

Publishing Title:

Candidate Vaccine Against Extra-Intestinal Pathogenic *E. coli*: A Multicenter Phase I, First-in-Human, Randomized, Placebo-Controlled Trial in Women with a History of Recurrent Urinary Tract Infection

Author Block:

A. Huttner[1], S. Harbarth[1], C. Hatz[2], K. Fae[3], T. Davies[4], G. van den Dobbelsteen[3], A. Hornacek[5], A. Dreyer[5], P. Martin[5], C. Alaimo[5], V. Gambillara[5]; [1]Infection Control Program, Univ. Hosp. of Geneva, Geneva, Switzerland, [2]Epidemiology, Biostatistics and Prevention Inst., Zürich, Switzerland, [3]Janssen Res. and Dev., Leiden, Netherlands, [4]Janssen Res. and Dev., Raritan, NJ, [5]LimmaTech Biologics, Schlieren, Switzerland

Abstract Body:

A novel bioconjugate candidate vaccine containing O-antigen polysaccharides from 4 *E. coli* serotypes (O1A, O2, O6A, O25B) was tested in a multicenter phase I randomized, single-blind, placebo-controlled study assessing its safety, immunogenicity and effect on the incidence of urinary tract infections (UTI) in non-pregnant healthy women with a history of recurrent UTI.Primary outcome was safety; secondary outcomes were immunogenicity and UTI incidence 9 months after injection.In total, 194 women were randomized to reduced-dose vaccine (n=6; 4ug polysaccharide), target-dose vaccine (n=93; 16ug polysaccharide) or placebo (n=95; vehicle). There were no vaccine-related serious adverse events (AE). Though local solicited AE (injection site pain and/or erythema) were slightly more frequent in the target-dose group compared to placebo (37/93 [39.8%] vs 32/95 [33.7%], p=0.386), there were no differences in the frequency of unsolicited AE, or changes in laboratory parameters among groups. Target-dose vaccine triggered significant increases (all $p<0.01$) in geometric mean titers for antibodies towards the 4 serotypes compared to baseline and to placebo. Antibody durability was observed in the target-dose group at 9 months. There were no significant differences in single-pathogen *E. coli* mean UTI (total number of UTIs/number of subjects) between target-dose group and placebo in ITT analyses (p=0.146), while a decrease was observed in the per-protocol analyses (p=0.038). No reduction in vaccine-specific mean UTI was observed in either ITT or PP analyses.In conclusion, *E. coli* bioconjugate vaccine was safe and immunogenic. Larger trials are needed to evaluate its clinical efficacy.**Study Support**: Janssen Research & Development, LLC, Raritan, NJ, USA**Registration**: ClinicalTrials.gov: NCT02289794

Author Disclosure Block:

A. Huttner: None. **S. Harbarth:** None. **C. Hatz:** None. **K. Fae:** None. **T. Davies:** None. **G. van den Dobbelsteen:** None. **A. Hornacek:** None. **A. Dreyer:** None. **P. Martin:** None. **C. Alaimo:** None. **V. Gambillara:** None.

Poster Board Number:

SATURDAY-469

Publishing Title:

Extracellular Vesicles Purified from a Recombinant *Staphylococcus aureus* Isolate Elicit Protection Against Experimental Staphylococcal Infection

Author Block:

X. Wang, X. Li, J. Lee; Brigham and women's Hosp. and Harvard Med. Sch., Boston, MA

Abstract Body:

In the past decade, the high prevalence of methicillin-resistant *Staphylococcus aureus* in healthcare and community settings has become a serious public health concern due to treatment failures with a number of antimicrobial drugs. Conventional approaches toward *S. aureus* vaccine development have failed, and new innovative approaches are needed. The secretion of nano-scale extracellular vesicles (EVs) from *S. aureus* and other Gram-positive pathogens has been described recently. These EVs have been shown to package multiple antigens including lipoproteins, toxins, and enzymes, and the vesicle contents are protected from extracellular proteases by a hydrophobic membrane. Thus, it is feasible to develop EVs as a multivalent *S. aureus* vaccine candidate if we can further engineer them to improve safety and protective efficacy against *S. aureus* infections. To abrogate the toxicity of native EVs from USA300 strain JE2, we mutated the *agr* locus, which positively regulates the expression of exotoxins, and the *spa* gene, which encodes protein A that binds to the Fcγ domain of IgG and to the Fab domain of V_H3-type B cell receptors, disrupting the antibody response and inducing B cell apoptosis. EVs were isolated from culture supernatants of wild type JE2, Δ*agr*, and Δ*agr*Δ*spa* mutants with Opti-prep density gradient ultracentrifugation, and vesicle-like structures were observed by negative staining electron microscopy. As expected, EVs from both mutants exhibited negligible toxicity to rabbit blood cells, A549 lung epithelial cells, and neutrophil-like HL60 cells compared to EVs from the wild type JE2. In the absence of adjuvant, EVs were immunogenic in mice, and vaccination with EVs isolated from the Δ*agr*Δ*spa* mutant, but not EVs from the Δ*agr* mutant, significantly reduced lethality in a sepsis model following challenge with USA300 strain FPR3757. Our data indicate that EVs from the Δ*agr*Δ*spa* mutant offer potential as a vaccine against *S. aureus* infections. Moreover, by generating EVs from JE2Δ*agr*Δ*spa* engineered to express additional vaccine antigens like nontoxic Hla, vaccine efficacy may be enhanced.

Author Disclosure Block:

X. Wang: None. **X. Li:** None. **J. Lee:** None.

Poster Board Number:

SATURDAY-470

Publishing Title:

Widespread Prevalence of *Staphylococcus pseudintermedius* Vaccine Candidate Antigens Identified by Serological Proteomic Analysis

Author Block:

N. Couto[1], L. Fernandes[1], J. Martins[2], A. V. Coelho[3], **C. F. Pomba**[1]; [1]Faculty of Vet. Med. Univ. of Lisbon, LISBON, Portugal, [2]2ITQB António Xavier, OEIRAS, Portugal, [3]ITQB António Xavier, OEIRAS, Portugal

Abstract Body:

The emergence of methicillin-resistant *S. pseudintermedius* (MRSP) has impaired the treatment of infections caused by these bacteria. The objective of this study was to identify potential vaccine candidate using the SERological Proteome Analysis (SERPA) approach and determine the prevalence of these proteins in a *S. pseudintermedius* population.To detect immunogenic *S. pseudintermedius* proteins, a Western blot analysis of 2DE gels (cell-wall and membrane fractions) was carried out with serum from healthy dogs (H) and dogs infected with *S. pseudintermedius* (D). Only immunogenic areas identified by ≥ 50% of the D sera and identified by < 50% of the H sera were excised and identified by MALDI-TOF/TOF MS. The genes encoding the proteins of interest were identified by PCR. A population of 347 *S. pseudintermedius* isolates were tested. A Western blot analysis of 2DE gels (cell-wall and membrane fractions) was carried out with serum from healthy dogs (H) and dogs infected with S. pseudintermedius (D). Only immunogenic areas identified by ≥ 50% of the D sera and identified by < 50% of the H sera were excised and identified by MALDI-TOF/TOF MS. The genes encoding the proteins of interest were identified by PCR using primers designed by Primer3. A population of 347 S. pseudintermedius isolates were tested, of which 16 were MRSP.Four and one highly immunogenic areas (containing more than one spot) were detected in the cell wall fraction and in the membrane associated fraction, respectively. We were able to identify 13 unique proteins after in-gel digestion of the highly immunogenic areas. Based on the predicted surface localization and/or crucial function in bacterial pathogenesis or survival (e.g. adhesion), four of the identified proteins seem promising candidate therapeutic targets: fibronectin binding protein SpsD, putative lipoprotein, ATP synthase subunit alpha AtpA and enoyl-[acyl-carrier-protein] reductase [NADPH] FabI. The gene encoding SpsD was present in 84%, AtpA gene in 97%, the putative lipoprotein gene in 97% and the FabI gene in 96 % of the isolates.The SERPA approach revealed 4 novel and highly prevalent candidate therapeutic targets for the control of *S. pseudintermedius* infections.

Author Disclosure Block:

N. Couto: None. **L. Fernandes:** None. **J. Martins:** None. **A.V. Coelho:** None. **C.F. Pomba:** None.

Poster Board Number:

SATURDAY-471

Publishing Title:

Oral Delivery of a Novel Recombinant *Streptococcus mitis* Vector Elicits Robust Vaccine Antigen-specific Oral Mucosal and Systemic Antibody Responses and T Cell Tolerance

Author Block:

M. Cayabyab, E. Xie, A. Kotha, T. Biaco, S. Nikita, J. Zou, P. Stashenko, M. Duncan, A. Campos-Neto; Forsyth Inst., Cambridge, MA

Abstract Body:

The pioneer human oral commensal bacterium *Streptococcus mitis* has unique biologic features that make it an attractive mucosal vaccine or therapeutic delivery vector. *S. mitis* is safe as a natural persistent colonizer of the mouth, throat and nasopharynx and the oral commensal bacterium is capable of inducing mucosal antibody responses. A recombinant *S. mitis* (*rS. mitis*) that stably expresses HIV envelope protein was generated and tested in the germ-free mouse model to evaluate the potential usefulness of this vector as a mucosal vaccine against HIV. Oral vaccination led to the efficient and persistent bacterial colonization of the mouth and the induction of both salivary and systemic antibody responses. Interestingly, persistently colonized animals developed antigen-specific systemic T cell tolerance. Based on these findings we propose the use of *rS. mitis* vaccine vector for the induction of mucosal antibodies that will prevent the penetration of the mucosa by pathogens such as HIV. Moreover, the first demonstration of *rS. mitis* having the ability to elicit T cell tolerance suggest the potential use of *rS. mitis* as an immunotherapeutic vector to treat inflammatory, allergic and autoimmune diseases.

Author Disclosure Block:

M. Cayabyab: None. **E. Xie:** None. **A. Kotha:** None. **T. Biaco:** None. **S. Nikita:** None. **J. Zou:** None. **P. Stashenko:** None. **M. Duncan:** None. **A. Campos-Neto:** None.

Poster Board Number:

SATURDAY-472

Publishing Title:

Antibody Response Measured by Elisa and Opsonophagocytic Killing Assays Elicited by a Vaccine Targeting Extraintestinal Pathogenic *Escherichia coli*

Author Block:

D. R. Abbanat[1], K. Fae[2], T. Davies[1], K. Amsler[1], W. He[1], K. Contractor[1], C. Alaimo[3], A. Dreyer[3], V. Gambillara[3], J. Poolman[2], G. van den Dobbelsteen[2]; [1]Janssen Res. & Dev., Raritan, NJ, [2]Janssen Res. & Dev., Leiden, Netherlands, [3]LimmaTech Biologics AG, Schieren, Switzerland

Abstract Body:

Background: ExPEC4V, a vaccine targeting extraintestinal pathogenic *E. coli* (ExPEC) O-antigen serotypes O1A, O2, O6A, and O25B, is being evaluated for the treatment of ExPEC invasive disease. This study investigated the safety and immunogenicity of ExPEC4V in healthy women with a history of recurrent urinary tract infection.**Methods:**I n this Phase I staggered, single blind, placebo-controlled, multicenter study, 188 women were randomized (1:1) to receive either placebo, or the EXPEC4V vaccine consisting of four *E. coli* O-antigens bioconjugated to detoxified Exoprotein A from *P. aeruginosa*. Sera from all participants were tested for antibodies on day 1 (prevaccine), and days 30 or 270 postvaccine. IgG titers were measured by ELISA and functional antibodies by an Opsonophagocytic Killing (OPK) assay with differentiated HL60 cells and heterologous human complement.**Results:** Day 30 geometric mean (GM) IgG EC_{50} values (ELISA) were O1A: 9460, O2: 27973, O6A: 4475, and O25B: 2164, a 4.9 to 9.8 fold increase over respective baseline values. For each ExPEC serotype, ≥80% of participants (O1A: 81%, O2: 93%, O6A: 80%, O25B: 82%) showed a ≥2-fold increase in IgG antibodies from day 1 to 30. The proportion of participants with a ≥4-fold increase in IgG titers ranged from 57% (O1A and O6A) to 80% (O2). With the OPK assay, day 30 GM titers were O1A: 951, O2: 4133, O6A: 1542, and O25B: 415, a 2.0- to 14-fold increase over baseline values. The proportion of participants with a ≥2-fold increase in antibodies per serotype was: O1A: 63%, O2: 90%, O6A: 33%, and O25B: 55%; the proportion of participants with a ≥4-fold increase ranged from 20% (O6A) to 82% (O2). Day 270 antibody titer increases were slightly lower compared to day 30, with per serotype GM fold increases from baseline ranging from 3.8 - 7.3 for ELISA and 1.8 - 5.6 for OPK.**Conclusion:** The ExPEC4V vaccine induced an IgG immune response for all 4 serotypes, with functionality demonstrated by associated OPK activity. A durable vaccine response to the 4 serotypes was demonstrated over 270 days.

Author Disclosure Block:

D.R. Abbanat: D. Employee; Self; Janssen Research & Development. K. Shareholder (excluding diversitied mutual funds); Self; Janssen Research & Development. **K. Fae:** D.

Employee; Self; Janssen Research & Development. K. Shareholder (excluding diversitied mutual funds); Self; Janssen Research & Development. **T. Davies:** D. Employee; Self; Janssen Research & Development. K. Shareholder (excluding diversitied mutual funds); Self; Janssen Research & Development. **K. Amsler:** D. Employee; Self; Janssen Research & Development. K. Shareholder (excluding diversitied mutual funds); Self; Janssen Research & Development. **W. He:** D. Employee; Self; Janssen Research & Development. K. Shareholder (excluding diversitied mutual funds); Self; Janssen Research & Development. **K. Contractor:** D. Employee; Self; Janssen Research & Development. K. Shareholder (excluding diversitied mutual funds); Self; Janssen Research & Development. **C. Alaimo:** D. Employee; Self; LimmaTech Biologics AG. **A. Dreyer:** D. Employee; Self; LimmaTech Biologics AG. **V. Gambillara:** D. Employee; Self; LimmaTech Biologics AG. **J. Poolman:** D. Employee; Self; Janssen Research & Development. K. Shareholder (excluding diversitied mutual funds); Self; Janssen Research & Development. **G. van den Dobbelsteen:** D. Employee; Self; Janssen Research & Development. K. Shareholder (excluding diversitied mutual funds); Self; Janssen Research & Development.

Poster Board Number:

SATURDAY-473

Publishing Title:

Inactivated *Shigella* as Effective Vaccines and Vaccine Vectors

Author Block:

I. Albino-Flores, E. S. Stibitz, **M. Osorio**; FDA, Silver Spring, MD

Abstract Body:

Shigellae and Enterotoxigenic *Escherichia coli* (ETEC) remain major causes of diarrhea among children in developing countries and travelers to these areas. However, currently there are no effective vaccines against these enteric pathogens. Previously we demonstrated the vaccine and vector potential of formalin inactivated *Shigellae*[1] by demonstrating the strong protective immune responses elicited in the mouse model. In addition we showed that these inactivated strains can serve as effective vaccine carriers for fimbrial antigens (CFA/I and CS3) of ETEC. However, we have observed that the harsh formalin treatment can negatively affect the immunogenicity of the ETEC fimbrial antigens. We therefore explored alternative methods of inactivating *Shigella*. Here we present our results examining the vaccine and vector potential of *Shigella* ghost cells expressing ETEC antigens (CVD1203). Bacterial ghost cells in general have been shown to be immunogenic and possess inherent adjuvant properties[2]. We have found that mice immunized orally or intranasally with ghost cells of *S. flexneri* expressing CFA/I and CS3 induced strong IgG titers to the homologous LPS and to the ETEC antigens. We show that these immune responses are protective as 100% of the vaccinated animals can be protected from challenge with the live homologous *Shigella* strain compared to negligible survival in mice given PBS. More recently, we have explored gamma-irradiation as an alternative method to inactivate *Shigella* cells, and have optimized conditions that result in complete inactivation of *Shigella* cells following irradiation. We are currently performing a comparative immunogenicity study with the various inactivated cell formulations to determine their ability to induce strong and protective immune responses to *Shigella* pathogens. These studies demonstrate that inactivated *Shigella* cells are highly immunogenic and can serve as effective carriers for exogenous antigens.

Author Disclosure Block:

I. Albino-Flores: None. **E.S. Stibitz:** None. **M. Osorio:** None.

Poster Board Number:

SATURDAY-474

Publishing Title:

Development of a Multivalent Subunit Vaccine Against Tularemia Using Tobacco Mosaic Virus (TMV) Based Delivery System

Author Block:

A. A. Mansour[1], S. Banik[1], S. M. Rabadi[1], A. A. McCormick[2]; [1]New York Med. Coll., Valhalla, NY, [2]Touro Univ., Vallejo, CA

Abstract Body:

Background: *Francisella tularensis* (*Ft*); the causative agent of a fatal human disease tularemia is classified as a Category A Select Agent. No licensed vaccine is available for prevention of tularemia in the U.S.A. In this study, we used a novel Tobacco Mosaic Virus (TMV) based delivery platform for development of a fully protective multi-antigen subunit tularemia vaccine. Previously we have published that a trivalent TMV-conjugate vaccine confers 50% protection in immunized mice against respiratory *Ft* LVS challenge. In this study, we refined TMV-conjugate vaccine formulation to improve the level of protection in immunized C57BL/6 mice against respiratory tularemia.**Methods:** We refined our trivalent vaccine formulation (OmpA, DnaK and Tul4) by including dihydrolipoamide succinyl transferase (SucB). To improve Th1 type humoral response, we also included CpG adjuvant in the vaccine formulation. Each recombinant protein was conjugated individually to TMV, blended in equimolar concentration to yield a TMV-tetravalent conjugate vaccine. C57BL/6 mice were immunized intranasally (i.n.) on days 0, 14 and 28. Mice were challenged i.n. with $10LD_{100}$ of *Ft* LVS on day 45 post-primary immunization. Humoral, cell mediated and recall memory responses were evaluated in immunized mice to determine the vaccine efficacy.**Results:** 100% of immunized mice were protected against a $10LD_{100}$ respiratory challenge dose of *Ft* LVS. Mice vaccinated with TMV-tetravalent vaccine showed high levels of IgG1, IgG2a, 2b and 2c antibodies than those observed in mice vaccinated with trivalent vaccine formulation. A strong cell mediated immune response was observed against each individual recombinant protein. The splenocytes from immunized mice isolated on day 84 post-primary immunization induced high levels of both IL-17 and IFN-γ in co-culture assays, indicating generation of potent memory response in immunized mice.**Conclusions:** TMV serves as a suitable platform for delivery of multiple *Ft* antigens and provide 100% protection in mice against respiratory tularemia caused by *Ft* LVS. Immunization with TMV-tetravalent vaccine induces potent humoral and cell medicated immune responses. Study to evaluate the overall efficacy of TMV-tetravalent conjugate vaccine against virulent *Ft* SchuS4 is currently underway.

Author Disclosure Block:

A.A. Mansour: None. **S. Banik:** None. **S.M. Rabadi:** None. **A.A. McCormick:** None.

Poster Board Number:

SATURDAY-475

Publishing Title:

Intradermal Vaccination against *Pseudomonas aeruginosa* Induces Antibody and Cellular Immune Responses in the Lung

Author Block:

S. M. Baker, J. D. Clements, L. A. Morici; Tulane Univ., New Orleans, LA

Abstract Body:

Pseudomonas aeruginosa is a Gram-negative opportunistic bacterium that causes chronic lung infection in individuals with cystic fibrosis. Growing evidence suggests that a successful *P. aeruginosa* vaccine may need to elicit antibody and Th1- and Th17-$CD4^+$ T cells both systemically and in the lung to prevent chronic infection. The majority of *P. aeruginosa* vaccine studies in mice utilize intramuscular (IM) vaccination. However, IM immunization may not be the optimal route for eliciting mucosal immune responses. Recent work suggests that intradermal (ID) immunization in the context of certain classes of adjuvants can induce a mucosal immune response, as dermal dendritic cells process antigen and migrate to the draining lymph nodes where they induce the expression of mucosal homing markers on B and T cells. In order to determine if ID immunization could induce mucosal immune responses to *P. aeruginosa*, we immunized groups of five C57Bl/6 mice with 1-10 ug of a *P. aeruginosa* outer membrane protein (OMP) preparation combined with 1 ug of a double mutant of *E. coli* heat-labile toxin (dmLT) adjuvant. Control mice received OMPs or dmLT only. Mice were immunized ID at two anatomical locations, subcutaneously (SC), or IM. Antibody and T cell responses were assessed by ELISA and flow cytometry, respectively. ID vaccination with 1 ug of OMPs plus dmLT induced significantly more anti-pseudomonal serum IgG than the same formulation administered SC and significantly greater anti-pseudomonal IgG in the bronchoalveolar lavage fluid (BALF) compared to SC or IM vaccination. ID administration of 1 ug OMPs plus dmLT on the side of the mouse below the axilla induced significantly more anti-pseudomonal IgG in the BALF compared to ID vaccination on the ventral surface. ID immunization at both locations induced the production of IFN-gamma-producing CD4+ T cells in the lungs and mediastinal lymph nodes, however only ID vaccination below the axilla induced IL-17-producing CD4+ T cells in both tissues. These results demonstrate the potential of ID vaccination with OMPs plus dmLT to stimulate protective mucosal antibody and cellular immune responses in the lung. Studies are currently underway to assess protection in a mouse model of *P. aeruginosa* pulmonary infection.

Author Disclosure Block:

S.M. Baker: None. **J.D. Clements:** None. **L.A. Morici:** None.

Poster Board Number:

SATURDAY-476

Publishing Title:

Identifying Protective Immune Responses to Oligopeptide Permease A for the Development of a Vaccine Against *Moraxella catarrhalis*

Author Block:

A. C. Perez, T. F. Murphy; Univ. at Buffaly, SUNY, Buffalo, NY

Abstract Body:

Moraxella catarrhalis infections are a significant cause of morbidity and a huge financial burden globally. Development of a vaccine against this pathogen has the potential to prevent otitis media in children and respiratory infections in adults with chronic obstructive pulmonary disease. Oligopeptide permease A (OppA) is a potential candidate vaccine antigen that has been studied extensively. However, assessing the effectiveness of a potential vaccine in humans is challenging because of the lack of an identified correlate of protection against *M. catarrhalis* infections. Therefore, the overall goal of these studies is to identify protective immune responses to OppA in vaccinated animal models. Eight truncated constructs of the protein were designed, engineered and purified to identify B cell epitopes on OppA. Using serum from vaccinated rabbits, antibody titers to each construct were assessed by immunoblot assay and ELISA. The results from these studies showed that epitopes were present on each construct of the protein. Additionally, mice were immunized using multiple immunization strategies with OppA to identify an optimal vaccine formulation and schedule. Mice were subjected to aerosol challenge with *M. catarrhalis* and pulmonary clearance was determined. Overall, OppA induced enhanced pulmonary clearance, a potentially protective response, when OppA is given with incomplete Freund's adjuvant, alum, or AS04 adjuvants. While all formulations tested induced high antibody titers, the epitopes to which the antibody response is directed differed depending on the adjuvant and schedule. All together, these studies reveal that (1) OppA is highly immunogenic in vaccinated animals, (2) OppA has multiple B cell epitopes, (3) it induces protective responses in mice, and (4) adjuvant choice influences B cell epitope preference, which may aid in identification of the most protective regions of the protein. In conclusion, OppA is highly immunogenic and has great potential for use in a vaccine against *M. catarrhalis*.

Author Disclosure Block:

A.C. Perez: None. **T.F. Murphy:** None.

Poster Board Number:

SATURDAY-477

Publishing Title:

Evaluating the Inclusion of Adenylate Cyclase Toxin Antigen into the Acellular Pertussis Vaccine

Author Block:

D. T. Boehm[1], J. Bevere[1], E. Sen[1], J. A. Maynard[2], E. L. Hewlett[3], M. Barbier[1], F. H. Damron[1]; [1]West Virginia Univ., Morgantown, WV, [2]Univ. of Texas at Austin, Austin, TX, [3]Univ. of Virginia, Charlottesville, VA

Abstract Body:

Bordetella pertussis is the causative agent of pertussis (whooping cough), a respiratory infection leading to a violent cough, which can be fatal. Acellular pertussis vaccines (ACV) replaced whole cell vaccines (WCV) in the US immunization schedule in the early 1990s, and for multiple reasons, pertussis is re-emerging despite 95% vaccine coverage. Adenylate Cyclase Toxin (ACT) is a major virulence factor that has been shown to be: required for the establishment of infection, an effective immunogen, and a protective antigen. In this study, we used a murine vaccination/infection model to evaluate the effectiveness and immunological profiles of ACV vaccines (1/5 human dose) +/- ACT compared WCV and unvaccinated control groups.CD1 mice received initial and booster vaccines consisting of either: PBS, WCV, ACV, ACV+ACT, ACV + a truncated ACT-RTX (residues 751-1706). Mice were infected with *B. pertussis* strain UT25 and were euthanized at 1, 3, and 8 days post challenge, and samples were collected and processed. Bacterial burden was measured and cytokine quantification was performed with arrays and ELISA.Vaccination with ACVs or WCV significantly decreased bacterial burden compared to the unvaccinated control group at all time points. The WCV group at all days post infection had noticeable splenomegaly, which appeared by flow cytometry to consist of an increased number of all cell types compared to all other groups. Addition of ACT or ACT-RTX did not decrease bacterial burden when provided with the $1/5^{th}$ human ACV dose. In the lung, WCV vaccinated mice had increased INF-γ and IL-17 compared to all other groups. Serum IL-17 was also observed to be increased in WCV compared to control infected mice, while ACV mice had similar IL-17 levels to uninfected mice. Vaccination with ACV-RTX induced IL-17 expression at 8 days post infection compared to ACV alone. Vaccination with ACT or ACT-RTX resulted in production of antibodies that could neutralize ACT in vitro. These promising observations suggest that inclusion of ACT in the ACV may alter the nature of the immunological response and potentially elicit more effective protection. Future studies, are underway to test different adjuvants with ACT, other antigen ratios, and additional time points post challenge.

Author Disclosure Block:

D.T. Boehm: None. **J. Bevere:** None. **E. Sen:** None. **J.A. Maynard:** None. **E.L. Hewlett:** None. **M. Barbier:** None. **F.H. Damron:** None.

Poster Board Number:

SATURDAY-478

Publishing Title:

Linear Epitope Mapping of Anthrax Toxin Lethal Factor Using Anti-Rlf Rhesus Macaque Sera

Author Block:

V. A. Semenova, E. Steward-Clark, P. Svoboda, J. Pohl; CDC, Atlanta, GA

Abstract Body:

Introduction: Lethal factor (LF) is a 90-kDa zinc-dependent metallo-protease which is a part of the tripartite anthrax toxin protein complex together with protective antigen (PA) and edema factor (EF). The licensed anthrax vaccine (AVA, BioThrax®) elicits antibodies to PA, however, anti-LF antibodies alone and in combination with anti-PA antibodies can contribute to neutralization of anthrax toxin. Little information is available about LF sequences which induce neutralizing antibodies. This study used overlapping synthetic peptide sequences to identify linear epitopes in LF determined by their reactivity to sera from recombinant LF (rLF) vaccinated rhesus macaques. **Methods:** Fmoc synthesis was used to prepare 39 N-terminally biotinylated peptides of 30 amino acid (AA) residues, overlapping by 10 AA. Sequences were synthesized as C-terminal amides, HPLC-purified and prepared as trifluoracetic acid salts. Peptides were captured on streptavidin coated (2 µg/ml) microtiter plates and screened for antibody binding by enzyme-linked immunosorbent assay (ELISA). Seroreactivity was evaluated using anti-rLF macaque standard AVR2079, anti-LF proficiency sera AVR2084-AVR2093 (n=11) and sera from individual macaques AVR1315 -1321 that received 5 injections of 50µg rLF (n=6). A peptide was considered antigenic if its ELISA Optical Density (OD) value was greater than the mean OD plus 3 Standard Deviations (SD) of controls.**Results:** Sera from rLF vaccinated macaques reacted with peptide sequences in all 4 domains of LF. Despite individual variations in response, all macaques showed consensus reactivity with peptides corresponding to 7 LF sequences: AA41-47, AA81-130, AA261-290, AA301-390, AA400-430, AA641-670, and AA681-710.**Conclusions:** Vaccination of macaques with rLF elicits antibody responses to linear peptide sequences in all 4 LF domains. There were several sequences that were recognized by antisera from all animals tested. The developed method will be used for developing serologic correlates of protection using sera from macaques that survived after challenge with aerosolized *Bacillus anthracis* Ames spores.*The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.*

Author Disclosure Block:

V.A. Semenova: None. **E. Steward-Clark:** None. **P. Svoboda:** None. **J. Pohl:** None.

Poster Board Number:

SATURDAY-479

Publishing Title:

Characterization of a Live Attenuated Vaccine for Protection Against Multi-Drug Resistant *Acinetobacter baumannii*

Author Block:

S. Ainsworth[1], P. Ketter[2], J-J. Yu[1], M. Guentzel[1], B. Arulanandam[1]; [1]Univ. of Texas at San Antonio, San Antonio, TX, [2]US Army Inst. for Surgical Res., JBSA-Fort Sam Houston, TX

Abstract Body:

Background: Multi-drug resistant *Acinetobacter baumannii* (MDR-Ab) is an opportunistic pathogen associated with nosocomial and combat related infections sustained by military personnel. This emerging infectious disease is difficult to control due to enhanced multi-drug resistance limiting treatment options. **Objective**: Deletion of the thioredoxin gene (*trxA*) from a clinical isolate of MDR-Ab resulted in a 100-fold increase in LD_{50} relative to the wild type strain. Thus, the objective of this study was to test the efficacy of this attenuated strain as a vaccine against MDR-Ab. **Methods:** Mice were vaccinated by either intraperitoneal (i.p.) or subcutaneous (s.c.) injection of 2×10^5 CFU of the *trxA* mutant. Mice were then given a booster of equivalent inoculum 14 days later and challenged at 30 days post-vaccination by i.p. injection with a lethal dose of the WT Ci79 strain ($10 \times LD_{50}$). Serum was collected on days 14 and 28 post-vaccination to monitor antibody titers. Spleens were collected from vaccinated mice on day 28 and splenocytes were stimulated with UV killed Ci79 to assess T-cell responses. Livers, spleens, and kidneys were extracted from vaccinated mice 24 hours post lethal infection and observed for pathology.**Results:** Mice vaccinated with the *trxA* mutant were 100% protected against a lethal challenge of Ci79 regardless of vaccination route. Surprisingly, little if any immunoglobulin class switching was observed with IgM predominating. Spleens harvested from vaccinated mice exhibited negligible levels of IFNγ and IL-4 production when stimulated with UV killed WT Ci79. Additionally, organs collected from vaccinated mice displayed reduced pathology compared to organs from non vaccinated mice.**Conclusions:** The attenuated *trxA* mutant provides protection against a lethal dose of the WT strain through a T-cell independent mechanism. Additional studies are currently underway to evaluate cross strain protection and to further elucidate the exact mechanism of protection.

Author Disclosure Block:

S. Ainsworth: None. **P. Ketter:** None. **J. Yu:** None. **M. Guentzel:** None. **B. Arulanandam:** None.

Poster Board Number:

SATURDAY-480

Publishing Title:

Contributions of *Clostridium difficile* Toxin A and Toxin B Regions in Antibody-specific Binding and Neutralizing Responses to a *Clostridium difficile* Toxoid Vaccine

Author Block:

N. Anosova[1], L. Li[1], U. Jetley[1], L. Barone[1], S. Liu[1], L. Cole[1], Y. Yan[1], S. Mundle[1], C. Rogers[1], J. Totman[2], R. B. Hunter[2], L. Quemeneur[3], P. Pietrobon[4], H. Kleanthous[1], S. Anderson[1]; [1]Sanofi Pasteur, Cambridge, MA, [2]Sanofi Genzyme, Cambridge, MA, [3]Sanofi Pasteur, Marcy l'Etoile, France, [4]Sanofi Pasteur, Swiftwater, PA

Abstract Body:

Background: *Clostridium difficile* infection (CDI) is the principal cause of nosocomial diarrhea and pseudomembranous colitis associated with antibiotic therapy. CDI is mediated by two large exotoxins, toxin A (TcdA) and toxin B (TcdB). There is ongoing discussion focused on the relative importance of antibodies against the different regions of TcdA and TcdB in mediating a protective response. While neutralizing antibodies to the C-terminal domain (CTD) of both toxins have long been implicated in efficacy, the ability of other domains of TcdB to elicit neutralizing antibodies has also been reported. Sanofi Pasteur's *C. difficile* candidate vaccine which contains highly purified formalin-inactivated preparations of full length TcdA and B is being developed for prevention of symptomatic disease. **Methods:** Here hamsters were immunized trice with a *C. difficile* vaccine and immune sera were collected 7 days post final immunization but prior to the lethal *C. difficile* challenge. The binding profiles of the sera collected from protected versus non-protected hamsters were generated in ELISA using recombinant toxin fragments. Neutralizing activity of the sera was tested in the Vero cell-based cytotoxicity assay. **Results:** A strong binding response towards C-terminal domain of TcdB was detected, however, other regions, such as N-terminal and central, also generated notable responses in sera of protected animals. Contributions of CTD-specific to the overall toxin-specific binding and neutralizing antibody responses were assessed. Augmentation of potency of antibody response to the multiple domains in contrast to the CTD only was studied. **Conclusions:** In this pre-clinical study we have attempted to identify differences in antibody binding as well as function profiles between vaccine candidate immunized hamsters that were protected against subsequent challenge and those that succumbed such that we could gain insight into the relative contributions of TcdA and TcdB domains in generating binding and neutralizing antibody responses to the vaccine.

Author Disclosure Block:

N. Anosova: D. Employee; Self; Sanofi Pasteur. **L. Li:** D. Employee; Self; Sanofi Pasteur. **U. Jetley:** H. Research Contractor; Self; Sanofi Pasteur. **L. Barone:** H. Research Contractor; Self;

Sanofi Pasteur. **S. Liu:** H. Research Contractor; Self; Sanofi Pasteur. **L. Cole:** D. Employee; Self; Sanofi Pasteur. **Y. Yan:** D. Employee; Self; Sanofi Pasteur. **S. Mundle:** D. Employee; Self; Sanofi Pasteur. **C. Rogers:** H. Research Contractor; Self; Sanofi Pasteur. **J. Totman:** D. Employee; Self; Sanofi Genzyme. **R.B. Hunter:** D. Employee; Self; Sanofi Genzyme. **L. Quemeneur:** D. Employee; Self; Sanofi Pasteur. **P. Pietrobon:** D. Employee; Self; Sanofi Pasteur. **H. Kleanthous:** D. Employee; Self; Sanofi Pasteur. **S. Anderson:** D. Employee; Self; Sanofi Pasteur.

Poster Board Number:

SATURDAY-481

Publishing Title:

Lipooligosaccharide Structural Variations Affect Efficacy of *Neisseria gonorrhoeae* Vaccine Antibody

Author Block:

S. Chakraborti, L. A. Lewis, P. Rice, S. Ram; Univ. of Massachusetts Med. Sch., Worcester, MA

Abstract Body:

Background: *Neisseria gonorrhoeae* (*Ng*), the pathogen causing gonorrhea, a sexually transmitted infection is resistant to most antibiotics. The lack of a safe and effective vaccine makes gonorrhea a major public health challenge. Antibodies against a *Ng* lipooligosaccharide (LOS) epitope recognized by mAb 2C7 (2C7 epitope) are bactericidal. This epitope is expressed by >95% of clinical *Ng* isolates. Immunization with a peptide mimic of the 2C7 epitope attenuates *Ng* infection in a mouse vaginal colonization model. Binding of mAb 2C7 to *Ng* LOS requires lactose on heptose (Hep) II; expression of which is initiated by the phase-variable (pv) LOS glycosyltransferase (*lgt*) G. Glycan extensions from HepI are modified by three pv genes; *lgtA, lgtC* and *lgtD*. We aimed to determine effects of HepI glycan variation on mAb 2C7 function. **Methods:** A *Ng* mutant with HepII lactose (lgtG-ON) was used to engineer four mutants with varying HepI glycan extensions; *lgtA, C, and D* were inactivated by deletion (OFF) or locked-ON by synonymous point mutations in their homopolymer tracts. All mutants had HepII lactose and HepI with either: i) Lac (2-Hex), ii) Gal→Lac (3-Hex), iii) Lacto-N-neotetraose (LNT) (4-Hex), or iv) GlcNAc→LNT (5-Hex). Human complement (C′) was prepared by depleting IgG and IgM from normal human serum. C′-dependent killing by mAb 2C7 was measured by serum bactericidal assays (SBA) and opsonophagocytosis (OPA) using freshly isolated human polymorphonuclear leukocytes (PMNs). mAb 2C7 and C3 on bacteria were measured by flow cytometry.**Results:** HepI chain extensions modulate 2C7 binding (2-Hex >> 4-Hex ≈ 5-Hex > 3-Hex). While mAb 2C7 completely killed *Ng* with HepI 2-Hex in a SBA with 0.2 μg/mL, the 3-Hex mutant was fully resistant to 10 μg/mL. The 4- and 5-Hex mutants showed intermediate resistance and were killed by 4, but not 2 μg/mL of 2C7. In presence of 2C7 and C′, the 3-Hex mutant showed the least C3 deposition. The low level of C3 deposited of the 3-Hex mutant was sufficient to mediate ~64% killing by PMNs in an OPA.**Conclusions:** HepI glycan extensions modulate the binding and function of mAb 2C7. While three of four HepI mutants were killed in a SBA with mAb 2C7, the fourth and only SBA resistant mutant, HepI 3-Hex, was killed by PMNs. Thus, the 2C7 LOS epitope is a promising vaccine candidate that may surmount challenges posed by pv of HepI glycans that are present in infected humans.

Author Disclosure Block:

S. Chakraborti: None. **L.A. Lewis:** None. **P. Rice:** None. **S. Ram:** None.

Poster Board Number:

SATURDAY-482

Publishing Title:

Passive Targeting of Dendritic Cells by Poly (Lactic Acid)-B-Poly (Ethylene Glycol) Nanoparticles Encapsulated Outer Membrane Peptide of *Chlamydia trachomatis* for Enhanced Adaptive Immune Responses

Author Block:

S. Dixit, R. Sahu, S. Duncan, S. R. Singh, V. A. Dennis; Alabama State Univ., Montgomery, AL

Abstract Body:

In quest of a vaccine against *Chlamydia trachomatis*, the most reported sexually transmitted bacterial infection globally, we previously published the successful encapsulation and slow release of M278 (a peptide derivative of *C. trachomatis* major outer membrane protein) from PLA-PEG, and its elicitation of enhanced adaptive immune responses in immunized mice. However, it is important to investigate the mechanisms of immuno-stimulation resulting in induction of adaptive immune responses and the role of passively targeted dendritic cells (DCs) as effective antigen presentation cells (APCs) for bolstering these responses. Pro-inflammatory responses in the presence of IL-6 activate expression of costimulatory molecules (CD40, CD80, and CD86) and cytokine (IL-12p40) by DCs for skewing Th1 adaptive immune responses. In contrast, low expression of CD40, CD80, CD86 and IL-10 is skewed towards Th2 responses. In this study, we investigated the mechanisms of PLA-PEG-encapsulated M278 uptake and processing by DCs and focused on expressions of CD40, CD80, CD86; cytokines (IL-6, IL-12p40 and IL-10), and MHC expression profiles. DCs derived from mouse bone marrow cells were exposed to naked M278 as well as encapsulated M278 nanoparticles (NPs) for different time-points (4-72 hours) and doses (1.25-10 µg/mL) to determine their role in antigen presentation and cellular uptake. Results from flow cytometry revealed that encapsulated M278 enhanced the expression levels of CD86 and CD40 on DCs by 2-fold in comparison to naked M278. Cytokine ELISA data showed increased production levels of IL-6 and IL-12p40 over a 72-hour time-period, which are prerequisites for maturation of DCs as potent APCs. Lower dose of 1.25 µg/mL of NPs was as effective as 10 µg/mL of naked M278, suggesting the adjuvanticity of the NPs. Concomitantly, we observed low production of IL-10, with further decrease over time. Collectively, these data suggests that our encapsulated M278 vaccine comprises an effective antigen and adjuvant that can drive maturation of DCs by IL-6 and stimulation of IL-12p40 for elicitation of enhanced Th1 adaptive immune responses.

Author Disclosure Block:

S. Dixit: None. **R. Sahu:** None. **S. Duncan:** None. **S.R. Singh:** None. **V.A. Dennis:** None.

Poster Board Number:

SATURDAY-483

Publishing Title:

Characterization and Intracellular Tracking of *Chlamydia trachomatis* Recombinant Momp Encapsulated in Biodegradable Plga 85/15 Nanoparticles in Mouse Primary Dendritic Cells

Author Block:

R. Sahu, S. Dixit, S. Duncan, E. Nyairo, S. R. Singh, V. A. Dennis; Alabama State Univ., Montgomery, AL

Abstract Body:

Chlamydia is a sexually transmitted disease caused by the intracellular bacterium, *Chlamydia trachomatis* (CT). Vaccine development and alternative therapeutics are inimitable ways to eradicate or control its infection. Development of vaccine using nanoparticles for therapeutic use is advancing in the infectious disease field. Applicability of nanoparticles depends on their physical properties for their uptake, intracellular processing and degradation, which are vital for biosafety and therapeutic efficacy studies. Herein, we have developed a CT nanovaccine by encapsulating its immunogenic MOMP (major outer membrane protein) in biodegradable PLGA [poly (D, L-lactic-co-glycolic acid); 85:15 lactide: glycolide ratio] nanoparticles. We first subjected the encapsulated rMOMP to physio-structural techniques followed by its uptake and intracellular distribution by antigen presenting cells, specifically dendritic cells (DCs). Zeta sizing, Zeta potential, DSC (Differential Scanning Calorimetry), UV spectroscopy and FT-IR (Fourier Transform Infrared Spectroscopy) were used to determine the size, stability and successful encapsulation of rMOMP. Encapsulated rMOMP was small (~200 nm), thermally stable (90ºC), negatively charged (-13 mV) with > 90% encapsulation efficiency. Using Taqman qPCR we show that exposing mouse primary DCs to encapsulated rMOMP resulted in higher upregulation of the mRNA gene transcripts of TLR-2 and the co-stimulatory molecules, CD80, CD86 as compared with naked rMOMP. To validate the allocation of nanoparticles and released rMOMP from PLGA85/15 within DCs, intracellular localization was studied by fluorochrome labeling multiple organelles (cell membrane, mitochondria, endoplasmic reticulum and lysosomes). Each organelle was identified with specific primary antibody followed by secondary antibody tagged with distinct fluorescent dyes. Anti-MOMP antibody with divergent fluorescence was used for discerning the presence of naked rMOMP and encapsulated rMOMP within the labeled organelles. Fluorescence and overlapping analyses of images showed that fluorescent labeling using target-specific antibodies were advantageous in locating the rMOMP and encapsulated rMOMP within the intracellular compartments of DCs.

Author Disclosure Block:

R. Sahu: None. **S. Dixit:** None. **S. Duncan:** None. **E. Nyairo:** None. **S.R. Singh:** None. **V.A. Dennis:** None.

Poster Board Number:

SATURDAY-484

Publishing Title:

Novel Fusion Protein as a Potential Vaccine against Chlamydial Infection

Author Block:

S. Liang, D. C. Bulir, E. Simms, J. B. Mahony; McMaster Univ., Hamilton, ON, Canada

Abstract Body:

Background: Sexually transmitted *C. trachomatis* infections can lead to a number of complications, including pelvic inflammatory disease and hydrosalpinx, a correlate of tubal infertility. However, up to 90% of women with *C. trachomatis* infections experience subclinical infections. Women with asymptomatic infections do not have an impetus to seek medical help, making them even more susceptible to developing serious complications. The high rate of asymptomatic infections, taken together with the severity of the infection-induced pathology, suggests that controlling *C. trachomatis* infections requires a vaccine, despite the availability of antibiotics. We previously demonstrated that mice vaccinated with a fusion antigen (BD584) consisting of chlamydial type III secretion components (CopB, CopD, and CT584) were protected from infection and pathology. Here, we compare the relative protective efficacy of the individual components of BD584 against *Chlamydia* infection.**Methods:**Five groups (n=5) of female C57BL/6 mice were vaccinated intranasally with 20 µg of the fusion protein BD584, CopB, CopD, CT584, or CopB+CopD+CT584 together with 10 µg of CpG in PBS. A group (n=5) of control mice were vaccinated with PBS only. All mice were vaccinated at -6 and -3 weeks prior to challenge with 4 x 10^5 inclusion forming units of *C. muridarum*. Five days after challenge, vaginal swabs were collected to quantify bacterial shedding via qPCR. Mice were sacrificed 49 days after challenge and upper genital tracts were harvested to assess incidence of hydrosalpinx.**Results:**Mice vaccinated with BD584, CopB, CopD, CT584, and CopB+CopD+CT584 displayed similar bacterial load upon challenge with *C. muridarum*. However, mice vaccinated with BD584 were best protected from the development of hydrosalpinx.**Discussion:**A *C. trachomatis* vaccine is urgently needed. Here we compared the relative protective efficacy of a fusion protein against that of its individual components in a mouse model of *Chlamydia* infection. Although mice vaccinated with each individual component demonstrated similar levels of protection against infection, mice vaccinated with BD584 were best protected from hydrosalpinx. Further studies are needed to elucidate the mechanism underlying this disconnection between infection and pathology, which will aid in the development of a *Chlamydia* vaccine that primarily aims to prevent pathology.

Author Disclosure Block:

S. Liang: None. **D.C. Bulir:** None. **E. Simms:** None. **J.B. Mahony:** None.

Poster Board Number:

SATURDAY-485

Publishing Title:

Construction of a Vaccine to Protect Against Food Poisoning From *Campylobacter jejuni* Colonization of Poultry Meat

Author Block:

R. Korba, **L. M. Temple**; James Madison Univ., Harrisonburg, VA

Abstract Body:

Bordetella avium is a gram negative bacterium that causes an upper respiratory infection in poultry. Though the mortality rate for bordetellosis is low, it weakens their immune system leading to secondary infection. *Campylobacter jejuni* is often found in the flora of poultry, but causes food poisoning in humans when contaminated meat is ingested. There is no vaccine to prevent colonization of poultry by *C. jejuni,* so a vaccine that would lower the level of colonization could have a major impact on this significant food safety problem. We hypothesized that a characterized autotransporter in *B. avium* called Baa1 could serve as a genomic location for insertion of an antigen region from *C. jejuni*, replacing a portion of this non-essential gene. The autotransporter has three regions: a promoter, passenger, and transporter. The passenger domain is translocated through the barrel formed by the transporter and expressed on the outer surface of the bacterial cell. Mutants lacking *baa1* are non-virulent. Our constructs were made using the TA cloning vector in *E. coli* that does not replicate in *B. avium.* In our research, we have successfully cloned two separate genes from *C. jejuni* to replace the passenger domain in between the *B. avium* promoter and transporter regions. The transporter region was retained to provide the secretion mechanism for the antigenic region encoded by two genes, *flaA* and *cjaA*. The promoter and transporter regions were cloned together, then the *C. jejuni* genes were separately ligated using unique restriction sites between the two *B. avium* regions to restore the approximate length of the native gene. Cloning was verified through PCR. Multiple positive clones containing each *C. jejuni* genes were found. Because the TA cloning vector does not replicate in *B. avium,* it serves as a suicide vector to deliver the heterologous construct into the host. We have begun the allelic replacement process using tri-parental mating or electroporation and have strains with putative single crossover events that contain the entire plasmid. When these are confirmed, antibiotic selection will be removed so the second crossover can occur. Once this step is completed, expression of the antigenic region of *C. jejuni* will be accomplished using Western blots with available antibodies. Future studies include testing for virulence, determination of ID_{50} in chickens, and protection tests.

Author Disclosure Block:

R. Korba: None. **L.M. Temple:** None.

Poster Board Number:

SATURDAY-486

Publishing Title:

Developing Peptide Mimotope Vaccines for *Burkholderia*

Author Block:

R. C. Bernhards[1], **C. H. Weaver**[1], P. Guo[2], J. Zhang[2], B. Li[2], S-C. Lo[2], S. L. Welkos[1];
[1]USAMRIID, Fort Detrick, MD, [2]FDA, Silver Spring, MD

Abstract Body:

Burkholderia pseudomallei and *Burkholderia mallei* represent bacterial biothreats and are classified as Tier 1 select agents. *B. pseudomallei* causes melioidosis which is endemic to Southeast Asia and Northern Australia, while *B. mallei* causes glanders primarily in horses but is also capable of infecting humans. Both of these diseases have high mortality rates and there are currently no vaccines available for either of them. The capsular polysaccharide (CPS) and lipopolysaccharide (LPS) expressed by these pathogens are important virulence factors and have been evaluated as potential protective vaccine antigens in animals. However, such polysaccharides usually induce short term immune responses but not longer-term immune memory, and are often associated with significant toxicity in the vaccinated host. Therefore, development of safer and more defined and immunogenic derivatives of CPS and LPS antigens is a priority. Peptides or other small analogs which mimic the immunogenic epitopes of CPS or LPS are possible candidates for safer and more effective vaccines. Phage display panning was used to discover peptide mimotopes of CPS and LPS using highly specific and protective monoclonal antibodies. The peptides were then tested for specific binding to their respective monoclonal antibodies using ELISA and bio-layer interferometry (BLI). Using BLI, the dissociation constants were determined for each peptide to help rank them according to binding strength and dissociation properties. The best peptide candidates are being conjugated to carrier proteins and the protein conjugates will be re-evaluated using BLI and assessed for their ability to elicit protection from *Burkholderia* infection in mice and nonhuman primates. The goal is to develop a multivalent CPS/LPS vaccine that is highly protective against both *B. pseudomallei* and *B. mallei*.

Author Disclosure Block:

R.C. Bernhards: None. **C.H. Weaver:** None. **P. Guo:** None. **J. Zhang:** None. **B. Li:** None. **S. Lo:** None. **S.L. Welkos:** None.

Poster Board Number:

SATURDAY-487

Publishing Title:

A Complex Problem: Targeting Meningococcal Proteins for Serogroup B Vaccine Design

Author Block:

K. A. Matthias[1], M. B. Strader[1], J. Lee[2], D. S. Patel[2], W. Im[2], M. C. Bash[1]; [1]Food and Drug Admin., Silver Spring, MD, [2]Univ. of Kansas, Lawrence, KS

Abstract Body:

The development and implementation of vaccines derived from the purified capsular polysaccharide (Ps) of *Neisseria meningitidis* serogroups A, C, W, and Y have been highly successful at preventing invasive disease. Use of Ps vaccines is not feasible for elimination of serogroup B, however, as the capsule resembles human Ps moieties and is poorly immunogenic. For this reason, group B vaccine design has targeted outer membrane proteins (OMPs). Vaccine effectiveness correlates with the presence of serum bactericidal activity, suggesting a dominant role for antibody-mediated bacterial lysis in protection against *N. meningitidis in vivo*. Yet, meningococcal OMPs can be subject to phase and antigenic variation, making elicitation of broadly protective bactericidal antibodies difficult. The essential protein PorB, which is comprised of eight surface-exposed loops (L1-L8), is a vaccine candidate and a useful model for testing the impact of antigenic variability on antibody binding, cross-protection, and OMP complex formation. We generated a panel of isogenic strains expressing chimeric serogroup 15 (MC58) and 4 (Cu385 and BB1350) PorB types. We then assessed the effect of sequence mutation on antibody binding using PorB-specific monoclonal (mAbs) and polyclonal antibodies (pAbs). L1-specific mAb binding to PorB was affected by sequence changes to other loop regions, such that mAb binding to wild type PorB > PorB with heterologous L5-L6 > PorB with heterologous L7-L8 > PorB with heterologous L5-L8; binding levels correlated with bactericidal activity against the same strains. Strains exhibiting heterologous PorB sequence were likewise decreased in binding affinity of pAbs specific for L5-L8, demonstrating the observed effect was not relegated to L1 alone. Next, we examined the role of OMPs known to interact with PorB in affecting epitope recognition. Deletion of two of these, PorA and RmpM, had no effect on the ability of antibodies to bind to PorB, but did alter the size and composition of the OMP complexes formed. These data suggest that genetic mosaicism may result in antigenic diversity through interactions between loops and that vaccine candidates targeting individual epitopes may be more limited than suggested by protein sequence alone.

Author Disclosure Block:

K.A. Matthias: None. **M.B. Strader:** None. **J. Lee:** None. **D.S. Patel:** None. **W. Im:** None. **M.C. Bash:** None.

Poster Board Number:

SATURDAY-488

Publishing Title:

Mosquitocidal Properties of Igg Targeting the Voltage Gated Sodium Channel in Malaria Vector *Anopheles gambiae*

Author Block:

J. Donkoh[1], J. Meyers[2], B. Foy[1]; [1]Colorado State Univ., Fort Collins, CO, [2]Texas A&M Univ., College Station, TX

Abstract Body:

Insecticides are crucial for controlling the transmission of malaria parasites and the disease they cause. Voltage gated sodium channels (VGSC) are large transmembrane proteins that are responsible for generating action potentials in the neurons of malaria vector mosquitoes, facilitating neurophysiologic function. They are also targets of the pyrethroid and organochlorine classes of insecticides. VGSCs are important insecticide targets for malaria control, but often have mutations which make them resistant to chemical insecticides. We are attempting to target the VGSC of malaria vectors with specific immunoglobulins that could be ingested in the mosquito's blood meals from vaccinated hosts. VGSCs have many transmembrane domains that are connected by extracellular loops of amino acids that vary in length and importance relative to the molecular action of the channel function. We developed antibodies against extracellular loops in VGSC domains 1, 2 and 4 from the malaria vector mosquito *Anopheles gambiae*. Immunohistochemical staining of adult *An. gambiae* tissue sections with our antibodies showed binding in in tissues of the head and thoracic ganglia. Surprisingly, two our antibodies also showed unique tissue staining relative to the others in Malpighian tubules, ovaries and the flight muscles. To determine if our antibodies could kill An. gambiae, we injected a physiological dose (958ng/mL) of our anti-VGSC IgGs into the thorax of female mosquitoes and measured their survivorship compared to control-injected mosquitoes. These injections reduce mosquito survivorship compared to controls, suggesting that the antibodies bind to the channels *in vivo* and disrupt VGSC function. We are preparing to blood feed these same antibodies to groups of mosquitoes and expect that they will kill them relative to controls. This work constitutes the first steps in developing mosquitocidal vaccines that could be used to combat malaria transmission and disease in many parts of the world.

Author Disclosure Block:

J. Donkoh: None. **J. Meyers:** None. **B. Foy:** None.

Poster Board Number:

SATURDAY-489

Publishing Title:

Resistance Incidence to Spr741 in Combination with Rifampin in Gram-Negative Isolates

Author Block:

N. COTRONEO, A. Rubio, T. R. Parr, Jr, T. Lister; Spero Therapeutics, Inc., Cambridge, MA

Abstract Body:

Background: SPR741 is a novel polymyxin B (PMB) analog that interacts with the outer membrane of Gram-negative bacteria, compromising the integrity of the lipopolysaccharide (LPS) barrier and enabling entry of antimicrobial compounds which otherwise lack significant activity, such as rifampin (RIF).**Methods:** The spontaneous resistance incidence (RI) of SPR741 in combination with RIF was assessed for seven isolates of *Escherichia coli* (Ec, n=3), *Acinetobacter baumannii* (Ab, n=3) and *Klebsiella pneumoniae* (Kp, n=1) in triplicate using an agar plating method with SPR741 at 4-32 ug/mL in combination with RIF at 0.1-64 ug/mL in comparison to RIF at 8x MIC. *Staphylococcus aureus* (Sa) RIF RI was also tested. A subset of isolates identified in the RI was passed 3x on non-selective agar and tested in modified CLSI-based agar dilution and broth microdilution MIC methods.**Results:** The RI to RIF ranged from 10^{-7} to 10^{-9} for all strains tested while SPR741 at 4-32 ug/mL in combination with RIF at 0.1-64 ug/mL RI was found to range from 10^{-6} to $\leq 10^{-9}$. An 88% resistance confirmation rate was obtained based on 215 colonies taken from multiple conditions tested using an agar based screening method. Two resistance phenotypes were apparent using this agar method:

Mutant Phenotype:	**Decreased RIF Susceptibility (%):**	**Decreased PMB Susceptibility (%):**	**Not Confirmed (%):**	**# Colonies Tested:**
***Ec* (n=3)**	66%	7%	27%	104
***Ab* (n=3)**	95%	0%	5%	62
***Kp* (n=1)**	67%	29%	4%	49

When elevated, RIF MICs were $\geq$128 ug/mL. Broth MICs of a subset of Ec isolates found to have elevated PMB MICs also demonstrated decreased susceptibility to 741 + clarithromycin (CLR), while susceptibility to amikacin, meropenem, and ciprofloxacin was unchanged for all isolates tested.**Conclusions:** RIF and RIF + SPR741 RI values in Ec, Ab and Kp were generally comparable and were similar to the RIF RI in Sa. Isolates with reduced susceptibility to RIF had RIF MICs $\geq$128 ug/mL, consistent with high level resistance conferred by *rpoB* mutations [AAC, **44**, 11 (2000)]. Cross-resistance to clinically relevant antibiotics aside from PMB was not

observed. Isolates with increased PMB MICs also had elevated MICs to SPR741 + CLR, however their virulence and potential clinical relevance has not been established.

Author Disclosure Block:

N. Cotroneo: None. **A. Rubio:** None. **T.R. Parr:** None. **T. Lister:** None.

Poster Board Number:

SATURDAY-490

Publishing Title:

Synergistic Effect of Gram-positive Agents Tested in Combination with a New Polymyxin Derivative (SPR741) against Multidrug-resistant (MDR) Gram-negative (GN) Pathogens

Author Block:

R. E. Mendes[1], P. R. Rhomberg[1], H. K. Becker[1], A. P. Davis[1], T. Lister[2], T. R. Parr[2], M. Vaara[3], R. K. Flamm[1]; [1]JMI Lab., North Liberty, IA, [2]Spero Therapeutics, Cambridge, MA, [3]Northern Antibiotics, Ltd, Espoo, Finland

Abstract Body:

Background: Few options are available for treating MDR GN infections and combination therapy is used to obtain activity greater than each individual component. The activity of clarithromycin (CLA) and rifampin (RIF) combined with SPR741 was assessed against wildtype (WT) and MDR GN isolates. **Methods:** WT and MDR *E. coli* (EC), *E. cloacae* (ECL), *K. pneumoniae* (KPN) and *A. baumannii* (ACB) were selected. CLA, RIF and SPR741 were tested by CLSI methods alone and in combination with SPR741 at fixed concentrations of 2 (F2), 4 (F4) and 8 (F8) μg/ml. Isolates with CLA-SPR741 (F8) MICs of ≥8 μg/ml were subjected to whole genome sequencing (WGS) for screening of MLS_B genes. **Results:** CLA had MIC_{50} values of 16-128 μg/ml against WT GN isolates, while CLA-SPR741 at F2 (MIC_{50}, 1-16 μg/ml), F4 (MIC_{50}, 0.12-1 μg/ml) and F8 (MIC_{50}, 0.12 μg/ml) showed MIC_{50} 8- to 2,048-fold lower than CLA. SPR741 at F8 had lowest MICs against MDR ECL ($MIC_{50/90}$, 0.12/2 μg/ml) and EC ($MIC_{50/90}$, 0.5/8 μg/ml), whereas this combination was less active against MDR KPN ($MIC_{50/90}$, 1/>32 μg/ml) and ACB ($MIC_{50/90}$, 32/>32 μg/ml). RIF tested alone had MIC_{50} values of 2-32 μg/ml against WT GN isolates, while RIF-SPR741 tested at F2 (MIC_{50}, 0.5-4 μg/ml), F4 (MIC_{50}, 0.06-0.25 μg/ml) and F8 (MIC_{50}, 0.015-0.12 μg/ml) decreased the RIF MIC_{50} values 4- to 2,048-fold. RIF-SPR741 at F8 had lowest MICs against MDR EC ($MIC_{50/90}$, 0.015/0.12 μg/ml) and ECL ($MIC_{50/90}$, 0.03/0.12 μg/ml), representing a 128- to 1,024-fold reduction in MICs compared to RIF. RIF had MICs 4- to 16-fold higher than the RIF-SPR741 combinations against MDR ACB. 14 GN were selected for WGS and showed *mph*(A or E), except for one EC that carried *erm*(B). **Conclusions**: CLA, RIF and SPR741 did not show direct *in vitro* activity against GN isolates. However, potent synergistic activity was observed when CLA, especially RIF, were combined with SPR741, which decreased CLA and RIF MIC up to 2048-fold.

	E. coli (No.)		*E. cloacae* (No.)		*K. pneumoniae* (No.)		*A. baumannii* (No.)	
	WT (10)	MDR (28)	WT (11)	MDR (10)	WT (10)	MDR (26)	WT (10)	MDR (51)

Antimicrobial (tested concentration)	$MIC_{50/90}$	$MIC_{50/90}$	$MIC_{50/90}$	$MIC_{50/90}$	$MIC_{50/90}$	$MIC_{50/90}$	$MIC_{50/90}$	$MIC_{50/90}$
CLA alone	32/128	256/>256	128/256	256/>256	128/128	128/>256	16/16	>256/>256
CLA + SPR741 (4 µg/ml)	0.12/0.5	2/16	0.5/1	1/8	1/1	16/>32	0.5/1	>32/>32
CLA + SPR741 (8 µg/ml)	0.12/0.12	0.5/8	0.12/0.12	0.12/2	0.12/0.25	1/>32	0.12/0.25	32/>32
RIF alone	8/16	16/16	32/32	32/128	16/32	32/>256	2/8	4/16
RIF + SPR741 (4 µg/ml)	0.06/0.12	0.03/0.12	0.12/1	0.12/0.25	0.12/0.12	0.25/>8	0.25/1	0.5/4
RIF + SPR741 (8 µg/ml)	0.03/0.03	0.015/0.12	0.015/0.03	0.03/0.12	0.06/0.06	0.06/>8	0.12/0.5	0.25/4
CLA = clarithromycin; RIF = rifampin.								

Author Disclosure Block:

R.E. Mendes: H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Spero Therapeutics. **P.R. Rhomberg:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Spero Therapeutics. **H.K. Becker:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Spero Therapeutics.. **A.P. Davis:** None. **T. Lister:** D. Employee; Self; Employee of Spero Therapeutics. **T.R. Parr:** D. Employee; Self; Employee of Spero Therapeutics. **M. Vaara:** B. Collaborator; Self; Collaborator with Spero Therapeutics.. **R.K. Flamm:** None.

Poster Board Number:

SATURDAY-491

Publishing Title:

Mechanism of Action of Spr-741, a Potentiator Molecule for Gram-Negative Pathogens

Author Block:

B. Murray[1], C. Pillar[1], M. Pucci[2], **D. Shinabarger**[1]; [1]Micromyx, LLC, Kalamazoo, MI, [2]Spero Therapeutics, Cambridge, MA

Abstract Body:

Background: Few options are available for treating multidrug resistant infections caused by Enterobacteriaceae. SPR-741 is a polymyxin (PMB) derived potentiator molecule under development for use in combination therapies against Gram-negative infections. To understand the mechanism of action, membrane leakage and inhibition of macromolecular synthesis (MMS) pathways were studied using *Escherichia coli* ATCC 25922.**Methods:** The MIC of SPR-741 and PMB was determined by standard broth microdilution standard methods (CLSI M7). Membrane damage was examined by exposing *E. coli* ATCC 25922 to various concentrations of SPR-741, PMB, or tetracycline (TET) and measuring the release of ATP from cells after 30 min and 2 hr exposures. Inhibition of DNA, RNA, protein, cell wall, and lipid MMS using radiolabeled precursors was studied using the same concentrations of SPR-741, and included positive control drugs for each pathway.**Results:** The MIC for SPR-741 was 64 µg/mL for *E. coli* ATCC 25922, and the MIC for PMB was 0.25 µg/mL. Results from the ATP release assays are shown in the table below.

Drug/Incubation Time	ATP Release (µM) with Drug Exposure (µg/mL)				
	16 µg/mL	32 µg/mL	64 µg/mL	128 µg/mL	512 µg/mL
SPR-741 (30 min)	0.01	0.00	0.00	0.02	0.00
PMB (30 min)	0.10	0.12	0.16	-	-
SPR-741 (120 min)	0.00	0.00	0.00	0.00	0.03
PMB (120 min)	0.12	0.10	0.09	-	-

For evaluation of membrane damage, it was determined that very little intracellular ATP (0.03 µM) was released into the extracellular medium after a 2 hr exposure with up to 512 µg/mL (8-fold the MIC) of SPR-741. When tested at 8-fold the MIC, the negative control TET released 0.03 µM of ATP. Incubation of cells with 16-64 µg/mL of PMB (64-256 fold the MIC) resulted in the release of 3-5 times more ATP (0.10-0.16 µM). There was little inhibition of DNA, RNA or cell wall synthesis by SPR-741. Slight dose-dependent inhibition of protein synthesis occurred from 1-4 fold the MIC, and there was constant (25-46%) inhibition of lipid synthesis from 0.25-8

fold the MIC.**Conclusions:** SPR-741 had an MIC of 64 μg/mL against *E. coli* ATCC 25922. Compared to PMB, very little ATP was released from actively growing cells when exposed to high concentrations (512 μg/mL) of SPR-471 representing 8-fold the MIC. No clear dose-dependent inhibition of the 5 MMS pathways was detected for SPR-471.

Author Disclosure Block:

B. Murray: H. Research Contractor; Self; Spero Therapeutics. **C. Pillar:** H. Research Contractor; Self; Spero Therapeutics. **M. Pucci:** D. Employee; Self; SperoTherapeutics. **D. Shinabarger:** H. Research Contractor; Self; Spero Therapeutics.

Poster Board Number:

SATURDAY-492

Publishing Title:

Potentiation of Antibiotic Activity by a Novel Cationic Peptide, Spr741

Author Block:

D. Corbett[1], A. Wise[1], S. Birchall[1], E. Trimby[1], J. Smith[1], T. Lister[2], M. Vaara[3]; [1]Evotec (UK) Ltd, Manchester, United Kingdom, [2]Spero Therapeutics, Cambridge, MA, [3]Northern Antibiotics Ltd, Espoo, Finland

Abstract Body:

Background: Novel approaches to the treatment of multi drug resistant (MDR) Gram-negative bacterial (GNB) infections are urgently required. One approach is to potentiate the efficacy of existing antibiotics whose spectrum of activity is limited by the permeability barrier presented by the GNB outer membrane (OM). Cationic peptides derived from polymyxin B have been used to permeabilise the OM of GNB, granting antibiotics that would otherwise be excluded access to their targets. We assessed the *in vitro* efficacy of combinations of SPR741 with conventional antibiotics against *Escherichia coli* (*Ec*), *Klebsiella pneumoniae* (*Kp*), and *Acinetobacter baumannii* (*Ab*).**Methods:** Efficacy was assessed in checkerboard assays. The MIC of SPR741, antibiotics, and combinations thereof was defined as the lowest concentration that inhibited growth of *Ec* ATCC 25922, *Ab* NCTC 12156 and *Kp* ATCC 43816. *Ec* BW25113, *ΔtolC* and *ΔacrA* were used to assess the contribution of the multi drug efflux pump AcrAB-TolC to susceptibility to the combinations. Interactions were assessed by calculating fractional inhibitory concentration indices (FICI) for each combination in which the MIC differed from compounds in isolation. Interactions were defined as: FICI > 4, antagonism; 0.5-4, no interaction; <0.5, synergy. The minimum bactericidal concentration of combinations in the presence of 5% surfactant (Survanta) was also determined.**Results:** Of 22 antibiotics tested, the MIC of 7 - azithromycin, clarithromycin (CLR), fusidic acid (FA), mupirocin, retapamulin (RET) rifampicin (RIF), telithromycin - against *Ec* and *Kp* was reduced 32 - 8,000-fold in the presence of 8-16 µg/mL SPR741; against *Ab*, similar potentiation was achieved with CLR, FA, RET, and RIF. SPR741 was able to potentiate antibiotics that are substrates of AcrAB-TolC, effectively circumventing the pump's contribution to intrinsic antibiotic resistance. RIF or CLR in combination with SPR741 were bactericidal at concentrations similar to the MIC irrespective of the presence of Survanta.**Conclusions:** The efficacy of several antibiotics with diverse targets was substantially increased when combined with SPR741. These studies support the development of SPR741 in combination with antibiotics for treatment of MDR GNB.

Author Disclosure Block:

D. Corbett: H. Research Contractor; Self; Spero. **A. Wise:** H. Research Contractor; Self; Spero. **S. Birchall:** H. Research Contractor; Self; Spero. **E. Trimby:** H. Research Contractor;

Self; Spero. **J. Smith:** H. Research Contractor; Self; Spero. **T. Lister:** D. Employee; Self; Spero Therapeutics. **M. Vaara:** B. Collaborator; Self; Spero. C. Consultant; Self; Spero. J. Scientific Advisor (Review Panel or Advisory Committee); Self; Spero.

Poster Board Number:

SATURDAY-493

Publishing Title:

Bacterial Cytological Profiling of Spr741 Mechanism of Action Is Consistent with Membrane Permeabilization That Allows Penetration of Antibiotics into Gram-Negative (G-) Bacteria

Author Block:

J. Pogliano[1], M. Sharp[1], T. Lister[2], A. RUBIO[2]; [1]Linnaeus BioSci.s, San Diego, CA, [2]Spero Therapeutics, Cambridge, MA

Abstract Body:

Background: SPR741 is a derivative of polymyxin B that sensitizes G- bacteria to antibiotics that are normally excluded by the outer membrane. To understand the mechanism by which SPR741 enhances sensitivity, we used bacterial cytological profiling (BCP), a microscopy based technique that provides insight into the mechanism of action for antibacterial compounds. Here, we assess SPR741 alone and in combination with a fluorescently labeled macrolide or penicillin, NBD-Azithromycin or Bocillin, respectively, along with other fluorescent stains to observe uptake into *Escherichia coli* (Ec). **Methods:** For profiling experiments, overnight cultures were diluted and grown to an OD_{600} 0.15, at 30^{o}C. Cultures were then split into 500 uL samples, mixed with increasing concentrations of SPR741 and incubated at 30^{o}C for 2 hours. Samples were stained with FM4-64, DAPI or Sytox Green, concentrated by centrifugation and examined by microscopy. For uptake experiments, Ec ATCC25922 cultures were grown to early logarithmic phase, diluted to ~10^7 cfu/mL and exposed to increasing concentrations of SPR741 for 2 hours. Cultures were then treated with 5 ug/mL of NBD-Azithromycin or Bocillin for an additional hour. Then samples were stained with FM4-64 and DAPI, washed three times, examined by microscopy and quantified using flow cytometry. **Results:** Profiling results demonstrate that SPR741 affects the cell envelope, causing subtle cell shape changes and increasing permeability to stains normally excluded by the outer membrane of *E. coli*. Quantitative flow cytometry experiments show that Ec is largely impenetrant to NBD-Azithromycin or Bocillin. Addition of 4 µg/mL or 8 µg/mL SPR741 increased the uptake of NBD-Azithromycin 4.7-fold and 6.9-fold, respectively. Preincubation with 4 µg/mL or 8 µg/mL of SPR741 also increased the accumulation of the fluorescent penicillin, Bocillin by 2.5-fold, and at higher doses (up to 32 µg/mL SPR741) increased Bocillin staining more than 5-fold. **Conclusions:** Results shown herein are consistent with SPR741 permeabilizing the outer membrane to allow penetration of normally impenetrant G+ antibacterials to the periplasm and cytoplasm of G- bacteria, like Ec.

Author Disclosure Block:

J. Pogliano: D. Employee; Self; Linnaeus Biosciences. **M. Sharp:** D. Employee; Self; Linnaeus Biosciences. **T. Lister:** D. Employee; Self; Spero Therapeutics. **A. Rubio:** D. Employee; Self; Spero Therapeutics.

Poster Board Number:

SATURDAY-494

Publishing Title:

Projection of Spr741 Human Pharmacokinetics and Efficacious Dose Using Three Species Allometric Scaling

Author Block:

P. Shastri[1], **S. Coleman**[2]; [1]WIL Res., Ashland, OH, [2]Spero Therapeutics, Cambridge, MA

Abstract Body:

Background: SPR741 is a polymyxin derivative that is currently being investigated as a partner in combination with other antibiotics in the treatment of multi-drug resistant Gram-negative infections. Estimation of the efficacious human dose was made using a three species allometric scaling analysis. **Methods:** Human pharmacokinetic (PK) parameters for SPR741 were estimated using both fixed and floating exponent allometric scaling methods. SPR741 PK data from mouse, rat, and monkey studies were fit to a 1-compartmental model and the same model was used for human simulations. PK analysis was performed using Phoenix® WinNonlin® Version 6.3 (Pharsight Corp. [Mountain View, CA]). Mean plasma concentrations of SPR741 were used to derive PK parameters from preclinical studies. A one-compartment model was fitted to mean SPR741 PK profiles from mouse, rat, and monkey PK studies. A MixRatio (additive and multiplicative) weighting was applied for all PK modeling. Two sets of scaled human PK parameters for SPR741 that yielded a wide range of estimates were used to calculate Human Equivalent Dose (HED) and to simulate SPR741 concentrations in healthy subjects after a single one-hour IV infusion dose.**Results:** Human clearance and half-life estimates for SPR741 from various allometric approaches ranged from 51 to 103 mL/h/kg and 2.2 to 2.4 hours, respectively. Efficacy studies in mice with SPR741 demonstrated pharmacological activity with an AUC value of 60 µg*h/mL. The projected human efficacious dose for SPR741 ranges from approximately 200 to 400 mg administered three times per day using the scaled clearance and the AUC that demonstrated efficacy in the mouse studies. Human PK simulations were performed after a single IV infusion administration of SPR741 with the AUC ranging from 14 to 28, 28 to 56, 55 to 112, and 110 to 225 µg*h/mL at 100, 200, 400, and 800 mg, respectively.**Conclusions:** The projected human PK profile and efficacious dose of SPR741 are consistent with potential partner antibiotics. Further, these data in combination with the current nonclinical studies for SPR741 support advancement into more thorough nonclinical studies to enable clinical development.

Author Disclosure Block:

P. Shastri: H. Research Contractor; Self; WIL Research. **S. Coleman:** D. Employee; Self; Spero Therapeutics.

Poster Board Number:

SATURDAY-495

Publishing Title:

***In Vitro* Activity of Faddi-287, a Representative of a Novel Series of Polymyxins (Pm) with Reduced Nephrotoxic Potential**

Author Block:

O. Lomovskaya[1], D. Rubio-Aparicio[1], K. Nelson[1], K. D. Roberts[2], P. E. Thompson[2], R. L. Nation[2], T. Velkov[2], J. Li[2], S. J. Hecker[1], D. C. Griffith[1], M. N. Dudley[1]; [1]The Med.s Company, San Diego, CA, [2]Monash Inst. of Pharmaceutical Sci., Melbourne, Australia

Abstract Body:

Background: The major objective of our PM discovery program is to develop novel compounds that retain PM-like potency but have significantly improved safety profiles. In this study we evaluated in vitro potency of FADDI-287, a representative of a series of novel PM(s) with much reduced nephrotoxic potential, against the panels of *Pseudomonas aeruginosa* (PA), carbapenem-resistant *Acinetobacter baumannii* (CRAB), and carbapenemase-producing, carbapenem-resistant *Enterobacteriaceae* (CP-CRE).**Methods:** Clinical isolates of PA (N=200), CRAB (N=210) and CP-CRE (N=176, 73 KPC, 78 MBL and 25 OXA-48-like producing strains) were tested by the reference broth microdilution method for susceptibility to FADDI-287, polymyxin B (PMB) and other comparators.**Results:** FADDI-287 ($MIC_{50/90}$, 0.25/0.5 µg/ml) was 4-fold more potent than PMB ($MIC_{50/90}$, 1/2 µg/ml) against CRAB isolates, and had much higher potency compared to other tested antibiotics ($MIC_{50/90}$ of tigecycline, minocycline, doxycycline, levofloxacin and meropenem of 4/8, 8/16, 64/>64, 16/64 and 32/>64 µg/ml, respectively). FADDI-287 ($MIC_{50/90}$, 1/1 µg/ml) had excellent activity when tested against PA: it was slightly more potent than PMB ($MIC_{50/90}$, 1/2 µg/ml) and much more potent that comparator agents ($MIC_{50/90}$ of levofloxacin, imipenem, aztreonam, cefepime, ceftazidime and amikacin of 0.5/32, 2/32, 8/32, 4/32, 4/64 and 2/32 µg/ml, respectively). The potency of FADDI-287 ($MIC_{50/90}$, 0.5/16 µg/ml) against the challenge panel of CP-CRE isolates was similar to that of PMB ($MIC_{50/90}$, 1/16 µg/ml). This panel was highly resistant to comparator antibiotics with MIC_{90} >64 µg/ml for levofloxacin, gentamicin, and all tested beta-lactams (meropenem, ceftazidime, aztreonam). The CRE panel contained 21 strains with MIC for PMB >4 µg/ml and all these strains had increased FADDI-287 MICs. Cross-resistance between PMB and FADDI-287 was also observed in PA and CRAB.**Conclusions:** FADDI-287 was very active against CRAB, PA and CP-CRE isolates warranting further preclinical evaluation of the promising series of novel PMs.

Author Disclosure Block:

O. Lomovskaya: D. Employee; Self; The Medicines Company. **D. Rubio-Aparicio:** D. Employee; Self; The Medicines Company. **K. Nelson:** D. Employee; Self; The Medicines

Company. **K.D. Roberts:** None. **P.E. Thompson:** None. **R.L. Nation:** None. **T. Velkov:** None. **J. Li:** None. **S.J. Hecker:** D. Employee; Self; The Medicines Company. **D.C. Griffith:** D. Employee; Self; The Medicines Company. **M.N. Dudley:** D. Employee; Self; The Medicines Company.

Poster Board Number:

SATURDAY-496

Publishing Title:

Impact of Dosing Regimens on the *In Vivo* Efficacy of Combinations of Novel Antimicrobial Cationic Peptide Spr741 and Rifampicin in Murine Thigh Infection Models

Author Block:

P. Warn[1], D. Corbett[1], J. Gould[1], G. Parker[1], G. Daws[1], P. Thommes[1], P. Evenden[1], T. Lister[2], T. R. Parr[2]; [1]Evotec (UK) Ltd, Manchester, United Kingdom, [2]Spero Therapeutics, Cambridge, MA

Abstract Body:

Background: Potentiation of new or existing antibiotics may be an alternative approach to bridge the gap in the pipeline of therapeutic options for MDR Gram-negative infections. In these studies we assessed the impact of different dosing regimens on the efficacy of combinations of a polymyxin derivative, SPR741, with rifampicin (Rif) in murine models of thigh muscle infection**Methods:** Male ICR mice were rendered neutropenic using 2 doses of cyclophosphamide. Thighs of the mice were infected by intramuscular injection on day 0 with *E. coli* IR60 [Bla_{NDM-1}]. Subcutaneous treatment was initiated 1h post infection with 120mg/kg total dose SPR741 fractionated as 1, 2 or 3 doses administered at 1, 4 and 8h post infection plus 60mg/kg/dose of subcutaneously Rif at 1 and 5h PI to determine the post antimicrobial response. Thigh burdens were quantitatively assessed at 5, 9, 13, 19 and 25h PI.In a second study 120mg/kg total dose of SPR741 was fractionated as 1, 2, 3, or 4 doses administered subcutaneously in combination with 60, 120, 180 or 240mg/kg total dose Rif subcutaneously at 1h; 1 and 13h; 1, 9, and 17h; or 1, 7, 13, and 19h post infection. Thigh burdens were quantitatively assessed at 25h post infection.**Results:** SPR741 and Rif were well tolerated by the animals in both studies. The isolate demonstrated vigorous *in vivo* growth of 2.7 - 3.6Log_{10}cfu/g thigh tissue in vehicles across studies. Following a single dose of the SPR741-Rif combination all burdens were reduced to below stasis for >9h, a second dose of Rif or a more fractionated regimen of SPR741 reduced burdens below stasis for >19h. When the SPR741-Rif combination was administered less frequently the only regimen to achieve burdens below stasis was a total dose of 40mg/kg SPR741 plus 60mg/kg Rif administered every 8h**Conclusions:** The combination of SPR741 with Rif was effective at reducing the thigh burden of mice infected with *E. coli* expressing Bla_{NDM-1}. The data from these two studies suggest that a minimum area under the curve (AUC) must be maintained over time to demonstrate efficacy for this combination and support continued development of SPR741 combinations for the treatment of MDR-GNB.

Author Disclosure Block:

P. Warn: H. Research Contractor; Self; Spero Therapeutics. **D. Corbett:** H. Research Contractor; Self; Spero Therapeutics. **J. Gould:** H. Research Contractor; Self; Spero Therapeutics. **G. Parker:** H. Research Contractor; Self; Spero Therapeutics. **G. Daws:** H. Research Contractor; Self; Spero Therapeutics. **P. Thommes:** H. Research Contractor; Self; Spero Therapeutics. **P. Evenden:** H. Research Contractor; Self; Spero Therapeutics. **T. Lister:** D. Employee; Self; Spero Therapeutics. **T.R. Parr:** A. Board Member; Self; Spero Therapeutics. D. Employee; Self; Spero Therapeutics.

Poster Board Number:

SATURDAY-497

Publishing Title:

***In Vivo* Efficacy of Combinations of Novel Antimicrobial Peptide Spr741 and Rifampicin in Short-Duration Murine Lung Infection Models of *Klebsiella pneumoniae* Infection**

Author Block:

P. Warn[1], J. Teague[1], E. Burgess[1], L. Payne[1], D. Corbett[1], A. Sharp[1], T. Lister[2], T. R. Parr[2]; [1]Evotec (UK) Ltd, Manchester, United Kingdom, [2]Spero Therapeutics, Cambridge, MA

Abstract Body:

Background: Infections due to carbapenem-resistant Enterobacteriaceae (CRE) (*Klebsiella pneumoniae* carbapenemase [KPC] and (New Delhi Metallo-β-lactamase [NDM-1]) are being increasingly reported. Following infection, treatment options are restricted to drugs with modest efficacy or dose-limiting toxicity leading to sub-optimal outcomes. Potentiation of antimicrobial agents to increase potency and extend spectrum of activity has shown utility in pre-clinical studies. In these studies we assessed the efficacy of combinations of a novel antimicrobial cationic peptide (SPR741) with rifampicin (Rif) in murine models of acute pneumonia due to *K. pneumoniae*.**Methods:** Male ICR mice were rendered neutropenic using two doses of cyclophosphamide on days -4 and -1. Mice were infected by intranasal instillation on day 0 with either *K. pneumoniae* (Kp114 [Bla_{KPC}] or ATCC BAA 2146 [Bla_{NDM-1}]). Treatment was started 2h post infection (PI) with SPR741 administered at 2, 4.5 and 8h PI and Rif administered at 2 and 6h PI). SPR741 was administered at 10, 20 and 40mg/kg/dose, Rif was administered at 40 and 64 mg/kg/dose for Kp114 and ATCC BAA 2146 respectively. Mice were euthanized 10h post infection and the lungs quantitatively cultured.**Results:** SPR741 and Rif were well tolerated and all animals continued to the study end. Isolates demonstrated robust *in vivo* growth of 0.6 and 0.75Log_{10}cfu/g lung tissue between pre-treatment and harvest samples (Kp114 and ATCC BAA 2146 respectively). Monotherapy with SPR741 at 20mg/kg/dose or Rif had little effect on the burdens and did not achieve stasis against either isolate. In contrast, for Kp114 the combination of ≤10mg/kg/dose SPR741 with 40mg/kg/dose Rif led to reductions in burden of ≥1.6Log10cfu/g below stasis. For ATCC BAA 2146 the combination of 40mg/kg/dose SPR741 with 64mg/kg/dose Rif led to reductions in burden of 0.85Log10cfu/g below stasis.**Conclusions:** The combination of SPR741 with Rif was highly effective at reducing the lung burden of mice infected with *K. pneumonia* including strains expressing Bla_{KPC} and Bla_{NDM-1}. These studies support continued development of novel antimicrobial cationic peptides for the treatment of MDR Gram-negative infections.

Author Disclosure Block:

P. Warn: H. Research Contractor; Self; Spero Therapeutics. **J. Teague:** H. Research Contractor; Self; Spero Therapeutics. **E. Burgess:** H. Research Contractor; Self; Spero

Therapeutics. **L. Payne:** H. Research Contractor; Self; Spero Therapeutics. **D. Corbett:** H. Research Contractor; Self; Spero Therapeutics. **A. Sharp:** H. Research Contractor; Self; Spero Therapeutics. **T. Lister:** D. Employee; Self; Spero Therapeutics. **T.R. Parr:** A. Board Member; Self; Spero Therapeutics. D. Employee; Self; Spero Therapeutics.

Poster Board Number:

SATURDAY-498

Publishing Title:

***In Vivo* Efficacy of Combinations of Novel Antimicrobial Cationic Peptide Spr741 and Clarithromycin (Clr) in Short-duration Murine Thigh and Lung Models of Gram-negative Infection**

Author Block:

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Abstract Body:

Background: Due to an increasing incidence of multi-drug-resistant Gram-negative infections (MDR-GNB) new therapeutic options are urgently required. SPR741 is cyclic peptide that permeabilizes the outer membrane of GNB. When used in combination, in some cases, it can increase the intracellular exposure of inpermeant antimicrobials. In these studies we assessed the efficacy of combinations of SPR741 with CLR in murine models of thigh and lung infection**Methods:** Male ICR mice were rendered neutropenic using 2 doses of cyclophosphamide. Mice were infected either IM into the lateral thigh muscle or by IN instillation on day 0 with either *E. coli* (*E.c*.) (ATCC 25922 and IR60 [Bla_{NDM-1}]) (thigh only), *Klebsiella pneumoniae* (*K.p*.) ATCC BAA 2146 [Bla_{NDM-1}] or Kp114 [Bla_{KPC-3}] (lung only) or *Acinetobacter baumannii* (*A.b.)* (ATCC BAA 747). Treatment was initiated 1h (thigh) or 2h (lung) post infection (PI) with SPR741 given subcutaneously at 1, 3.5 and 7h (thigh) or 2, 4.5 and 8h (lung) PI and CLR given IV at 1h (thigh) or 2h (lung) PI. SPR741 was administered at 10, 20 and 40 mg/kg/dose, and CLR was administered at 125 mg/kg (E.c. ATCC 25922 only) or 100 mg/kg. Mice were euthanized 9h (thigh) or 10h (lung) PI and the thigh or lung quantitatively cultured**Results:** SPR741 was well tolerated. Isolates demonstrated robust *in vivo* growth between pre-treatment and harvest samples. Monotherapy with SPR741 at 20 mg/kg/dose or CLR had little effect on the burdens. In contrast in all thigh burden models combinations with >20 mg/kg/dose thigh SPR741 with CLR led to reductions in burden (1.8, 0.4, 3.3 and 1.6 Log_{10}cfu/g below stasis for ATCC 25922, IR60, ATCC BAA 2146 and ATCC BAA 747, respectively), In lung burden models combinations with 40mg/kg/dose SPR741 with CLR led to reductions in burden (1.8, 1.2Log_{10}cfu/g below stasis for Kp114 and ATCC BAA 747 respectively) but was ineffective against ATCC BAA 2146**Conclusions:** The combination of SPR741 with CLR was effective at reducing the thigh burden of mice infected with *E.c., K.p.* and *A.b* including strains expressing Bla_{NDM-1}. It was also effective in pneumonia models due to a *K.p.* strain expressing Bla_{KPC-3} and *A.b.* These studies support continued development of SPR741 for the treatment of MDR-GNB

Author Disclosure Block:

P. Warn: H. Research Contractor; Self; Spero Therapeutics. **A. Sattar:** H. Research Contractor; Self; Spero Therapeutics. **P. Thommes:** H. Research Contractor; Self; Spero Therapeutics. **D. Corbett:** H. Research Contractor; Self; Spero Therapeutics. **K. Holden:** H. Research Contractor; Self; Spero Therapeutics. **T. Lister:** D. Employee; Self; Spero Therapeutics. **T. Parr:** A. Board Member; Self; Spero Therapeutics. D. Employee; Self; Spero Therapeutics.

Poster Board Number:

SATURDAY-499

Publishing Title:

Pharmacology Of The Novel Polymyxin Faddi-287 In Preclinical Models

Author Block:

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Abstract Body:

Background: The widespread dissemination of antibiotic resistance in gram negative pathogens has necessitated the increased use of potentially nephrotoxic polymyxins. We have developed a new series of polymyxin B (PMB) derivatives with improved safety profiles and activity against major MDR bacteria. This study aimed to demonstrate the activity of FADDI-287 in animal models of infection. M**ethods:** The minimum lethal dose (MLD) and nephrotoxicity of FADDI-287 and PMB were determined in Swiss mice. PK studies were conducted to compare drug exposure in mice and rats. For the neutropenic mouse thigh infection using *A. baumannii* or *P. aeruginosa*, Swiss mice were infected with ~10^6 CFU/thigh. Doses were administered IP at various intervals starting 2 h post-infection and continued over 24 h. For the rat lung infection model using *A. baumannii*, Sprague-Dawley rats were infected with ~10^7 CFU/lung. FADDI-287 and PMB were administered IV BID starting 2 h post-infection and continued over 24 h. For both infection models, untreated control groups were sacrificed at the start of treatment and both untreated and treated groups were sacrificed 24 h after the start of treatment, infected tissues harvested, homogenized, and plated to determine colony counts. **Results:** The table summarizes the toxicity, PK, and efficacy in mice.

Compounds	MLD IV (mg/kg)	Kidney Changes (6 x 10 mg/kg; IP)	*f*AUC (mg*hr /L) 5 mg/kg (IP)	24h *f*AUC at EC_{50} vs. *P. aeruginosa*
PMB	7.5	Minimal to Severe Nephrosis	1.39	5.72
FADDI-287	15	No change	0.88	5.33

In the mouse thigh infection model, equivalent exposures of FADDI-287 and PMB produced similar bacterial killing against *P. aeruginosa*. In the mouse thigh and rat lung infection models against *A. baumannii*, FADDI-287 showed much better bacterial killing (-1.98 [mouse] and -0.79 [rat] log CFU/lung) than PMB (-0.23 [mouse] and +1.21 [rat] log CFU/lung) at similar exposures. **Conclusion:** FADDI-287 was less acutely toxic, less nephrotoxic, but with

comparable to superior efficacy compared to PMB at similar exposures. Further studies on the activity of FADDI-287 are warranted.

Author Disclosure Block:

M. Sabet: D. Employee; Self; The Medicines Company. **Z. Tarazi:** D. Employee; Self; The Medicines Company. **T. Nolan:** D. Employee; Self; The Medicines Company. **J. Parkinson:** D. Employee; Self; The Medicines Company. **D. Rubio-Aparicio:** D. Employee; Self; The Medicines Company. **K.D. Roberts:** None. **P.E. Thompson:** None. **R.L. Nation:** None. **T. Velkov:** None. **J. Li:** None. **S.J. Hecker:** D. Employee; Self; The Medicines Company. **O. Lomovskaya:** D. Employee; Self; The Medicines Company. **M.N. Dudley:** D. Employee; Self; The Medicines Company. **D.C. Griffith:** D. Employee; Self; The Medicines Company.

Poster Board Number:

SATURDAY-500

Publishing Title:

In Vitro* Activity of Spr741 Against Recent Clinical Isolates of *Escherichia coli* and *Klebsiella pneumoniae

Author Block:

M. Hackel[1], T. Lister[2], T. R. Parr, Jr.[2], M. Vaara[3], D. Sahm[1]; [1]IHMA, Inc., Schaumburg, IL, [2]Spero Therapeutics, Cambridge, MA, [3]Northern Antibiotics, Espoo, Finland

Abstract Body:

Background: SPR741 is a potentiator compound currently under development by Spero Therapeutics. The objective of the present study was to investigate the *in vitro* activity of SPR741 in combination with rifampin or clarithromycin against 199 recent clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae*.**Methods**: Minimal inhibitory concentrations (MICs) were determined following CLSI microdilution guidelines against a global collection of 100 *E. coli* and 99 *K. pneumoniae* from 2014-2015. Compounds tested included rifampin and clarithromycin alone, and in combination with SPR741 at fixed concentrations of 2, 4 and 8 µg/mL.**Results**: Addition of fixed concentrations of SPR741 reduced the MIC values of rifampin and clarithromycin at all concentrations tested, with the greatest effect seen at a concentration of 8 µg/mL. Clarithromycin and rifampin MICs for *E. coli* were reduced ≥5 dilutions for 94% and 100% of isolates, respectively. For *K. pneumoniae*, clarithromycin and rifampin MICs were reduced ≥5 dilutions for 58% and 86% of isolates, respectively. Results are shown in the Table with MIC_{50} values shaded and MIC_{90} values bolded.Frequency distribution (n) of 199 *E. coli* and *K. pneumoniae* at each MIC.

Organism (n)	Compound	MIC (µg/mL)											
		≤0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	>64
E. coli (100)	CLR										1	2	11	**86**
	CLR SPER 8	1	1	5	19	**39**	19	7	3	3	2	1		
	RIF								6	35	**47**	5	2	5
	RIF SPER 8	**90**	4			2	1	2		1				
K. pneumoniae (99)	CLR													**99**
	CLR SPER 8					4	8	23	**22**	10	8	6	5	**13**
	RIF										12	**69**	8	**12**
	RIF SPER 8	6	22	**23**	18	5	4	5	1	4	4		1	2

CLR, clarithromycin; SPER, SPR741; RIF, rifampin

Conclusions: SPR741 exhibited strong potential for increasing the *in vitro* activity of clarithromycin and rifampin against many *E. coli* and *K. pneumoniae*. Further development of this potentiator compound could provide a valuable therapeutic option for treating infections caused by resistant gram-negative bacilli.

Author Disclosure Block:

M. Hackel: M. Independent Contractor; Self; IHMA, Inc. **T. Lister:** D. Employee; Self; Spero Therapeutics. **T.R. Parr:** D. Employee; Self; Spero Therapeutics. **M. Vaara:** D. Employee; Self; Northern Antibiotics. **D. Sahm:** M. Independent Contractor; Self; IHMA, Inc..

Poster Board Number:

SATURDAY-501

Publishing Title:

In Vitro* Activity of Spr741 Against Recent Clinical Isolates of *Acinetobacter baumannii

Author Block:

M. Hackel[1], T. Lister[2], T. R. Parr, Jr.[2], D. Sahm[1]; [1]IHMA, Inc., Schaumburg, IL, [2]Spero Therapeutics, Cambridge, MA

Abstract Body:

Background: SPR741 is a potentiator compound currently under development by Spero Therapeutics. The objective of the present study was to investigate the *in vitro* activity of SPR741 in combination with rifampin, clarithromycin or meropenem against 100 recent clinical isolates of *Acinetobacter baumannii*. **Methods:** Minimal inhibitory concentrations (MICs) were determined following CLSI microdilution guidelines against a global collection of 100 *A. baumannii* from 2014-2015. Compounds tested included rifampin, clarithromycin and meropenem alone, and in combination with SPR741 at fixed concentrations of 2, 4 and 8 µg/mL.**Results**: Addition of fixed concentrations of SPR741 reduced the MIC values of clarithromycin, rifampin, and meropenem at all concentrations tested, with the greatest effect seen at a concentration of 8 µg/mL. Reductions in MIC of 3 doubling dilutions and 5 doubling dilutions, respectively, with the addition of 8 µg/mL of SPR741 were: clarithromycin: 26% and 56%; rifampin: 63% and 45%; meropenem: 9% and 0%. Results are shown in the Table with MIC_{50} values shaded and MIC_{90} values bolded.Frequency distribution (n) of 100 *A. baumannii* at each MIC.

Compound	MIC (µg/mL)												
	≤0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	>64
CLR							1		5	4	9	15	**66**
CLR SPER 2				1		4	16	17	9	4	6	5	**38**
CLR SPER 4		1		5	13	17	7	8		4	3	5	**37**
CLR SPER 8		4	3	16	20	6	2	3	1	2	10	2	**31**
RIF			1		1	4	41	28	**17**	1	3	4	
RIF SPER 2		1	1	9	32	17	23	**11**	1	2	3		
RIF SPER 4	1	1	17	27	14	10	**22**	4	1		3		
RIF SPER 8	3	32	13	10	11	12	**15**	1		2	1		
MEM		1	8	6	3	2	1	2	1	3	14	29	**30**
MEM SPER 2		4	9	4	3	1	1	2	1	6	21	29	**19**
MEM SPER 4	1	6	9	4		1	2	2	2	10	27	23	**13**
MEM SPER 8	1	1	11	6	1	3	2	1	9	17	23	**20**	5

CLR, clarithromycin; SPER, SPR741; RIF, rifampin; MEM, meropenem

Conclusions: SPR741 exhibited strong potential for increasing the *in vitro* activity clarithromycin and rifampin against many *A. baumannii*, while the effect on meropenem was more modest. Further development of this potentiator compound could provide a valuable therapeutic option for treating infections caused by this difficult to treat pathogen.

Author Disclosure Block:

M. Hackel: M. Independent Contractor; Self; IHMA, Inc. **T. Lister:** D. Employee; Self; Spero Therapeutics. **T.R. Parr:** D. Employee; Self; Spero Therapeutics. **D. Sahm:** M. Independent Contractor; Self; IHMA, Inc..

Poster Board Number:

SATURDAY-502

Publishing Title:

Potentiation of Antibiotic Activity by Novel Antimicrobial Cationic Peptides: Potency and Spectrum of Activity of Spr741

Author Block:

A. Wise[1], D. Corbett[1], A. Dorali[1], T. Langley[1], K. Skinner[1], L. Payne[1], T. Lister[2]; [1]Evotec (UK) Ltd, Manchester, United Kingdom, [2]Spero Therapeutics, Cambridge, MA

Abstract Body:

Background: Novel approaches to the treatment of multi drug resistant (MDR) Gram negative bacterial (GNB) infections are urgently required. Cationic peptides derived from polymyxin B have previously been shown to permeabilize the outer membrane (OM) of GNB, thus granting antibiotics that would otherwise be excluded access to their targets when administered in combination. We assessed the *in vitro* potency of combinations of the cationic peptide, SPR741 with selected antibiotics against MDR and clinical isolates of *Escherichia coli* (*Ec*), *Klebsiella pneumoniae* (*Kp*), and *Acinetobacter baumannii* (*Ab*).**Methods:** The potency of SPR741 in combination with azithromycin (AZ), aztreonam (AZT), clarithromycin (CLR), fusidic acid (FA), meropenem (MEM), mupirocin (MUP), rifampicin (RIF), and retapamulin (RET) was assessed using a susceptibility testing method based on CLSI guidelines M7-A10. Assays were performed against 25 *Ec*, 25 *Kp*, and 17 *Ab* MDR and clinical isolates, in Mueller-Hinton broth-II (cation adjusted) containing 0, 2 or 8 µg/mL SPR741. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of antibiotic required to inhibit visible bacterial growth. MIC values were determined for each antibiotic/SPR741 combination and maximum reductions in MIC of the antibiotic in the combination were determined.**Results:** Combination with SPR741 resulted in potentiation of test antibiotics against isolates of *Ab*, *Ec*, and *Kp*. For *Ab*, potentiation followed the order FA > RIF > CLR > RET > MEM > AZ > AZT > MUP, with 128 fold reductions in MIC relative to antibiotic alone (MIC reduced from >128 to ≤ 2 µg/mL in the combination). For *Ec*, potentiation followed the order RIF > MUP = FA > AZ = RET > AZT > MEM = CLR; maximum RIF MIC reduction of >2000 fold (MIC reduced from >128 to ≤0.125 µg/mL). For *Kp*, potentiation followed the order RET > RIF = AZ = CLR > MUP > MEM = FA > AZT; the MIC of RET was reduced 32 fold (MIC reduced from 128 to 4 µg/mL).**Conclusions:** SPR741 effectively reduced the MIC of currently utilized antibiotics against MDR isolates of *Ab*, *Ec*, and *Kp*, extending the promising spectrum of activity of these combinations, and supporting continued development of SPR741 for the treatment of MDR GNB.

Author Disclosure Block:

A. Wise: H. Research Contractor; Self; Spero. **D. Corbett:** H. Research Contractor; Self; Spero. **A. Dorali:** H. Research Contractor; Self; Spero. **T. Langley:** H. Research Contractor;

Self; Spero. **K. Skinner:** H. Research Contractor; Self; Spero. **L. Payne:** H. Research Contractor; Self; Spero. **T. Lister:** D. Employee; Self; Spero Therapeutics.

Poster Board Number:

SATURDAY-503

Publishing Title:

Effect Of An Antimicrobial Stewardship Intervention On Outcomes For Patients With Severe *clostridium Difficile* Infection

Author Block:

G. Eschenauer, T. S. Patel, T. Gandhi, C. Chenoweth, L. Washer, B. Chen, H. Welch, J. DeLeon, K. Rao, **J. Nagel**; Univ. of Michigan, Ann Arbor, MI

Abstract Body:

Background: Treatment of *Clostridium difficile* infection (CDI) is an ideal target for antimicrobial stewardship programs (ASP), as ASPs have been effective in improving care in patients with a variety of infections. Unfortunately, studies to date have not rigorously evaluated the impact of ASP involvement on complications attributed to CDI.**Methods**: We performed a quasi-experimental study of adult patients with severe CDI prior to (n= 231) and after (n=227) a real-time ASP review was initiated. In the ASP review (ASPR) group, a pharmacist member of the ASP was notified in real time of positive CD results and consulted with the care team to initiate optimal therapy, minimize concomitant antibiotic and acid-suppressive therapy, and recommend surgical/infectious diseases consultation (ID) in complicated cases. The primary outcome was a composite of 30-day mortality, ICU admission, colectomy/loop ileostomy, and/or recurrence. A blinded review panel of ID physicians determined whether outcomes were attributed to CDI.**Results**: A significantly higher percentage of patients in the ASPR group received vancomycin (87% vs. 59%, respectively, $p<0.0001$) and vancomycin therapy was initiated earlier (mean 1.05 days vs. 1.70 days, respectively, $p=0.04$), compared to the pre-ASPR group. The incidence of the composite outcome was not significantly different between the ASPR and pre-ASPR groups (13.7% vs. 15.2%, respectively, $p=0.65$).**Conclusions**: ASP review and intervention in patients with severe CDI improved process measures. A decrease in composite outcomes was not found, which may be due to low baseline rates of attributable surgery (2%), mortality (3%) and ICU admission (6%) in our institution.

Author Disclosure Block:

G. Eschenauer: E. Grant Investigator; Self; Merck. **T.S. Patel:** None. **T. Gandhi:** None. **C. Chenoweth:** None. **L. Washer:** None. **B. Chen:** None. **H. Welch:** None. **J. DeLeon:** None. **K. Rao:** None. **J. Nagel:** E. Grant Investigator; Self; Merck, Astellas.

Poster Board Number:

SATURDAY-504

Publishing Title:

Customizing an Electronic Medical Record to Automate the Workflow and Tracking of an Antimicrobial Stewardship Program

Author Block:

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Abstract Body:

Background: Documenting the actions and effects of an antimicrobial stewardship program (ASP) is essential for quality improvement and continued support by hospital leadership. Thus, our ASP (reference 1) tallies the number of charts reviewed, types of recommendations in 16 categories, to whom and how recommendations were communicated, whether they were followed, and any resultant days of antimicrobials avoided or added. Before 2014 we used a secure spreadsheet, but since then we have used a customized adaptation within Cerner PowerChart, the electronic medical record (EMR) at our institution, to track these data, facilitate our workflow, and enhance our ASP. **Methods and Results:** Our system involves the creation of a novel and intuitive ASP form in each patient EMR reviewed by the ASP, and two mutually exclusive tracking systems: one for active forms (to facilitate the daily ASP workflow) and one for finalized forms (to generate cumulative data reports). The ASP form combines autopopulated fields, checkbox options, spaces for concise entry of relevant text, and built-in logic to open portions of the form as appropriate and require certain entries before it can be closed. Forms are created by the ASP pharmacist, edited by the ASP physician, then reaccessed to record whether the recommendation was followed. The pharmacist subsequently records any antimicrobial days added or avoided, and the physician performs a final reconciliation and proofing of the form to bring it to final status. All ASP forms that have not reached final status are visible to ASP personnel on a real-time "MPage" that displays the information from each form in a single row that also contains two hyperlinks: one to access the patient chart and one to open the ASP form for updating. In contrast, our ASP Reports Program scans PowerChart every night to compile the data from all finalized ASP forms so they can be converted into sets of 63 graphs that can display a wealth of data — over any selected time span — by month, quarter, semiannually, or annually. **Conclusions:** We have customized Cerner PowerChart to facilitate the daily workflow and track the activities of our ASP. This user-friendly system has automated much of the documentation, enhanced the completeness and validity of the data recorded, facilitated data analysis, and freed up time to expand the activities of our ASP.

Author Disclosure Block:

M. Katzman: None. **J. Kim:** None. **M. Lesher:** None. **M. Loser:** None. **M. Ward:** None. **F. Glasser:** None.

Poster Board Number:

SATURDAY-505

Publishing Title:

Antibiotic Use for Surgical Prophylaxis as a Risk Factor for Surgical Site Infections at Three Egyptian Hospitals

Author Block:

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Abstract Body:

Background: Data on antibiotic use for surgical prophylaxis (SP) are limited in Egypt. The objective of this study is to assess the risk factors for surgical site infections (SSI) including antibiotic use for SP.**Methods:**Surveillance of SSI was conducted through prospective active surveillance at three surgical hospitals. Collected data included: patient demographics (age and gender), surgery related risk factors (type, duration, wound classification, use of implants, and use of drains) and surgical prophylaxis (antibiotic type, timing of preoperative dose, and post-operative duration). SSIs were identified using case definitions of CDC's National Healthcare Safety Network (NHSN). Antibiotic use for SP was evaluated using clinical practice guidelines for antimicrobial prophylaxis in surgery developed jointly by the American Society of Health-System Pharmacists, the Infectious Diseases Society of America, the Surgical Infection Society, and the Society for Healthcare Epidemiology of America.**Results:**A total of 2144 surgeries were included in the study during the period of September 2014-August 2015. SSI rates varied between hospitals (Hospital A: 2.8%, Hospital B: 6.3 %, Hospital C: 6.5%) with an overall rate of 4.5 % (96/2144). Univariate analyses showed significantly higher risk of SSI in surgeries at hospitals B and C, abdominal surgeries, emergency operations, clean-contaminated wounds, use of drains, administering SP > 1 hour before incision, use of antibiotics other than those recommended by the guidelines, and extending SP > 24 hours after operation ($p < 0.05$). Independent risk factors identified by logistic regression were surgeries at hospital B (adjusted Odds Ratio [OR], 2.3; 95% Confidence Interval [CI], 1.3-4.08), administering SP > 1 hour before incision (adjusted OR, 2.3; 95% CI, 1.5-3.7), and extending SP > 24 hours after operation (adjusted OR, 2.06; 95% CI, 1.3-3.3).**Conclusions:**Noncompliance with the guidelines regarding timing of the preoperative dose and duration of post-operative SP contribute to acquiring SSI. Enforcing policies for SP is an essential measure to prevent SSIs in the study hospitals.

Author Disclosure Block:

T. Saied: None. **S. Hafez:** None. **S. Girgis:** None. **H. Morsi:** None. **T. Youssef:** None. **E. Abdou:** None. **M. Talaat:** None.

Poster Board Number:

SATURDAY-506

Publishing Title:

Impact of an Antimicrobial Stewardship Program Using the Bedside Teaching Method Targeting Trainees of the Hematopoietic Stem Cell Transplantation Division: A Quasi-experimental Study at a Tertiary Care Cancer Center

Author Block:

K. Okinaka, T. Fukuda; Natl. Cancer Ctr. Hosp., Tokyo, Japan

Abstract Body:

Background: Very few studies have reported regarding the efficacy of an antimicrobial stewardship program (ASP) focusing on hematopoietic stem cell transplantation (HSCT). This study aimed to assess the effect of ASP using the bedside teaching method.**Methods:** We retrospectively reviewed the medical records of patients who were admitted to the HSCT division at the National Cancer Center Hospital from June 2013 to June 2015. The study period was divided into three phases: pre-intervention (Phase 1: June-December 2013), early-intervention (Phase 2: January-June 2014), and late-intervention phases (Phase 3: July 2014-June 2015). During Phases 2 and 3, an infectious disease (ID) physician examined all the patients who had fever during the pre-engraftment phase. Subsequently, the ID physician discussed the treatment strategy with the residents in charge of the patients as necessary.**Results:** A total of 205 HSCT (allogeneic-HSCT: 177) were performed during the study period and were distributed evenly among each phases. Eleven patients died because of infections within 100 days post-HSCT, and 3 of them died due to *Stenotrophomonas maltophilia* bacteremia. ASP intervention did not alter the number of beta-lactam (BL) prescriptions, the number of carbapenem prescription, the median treatment duration with BLs, and the number of BL represcription within 7 days. However, the median treatment duration with carbapenems was significantly decreased at each phase (Kruskal-Wallis test: 13 vs. 10 vs. 7 days; $p < 0.01$). The rate of de-escalation in BL prescriptions was significantly increased at each phase (Fisher's test: 9% vs. 14% vs. 19%; $p < 0.01$), and 34% of de-escalation was performed during neutropenia. The incidence density rates of *S. maltophilia* bacteremia tended to decrease (Fisher's test: 0.94 vs. 0.67 vs. 0.18 bacteremia/1000 patient-day; $p = 0.08$). The all-cause and infectious disease mortality rate during 100 days post-HSCT were similar among each phase (Log rank test: $p = 0.93$, and $p = 0.60$, respectively).**Conclusions:** Our findings suggested that ASP using the bedside teaching method has the potential to be effective in the HSCT setting wherein the problems associated with multi-drug resistant pathogens, such as *S. maltophilia*, are critical.

Author Disclosure Block:

K. Okinaka: None. **T. Fukuda:** None.

Poster Board Number:

SATURDAY-507

Publishing Title:

Evaluation of Appropriate Use of Antifungal Agents in a Tertiary Care Hospital in Greece

Author Block:

K. Korantanis[1], A. Markogiannakis[1], M. Gamaletsou[2], M. Samarkos[1], G. Daikos[1], **N. V. Sipsas**[1]; [1]Natl. and Kapodistrian Univ. of Athens, Athens, Greece, [2]Univ. of South Manchester, Athens, United Kingdom

Abstract Body:

Background: Studies have shown that misuse of antifungals (AF) is common in the hospital setting. Our aim was to the assess the quality of antifungal use in our institution as the first step for the implementation of a targeted antifungal stewardship program**Methods:** This study was conducted at a 550 bed tertiary teaching hospital inAthens, Greece. Starting in September 2014 and for 13 months all prescriptions of AF were prospectively assessed by using a pre-established protocol. All patients receiving AF were prospectively evaluated by the study team when the drug was first administered and at discharge. The adequacy of AF use was evaluated by using a predefined point score that considered indication, drug selection, dosage, adjustments after microbiology results, switching to an oral agent, length of treatment, serum levels monitoring, and interactions. The criteria used to define the appropriateness of AF prescription were adopted from the existing treatment guidelines. Each prescription was characterized as “appropriate”, “appropriate but not optimal,” and “inappropriate”, by consensus.**Results:** We assessed 208 consecutive prescriptions of antifungal agents for the treatment of 147 in-patients (male 89, mean age 58.7 y). AF were prescribed by the attending physicians in 69.3% of cases, and by infectious diseases specialists in 30.7%. The main underlying diseases were hematological malignancy (124, 59.6%), solid tumor (35, 16.8%), and HIV infection (9, 4.3%). Overall, 101 (48.5%) of prescriptions came from medical, 53 (25.5%) from hematology, and 36 (17.3%) from surgery departments and 18 (8.6%) from the intensive care unit. The main reasons for starting antifungals were prophylaxis 65 (31.25%); empirical 120(57.69%); pre-emptive 7 (3.36 %) and targeted treatment 19 (9.13%). Overall, 94 (45.1%) of the prescriptions have been characterized as appropriate, 25 (12%) appropriate but not optimal and 89 (42.7%) as inappropriate. The most common mistakes were no indication for AF (54.4%), selection of an inappropriate AF (27.2%), incorrect dosage (11.2%) or duration of treatment (12%).**Conclusions:** Inappropriate use of AF is unacceptably common in our institution, in accordance with similar findings from many hospitals around the world.

Author Disclosure Block:

K. Korantanis: None. **A. Markogiannakis:** None. **M. Gamaletsou:** None. **M. Samarkos:** None. **G. Daikos:** None. **N.V. Sipsas:** None.

Poster Board Number:

SATURDAY-508

Publishing Title:

Impact of a Clinical Decision Support System (Cdss) on Time to Appropriate Empiric and Targeted Antibiotics in Gram Negative Bacteremia

Author Block:

T. Vo, S. Kuhn, S. Fogg; Wesley Med. Ctr. (WMC), Wichita, KS

Abstract Body:

Background: Bloodstream infections (BSI) are a common infection in the United States and have been associated with increased mortality, hospital stay, and costs. Delaying the time to appropriate therapy in gram negative BSI has been associated with increased rates of mortality. CDSS have demonstrated to improve antibiotic selection, reduce antibiotic costs, and shorten hospital length of stay (LOS). At WMC, a CDSS was implemented in June of 2014.The purpose of this study is to compare time to ordered appropriate empiric antibiotic (EA) and targeted antibiotic (TA) therapy in gram negative bacteremia before and after the implementation of a CDSS. **Methods:** This is a retrospective, single site, observational study comparing time to ordered appropriate empiric and targeted antibiotic therapy in gram negative bacteremia before and after the implementation of a CDSS. Patients (pts) identified with positive blood cultures (PBC) as one or more of the following- *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae, Enterobacter cloacae, Proteus mirabilis,* or *Acinetobacter* species between the dates of January 1, 2013 to June 30, 2013 for the pre-intervention (pre-I) period and January 1, 2015 to June 30, 2015 for the post-intervention (post-I) period will be included. The primary outcome is the time to ordered appropriate empiric and targeted antibiotic therapy. Secondary outcomes include intensive care unit (ICU) and overall hospital LOS. **Results:** Pre-I (n=50) vs post-I (n=33): Thirty-one pts (62%) vs. 25 (76%) pts were on appropriate EA therapy prior to PBC. Of the pts not on appropriate EA prior to PBC, the mean time ± standard deviation (SD) to appropriate EA therapy was 8.64 ± 9.78 hours (hr) vs. 1.50 ± 1.05 hr (p = 0.005). Ten (20%) vs. six pts (18%) were on appropriate TA, respectively. Of the pts on inappropriate TA therapy, the mean time ± SD to appropriate TA therapy was 59 ± 22 hr vs 57 ± 26 hr (p = 0.81). Mean overall hospital LOS was 200 hr ± 134 hr vs 334 ± 667 hr (p = 0.26). Mean ICU LOS (pre-I n=29; post-I n=15) was 92 ± 75 hr vs 474 ± 981 hr (p = 0.15). **Conclusions:** At WMC, CDSS made a statistical difference in time to appropriate EA but no difference in time to appropriate TA, overall hospital LOS, or ICU LOS. The decreased time to appropriate EA may improve patient outcomes.

Author Disclosure Block:

T. Vo: None. **S. Kuhn:** None. **S. Fogg:** None.

Poster Board Number:

SATURDAY-509

Publishing Title:

The PK-PD Compass: Transforming the Landscape of Antimicrobial Stewardship

Author Block:

C. C. Bulik[1], S. M. Bhavnani[1], J. C. Bader[1], L. Zhang[1], D. R. George[1], S. Ahmed[2], S. Sajid[2], M. Trang[1], K. L. Sweeney[1], P. G. Ambrose[1], C. M. Rubino[1]; [1]ICPD Tech., Latham, NY, [2]Appstronomy, Herndon, VA

Abstract Body:

Antimicrobial stewardship programs face many challenges, including a lack of antibiotic dose, interval, and duration guidance. There is no one method or tool which accounts for patient demographic data, pathogen susceptibility, and PK-PD data when advising clinicians on the selection of antimicrobials and dosing regimens. We developed the PK-PD Compass, an educational mobile device application (APP), to address these deficiencies. The APP consists of a Monte Carlo simulation (MCS) algorithm which integrates PK data, PK-PD targets, patient-specific characteristics, and pathogen susceptibility data. A total of 29 infection categories, 33 drugs, and MIC surveillance data for associated pathogens (SENTRY) were included. Population PK models for each drug were identified, evaluated, and refined as needed. Susceptibility breakpoints utilized were based upon FDA and CLSI criteria. Results were validated by comparing APP- and model-derived percentage probabilities of PK-PD target attainment (%PTA) with a maximum tolerated difference of 10%. Incorporating these data into one interface allows clinicians to select the infection, pathogen, and antimicrobials of interest and obtain the %PTA for each regimen based upon patient characteristics (see **Figure**). As evidenced by the absolute median (min-max) difference of 4.3 (2.0-7.8), APP- and model-derived %PTA values across drugs were highly concordant. Using a MCS algorithm, an APP was developed to serve as an educational tool to support the implementation of PK-PD-driven antimicrobial stewardship programs. Using this APP, clinicians can now confidently answer the questions of "what drug, what dose, and what duration" at the patient's bedside.

Author Disclosure Block:

C.C. Bulik: C. Consultant; Self; ICPD Tech. **S.M. Bhavnani:** G. Member; Self; ICPD Tech. **J.C. Bader:** C. Consultant; Self; ICPD Tech. **L. Zhang:** C. Consultant; Self; ICPD Tech. **D.R. George:** C. Consultant; Self; ICPD Tech. **S. Ahmed:** C. Consultant; Self; ICPD Tech. **S. Sajid:** C. Consultant; Self; ICPD Tech. **M. Trang:** C. Consultant; Self; ICPD Tech. **K.L. Sweeney:** C. Consultant; Self; ICPD Tech. **P.G. Ambrose:** G. Member; Self; ICPD Tech. **C.M. Rubino:** G. Member; Self; ICPD Tech.

Poster Board Number:

SATURDAY-510

Publishing Title:

A Cardiology and Antimicrobial Stewardship Team Collaboration to Identify and Improve the Management of High-Risk Antimicrobial-Warfarin Drug-Drug Interactions

Author Block:

N. Ha, S. Hanigan, K. Yang, H. Mak, B. Kurtz, M. Dorsch, **J. Nagel**; Univ. of Michigan, Ann Arbor, MI

Abstract Body:

Background: Drug-drug interactions (DDIs) with warfarin and antimicrobial agents frequently cause INR instability, which can affect the risk for bleeding and thrombotic events. The purpose of this study is to assess the impact of a comprehensive guideline for the management of 16 significant warfarin and antibiotic, antifungal or antiviral drug interactions. The guideline emphasizes improving identification of high-risk antimicrobial-warfarin DDIs during hospitalization; empiric modification of warfarin dosing based on the individual antimicrobial interaction and baseline INR; providing patient education; documentation of the DDI in the medical chart; communication with outpatient providers regarding the DDI and anticipated antimicrobial stop date; and providing warfarin dosing recommendations upon discontinuation of antimicrobial agent. **Methods:** This retrospective, single-center, quasi-experimental, pre-post study compared endpoints 3 months before and after guideline implementation, into an established pharmacists-run anticoagulation service. The primary outcome was time within therapeutic range (TTR), which represents the proportion of the time the INR was therapeutic during the DDI. **Results:** 78 pre-guideline and 31 post-guideline patients were included, and baseline characteristics were similar between groups. Implementation of the guideline resulted in greater in-hospital TTR (72% vs. 50%, p=0.04), and improved TTR across transitions of care (70% vs. 46%, p=0.01). Documentation of DDI in the pharmacy anticoagulation discharge summary significantly improved in the post-guideline group (40% vs. 14%, p=0.02) and numerically improved within the daily pharmacy progress notes (94% vs. 82%, p=0.13). The implementation of the guideline was associated with a numeric reduction in bleeding events compared to the pre-guideline group (0 events vs. 4 events, p=0.11). **Conclusion:** This antimicrobial stewardship initiated intervention represents a novel single-center approach to optimize the comprehensive management of significant antimicrobial-warfarin drug-drug interactions, which resulted in improved communication with outpatient providers via the discharge summary and improved INR time within therapeutic range.

Author Disclosure Block:

N. Ha: None. **S. Hanigan:** None. **K. Yang:** None. **H. Mak:** None. **B. Kurtz:** None. **M. Dorsch:** None. **J. Nagel:** None.

Poster Board Number:

SATURDAY-511

Publishing Title:

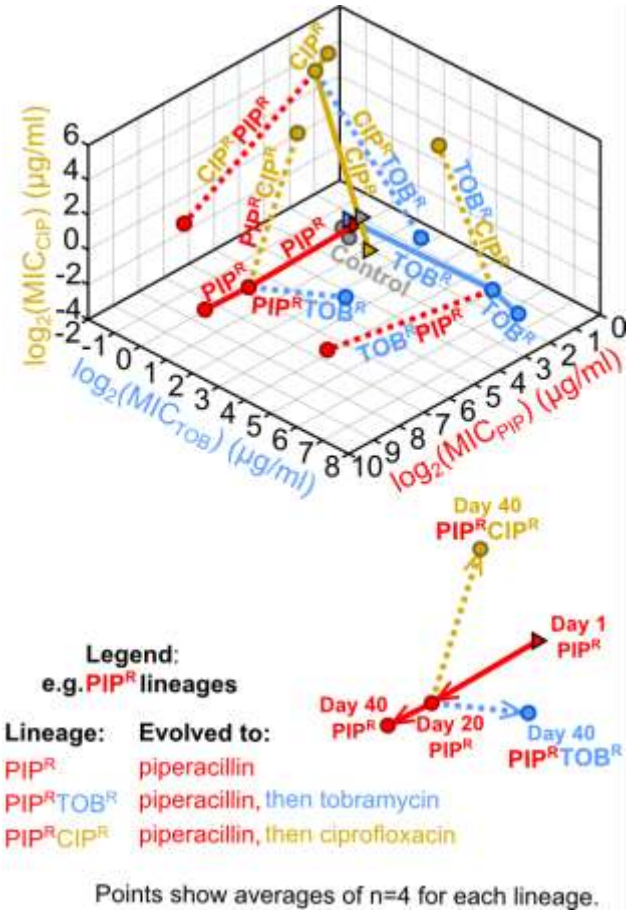
Antibiotic History Influences Microbial Evolutionary Dynamics in Subsequent Treatment

Author Block:

P. Yen, J. Papin; Univ. of Virginia, Charlottesville, VA

Abstract Body:

It remains unclear how history of antibiotic exposure affects subsequent resistance evolution trajectories in sequential therapies. Knowledge of potential evolutionary dynamics of resistance given prior history of drug exposure can help clinicians prescribe regimens that minimize resistance levels. We studied how history of past drug exposure affects the development of resistance during subsequent drug therapy in *Pseudomonas aeruginosa*. We serially passaged replicates of *P. aeruginosa* to increasing concentrations of piperacillin (PIP), tobramycin (TOB) and ciprofloxacin (CIP) daily for 20 days. These single-drug resistant mutants were then passaged to the other two drugs for 20 days, resulting in six sets of mutants evolved to two drugs each. Minimum inhibitory concentrations (MICs) of all drugs were measured for all lineages during the adaptive evolution. We observed drug sequence-specific effects where: adaptation to the first drug limits subsequent adaptation to the second drug (e.g. past PIP adaptation limits subsequent development of TOB resistance), adaptation to the second drug decreases the resistance to the first drug (e.g. evolution to CIP after PIP or TOB result in loss of PIP or TOB resistance, respectively), or final resistance levels depend on the order of the sequence (e.g. PIP resistance is less when evolved to PIP then CIP, compared to the reverse order). The figure shows the MICs of the three drugs on days 1, 20, and 40 for the different lineages and depicts the sequence-specific effects. These effects show how adaptation history to antibiotics can complicate strategies for mitigating resistance of bacterial pathogens. Effective antibiotic stewardship must account for these effects in the development of antibiotic resistance in order to deter the onset of the post-antibiotic era.



Author Disclosure Block:

P. Yen: None. **J. Papin:** None.

Poster Board Number:

SATURDAY-512

Publishing Title:

Establishing a Long-term Model for Improvement of Blood Culture Bottle Fill Volumes

Author Block:

P. A. Erdman[1], A. Michael[1], E. Floyd[2], W. Hess[1], D. Myers[1], M. England[1], J. Snyder[2], D. Craft[1]; [1]Hershey Med Ctr, Hershey, PA, [2]Univ of Louisville Hosp., Louisville, KY

Abstract Body:

Background: CAP requires clinical microbiology labs to have a system for monitoring blood culture volumes (MIC.22640). Fill volume and diagnostic yield are correlated; according to the manufacturer, 8-10 mls is the recommended fill volume. In response to this initiative, we developed a program to monitor fill volumes to include partnering with the hospital staff. Methods: The BD Epicenter (BD, Sparks, MD) is an add-on software system to automatically monitor blood culture volumes in BD BACTEC™ Plus Aerobic/F bottles throughout the hospital. To validate, we filled 50 bottles with known volumes of donor blood and placed them in the BD BACTEC FX blood culture incubator. We also partnered with ICUs in which we held educational sessions and sent a monthly lab "report card" to the nurse manager with trends in blood volume. Results: The mean software generated volume of blood per bottle was 6.1 mls (n=50) and the mean manual volume of blood per bottle was 6.4 mls (n=50) with a standard deviation of 1.5 and 1.9, respectively. The microbiology lab began monitoring hospital-wide blood volumes in 2014. Specific discussions and partnership with the SICU began in January 2015. Educational sessions took place on 04/08/15 and 10/15/15 which resulted in an increase in blood volume trends following each session.

Conclusions: We validated the BD Epicenter blood culture volume software and demonstrated its use as an automated system for monitoring blood culture bottle volume. In addition, setting up a partnership with a specific patient unit and the use of 'report cards' had a direct positive correlation on increasing fill volumes. Increasing blood volumes should equate to a higher yield of positive blood cultures and fewer false negative cultures.

Author Disclosure Block:

P.A. Erdman: None. **A. Michael:** None. **E. Floyd:** None. **W. Hess:** None. **D. Myers:** None. **M. England:** None. **J. Snyder:** None. **D. Craft:** None.

Poster Board Number:

SATURDAY-513

Publishing Title:

Predictors of Readmission and Failure of Outpatient Parenteral Antimicrobial Therapy Completion Across a Large Integrated Healthcare Network

Author Block:

M. Schmidt, L. McCurdy, S. Abraham, F. Gohs, B. E. Hearn, M. Spencer; Carolinas Hlth.care System, Charlotte, NC

Abstract Body:

Background: Outpatient parenteral antimicrobial therapy (OPAT) prescribing has increased as there has been a trend toward early discharge of hospitalized patients with infections. There is limited literature on readmission and failure of OPAT completion, key measures of successful treatment. This study aims to elucidate the predictors of readmission and non-completion of therapy in patients seen across facilities within Carolinas HealthCare System (CHS). Understanding these predictors may inform future interventions that improve treatment efficacy and patient outcomes. **Methods:** Data were collected electronically between 2014 and 2015 for patients initiating OPAT. Patient demographics, treatment indication, complications, therapy completion, readmission and other variables were collected using RedCap. Patients >18 years of age with complete follow-up data were included (n=1,681) while those who died were excluded. Predictors were identified using readmission and non-completion as outcomes in a Poisson model that reported incident risk ratios and controlled for age, race, gender and indication for OPAT utilization. **Results:** The study cohort readmission rate was 13.6%, and 25.0% of patients that did not complete OPAT as initially prescribed. In adjusted analyses for readmission, when OPAT treatment was modified due to failure or worsening infection, readmission risk increased by 62.3% (IRR 1.623; 95% CI 1.24-2.12). Having OPAT performed at an infusion center decreased risk of readmission by 70.0% (IRR 0.30; 95% CI 0.12-0.73) when compared to therapy at home. Having an adverse drug reaction increased the risk of non-completion by 7-fold (IRR 7.6; 95% CI 4.8-12.1). Other predictors of non-completion were: change in antimicrobial mid-course (IRR 2.55; 95% CI 1.8-3.6), financial issues (IRR 3.52; 95% CI 1.2-10.4), access complications (IRR 1.9; 95% CI 1.3-2.7) and readmission (IRR 7.5; 95% CI 6.0-9.1). **Conclusions:** These results suggest that having OPAT performed at an infusion center versus home may decrease the risk of readmission. Testing interventions to prevent non-completion, such as mitigating adverse drug reactions, providing financial assistance or improved monitoring to prevent IV access complications, may identify ways to improve outcomes.

Author Disclosure Block:

M. Schmidt: None. **L. McCurdy:** J. Scientific Advisor (Review Panel or Advisory Committee); Self; Cubist. **S. Abraham:** None. **F. Gohs:** None. **B.E. Hearn:** None. **M. Spencer:** None.

Poster Board Number:

SATURDAY-514

Publishing Title:

Antimicrobial Prescribing Practices in the Outpatient Setting Across a Large Healthcare System

Author Block:

M. Schmidt, M. Spencer, J. Roberge, H. Petruso, L. Davidson; Carolinas Hlth.care System, Charlotte, NC

Abstract Body:

Background: The CDC estimates that 30-50% of antibiotics prescribed for hospitalized patients are either unnecessary or inappropriate, contributing to increases in multidrug resistant organisms and infections. In the outpatient setting, prescribing behavior has not been well-described. This study investigates provider and practice characteristics in the outpatient setting to understand patterns of prescribing behavior and to inform new approaches for outpatient antibiotic stewardship. **Methods:** Data on outpatient provider and practice antimicrobial prescribing was collected from electronic medical records at Carolinas HealthCare System between Jan 2014 and Aug 2015 (n=2,318). The primary outcome was the number of antimicrobials prescribed per 1000 patient encounters. Unadjusted analyses of provider and practice characteristics used Wilcoxon rank-sum trend analysis. An ordinary least squares model was used for adjusted analyses. **Results:** Unadjusted analysis of prescribing by practice type showed that urgent care practices had significantly higher antimicrobial prescribing than all other practice types ($p<0.001$). After adjusting for provider gender, race, year, PCMH recognition and provider level, providers in urgent care practices prescribed 371 (95% CI 55-689; $p=0.021$) and primary care practices prescribed 201 (95% CI 101-301; $p<0.001$) more antimicrobials per 1000 encounters compared to all other practice types. Among all types of providers, mid-level providers prescribed antimicrobials more often than did physician providers ($p<0.001$). The adjusted model identified that mid-level providers prescribed 141 more antimicrobials per 1000 encounters than did physician providers (95% CI 45-237; $p=0.004$). **Conclusions:** There are significant differences between types of providers and practices in antimicrobial prescribing patterns in the outpatient setting. One of the key limitations of this analysis is the need for indication information to determine when prescribing is appropriate. Further study is warranted to investigate key drivers of provider and practice prescribing patterns and to test interventions that improve appropriate prescribing and patient outcomes. These interventions may reduce overuse of antimicrobials and mitigate the increasing prevalence of multidrug resistant organisms.

Author Disclosure Block:

M. Schmidt: None. **M. Spencer:** None. **J. Roberge:** None. **H. Petruso:** None. **L. Davidson:** None.

Poster Board Number:

SATURDAY-515

Publishing Title:

Outpatient Parenteral Antimicrobial Therapy (OPAT) for Infective Endocarditis (IE) in Patients Aged Over 80 Years

Author Block:

X. Kortajarena, **M. Á. Goenaga**, H. Azkune, M. J. Bustinduy, M. Ibarguren, A. Fuertes, R. Garcia, O. Ibarguren; Hosp. Univ.rio Donostia, Donostia, Spain

Abstract Body:

Background: OPAT is becoming more frequent in recent years. It is unclear whether the age could be a risk factor per se. The aim of this research is to describe our experience in OPAT for IE patients and to compare the differences between patients older and younger than 80 years old.**Methods:** We included all IE patients treated with OPAT in our hospital from 1996 to 2015. Demographic, diagnostic, treatment and outcome data were collected.**Results:** 194 cases were included. Mean age: 65 (range 25-92).163 (84%) under 80 and 31 (16%) over 80 years old. Etiology in younger and older subgroup respectively: S. aureus (11.7/6.5%), enterococcus species (15.3/9.7%), coagulase-negative staphylococcus (17.8/9.7%), viridans group streptococci (35/51.6%), other (15.3/12.9%) and negative cultures (4.9/9.7%). Type of affected valve: native (49.7/48.4%), prosthetic (33.1/22.6%), electrocatheter (9.2/16.1%) and unknown (8/12.9%). Affected valve: aortic (42.3/41.9%), mitral (30.7/19.4%), right-sided valves (3.1/9.7%), multivalvular (6.7/0%), electrocatheter (9.2/16.1%) and unknown (8/12.9%).Used antibiotics: vancomycin (4.9/3.2%), daptomycin (12.9/12.9%), cloxacilin (14.7/6.5%), ampicilin (24.5/22.6%), ceftriaxone (31.9/48.4%) and other antibiotics (11/6.5%). We found no statistical differences in the etiology (p= 0.376), type of affected valve (p=0.413), affected valve (p=0.15) and used antibiotics (p=0.533).**Conclusions:** An increasing trend in the prevalence of viridans group streptococci was seen in over 80 years subgroup, as well as an increase in the use of ceftriaxone. Surgical therapy rate is higher in younger patients subgroup, which probably justifies the longer hospital stay. Despite this fact, total length of treatment was similar in both groups. No statistical differences were seen in complications, re-admissions and mortality.In conclusion, elderly patients would be good candidates for OPAT in IE, as long as they were correctly selected and with an adequate monitoring.

	<80	>80	p
n	163	31	
Male	73%	67.7%	.549
Definite IE (Duke)	71.8%	58%	.182
Surgery	39.9%	9.7%	.001

Infusion pump	55.2%	35.5%	.044
Catheter related complications	14.8%	9.7%	.507
Length of treatment (days)	39	36	.182
Inpatient length	17	12	.003
Outpatient length	22	24	.375
Re-admission	16.6%	25.8%	.22
Mortality	0.61%	0%	.66

Author Disclosure Block:

X. Kortajarena: None. **M.Á. Goenaga:** None. **H. Azkune:** None. **M.J. Bustinduy:** None. **M. Ibarguren:** None. **A. Fuertes:** None. **R. Garcia:** None. **O. Ibarguren:** None.

Poster Board Number:

SATURDAY-516

Publishing Title:

Consumption of Antibiotics in the Community by Adults in Greece

Author Block:

H. Maltezou[1], G. Adamis[2], P. Tsonou[1], P. Katerelos[1], P. Gargalianos[3]; [1]Hellenic Ctr. for Disease Control and Prevention, Athens, Greece, [2]Gennimatas Gen. Hosp., Athens, Greece, [3]Gennimatas general Hosp., Athens, Greece

Abstract Body:

Background: Greece is among the European countries with the highest consumption of antibiotics. We studied the antibiotic consumption of adults in the community in this country.**Methods:** Cross-sectional study conducted during March-July 2014. **Results:** We studied 309 adults (mean age: 71.8 years, range: 16-98). Of them, 293 (94.8%) had received ≥1 antibiotic course the past year (mean duration: 11.6 days). In total, 419 antibiotic courses were consumed by them (mean number of courses the past year: 1.4), including 285 (68%) following examination by a physician, 72 (17.2%) following phone consultation, 30 (7.2%) following suggestion by a pharmacist and 16 (3.8%) as self-medication (the antibiotic was already available at home). Prevalent reasons for consumption were urinary tract infection (34.6%), pneumonia (17.7%), bronchitis (11%), skin infection (10.7%), gastroenteritis (8,1%), and phanyrnotonsilitis (7,2%). Prevalent antibiotics were amoxicillin-clavulanate for sinusitis, skin infection and pharyngotonsillitis (72,7%, 45% and 43.4%, respectively), amoxicillin for acute otitis media (30%), clarithromycin for bronchitis (34.8%), moxifloxacine for pneumonia (36.9%), and ciprofloxacin for gastroenteritis and urinary tract infection (66.7% and 47.3%, respectively). In the multivariate analysis, an older age and asthma were statistically significantly associated with a higher antibiotic consumption (p-values <0.001 and 0.002, respectively).**Conclusions**: Antibiotic consumption of adults in Greece is mainly driven by physicians. Good compliance of physicians with the international antibiotic guidelines was found in several clinical indications. Continuing medical education will improve antibiotic prescription practices.

Author Disclosure Block:

H. Maltezou: None. **G. Adamis:** None. **P. Tsonou:** None. **P. Katerelos:** None. **P. Gargalianos:** None.

Poster Board Number:

SATURDAY-517

Publishing Title:

Cultivation in Consortia and Subsequent Isolation of *Stomatobaculum Sp.* hot-097, a Member of the Human Oral Microbiome

Author Block:

A. J. Collins, F. E. Dewhirst; The Forsyth Inst., Cambridge, MA

Abstract Body:

Determining the roles of species comprising the oral microbiome in human health and disease is greatly facilitated by having isolates of each species in pure culture. Of the approximately 700 species comprising the human oral microbiome, investigators have succeeded in cultivating 65% of them in pure culture. About 3% of recently cultivated oral taxa are auxotrophic for factors not present in commonly-used rich media. They were successfully cultivated by cross streaking with a helper strain (such as *Staphylococcus aureus* or *Propionibacterium acnes*) which supplied the required factor(s). Other oral bacteria, such as *Fretibacterium fastidiosum,* required repeated laboratory passage in consortia before it would form macroscopic colonies adjacent to consortia members. Presumably genetic changes were selected for during passage that eventually permitted it to grow under laboratory conditions, a process that has been termed "domestication". The previously uncultivated oral bacteria *Stomatobaculum* sp. HOT-097 does not appear to be a simple auxotroph, but rather prefers growth in broth to growth on plates. In a study of the abundance of bacterial species at 9 oral sites in 20 subjects, *Stomatobaculum* sp. HOT-097 was found by Illumina 16S sequencing to be present in highest numbers (9% of reads) on the tongue of subject 3. A sample from the enriched subject-site was grown in enrichment broth. 16S qPCR suggested *Stomatobaculum* was present as about 0.1% in this initial consortium. The culture was densely plated on BHI-0.2% citrate blood agar and small regions of the consortia were picked and subcultured. Presence of Stomatobaculum was confirmed by PCR with taxon-specific primers and these cultures were further passaged. In these consortia on agar plates, *Stomatobaculum* was enriched to approximately 25% of the consortia, however *Stomatobaculum* colonies could not be isolated or passaged to fresh plates. After transferring a consortium to broth culture and selecting with the antibiotics sulfamethoxazole and trimethoprim, a nearly-pure culture of *Stomatobaculum* was successfully obtained. The *Stomatobaculum* could readily be grown and passaged in broth culture, but growth is inhibited on agar plates. A class of as-yet-uncultivated bacteria may share *Stomatobaculum* sp. HOT-097's reticence to grow on solid surfaces and therefore be cultivated using broth or other methods.

Author Disclosure Block:

A.J. Collins: None. **F.E. Dewhirst:** None.

Poster Board Number:

SATURDAY-518

Publishing Title:

Early Life Colonization of the Nasopharyngeal Microbiome: A Longitudinal Study in the Gambia

Author Block:

B. M. Hanson[1], B. Kwambana[2], A. Worwui[2], M. Antonio[2], J. S. Brown[1], D. S. Phillips[1], E. Sodergren[1], G. Weinstock[1]; [1]The Jackson Lab. for Genomic Med., Farmington, CT, [2]Med. Res. Council Unit, Banjul, Gambia

Abstract Body:

Background: *Streptococcus pneumoniae* is the most common cause of pneumonia in children under 5 worldwide, killing an estimated 1 million each year with the majority of deaths in developing countries. To mitigate the morbidity and mortality of this disease, pneumococcal conjugate vaccine (PCV) is administered. Most validation and investigative studies of these PCVs were performed in developed countries, and now national vaccine programs are being implemented throughout the world. In The Gambia, such a national program began several years ago with the implementation of the 7-valent PCV, moving to the 13-valent PCV to cover additional serotypes relevant within The Gambia. This study enrolled infants within The Gambia to 1) assess the appropriateness of the strains included within the PCV13 vaccine, determining the patterns of pneumococcal carriage in the first two years of life with widespread use of PCV13, 2) To characterize the normal development of the nasopharyngeal microbiome in the first two years of life, and 3) study the greater environmental context for the child, including the microbes on the mother and household contacts, to reveal routes of colonization. **Methods:** We conducted a longitudinal study, enrolling 120 newborns in The Gambia between March 2013 and September 2015 and followed them up for the first two years of life. Risk factor data and nasopharyngeal samples (NPS) were collected each month, including the three visits when the infants received the PCV13. In addition, NPS from household contacts and several samples from the mother including blood, milk, and NPS were collected.DNA was extracted from the samples and the V1-V3 region of the 16S ribosomal RNA (rRNA) gene was sequenced using the Illumina MiSeq platform. With this rich dataset, we will describe the overall characteristics of the nasopharyngeal microbiome among our cohort, as well as describe the variability of the microbiome during the first two years of life.**Results:** Sample collection was completed in September 2015. To date, 1844 infant, 1225 mother, and 796 household contacts samples have been extracted. Extraction, sequencing, and analysis is ongoing and results will be presented at the conference.

Author Disclosure Block:

B.M. Hanson: None. **B. Kwambana:** None. **A. Worwui:** None. **M. Antonio:** None. **J.S. Brown:** None. **D.S. Phillips:** None. **E. Sodergren:** None. **G. Weinstock:** None.

Poster Board Number:

SATURDAY-519

Publishing Title:

Bacterial Communities Vary over Multiple Spatial Scales in the Human Oral Cavity

Author Block:

D. M. PROCTOR[1], G. C. Armitage[2], S. A. Lee[2], N. M. Davis[3], S. P. Holmes[4], D. A. Relman[3]; [1]Stanford Univ. Sch. of Med., Palo Alto, CA, [2]UCSF Sch. of Dentistry, San Francisco, CA, [3]Stanford Univ. Sch. of Med., Stanford, CA, [4]Stanford Univ., Stanford, CA

Abstract Body:

Background: We have previously shown that microbial communities inhabiting tooth surfaces vary along a spatial gradient between the front and the back of the mouth. Based on this observation, we sought to test the hypothesis that microbial communities exhibit spatial structure not just on supragingival tooth surfaces but on oral mucosal tissues as well. **Methods:** To explore this question, we analyzed a dataset generated from 676 samples and 3 individuals, allowing for the high-resolution spatial mapping of communities inhabiting the buccal and lingual aspects of teeth, the keratinized gingiva, buccal mucosa, and alveolar mucosa adjacent to each tooth. DNA was extracted from each sample and used as template for barcoded amplification and sequencing of the V4-region of the bacterial 16S rDNA gene by Illumina HiSeq. **Results:** Tissue type (R^2= 0.15) accounts for as much variation as interpersonal variation (R^2 = 0.13), as revealed by permutational multivariate ANOVA on Bray Curtis distances. We then performed a multiple-tables analysis on tissue type in order to determine whether communities inhabiting each tissue share a common structure. Importantly, this analysis revealed that microbial communities vary along an ecological gradient between the anterior and posterior of the mouth across all tissues. Moreover, communities inhabiting the buccal and lingual surfaces of sites could be distinguished from one another in a principal coordinates analysis (PCoA), suggesting spatial segregation of communities at a spatial scale as small as opposing aspects of teeth. PCoA of neighbor matrices confirmed that microbial communities vary over multiple spatial scales from the local (by aspect within tissues) to the regional (between tissues and across the dental arches). **Conclusions:** These shared, non-random spatial structures imply that a common environmental variable, perhaps salivary flow, acts consistently across tissue types, shaping community structure across multiple spatial scales in the oral cavity.

Author Disclosure Block:

D.M. Proctor: None. **G.C. Armitage:** None. **S.A. Lee:** None. **N.M. Davis:** None. **S.P. Holmes:** None. **D.A. Relman:** None.

Poster Board Number:

SATURDAY-520

Publishing Title:

The Oral Microbiome of Pre-Columbian Caribbean Inhabitants

Author Block:

T. M. Santiago-Rodriguez[1], Y. M. Narganes-Storde[2], L. Chanlatte-Baik[2], G. A. Toranzos[2], R. J. Cano[1]; [1]California Polytechnic State Univ., San Luis Obispo, CA, [2]Univ. of Puerto Rico, San Juan, PR

Abstract Body:

IntroductionThe human oral microbiome is comprised of diverse bacteria; yet, little is known about the effects that culture may exert on this community structure and composition. Ancient microbiome studies may represent an opportunity to elucidate the possible effect of culture to the oral microbiome.**Methods**We investigated the microbiome of dental calculi from a pre-Columbian Caribbean culture using 16S rRNA gene high-throughput sequencing and compared it to the microbiome of coprolites from the same culture, and modern oral and gut samples.**Results**Bacterial relative abundances differed when compared to modern samples, suggesting that culture may have an effect on the bacterial composition of the human oral microbiome. Dental calculi and coprolites exhibited some resemblance in their microbiome composition, but there were also taxonomic differences that may be site-specific. While certain predicted functional profiles of dental calculi were similar to the coprolites, other resembled modern oral microbiomes. Specific functional categories associated with diseases were found to be more represented in dental calculi.**Conclusions**Results suggest that ancient oral microbiomes may reflect differences in culture. The present study also adds to the knowledge of ancient microbiomes by characterizing dental calculi from a Caribbean culture, opening the opportunity to reconstruct the ancestral state of the human oral microbiome.

Author Disclosure Block:

T.M. Santiago-Rodriguez: None. **Y.M. Narganes-Storde:** None. **L. Chanlatte-Baik:** None. **G.A. Toranzos:** None. **R.J. Cano:** None.

Poster Board Number:

SATURDAY-521

Publishing Title:

High-Throughput Its1 Sequencing Reveals Distinct Characteristics Of Skin Fungal Communities In Childhood

Author Block:

J-H. Jo, C. Deming, E. A. Kennedy, S. Conlan, E. C. Polley,W-l. Ng, NISC, J. A. Segre, H. H. Kong; NIH, Bethesda, MD

Abstract Body:

Background: Skin is colonized by various microbes, including fungi. Understanding the fungal component of the microbiome is necessary because several commensal fungi are also opportunistic pathogens and many pathogenic fungi can cause cutaneous disorders. Previous reports suggested fungal communities in children might differ from adults. For instance, the commensal *Malassezia* was shown to be less common in young children, and several cutaneous fungal infections such as tinea capitis are more frequently observed in children. However, differences in the fungal communities (mycobiomes) on the skin of healthy children versus adults are incompletely known.**Methods:** Here, we utilized next-generation sequencing (NGS) based internal transcribed spacer-1 (ITS1) profiling, to define mycobiomes of prepubertal children.**Results:** We determined that the mycobiome landscape of children is significantly different from adults. Lipid-dependent *Malassezia*, which was predominant in adults (98.8 ± 0.5%), was less frequent (73.4 ± 23.1% for children on sebaceous sites), and fungal diversity was increased on the skin of children. Moreover, the interpersonal variation of mycobiome is significantly higher among children, suggesting heterogeneity and permissivity of the skin of young hosts. Species-level interrogation showed specific bias towards *Malassezia globosa* in prepubertal skin. Most striking example was the forehead, where 74.5 ± 30.1% of *Malassezia* was *M. globosa* in children, in contrast to 5.9 ± 7.7% in adults.**Conclusions:** Our analyses demonstrated distinctive features of the prepubertal skin mycobiome, and suggested shifts in fungal communities during early sexual maturation. This pilot study highlights the need for longitudinal mycobiome studies will provide insights into understanding the interplay between the skin, commensal microbes, and clinically significant fungal infections.

Author Disclosure Block:

J. Jo: None. **H.H. Kong:** None.

Poster Board Number:

SATURDAY-522

Publishing Title:

Combat Wound Microbiota Profiling Using Pyrosequencing and Association with Wound Outcome and Clinical Factors

Author Block:

S. YILDIRIM[1], T. S. Brown[2], J. A. Forsberg[3], E. A. Elster[4], B. C. Kirkup[1]; [1]Walter Reed Army Inst. of Res., Silver Spring, MD, [2]Naval Med. Res. Ctr., Silver Spring, MD, [3]Walter Reed Natl. Military Med. Ctr., Rockville, MD, [4]Uniformed Services Univ. of the Hlth.Sci., Bethesda, MD

Abstract Body:

We characterized the microbiota colonizing blast-induced wounds using culture-independent pyrosequencing. 16S and 18S ribosomal RNA genes were sequenced to profile fungal and bacterial communities from 110 serial effluent samples, obtained from extremity wounds of 21 U.S. service members. Clinical metadata included closure outcomes, injury severity score, wound type, traumatic brain injury score, and heterotopic ossification. The majority of the samples had high relative abundances of *Acinetobacter spp.,* and *Pseudomonas spp*. Sequencing amplified housekeeping genes from a subset of samples revealed diversity within these genera. Fungal species of *Chaetomium, Malassezia,* and *Aspergillus* were frequently present in the wounds. Correlation between wound outcome and microbiome composition was not statistically significant.

Author Disclosure Block:

S. Yildirim: None. **T.S. Brown:** None. **J.A. Forsberg:** None. **E.A. Elster:** None. **B.C. Kirkup:** None.

Poster Board Number:

SATURDAY-523

Publishing Title:

Skin Microbiome Alterations Early In Infancy And Before Clinical Atopic Dermatitis

Author Block:

E. A. Kennedy[1], A. Gallagher[2], J. O. Hourihane[2], P. G. Fallon[3], D. Murray[2], J. A. Segre[1], H. H. Kong[1], A. D. Irvine[4]; [1]NIH, Bethesda, MD, [2]Univ. Coll., Cork, Ireland, [3]Trinity Coll., Dublin, Ireland, [4]Our Lady's Children's Hosp. Crumlin, Dublin, Ireland

Abstract Body:

Background: Disease flares of atopic dermatitis (AD, "eczema") are generally associated with a low-diversity skin microbiota dominated by *Staphylococcus aureus*. The relationship between this dysbiosis and the development of AD is unclear.**Methods:**We randomly selected 50 infants from the Cork BASELINE longitudinal birth cohort for microbiome sampling at three timepoints in the first six months of life at 4 skin sites relevant to AD. We identified 10 infants who developed AD and analyzed them alongside 10 control infants who did not develop AD. We performed 16S ribosomal RNA sequencing and analysis.**Results:**Bacterial community structures shifted over the different time points, as did within-sample (alpha) diversity, suggesting that age strongly affects the skin microbiome in infants. Overall, there was no clear clustering between patients and controls at each site and time point sampled. Unlike what is seen in older AD patients, these subjects did not have noticeably dysbiotic communities and were not colonized by *S. aureus*. However, infants who had affected skin at month 12 had statistically significant differences in bacterial communities on the inner elbow at month 2 as compared to infants who were unaffected at month 12. In particular, staphylococci were significantly less abundant in those patients that were affected at month 12, suggesting that this genus may protect against the later development of AD. Bacterial communities were similar between infants born by C-section or vaginally.**Conclusions:**This study suggests that young infants with AD are not colonized with *Staphylococcus aureus*, unlike older patients. Additional studies are needed to determine if colonization with other staphylococci modulates the skin immunity and later development of AD.

Author Disclosure Block:

E.A. Kennedy: None. **A. Gallagher:** None. **J.O. Hourihane:** None. **P.G. Fallon:** None. **D. Murray:** None. **J.A. Segre:** None. **H.H. Kong:** None. **A.D. Irvine:** None.

Poster Board Number:

SATURDAY-524

Publishing Title:

16s Sequencing Reveals Site-specific Signatures of the Paranasal Sinuses and Lower Airways of Cystic Fibrosis Patients

Author Block:

S. K. Lucas, R. Yang, A. Nicholson, J. M. Dunitz, H. Boyer, R. Hunter; Univ. of Minnesota, Minneapolis, MN

Abstract Body:

Chronic rhinosinusitis (CRS) is an inflammatory disorder of the paranasal sinuses that has nearly 100% prevalence in classical cystic fibrosis (CF) patients (mutation class I-III). Recent research suggests that the microbiome of the paranasal sinuses is developed through initial contact with the environment, and seeds the microbiota of the lower respiratory tract through an island biogeography model of colonization; however, this dynamic has not been thoroughly investigated in CF patients where lower respiratory infections contribute significantly to the mortality of the disease. Here we evaluated the microbiome of the upper and lower respiratory environments using matched sinus and lung sputum samples from CF patients. Expectorated sputum samples were obtained from 10 CF patients in the hours prior to undergoing functional endoscopic sinus surgery (FESS) for treatment of chronic rhinosinusitis and paired sinus mucus was obtained during surgery. DNA was extracted from the paired samples, the V4 hypervariable region of the 16S rRNA gene was sequenced using an Illumina MiSeq, and the sequences were analyzed using the Quantitative Insights Into Microbial Ecology (QIIME) pipeline. We found that the sinus and lung microbiome are distinct from one another when analyzing community alpha diversity metrics for richness and evenness. Additionally, ordination analyses point to sinus and lung environments as being stronger determinants of microbial community structure than the individual patient. Our findings indicate that while the paranasal sinuses and lungs may still comprise a unified airway, in which lower airway infections are seeded by the sinus, it is important to regard these two locations as discrete microbial environments. Further studies should target ecological pressures in the paranasal sinuses that may contribute to microbial community structure and potential pathogenicity.

Author Disclosure Block:

S.K. Lucas: None. **R. Yang:** None. **A. Nicholson:** None. **J.M. Dunitz:** None. **H. Boyer:** None. **R. Hunter:** None.

Poster Board Number:

SATURDAY-525

Publishing Title:

Longitudinal Analysis of Allograft Microbiota After Lung Transplantation in Cystic Fibrosis Patient

Author Block:

M. Beaume[1], V. Lazarevic[2], T. Köhler[1], N. Gaia[2], J. Schrenzel[2], O. Manuel[3], J-D. Aubert[3], P. Gasche[2], L. Baerlocher[4], L. Farinelli[4], C. van Delden[1], .. Swiss Transplant Cohort Study[5]; [1]Geneva Univ. Hosp. and Univ. of Geneva, Geneva, Switzerland, [2]Geneva Univ. Hosp., Geneva, Switzerland, [3]Lausanne Univ. Hosp., Lausanne, Switzerland, [4]Fasteris SA, Plan-les-Ouates, Switzerland, [5]., ., Switzerland

Abstract Body:

Backgroung: The airways of cystic fibrosis (CF) patients are chronically infected by various microorganisms including *Pseudomonas aeruginosa* which predominates in adults and contributes to lung destruction. Lung transplantation (LT) is a recognized treatment for CF patients with end-stage disease. However, CF- adapted *P. aeruginosa* residing in the sinuses of the recipient seed the new lung leading to infections and allograft damage. This occurs despite the preexisting healthy donor lung microbiota.Therefore the lung allograft of CF recipients represents an opportunity to study *in patient* the bacterial adaptation to a novel environment and its interactions with a well-established microbiota. **Methods:** We collected sequential airway samples from conductive and respiratory zones of CF and non-CF patients at regular time intervals during 2 years post-LT. 16S rRNA sequencing and taxonomic analyses were performed to evaluate the dynamics of bacterial populations during graft colonization.**Results:** Four hundred airway samples were collected from 12 CF and 51 non-CF patients and bacterial composition was determined for the first 192 samples. Our longitudinal analysis of the graft microbiota showed that: i) colonization by *P. aeruginosa* occurred in the first days post-LT despite intensive antibiotic treatments, ii) presence of *P. aeruginosa* was associated with a decrease of the microbial diversity and richness. A large-scale analysis allowed us to characterize the dynamics of the graft colonization by the microbiota. In addition, the influence of bacterial richness and diversity as well as particular microbial signatures on lung functions were evaluated.**Conclusion:** Increasing our understanding of the dynamics of *P. aeruginosa* invading the donor microbiota and adapting to its new non-CF environment might help to design new preventive strategies aiming to improve graft survival.

Author Disclosure Block:

M. Beaume: None. **V. Lazarevic:** None. **T. Köhler:** None. **N. Gaia:** None. **J. Schrenzel:** None. **O. Manuel:** None. **J. Aubert:** None. **P. Gasche:** None. **L. Baerlocher:** None. **L. Farinelli:** None. **C. van Delden:** None. **.. Swiss Transplant Cohort Study:** None.

Poster Board Number:

SATURDAY-526

Publishing Title:

Nasal Microbiome Features of *Staphylococcus aureus* Colonization and Infection During Critical Illness

Author Block:

B. J. Kelly, F. D. Bushman, R. G. Collman; Univ. of Pennsylvania, Philadelphia, PA

Abstract Body:

Background: Intranasal colonization with *Staphylococcus aureus* (SA) is a risk factor for nosocomial SA infection. Prior studies in healthy subjects have demonstrated features of intranasal bacterial community composition associated with the presence or absence of intranasal SA. We sought to evaluate the bacterial community features associated with SA colonization and infection in a cohort of critically ill subjects at high risk for nosocomial SA infection.**Methods:** We prospectively enrolled 15 intubated subjects from a medical intensive care unit at an academic medical center and collected flocked swab samples from the anterior nares at 24-48 hour intervals for the duration of intubation. DNA was extracted using the MoBio PowerSoil kit, and the V1-V2 hypervariable region of the 16S rRNA gene was amplified for sequencing on the Illumina MiSeq platform. Operational taxonomic units (OTUs) were clustered at 97% sequence similarity and taxonomy assigned using BLAST against the Living Tree Project database. Clinical bacterial culture and chart diagnosis data were also obtained.**Results:** From 15 subjects, we collected 42 samples. 31 samples had evidence of SA by 16S rRNA gene sequencing. Two subjects were diagnosed with SA pneumonia and a third with SA bloodstream infection. Differences in bacterial community composition (1) between SA-positive and SA-negative samples, and (2) between SA-infected and SA-uninfected subjects were evaluated. Principal coordinate analysis of pairwise unweighted Jaccard distances revealed clustering of SA-positive samples. Linear discriminant effect size (LEfSe) analysis identified *Corynebacterium tuberculostearicum* as most strongly associated with the absence of SA colonization (effect size 5.15, $p = 0.039$) and with the absence of SA infection (effect size 5.00, $p < 0.001$).**Conclusions:** The presence of *Corynebacterium tuberculostearicum* in the anterior nares bacterial community is associated with the absence of SA colonization and the absence of SA infection.

Author Disclosure Block:

B.J. Kelly: None. **F.D. Bushman:** None. **R.G. Collman:** None.

Poster Board Number:

SATURDAY-527

Publishing Title:

Sequence-Based Analysis Of Human Microrna Expression Associated With Dynamic Vaginal Microbiota

Author Block:

S. B. Smith[1], J. Ravel[2]; [1]Univ. of Maryland, College Park, MD, [2]Univ. of Maryland, Baltimore, MD

Abstract Body:

Bacterial Vaginosis (BV) is a condition of the human vagina characterized in part by a paucity of *Lactobacillus* spp. and the presence of a wide array of strict and facultative aerobes such as *Gardnerella vaginalis*, *Atopobium vaginae*, and *Mobiluncus* spp. In some women BV is associated with symptoms such as odor, itching and discharge, while in other it is asymptomatic. The etiology of BV remains unknown. Characterization of the vaginal microbiota sampled daily over 10 weeks has shown that some women experience transitions from *Lactobacillus*-dominated to BV-associated states independently of sexual or hygiene behaviors. Leveraging these daily samples, the types and abundance of a small RNA class known as microRNAs (miRNAs) are being used to gain insight into BV etiology and the host regulatory mechanisms that drive these transitions using miRNAseq. We hypothesize that specific miRNA are associated with transitions to a BV-associated Community State Type (CST) by affecting specific host functions. Results indicate the miRNASeq approach is sufficient to consistently detect miRNAs across human vaginal self-collected swab samples from different women and CSTs. Furthermore, the correlation of BV-associated bacteria and metadata with miRNA read counts revealed patterns that could be used to help understand BV recurrence and symptomology. The highest-ranking miRNAs are being validated and used to further develop predictive paradigms in BV.

Author Disclosure Block:

S.B. Smith: None. **J. Ravel:** None.

Poster Board Number:

SATURDAY-528

Publishing Title:

Severity of *Clostridium difficile* Infection Varies with the Type of Diets in a Murine Model of Infection

Author Block:

J. Scaria[1], S. Faisal[2], K. Jayaram[2], S. M. Lipkin[3], **Y. Chang**[2]; [1]South Dakota State Univ., Brookings, SD, [2]Coll. of Vet. Med., Cornell Univ., Ithaca, NY 14853, Ithaca, NY, [3]Cornell Univ., New York, NY

Abstract Body:

Clostridium difficile infection (CDI) is the leading cause of infectious diarrhea in the United States. Outcome of CDI varies from mild diarrhea to severe colitis and fatalities. Primary risk factor for CDI is the antibiotic induced gut microbiome dysbiosis. We hypothesized that type of patient's diet could shift the gut microbial population and such microbiota shift might influence the severity and outcome of infection. We tested this hypothesis in a murine model of infection. Experimental groups of five mice were maintained on two different isocaloric diets; high protein-low fiber diet or Low protein-high fiber diet for one month, further segregated into three treatment groups; control untreated mice, mice treated with antibiotic cocktail alone and mice treated with antibiotic cocktail plus *C. difficile* spore infection (intragastric administration of 1000 CFU). All mice including the control group received a single intra-peritoneal dose of clindamycin one day prior to initiation of the antibiotic/*C. difficile* challenge. Mice were euthanized 3 days post infection and cecum contents were collected for 16s rRNA based microbial population survey. Another fraction was flash frozen in liquid nitrogen and freeze dried and was used for LC-MS based metabolome analysis. Our results showed that mice receiving high protein-low fiber diet was more susceptible to CDI and there was 60% mortality in this group while all animals given low protein-high fiber diet survived. Microbiome population structure analyzed using 16s rRNA amplicon sequencing revealed that antibiotic treatment caused expansion of Enterobacteriacea in all groups while the maximum numbers were found in high protein-low fiber diet fed group. Metabolite analysis of cecal contents revealed that levels of secondary bile acids and short chain fatty acids were reduced in high protein-low fiber diet fed mice. Overall, our results indicate that type of diet particularly; high protein-low fiber diet may increase the severity of CDI.

Author Disclosure Block:

J. Scaria: None. **S. Faisal:** None. **K. Jayaram:** None. **S.M. Lipkin:** None. **Y. Chang:** None.

Poster Board Number:

SATURDAY-529

Publishing Title:

Dietary Zinc Alters the Gut Microbiota and Decreases Resistance to Clostridium Difficile

Author Block:

J. P. Zackular, J. L. Moore, L. J. Juttukonda, A. T. Jordan, Y. Zhang, W. J. Chazin, R. M. Caprioli, E. P. Skaar; Vanderbilt Univ., Nashville, TN

Abstract Body:

Clostridium difficile is the most commonly reported nosocomial pathogen in the United States and is an urgent public health concern worldwide. Over the past decade, incidence, severity, and costs associated with *C. difficile* infection (CDI) have increased dramatically. The primary risk factor for CDI is antibiotic use, which reduces colonization resistance to *C. difficile* by altering the resident gut microbiota. Interestingly, the number of non-antibiotic associated CDI cases is on the rise, suggesting that unexplored environmental, nutrient, and host factors likely play an important role in mediating susceptibility to *C. difficile.* One largely unexplored factor in both gut microbiota homeostasis and *C. difficile* pathogenesis is zinc (Zn) availability. Zn acquisition is an essential colonization factor for numerous pathogens and dietary Zn levels have been strongly linked to increased risk of gastrointestinal infections. In this study, we hypothesized that dietary Zn levels are a key factor shaping the ecology of the gut microbiota and in turn impact susceptibility to *C. difficile*. We used next generation sequencing to demonstrate that increased levels of dietary Zn significantly alter the structure and diversity of the gut microbiota in mice. In addition, we demonstrate that excess dietary Zn dramatically reduces the threshold of antibiotics needed to abolish colonization resistance to *C. difficile*. In mice colonized with *C. difficile*, increased dietary Zn also severely exacerbates *C. difficile*-associated disease (CDAD). Furthermore, the host metal binding protein calprotectin was determined to be essential for combating CDI by limiting Zn at the site of infection. Together, these data demonstrate that nutrient Zn levels play a key role in determining the outcome of the tripartite interaction between the host, *C. difficile*, and the gut microbiota. Furthermore, excess dietary Zn reduces community resistance to perturbation, which increases susceptibility to *C. difficile*. The profound impact of excess Zn on the gut microbiota may have broad implications for numerous microbiota-associated gastrointestinal disorders.

Author Disclosure Block:

J.P. Zackular: None. **J.L. Moore:** None. **L.J. Juttukonda:** None. **A.T. Jordan:** None. **Y. Zhang:** None. **W.J. Chazin:** None. **R.M. Caprioli:** None. **E.P. Skaar:** None.

Poster Board Number:

SATURDAY-530

Publishing Title:

Host-specific Fecal Microbiota Transplantation is More Effective in Treating Recurrent *Clostridium difficile* infection in a Murine Model

Author Block:

A. M. Seekatz[1], C. M. Theriot[2], K. C. Vendrov[1], V. B. Young[1]; [1]Univ. of Michigan Med. Sch., Ann Arbor, MI, [2]North Carolina State Univ. Coll. of Vet. Med., Raleigh, NC

Abstract Body:

Recurrent *Clostridium difficile* infection (CDI) occurs in 20-30% of patients following successful treatment of an initial episode of CDI. Defects in the gut microbiota, the indigenous bacterial community within the gastrointestinal tract, are hypothesized to contribute to the development of recurrence. Fecal microbiota transplantation (FMT) has emerged as an effective treatment for recurrent CDI, and although its success correlates to an increase in microbes associated with a healthy gut community, the specific microbes that enable recovery remain elusive. We have previously characterized a murine model of recurrent CDI, and observed that mouse fecal matter (mFMT) is sufficient to clear *C. difficile*. To identify potential bacterial members or community structures in humans that promote recovery from recurrent CDI, we treated recurrent animals with feces from a) healthy human donors (hdFMT) and b) pre-FMT human recipients (hrFMT) from previously successful FMT human donor-recipient pairs. We hypothesized that hdFMT would be successful in clearing *C. difficile* in our model. While mFMT from multiple mouse colonies was successful in clearing *C. difficile*, FMT from human sources was significantly less effective. We used 16S rRNA analysis to identify bacterial taxa associated with *C. difficile* clearance. Interestingly, we observed significant overlap between mFMT- and hdFMT-treated animals regardless of disease outcome. In particular, both FMT treatments resulted in an increase in several Bacteroidetes, such as the *Bacteroides* and *Porphyromonadaceae* groups, and Firmicutes, including several *Clostridiales* and *Lachnospiraceae* groups. These results emphasize the importance of the development of specific host-microbe interactions, and suggest that host specificity to gut microbial communities important in the dynamics of *C. difficile* colonization. Future studies that incorporate both human and murine models in identifying bacteria with a benefit to human health should consider how host specificity significantly impacts outcome.

Author Disclosure Block:

A.M. Seekatz: None. **C.M. Theriot:** None. **K.C. Vendrov:** None. **V.B. Young:** None.

Poster Board Number:

SATURDAY-531

Publishing Title:

Commensal *Bacteroidetes* Induce Expression of Reg3γ and Prevent *Clostridium difficile* Infection

Author Block:

H. Bangar, Yee-Shiuan Chen, George Weinstock, Heidi Andersen, D. Haslam; Cincinnati Children's Hosp. Med. Ctr., Cincinnati, OH

Abstract Body:

Background: *Clostridium difficile* infection (CDI) causes severe colitis that persists even after antibiotic treatment. Fecal microbiota transplant (FMT) has been demonstrated to be highly effective in treating protracted CDI. However, the identity of the protective organism(s) and the mechanism by which FMT attenuates CDI disease is not well understood. **Methods:** The fecal microbiome from susceptible and resistant mice before and after antibiotics was analyzed by whole metagenome shotgun sequencing. Either crude fecal microbiota from resistant mice or pooled protective bacterial species were transplanted to susceptible mice by oral gavage. Thereafter, mice were orally gavaged with *C. difficile* spores. Animals were observed for signs of disease and mortality. RNA-Seq analysis, flow cytometry, and histopathology were performed in each group to interrogate the mechanisms of protection.**Results:** Genetically identical C57BL/6 mice showed stark difference in susceptibility to CDI depending on the environment in which they were raised. Fecal material transplantation from CDI-resistant mice to CDI-susceptible mice resulted in protection from CDI, which correlated with an increased abundance of key *Bactoroidetes spp*. We demonstrated that oral inoculation with these key bacterial species prevented CDI-associated intestinal inflammation and stem cell injury by blocking access to colonic crypts from *C. difficile*. These protective effects were critically dependent on the crypt-associated antimicrobial peptide, Reg3γ.**Conclusions:** We identified the key *Bacteroidetes* spp. that provide protection from CDI by inducing production of antimicrobial peptides that are necessary for preventing *C. difficile* from accessing the intestinal crypts. Loss of these key species affects the production of antimicrobial peptides, which results in compromise to the physical barrier of the inner mucus layer.

Author Disclosure Block:

H. Bangar: None. **D. Haslam:** None.

Poster Board Number:

SATURDAY-532

Publishing Title:

Investigating *Clostridium difficile*-Microbiome Interactions to Characterize Potential Mechanisms for Colonization Resistance

Author Block:

J. M. Auchtung, R. A. Britton; Baylor Coll. of Med., Houston, TX

Abstract Body:

Clostridium difficile is the leading cause of antibiotic-associated diarrhea and nosocomial infections. Numerous studies have demonstrated that the gastrointestinal microbiome plays a key role in inhibiting infection by *C. difficile*. Multiple mechanisms by which the microbiome mediates resistance to *C. difficile* colonization have been proposed, including competition for resources and production of toxic metabolites such as short chain fatty acids, secondary bile acids, and bacteroicins. Recent research has focused on conversion of primary bile acids to secondary bile acids (e.g., deoxycholate) that inhibit growth of vegetative *C. difficile* cells as the primary mechanism through which the microbiota inhibits *C. difficile* colonization. We recently developed fecal mini-bioreactor arrays (MBRAs) that allow for anaerobic, continuous-flow cultivation of up to 96 communities simultaneously. We have shown these MBRA communities are resistant to *C. difficile* and disruption of community structure with antibiotics allows *C. difficile* to proliferate, produce toxins, and sporulate. We have now used this model to investigate the role of bile acid transformation in providing colonization resistance against *C. difficile*. Analysis of bile acid content in MBRA supernatants demonstrated that communities were capable of converting taurocholate to a mixture of cholate and deoxycholate. Treatment of communities with clindamycin reduced levels of deoxycholate and increased levels of cholate. We also asked whether communities could prohibit proliferation of vegetative *C. difficile* in the absence of bile acids. We found that bile acids were not required to mediate colonization resistance, indicating other mechanisms that prohibit *C. difficile* proliferation play a significant role in our model. Ongoing studies are focused on identifying and characterizing these other mechanisms that prevent *C. difficile* colonization. Understanding the mechanisms by which the microbiome inhibits *C. difficile* proliferation will likely play an important role in refining the composition of microbial therapeutics used to treat *C. difficile* infection.

Author Disclosure Block:

J.M. Auchtung: None. **R.A. Britton:** None.

Poster Board Number:

SATURDAY-533

Publishing Title:

Dietary Predictors of Successful Fecal Microbiota Transplant Treatment of *Clostridium difficile* Infection

Author Block:

J. J. Farrell[1], D. K. Martin[2], S. V. Thompson[3], A. Bogner[2], J. Bonello[2], H. M. Guetterman[3], K. S. Swanson[3], H. Holscher[3]; [1]Univ. of Illinois Coll. of Med., Peoria, IL, [2]OSF Med. Ctr., Peoria, IL, [3]Univ. of Illinois, Urbana, IL

Abstract Body:

Background: In healthy populations, diet contributes to gastrointestinal microbiota stability, diversity, and composition. But the impact of diet on recovery of intestinal microbial diversity following fecal microbiota transplantation (FMT) is not known. We hypothesized that patients with higher intakes of fermentable fibers would be more likely to establish intestinal microbial diversity following FMT. **Methods:** IRB approval was obtained to offer FMT for to patients with *Clostridium difficile* infection (CDI) refractory to conventional treatment begining November 2013. All FMT patients were invited to submit Diet History Questionnaires (DHQs) starting in October 2014. Participants completed the National Cancer Institute (NCI) Diet History Questionnaires (DHQII, Past Month with Portion Size) prior to FMT, and at 30 and 90 days post FMT. Questionnaire data were processed using NCI's Diet*Calc software and Healthy Eating Index scores were generated from the Diet*Calc output using SAS 9.4 and NCI macros. Success was defined as resolution of symptoms of CDI following FMT. Failure was defined as diarrhea with > 3 stools/day or retreatment (either oral vancomycin or a second transplant) within 30 days of FMT.**Results:** 16 patients and 2 donors completed FFQs (14 women, 2 men; mean age=53 years). FMT success was observed for 12/16 (75%) patients. Patients with successful FMT reported higher consumption of both fruit and fiber than patients with a failed FMT ($p < 0.05$). The percent of energy from carbohydrate was higher in patients with successful FMT ($p = 0.04$). Macronutrient percentages were also significantly different between FMT success and failure groups.**Conclusions:** Our objective was to study dietary factors predictive of FMT success. The data support our hypothesis that higher consumption of dietary fiber is predictive of microbiota transplantation success among patients presenting to our tertiary FMT referral center.

Diet and FMT Outcome		
Diet	Success	Failure
Total Fruit	3.39 ± 0.44	1.46 ± 0.77
Fiber (per Kcal)	0.88 ± 0.08	0.57 ± 0.15

Author Disclosure Block:

J.J. Farrell: None. **D.K. Martin:** None. **S.V. Thompson:** None. **A. Bogner:** None. **J. Bonello:** None. **H.M. Guetterman:** None. **K.S. Swanson:** None. **H. Holscher:** None.

Poster Board Number:

SATURDAY-534

Publishing Title:

***staphylococcus Aureus*-Induced Mucin Secretion By Goblet Cells: Dependency On Nlrp3 Inflammasome Activation And Release Of Mature Il-1β**

Author Block:

D. A. Dartt[1], D. LI[1], M. Lippestad[1], R. Hodges[1], M. Gilmore[2], M. Gregory-Ksander[1]; [1]Schepens Eye Res. Inst./Massachusetts Eye and Ear, Boston, MA, [2]Massachusetts Eye and Ear, Boston, MA

Abstract Body:

Purpose: Mucins protect mucosal surfaces the conjunctiva by entrapping bacteria and preventing bacterial invasion. We previously demonstrated that pathogenic *S. aureus* activates the NLRP3 inflammasome in ocular surface conjunctival goblet cells, resulting in secretion of the pro-inflammatory cytokine IL-1β and the mucins MUC5AC. This study was performed to determine if NLRP3 inflammasome activation is required for *S. aureus*-induced mucin secretion and if it is dependent upon the release of mature IL-1β.**Methods:** Cultured conjunctival human goblet cells were incubated with siRNA for NLRP3 or TLR 2 and stimulated with toxigenic *S. aureus* RN6390. Goblet cells were stimulated with IL-1β or *S. aureus* in the presence of an IL-1 receptor antagonist (IL-1Ra), an intracellular Ca^{2+} chelator, and an extracellular regulated kinase (ERK) 1/2 inhibitor. High molecular weight glycoconjugate (HMGC) secretion including MUC5AC was measured.**Results:** NLRP3 and TLR2 siRNA inhibited goblet cell HMGC secretion induced by *S. aureus*. Mature IL-1β stimulated goblet cell secretion in a dose-dependent manner. IL-1Ra blocked goblet cell HMGC secretion stimulated by *S. aureus* or IL-1β alone. IL-β-induced HMGC secretion was blocked by chelating intracellular Ca^{2+} or inhibiting ERK1/2 activityation.**Conclusions:** Goblet cell-derived mature IL-1β secretion is dependent upon both NLRP3 and TLR2 and mediates *S.aureus*-induced HMGC secretion by increasing intracellular [Ca^{2+}] and activating ERK1/2. These data reveal the pluripotent activities of goblet-cell-derived IL-1β secretion, induced by pathogenic *S. aureus,* could induce immune cell recruitment to clear pathogens andinduce HMGC secretion to enhance the mucosal ocular surface barrier to infection.

Author Disclosure Block:

D.A. Dartt: None. **D. Li:** None. **M. Lippestad:** None. **R. Hodges:** None. **M. Gilmore:** None. **M. Gregory-Ksander:** None.

Poster Board Number:

SATURDAY-535

Publishing Title:

NLRP3 Inflammasome Plays an Important Role in the Innate Immune Response to *Acinetobacter baumannii* Infection

Author Block:

B. Sukumaran, N. Dikshit; Duke-NUS Med. Sch., Singapore, Singapore

Abstract Body:

Background: *Acinetobacter baumannii* is an emerging multi- or pan-drug resistant nosocomial and community associated bacterium that is increasingly becoming a serious health threat world-wide. Pneumonia, bacteremia, urinary tract infections, and wound infections are some of the important clinical manifestations of *A.baumannii* infection. However, the innate immune pathways leading to various clinical outcomes associated with *A. baumannii* infections are not fully characterized yet. We identified that *A. baumannii* invades and survives within host cells and hypothesized that intracellular innate immune receptors might play a role in the host defense to *A. baumannii* infection. Inflammasomes are intracellular multiprotein complexes that play critical roles in innate immunity. But whether inflammasome pathway plays any role in the pathogenesis of *A. baumannii* infection is not studied yet.**Methods:** *In vitro* infection models using mouse bone marrow-derived macrophages and *in vivo* murine pneumonia infection models using NLRP3 knockout mice were used to assess the role of NLRP3 in controlling *A. baumannii* infection.**Results:** Our studies demonstrate that the most extensively studied inflammasome, NLRP3, is an important player in the host defense and inflammatory response associated with *A. baumannii* infection. We show that *A.baumannii* infection primes as well as activates the NLRP3 inflammasome assembly in mouse bone marrow-derived macrophages. *A.baumannii* infection induced IL-1β/IL-18 production is entirely dependent upon ASC/NLRP3/Caspase-1 axis. *A.baumannii* activates NLRP3 inflammasome by various overlapping mechanisms such as ROS generation/ Potassium ion imbalance.**Conclusions:** Thus we identified a previously unrecognized role for inflammasome components in the host innate immune response to *A. baumannii* infection and this information may provide alternative therapeutic approaches to tackle *A. baumannii* infections.

Author Disclosure Block:

B. Sukumaran: None. **N. Dikshit:** None.

Poster Board Number:

SATURDAY-536

Publishing Title:

Tir-Mediated Signaling Responses in the Social Amoeba Dictyostelium Discoideum

Author Block:

T. White, M. Fink, M. Berlett, K. Stephens, A. Zapf, M. Weichseldorfer, M. Snyder, 21252, M. Snyder, 21252; Towson Univ., Towson, MD

Abstract Body:

Cells of the innate immune system use pattern recognition receptors (PRRs) to detect conserved molecular motifs on a wide variety of microbial pathogens. The best characterized PRRs are the Toll-like receptors (TLRs), which utilize toll/interleukin-1 receptor (TIR) domains to initiate signaling responses. Among the signaling pathways activated downstream of mammalian TLRs are NF-kB, caspase and MAPK-mediated pathways, as well as the generation of reactive oxygen species (ROS). The social amoeba Dictyostelium discoideum phagocytizes bacteria for nutritional purposes. While its genome does not encodes for full-length TLRs, the model organism does express several TIR domain-containing proteins, including D. discoideum TirA, which previously has been shown to play a role in the growth of D. discoideum in the presence of Gram-negative bacteria. Furthermore addition of lipopolysaccharide (LPS), a component of the Gram-negative cell wall has been shown to enhance D. discoideum's bactericidal activities in a TirA- and ERK-dependent manner. While D. discoideum does not encode for NF-kB or caspases, exposure of D. discoideum to bacteria does result in the activation of MAPK-ERK signaling pathways as well as the generation of ROS. Here we find that both ERK phosphorylation, as well the generation of ROS, are reduced in TirA-deficient cells as compared with WT cells in response to Gram-negative, but not Gram-positive bacteria. In contrast, ERK phosphorylation in response to folic acid does not appear to be greatly affected by the absence of TirA. We are currently investigating the role of additional signaling molecules in the response of D. discoideum to Gram-negative bacteria. In addition to these procedures we are also attempting to solve the structure of D. discoideum TIR domains through the use of X-ray crystallography. By obtaining this structural information new insights into the conserved mechanisms of TIR domain signaling in may be revealed.

Author Disclosure Block:

T. White: None. **M. Fink:** None. **M. Berlett:** None. **K. Stephens:** None. **A. Zapf:** None. **M. Weichseldorfer:** None. **M. Snyder:** None. **M. Snyder:** None.

Poster Board Number:

SATURDAY-537

Publishing Title:

Cellular Mechanisms Of Alpha-Hemolysin-Mediated Activation Of The Nlrp3 Inflammasome

Author Block:

E. A. D. Ezekwe, Jr, J. A. Duncan; Univ. of North Carolina at Chapel Hill, Chapel Hill, NC

Abstract Body:

Background:*Staphylococcus aureus* toxin, α-hemolysin, is secreted as a soluble monomer that forms a heptameric pore in the membranes of a range of host cell types. Hemolysin binds and activates A Disintergin and Metalloprotease 10 (ADAM10) and is a well-chronicled virulence factor in staphylococcal disease. ADAM10 activity is important for toxin-mediated pathology in a number of cell types. In host monocytes, α-hemolysin activates the nucleotide-binding domain and leucine-rich repeat containing gene family, pyrin domain containing 3 (NLRP3) inflammasome leading to production of the pro-inflammatory cytokines (IL-1β, IL-18) and pyroptotic cell death. Human airway epithelial cells express the components of the NLRP3 inflammasome but the involvement of this signaling pathway in the cellular response to α-hemolysin is unknown. We hypothesized that both ADAM10 and NLRP3 are involved in host cellular responses to α-hemolysin in both monocytic and respiratory epithelial cells. **Methods:** To elucidate the role of ADAM10 and its protease activity in α-hemolysin-mediated activation of the inflammasome, we used the immortalized monocyte cell line, THP1. Cells were treated with siRNA against or chemical inhibitors of ADAM10, challenged with α-hemolysin, and NLRP3-inflamamsome activation assessed by measuring secreted IL-1β, cell death, and activation of caspase-1.To test for evidence of α-hemolysin –mediated inflammasome activation in respiratory epithelial cells, we used primary human tracheobronchial epithelial (hTBE) cells and measured their secretion of IL-1β in response to hemolysin challenge.**Results:** Loss of ADAM10 cell surface expression led to diminished α-hemolysin-mediated activation of the NLRP3 inflammasome and cell death. ADAM10 protease activity, however, was not required for NLRP3 activation in human monocytes. hTBEs secrete mature IL-1β in response to α-hemolsyin treatment, suggesting a role for the inflammasome in their response to α-hemolysin.**Conclusions:** This work demonstrates that ADAM10 is a receptor but not an active signaling participant in inflammasome activation by α-hemolysin in human monocytes. Preliminary evidence also suggests that the inflammasome may play a role in epithelial cell responses to α-hemolysin.

Author Disclosure Block:

E.A.D. Ezekwe: None. **J.A. Duncan:** None.

Poster Board Number:

SATURDAY-538

Publishing Title:

Amoebal Endosymbiont *Protochamydia* isolated from a Hospital Can Induce Il-8 In Human Immortal Hep-2 Cells

Author Block:

J. Matsuo[1], T. Fukumoto[2], T. Okubo[1], S. Nakamura[3], K. Akizawa[2], H. Shibuya[2], C. Shimizu[2], H. Yamaguchi[1]; [1]Hokkaido Univ., Sapporo, Japan, [2]Hokkaido Univ. Hosp., Sapporo, Japan, [3]Juntendo Univ., Tokyo, Japan

Abstract Body:

Obligate intracellular bacteria belonging to *Parachlamydiaceae*, such as *Protochlamydia* or *Parachlamydia*, survive in free-living amoebae, *Acanthamoeba*. Since the bacteria are considered to be a causative agent of respiratory tract infections in human, the infections are likely to be an emerging infection. Regarding this, we have previously shown that *Parachlamydia* DNA was widely distributed with *Acanthamoeba* DNA in a hospital, inferring from the finding that the bacteria may have an impact on hospital infections (1). In this study, we therefore attempted to isolate *Acanthamoeba* harboring endosymbionts from hospital environments and evaluated the bacterial infectivity and cytokine inducibility to human immortal HEp-2 cells. Fifty smear samples were collected from the floor (n = 30) and sink outlets (n = 20) in Hokkaido University Hospital. The survey was conducted repeatedly at different periods (February to March and August in 2012). The smear samples (1 m x 1 m) were suspended in Page's amoeba saline and centrifuged. The resulting pellets were cultured on a non-nutrient agar covered with heat-killed *E. coli*. Finally, *Acanthamoeba* was axenically cultured in PYG medium. Amoebal endosymbionts were detected by PCR and DAPI staining. Next, the bacteria were briefly purified by centrifugation and incubated with *Acanthamoeba* reference strain (C3) or HEp-2 cells for 5 days. The bacterial copy number was measured with qPCR. Proinflammatory cytokine IL-8 was also detected in culture supernatant of HEp-2 cells with ELISA. As a result, six *Acanthamoeba* strains were successfully cultured in PYG. Of them, three were found to harbor chlamydiae, *Protochlamydia* (W9) or *Neochlamydia* (Y20, Y23). Since *Protochlamydia* W9 exclusively could infect and propagate in C3 amoebae, the bacteria were used for the experiments with HEp-2 cells. However, the data showed that *Protochlamydia* W9 could not propagate in HEp-2 cells. Meanwhile, elevated IL-8 secretion was observed depending upon the bacterial stimulation. Taken together, these results suggested that *Protochlamydia* could spread within amoebae in hospital environments and induce inflammatory responses to human once the bacteria were brought to hospital environments.

Author Disclosure Block:

J. Matsuo: None. **T. Fukumoto:** None. **T. Okubo:** None. **S. Nakamura:** None. **K. Akizawa:** None. **H. Shibuya:** None. **C. Shimizu:** None. **H. Yamaguchi:** None.

Poster Board Number:

SATURDAY-539

Publishing Title:

Placental Macrophages Respond to *Streptococcus agalactiae* Infection by Releasing Matrix Metalloproteinase-Rich Extracellular Traps

Author Block:

R. S. Doster[1], L. M. Rogers[1], D. M. Aronoff[1], J. A. Gaddy[2]; [1]Vanderbilt Univ. Sch. of Med., Nashville, TN, [2]Tennessee Vally Hlth.care System, Nashville, TN

Abstract Body:

Background: Group B *Streptococcus* (GBS) infections are important causes of preterm birth and neonatal sepsis. Placental macrophages (PMs) are resident leukocytes present in gestational tissues. We sought to identify PM functions during GBS infection. **Methods:** PMs were incubated *ex vivo* with GBS clinical isolates (MOI 20:1 x 1 hr) before processing and imaging with scanning electron (SEM) or confocal microscopy. Extracellular traps (ETs) were quantified as percent of PMs producing ETs per field of SEM (750X magnification). Extracellular trap production was confirmed via confocal microscopy staining with Sytox Green for extracellular DNA and Hoechst 33342 for nuclei. ETs were then stained for matrix metalloproteinases (MMP) 7, 8 and 9 using polyclonal antibodies (abcam) followed by incubation with a fluorochrome conjugated secondary antibody. Transcriptional changes in MMP production were assessed using RT-PCR after infection of macrophages with GBS cells for 2 hours at MOI of 100:1.**Results:** PMs produced ETs in response to GBS infection as evidenced by characteristic appearance and extracellular DNA staining via SEM and confocal microscopy. DNAse treatment dissolved these structures. GBS infection also induced transcriptional upregulation of MMP 7,8 and 9, which have been implicated in premature rupture of membranes and preterm birth. We then examined if ETs could be a mechanism by which MMPs are released during infection. ETs from GBS infected cells stained strongly for MMP-7 and MMP-8, but not MMP-9.**Conclusions:** GBS infection induced transcription of MMPs and ET formation in PMs. These structures contained MMP-7 and 8. MMP-7 (matrilysin) and MMP-8 (human neutrophil collagenase) catalyze the breakdown of fibronectin, laminin, and collagens important for fetal membrane structural integrity. These MMPs are found to be elevated in amniotic fluid of patients with intra-amniotic infection and premature rupture of membranes. Thus this immune strategy thought to trap invading microbes may also result in localized damage to fetal membranes during infection, potentially contributing to premature membrane rupture. Future work will evaluate the impact of these MMP-rich ETs on membrane integrity.

Author Disclosure Block:

R.S. Doster: None. **L.M. Rogers:** None. **D.M. Aronoff:** None. **J.A. Gaddy:** None.

Poster Board Number:

SATURDAY-540

Publishing Title:

Group B *streptococcus* Infection Induces Siglec-14 Expression and M1 Activation in Human Monocyte-Derived and Mouse Decidual Macrophages

Author Block:

J. A. Sutton[1], L. M. Rogers[2], D. M. Aronoff[2]; [1]Meharry Med. Coll., Nashville, TN, [2]Vanderbilt Univ. Med. Ctr., Nashville, TN

Abstract Body:

Group B *Streptococcus* (GBS) is a leading cause of neonatal death and sepsis. The fundamental mechanisms by which GBS evades innate immune defenses in the gravid uterus are not fully elucidated. In response to stimuli, macrophages are activated along a spectrum ranging from a proinflammatory "M1" state to an anti-inflammatory "M2" state. Through molecular mimicry, sialic acid on the capsular surface of GBS binds to sialic acid binding immunoglobulin-like lectins, siglecs, on macrophages to modulate inflammatory responses. Polymorphisms in paired inhibitory and activating receptors, Siglec-5 and Siglec-14, respectively, are associated with preterm birth and GBS infection. This study aims to define changes in human MDMφ and mouse decidual macrophage activation and Siglec-5/Siglec-14 expression in response to GBS infection. MDMφs were infected with GBS or polarized *in vitro* with M1 (IFNγ + LPS) and M2 (IL-4 or IL-10) stimuli. Resulting phenotypes were characterized by qRT-PCR to evaluate siglec and polarization marker gene expression. Cytokines (TNFα, IL-1β, and IL-10) were evaluated by ELISA. To mimic human ascending vaginal infection *in vivo*, C57BL/6 mice were inoculated vaginally with GBS at day E13.5 for 48h. Subsequently, decidual macrophages were isolated and analyzed for polarization markers (CD11c and CD206) via flow cytometry. Our findings in MDMφ suggest that GBS infection upregulates expression of M1 genes (CCR7, CD80) and downregulates M2 genes (CD209, CD163). GBS infection induced release of M1 cytokines (TNFα, IL-1β) and M2 cytokine IL-10. Siglec-14 expression was upregulated in response to GBS while Siglec-5 exhibited little to no modulation. GBS infection in mouse decidual macrophages demonstrated a shift towards M1 polarization as evidenced by the decrease of surface CD206 and increase in surface CD11c. These results suggest that GBS induces M1 activation while activating Siglec-14 but not the inhibitory Siglec-5. Such responses might contribute to the inflammatory complications associated with chorioamnionitis such as membrane rupture, fetal inflammatory response syndrome, and premature labor. Taken together, our studies provide new insights into the role of macrophage polarization and siglec biology in GBS pathogenesis.

Author Disclosure Block:

J.A. Sutton: None. **L.M. Rogers:** None. **D.M. Aronoff:** F. Investigator; Self; Global Alliance to Prevent Prematurity and Stillbirth, Burrough's welcome Fund.

Poster Board Number:

SATURDAY-541

Publishing Title:

Dendritic Cell Involvement and Innate Defense Determinants in *Pseudomonas aeruginosa* Colonization of the Corneal Epithelium

Author Block:

M. M. Metruccio[1], C. K. P. Tam[1], M. E. Stern[2], **D. J. Evans**[3], S. M. J. Fleiszig[1]; [1]UC Berkeley, Berkeley, CA, [2]ImmunEyeZ LLC, Orange County, CA, [3]Touro Univ., Vallejo, CA

Abstract Body:

Healthy epithelial-lined tissue surfaces are remarkably resistant to bacterial colonization and infection. Nevertheless, contact lens wear can compromise corneal epithelial barrier function via unknown mechanisms leading to sight-threatening infections. Previously, we showed that corneal epithelial barrier function against *P. aeruginosa* requires MyD88, the TLR and IL-1R adaptor protein expressed by both epithelial cells and dendritic cells in the corneal epithelium. Here, we examined the roles of dendritic cells (DCs), specific TLRs and IL-1R. Intact, or superficially-injured (tissue-paper blotted) whole eyes from wild-type C57BL/6 and TLR or IL-1R gene knockout mice were imaged by confocal microscopy to compare *P. aeruginosa* colonization and traversal over 6 h. Transgenic diphtheria toxin receptor mice were used to selectively deplete corneal DCs, and qPCR used to assess corneal transcriptional changes with bacterial challenge. Intact corneas of TLR4 or IL-1R knockout mice were more susceptible to bacterial adhesion. TLR5 was required to prevent bacterial traversal, but not surface adhesion. Corneal DCs located in the stroma of uninjured corneas showed profound changes in shape (suggesting activation) after bacterial challenge, and extended long dendritic processes in close proximity to the epithelial surface. If corneas were superficially-injured before bacterial challenge, DC processes extending to the epithelial surface colocalized with adherent bacteria. Superficially injured DC-depleted corneas showed a 3-fold increase in *P. aeruginosa* adherence ($p < 0.05$, Mann-Whitney U test) and displayed differential mRNA expression for select innate immunity genes, including down-regulation of IL-6 (3-fold) and IL-1R (2-fold) in response to bacterial inoculation. These data show that multiple MyD88-dependent receptors contribute to corneal innate defense against bacterial adhesion and subsequent epithelial traversal. They also show that DCs in the stroma detect and respond to bacteria at the healthy corneal surface and protect it against bacterial adhesion. How DCs deep within the cornea detect bacteria at the surface to impact epithelial barrier function remains to be determined.

Author Disclosure Block:

M.M. Metruccio: None. **C.K.P. Tam:** None. **M.E. Stern:** D. Employee; Self; ImmunEyeZ LLC. **D.J. Evans:** None. **S.M.J. Fleiszig:** None.

Poster Board Number:

SATURDAY-542

Publishing Title:

Evaluation of Neutrophil Activity against *Nocardia brasiliensis* Hujeg-1 and Strains Obtained from Continuous *In Vitro* Passaging

Author Block:

L. Vera-Cabrera, J. Garza-Chapa, C. Molina-Torres, J. Ocampo-Candiani; Hosp. Univ.rio, U.A.N.L., Monterrey, Mexico

Abstract Body:

Background: *Nocardia brasiliensis* is the main causative agent of mycetoma in Mexico. Little is known about the virulence/host resistance events occurred in this neglected infectious disease. In order to study the physio pathogenic mechanisms involved in this infection, we have previously subcultured a N. brasiliensis isolate, obtained from a mycetoma human case, during 400 times. After 200 passages the bacteria presented several changes, including lost of virulence for mice, the growth in broth as an homogeneous suspension instead of filamented colonies, the decrease of acid-fastness, and the cell-wall density. Based on these findings, we hypothesized that important changes in the Nocardial cell-wall occurred, and this may explain the lost of virulence after 200 continuous passage. In this work we compared the susceptibility of the parental isolate to that of three passaged strains (P200, P300 and P400) to human PMN granulysins including HPN I-3, LL-37 and HBD-3; killing assays were also performed with these N. brasiliensis strains using human PMN, and rat neutrophils. The latter has been reported to be resistant to the *N. brasiliensis* infection. **Methods:** Bacterial unicellular suspensions from the original islate (P0), and the subcultured strains were prepared, and the susceptibility to destruction by granulosyns were determined. Human PMN were obtained from volunteer healthy donors, and rat neutrophils were obtained from female Wistar rats. In both cases , PMN were purified using the Ficoll/Hypaque method. PMN were infected at a 3:1 relationship and incubated for 180 min; CFU were determined at the beginning and the end of the experiment.**Results:** In the granulosyns susceptibility assays human neutrophils were unable to destroy P0 nocardial cells under these conditions, and no differences with the subcultured strains were observed. Neutrophils from rat were able to decrease 40% of the nocardial cells after 180 min of incubation. Interesently, P300 induced strongly the induction of PMN nets, even after a few seconds of incubation. **Conclusions:** The lack of virulence of the subcultured isolates do not seem to reside in an increased susceptibility to PMN killing.

Author Disclosure Block:

L. Vera-Cabrera: None. **J. Garza-Chapa:** None. **C. Molina-Torres:** None. **J. Ocampo-Candiani:** None.

Poster Board Number:

SATURDAY-543

Publishing Title:

Pre-exposure of Methicillin-resistant *Staphylococcus aureus* (Mrsa) to Subinhibitory Daptomycin Concentrations Enhances the Activity of Human Neutrophils Against Mature Biofilms

Author Block:

M. Simitsopoulou, P. Kadiltzoglou, I. Stamouli, C. Antachopoulos, **E. Roilides**; Aristotle Univ. of Thessaloniki, Thessaloniki, Greece

Abstract Body:

Background: Biofilms (BF) formed by MRSA on the surface of catheters or implanted biomaterials are associated with persistent infections. Neutrophils (PMN) have a central role in the innate host responses against pathogens. We hypothesized that sub-MIC daptomycin (DAP) concentrations can modify the activity of PMN against MRSA mature biofilms. **Methods:** 12 MRSA isolates from patients with sepsis were incubated at 10^6 CFU/mL in TSB growth media at 37°C for 24h. BF formation was assessed by staining with 1% safranin and measuring OD at 595nm. For MIC determination, BF were incubated with 2-fold dilutions of DAP at 0.007-256mg/L for 24h. MRSA strains were grown in TSB at 37°C for 24h in the presence of sub-MIC DAP concentrations of 1, 2 and 4mg/L. Pretreated and untreated MRSA were then grown in fresh TSB at 37°C for 24h to produce mature BF. PMN were isolated from healthy donors by dextran sedimentation/ficoll centrifugation. BF were then incubated with PMN for additional 24h at effector to target ratios (E:T) 2:1, 1:1, 1:3, 1:4, 1:5. Sub-MIC50 was determined as the minimum concentration that caused <50% biofilm damage (BD) compared to untreated controls. PMN-induced damage measured as % reduction of metabolic activity was assessed by XTT assay. All MRSA strains were tested in triplicate. ANOVA with Dunnett's post-test was used.**Results**Of the 12 MRSA strains, 3 were found to form intermediate BF. BF MIC50 for DAP of these 3 isolates was 32 mg/L. When PMN were added to drug-pretreated BF, there was a significant increase of PMN-induced % damage against MRSA exposed to 1, 2 and 4mg/L at 2:1 and 1:5 E:T ratios as compared to drug-untreated controls (2:1, 78%±5, 89%±4, 88%±4 vs 60%±7 and 1:5, 43%±8, 50%±8, 73%±3 vs 11.6%±6; $p<0.05$). A significant enhancement of PMN-induced % damage against MRSA was also observed at 1:1, 1:3 and 1:5 E:T ratios but with MRSA exposed to DAP concentrations of 2 and 4mg/L ($p<0.01$). The highest %BD (>86%) was achieved at 2:1 E:T at 2 and 4mg/L of DAP pretreatment.**Conclusion**Pre-exposure to sub-MIC DAP concentrations increases BF susceptibility to PMN antibacterial activity against MRSA. These findings may be clinically important in treatment of *S. aureus* biofilm-associated infections and warrant further study in animal models.

Author Disclosure Block:

M. Simitsopoulou: None. **P. Kadiltzoglou:** None. **I. Stamouli:** None. **C. Antachopoulos:** None. **E. Roilides:** None.

Poster Board Number:

SATURDAY-544

Publishing Title:

Activation, Impaired Tumor Necrosis Factor-α Production, and Deficiency of Circulating Mucosal-Associated Invariant T Cells in Scrub Typhus

Author Block:

S. Kang, H-M. Jin, Y-N. Cho, E. Won, B-S. Kim, S. Kim, U. Kim, J. An, H-C. Jang, S-I. Jung, S-J. Kee, Y-W. Park; Chonnam Natl. Univ. Med. Sch. and Hosp., Gwangju, Korea, Republic of

Abstract Body:

Background: Scrub typhus is a mite-borne bacterial infection of humans caused by *Orientia tsutsugamushi* prevalent in Asia, Northern Australia, and the Indian subcontinent. The dysregulation of immune responses is known to contribute to disease pathogenesis in scrub typhus, but the nature and mechanism of immune alterations are poorly define. The current study was aimed at examining the level and function of Mucosal-associated invariant T (MAIT) cells in patients with scrub typhus and evaluating the clinical relevance of MAIT cell levels.
Methods: Thirty-eight patients with scrub typhus and 53 health control (HC) subjects were enrolled in the study. The patients were further divided into subgroups according to disease severity. MAIT cell level and function in the peripheral blood were measured by flow cytometry.**Results:** Circulating MAIT cell levels were found to be significantly reduced in scrub typhus patients compared with HCs (median 0.69% versus 1.37%, $P < 0.05$). A linear regression analysis revealed that the level of MAIT cells was related to level of alanine aminotransferase, alkaline phosphatase positively ($P = 0.043$ and $P = 0.009$), and inversely correlated with age and disease severity ($P = 0.001$ and $P = 0.047$). A comparison of phorbol myristate acetate (PMA) and ionomycin (IM) - stimulated cytokine production in MAIT cells showed that the tumor necrosis factor-α (TNF-α) production was significantly impaired in acute phase of *O. tsutsugamushi* infection compared to HCs (median 15.6% versus 45.0%, $P < 0.05$). The impaired production of TNF-α was significantly recovered in convalescent phase compared to acute phase of infection (median 56.8% versus 34.1%, $P < 0.05$). In addition, this MAIT cell dysfunction was associated with upregulation of activation marker CD69, which was significantly higher in scrub typhus patients than in HCs (median 36.8% versus 6.0%, $P < 0.0001$). **Conclusions:** This study shows that circulating MAIT cells are activated, numerically and functionally deficient in patients with scrub typhus. These abnormalities possibly contribute to immune system dysregulation in scrub typhus infection.

Author Disclosure Block:

S. Kang: None. **H. Jin:** None. **Y. Cho:** None. **E. Won:** None. **B. Kim:** None. **S. Kim:** None. **U. Kim:** None. **J. An:** None. **H. Jang:** None. **S. Jung:** None. **S. Kee:** None. **Y. Park:** None.

Poster Board Number:

SATURDAY-545

Publishing Title:

Overactive Bladder Phenotype Observed in Urothelial Cells Treated with Bacterial Supernatant

Author Block:

E. E. Hilt, M. M. Pearce, S. Zhang, A. J. Wolfe, P. Le; Loyola Univ. Chicago, Maywood, IL

Abstract Body:

Background: The etiology of Overactive Bladder (OAB) remains poorly understood and diagnosis is primarily symptom-based. A new paradigm emerges that involves the contribution of a non-neuronal acetylcholine response in the urothelium. The recent discovery that a female urinary microbiota exists raises the question: could some bacterial product affect the acetylcholine response in the urothelium? **Materials:** Using an established urothelial cell line, we treated urothelial cells with bacterial cell-free supernatant from organisms associated with OAB and asked if there was a difference in the expression profiles of acetylcholine receptors and enzymes required for acetylcholine synthesis. **Results:** Treatment with bacterial cell-free supernatants of the OAB-associated bacterial species, *Streptococcus anginosus*, increased the urothelial cell expression of acetylcholine receptors and carnitine acetyltrandferase, the enzyme that synthesizes acetylcholine in urothelial cells. **Conclusion:** Our findings suggest that bacterial products from OAB-associated bacteria could target the urothelium to increase the production of non-neuronal acetylcholine. These data support the hypothesis that the FUM or components of the FUM may contribute to etiology of OAB.

Author Disclosure Block:

E.E. Hilt: None. **M.M. Pearce:** None. **S. Zhang:** None. **A.J. Wolfe:** None. **P. Le:** None.

Poster Board Number:

SATURDAY-546

Publishing Title:

Immunological Responses to Poly Glutamic Acid Polymers from *Bacillus* Species

Author Block:

T. M. Jelacic, W. J. Ribot, J. Chua, A. M. Friedlander; USAMRIID, Frederick, MD

Abstract Body:

Several *Bacillus* species produce poly-gamma-glutamic acid polymers (PGAs). The PGA produced by *Bacillus anthracis* is unique in that it is composed solely of D isomer glutamic acid while other *Bacillus* species produce mixed D, L isomer PGAs. We hypothesized that the exclusive use of the D isomer confers an advantage against the host immune system so we compared the responses of human innate immune cells to the pure D isomer PGA from *B. anthracis* to their responses to two mixed D, L isomer PGAs, a predominantly D isomer PGA from *B. subtilis chungkookjang* and a predominantly L isomer PGA from *B. subtilis*. Large differences were observed when the cytokine responses of differentiating monocytes were assessed. As the level of D isomer decreased, cytokine release increased and diversified. Pure D *B. anthracis* PGA elicited only very modest amounts of IL-8 and IL-6 while the predominantly D *B. subtilis chungkookjang* PGA elicited significantly larger amounts of IL-8 and IL-6 ($P < 0.01$, n = 5 donors for both) and also elicited IL-10 and TNF alpha. The predominantly L *B. subtilis* PGA in turn elicited significantly more IL-8 ($P < 0.01$), IL-6 ($P < 0.05$), IL-10 ($P < 0.01$), and TNF alpha ($P < 0.01$) than did *B. subtilis chungkookjang* PGA and elicited release of IL-1 beta as well. Similar differences in the magnitude and diversity of the cytokine response were observed when immature dendritic cells (iDCs) from the same group of donors were tested. *B. anthracis* PGA only elicited very modest amounts of IL-8, while *B. subtilis chungkookjang* PGA elicited larger amounts of IL-8 ($P < 0.001$) and also elicited IL-6. *B. subtilis* PGA in turn elicited significantly more IL-6 than did *B. subtilis chungkookjang* PGA ($P < 0.01$) and also elicited release of IL-12p70, IL-10, IL-1 beta and TNF alpha. Since a cocktail of TNF alpha, IL-6, IL-1 beta, and PGE_2 can be used to mature human iDCs *in vitro*, we assessed the PGA treated DCs for signs of maturation. *B. subtilis* PGA induced maturation of DCs as measured by increases in expression of CD83 and CCR7, and by CCR7 dependent chemotaxis. Neither *B. anthracis* nor *B. subtilis chungkookjang* PGA induced DC maturation. From these results, we conclude that the exclusive use of D isomer in *B. anthracis* PGA greatly blunts the magnitude and diversity of the cytokine response by monocytes and iDCs and furthermore, does not induce DC maturation, thus providing *B. anthracis* with an advantage against the host immune system.

Author Disclosure Block:

T.M. Jelacic: None. **W.J. Ribot:** None. **J. Chua:** None. **A.M. Friedlander:** None.

Poster Board Number:

SATURDAY-547

Publishing Title:

The O-Antigen of *Salmonella enterica* Serovar Paratyphi A Functions in Evasion of Complement Activation

Author Block:

T. Wangdi, H. Hiyoshi, A. J. Baumler; Univ. of California, Davis, Davis, CA

Abstract Body:

Paratyphoid fever, a severe systemic infection caused by Salmonella enterica serovar Paratyphi A (S. Paratyphi A) is a prevalent human disease with well-documented epidemiology but poorly understood pathogenesis. Paratyphoid fever is indistinguishable from typhoid fever (caused by S. enterica serovar Typhi) in its clinical presentation, but differs markedly from human gastroenteritis, which is caused by non-typhoidal Salmonella serovars, such as S. enterica serovar Typhimurium. We have previously shown that differences in the clinical presentation of typhoid fever and gastroenteritis are due in part to an evasion of complement activation mediated by the Vi capsular polysaccharide, a S. Typhi-specific virulence factor encoded by Salmonella pathogenicity island 7 (SPI7). The similarity in the clinical presentation suggests that S. Typhi and S. Paratyphi A use similar virulence strategies, however, SPI7 is not present in the latter. Here we show that the O-antigen of S. Paratyphi A lipopolysaccharide (LPS) fulfills a similar function during infection as the Vi capsular polysaccharide of S. Typhi. The S. Typhi Vi capsular polysaccharide inhibited a complement-dependent induction of reactive oxygen species (ROS) production in HL60 human neutrophil-like cells. Similarly to S. Typhi, S. Paratyphi A inhibited ROS production in HL60 cells while S. Typhimurium triggered a robust induction of ROS production. However, replacing paratose (O2 antigen) in the O-antigen of S. Paratyphi A with abequose (O4 antigen), a sugar present in the O-antigen of S. Typhimurium, markedly increased ROS production by HL60 cells. Furthermore, heterologous expression of the S. Typhimurium long O-antigen chain-length determinant gene wzzB in S. Paratyphi A resulted in kinetics of ROS production by HL60 cells that were similar to those induced by S. Typhimurium. These data suggest that unlike non-typhoidal Salmonella serovars, S. Typhi and S. Paratyphi A inhibit complement-dependent ROS production by neutrophils. However, while S. Typhi uses the Vi capsular polysaccharide to inhibit ROS production, S. Paratyphi A possesses O-antigen modifications that serve a similar function. We conclude similarities in the virulence strategies of typhoidal Salmonella serovars represents are the result of convergent evolution.

Author Disclosure Block:

T. Wangdi: None. **H. Hiyoshi:** None. **A.J. Baumler:** None.

Poster Board Number:

SATURDAY-548

Publishing Title:

Pneumococcal Serotype 35B Is a Novel Ficolin-2 Target, and Epidemiological Data Suggest That Ficolin-2 Protects Children from Serotype 35B Invasive Disease

Author Block:

K. A. Geno, B. L. Spencer, M. H. Nahm; Univ. of Alabama at Birmingham, Birmingham, AL

Abstract Body:

Background: Ficolin-2 (FCN2) is a pattern recognition molecule that activates complement via the lectin pathway. FCN2 binds many capsular types (serotypes) of the pathogen *Streptococcus pneumoniae* (pneumococcus) that encode the O-acetyl transferase *wcjE*. We previously reported that FCN2 protects children against invasive pneumococcal disease (IPD) by at least one such serotype, 11A. *wciG* is another O-acetyl transferase encoded by some serotypes, including 10C, which does not bind to ficolin-2. Surprisingly, we observed FCN2 binding to serotype 35B, which does not encode *wcjE* but encodes *wciG*. We studied the nature of FCN2 binding to serotype 35B and studied US epidemiology to determine whether FCN2 protects children against serotype 35B IPD. **Methods:** FCN2 and complement deposition were assayed by flow cytometry with specific antibodies. A serotype 35BΔ*wciG* strain was generated by allelic exchange mutagenesis of a serotype 35B clinical isolate. Epidemiological data were provided by the Centers for Disease Control and Prevention. **Results:** FCN2 bound to several isolates of serotype 35B pneumococcus. FCN2 binding was inhibited by known FCN2 inhibitors and purified 35B capsular polysaccharide, but not by phosphocholine. FCN2 opsonization was associated with complement opsonization in C1q-depleted serum. Deletion of *wciG* resulted in loss of FCN2 recognition. FCN2-opsonized serotypes 11A, 31, and 35B are largely absent from IPD among children in the US. **Conclusions:** We previously showed that *wcjE*-mediated O-acetylation is a ficolin-2 ligand on pneumococcal capsules. Serotype 35B, which does not encode *wcjE*, is also bound by FCN2, and thus WciG-mediated O-acetylation represents a novel FCN2 ligand. Epidemiological evidence supports the notion that children are protected against 35B infection, and infection models of FCN2-opsonized serotypes should be established for further study.

Author Disclosure Block:

K.A. Geno: None. **B.L. Spencer:** None. **M.H. Nahm:** None.

Poster Board Number:

SATURDAY-549

Publishing Title:

Graphen Induces Different Host Immune Response Before and After Deactivation of Endotoxin

Author Block:

K. Gokulan[1], M. Lahiani[2], K. Williams[1], M. Khodakovakaya[3], S. Khare[1]; [1]Food and Drug Adminstration, Jefferson, AR, [2]Univ. of Arkansan Little Rock, Little Rock, AR, [3]Univ. of Arkansas at Little Rock, Little Rock, AR

Abstract Body:

Engineered nanomaterials (ENM) offer considerable commercial potential and pharmaceutical applications. In pharmaceutical platforms, it is critical that ENM be carefully evaluated for their toxicity before use. Synthesis, storage, added surface coating, dispersants, and handling of the nanomaterial can result in the final product becoming contaminated with endotoxin,. The objective of this study was twofold: first, graphene nanomaterials were tested for the presence of endotoxin and strategies were developed to reduce the level of this contaminant. Second, a study was conducted to distinguish the specific immune response exhibited due to endotoxin contamination.Unaltered and functionalized graphene were used in this study. To detect endotoxin in the supplied material, the gel clot LAL assay and chromogenic-based LAL endotoxin system were used. Our results revealed that the graphene contained significant amounts of endotoxin. Comparative analysis of various depyrogenic strategies was conducted. However, the concentration of endotoxin remained above national regulatory authorities recommended limits at the graphene concentration >1ug/ml. Macrophages were incubated with depyrogenated and pristine graphene to test any differences in the phagocytosis of these material as well as gene expression of immune response related genes.The mechanism of uptake of depyrogenated graphene was different from that of the native graphene by macrophages. Both native and depyrogenated graphene caused down regulation of CD14 receptor on the macrophages; which may be due to the induction of a signal transduction cascade involved in the binding of endotoxin on CD14. Furthermore, there were several similarities, as well as differences, in the expression of Toll-like receptor signaling, NOD-like receptor signaling genes, and downstream signal transduction molecule during the exposure of depyrogenated graphene. Most interestingly, depyrogenated graphene-exposed macrophages dampened the expression of the NF-kB signaling pathway. This study affirms that endotoxin contamination should be assessed while evaluating the cellular toxicity, to differentiate nanomaterial-specific toxicity from the endotoxin effects.

Author Disclosure Block:

K. Gokulan: None. **M. Lahiani:** None. **K. Williams:** None. **M. Khodakovakaya:** None. **S. Khare:** None.

Poster Board Number:

SATURDAY-550

Publishing Title:

Molecular Regulation of Host Inflammation During Influenza a Virus Infection

Author Block:

R. Visconti[1], C. Williams[1], R. Wurth[1], J. A. Harton[2], **E. Yager**[1]; [1]Albany Coll. of Pharmacy and Hlth.Sci., Albany, NY, [2]Albany Med. Coll., Albany, NY

Abstract Body:

The innate immune system recognizes influenza A viruses through multiple mechanisms, with recent studies revealing a critical role for the cytoplasmic NLRP3 inflammasome complex which promotes the maturation of the pro-inflammatory cytokine interleukin 1-β (IL-1β). Virus-induced NLRP3 inflammasome activation is critical for host defense and tissue repair during flu infection. Conversely, it is also evident that excessive NLRP3 inflammasome activity, especially during infection by highly pathogenic or pandemic strains of virus, can result in dysregulated inflammation that is detrimental to the host. Recent studies have identified a small molecule (pyrin-only protein 2 or POP2) that blocks the formation and activation of inflammasome complexes in humans. Because POP2 can interact with the pyrin domain of NLRP3, we hypothesize that POP2 functions to regulate the robust NLRP3-dependent IL-1β responses elicited during human IAV infection. Data from our continuing studies indicate that POP2 gene expression and function "cycles" with the expression of pro-IL-1β in PMA-differentiated human THP-1 monocytic cells exposed to seasonal and pandemic strains of influenza A viruses. Additionally, POP2 gene expression was found to be up-regulated in human donor PBMCs exposed to influenza A viruses. These findings support our notion that POP2 plays a critical role in ensuring a controlled, inflammatory response during human influenza A virus infection to favor protection over pathology.

Author Disclosure Block:

R. Visconti: None. **C. Williams:** None. **R. Wurth:** None. **J.A. Harton:** None. **E. Yager:** None.

Poster Board Number:

SATURDAY-551

Publishing Title:

Nlrp10 Promotes Host Innate Immunity in Sepsis

Author Block:

P. Baral, S. Batra, S. Jeyaseelan; Louisiana State Univ., Baton Rouge, LA

Abstract Body:

Rationale: Sepsis is one of the leading causes of the death in Uniteed States and rest of world among infectious disease-caused death. It represents a complex systemic response to severe microbial infection. The molecular and cellular basis of immune response in sepsis is not fully understood. The NLRP10 has been implicated to regulate the inflammation and adaptive immunity. However, it remains unknown whether NLRP10 regulates the host innate defense mechanisms in response to microbial challenge.**Methods:** To test the hypothesis that NLRP10 plays an important role in regulating the host innate response, we employed a cecal ligation and puncture (CLP)-induced polymicrobial sepsis model in NLRP10 deficient mice (crossed in C57BL/6 mice background) and *in vitro* bacterial infections in primary mouse cells to demonstrate the importance of this molecule in sepsis.**Results:** NLRP10 found to be essential for restricting the bacterial outgrowth and the sepsis-induced lethality in mice following CLP. Moreover, NLRP10 is essential for the activation of NF-κB and MAPKs in sepsis. Further, it was observed to induce key host innate defense mechanisms during microbial sepsis, including phagocytosis, NADPH oxidase, autophagy and neutrophil extracellular trap formation.**Conclusions:** Our findings further a number of advancements in our understanding of the biological role of NLRP10 in innate host response against bacterial infection. Particularly, involvement of NLRP10 in regulating key hostinnate responses, including MAPK and NADPH oxidase activation and innate defense mechanisms, are novel findings which may be helpful in future for considering NLRP10-based therapy for the treatment of sepsis.

Author Disclosure Block:

P. Baral: None. **S. Batra:** None. **S. Jeyaseelan:** None.

Poster Board Number:

SATURDAY-552

Publishing Title:

Differences in Innate Immune Response in the Middle Ear of Otitis Prone and Non-Otitis Prone Children

Author Block:

R. Kaur[1], J. Casey[2], M. E. Pichichero[1]; [1]Rochester Gen. Hosp., Rochester, NY, [2]Legacy Pediatrics, Rochester, NY

Abstract Body:

Background: Acute Otitis Media (AOM) causes an inflammatory response in the middle ear. We assessed differences in innate immune responses in the middle ear at multi-cell levels involved in bacterial defense at onset of AOM in otitis prone (OP) (experienced ≥3 episodes of AOM within 6 months or ≥4 episodes within a year) and non-otitis prone children (NOP) (experienced 1 or 2 episodes of AOM only).**Methods:** Data reported here are from 24 children which were aged matched at 12±1 months old in two groups; OP children (n=12) and NOP children (n=12). Genes of TLRs. Nod-like and RIG-I-like receptors signal transduction molecules, downstream effectors important for inflammation and apoptosis including cytokines and chemokines were studied using real-time PCR array (SA Biosciences) from RNA of MEF samples collected by tympanocentesis. Changes in gene expression of >2 fold and $P<0.05$ were considered significant. Protein levels estimation of differentially regulated genes were determined using Luminex in MEF samples to confirm the real-time expression data.**Results:** Most of the 84 genes analyzed had similar expression in the two study groups. However, differential expression in two populations was identified for several important genes. OP children showed 4 fold higher IL8 expression compared to NOP children; significant difference ($p<0.05$) was also observed for IL8 at protein level estimation. Higher fold expression of secretory leukocyte protease inhibitor (SLPI) which is considered to be the predominant neutrophil elastase inhibitor in secretions was also significantly higher in OP children. Interferon regulatory factor 7 and its related signalling molecules of IFN-α, TICAM2, CCL5 showed significant downregulation in OP children compared to NOP indicates OP children are probably more prone to viral upper respiratory tract infections and consequent bacterial AOM. Although some pathogenic specific genes expression was observed in OP vs NOP chilldren but overall similar trend in differences is shown whether AOM was caused by *H. influenzae* or *S. pneumonia.***Conclusions:** Innate immune responses are differentially regulated in otitis prone compared to non-otitis prone children that likely contribute to otitis proneness condition in children.

Author Disclosure Block:

R. Kaur: None. **J. Casey:** None. **M.E. Pichichero:** None.

Poster Board Number:

SATURDAY-553

Publishing Title:

Expression of IFNG and TNFA in Inflammatory Cells from *Burkholderia pseudomallei* Chronically Infected BALB/c and C57BL/6 Mice

Author Block:

J. L. Dankmeyer, C. K. Cote, S. L. Welkos, S. R. Trevino, D. P. Fetterer, C. Soffler, P. L. Worsham, K. Amemiya; US Army Med. Res. Inst. of Infectious Diseases, Frederick, MD

Abstract Body:

Background: Melioidosis, which is caused by *Burkholderia pseudomallei* (Bp), is endemic in South East Asia and Northern Australia. Because of a wide number of strains that have been isolated from human melioidosis patients, we have been evaluating their virulence in a mouse model of melioidosis. We detected endogenous expression of TNFα and IFNγ in monocytes/macrophages, NK cells, and granulocytes in splenocytes from chronically infected mice but not in acutely infected mice. **Methods:** BALB/c and C57BL/6 mice were infected with the 12 different human isolates of Bp by aerosol exposure to determine their 21 day LD_{50}. No survivors were obtained after 21 days from two isolates. Blood was collected for antibody and cytokine/chemokine analysis, and spleens for colony forming units (CFU), changes in cell composition, cytokine/chemokine analysis, and *in vitro* cellular immune responses. Antibody responses to Bp K96243 were determined by ELISA. Cytokine/chemokine expression in serum, spleen extracts, and splenocyte cultures were determined by Luminex technology. Splenocyte composition and intracellular cytokine expression were analyzed by flow cytometry. Intracellular cytokine expression was detected by incubation of cells with IFNγ-APC or TNFα-APC. **Results:** More than 100 spleens were examined of which 26 had a pyogranuloma that were from chronically infected mice. Nine other spleens were swollen with little CFU and no pyogranulomas. In infected spleens there was an increase in monocytes/macrophages, NK cells, granulocytes in both types of mice, while in the swollen spleens there were fewer inflammatory cells. There was a significant increase in IL-1α and IL-1β expression in spleen extracts. We also detected the presence of endogenous IFNγ and TNFα in 16 of the infected spleens in the inflammatory cells. We saw low levels of TNFα in T cells but not IFNγ. In swollen spleens there was less endogenous TNFα and IFNγ than in infected spleens and none in normal appearing spleens. **Conclusions:** *B. pseudomallei* can cause a chronic infection in mice with a continuous production of IL-1α and IL-1β. Inflammatory cells but not T cells were producing IFNγ, while some TNFα could be seen in T cells that appeared to be associated with the presence of a pyogranuloma.

Author Disclosure Block:

J.L. Dankmeyer: None. **C.K. Cote:** None. **S.L. Welkos:** None. **S.R. Trevino:** None. **D.P. Fetterer:** None. **C. Soffler:** None. **P.L. Worsham:** None. **K. Amemiya:** None.

Poster Board Number:

SATURDAY-554

Publishing Title:

Serotypic Differences in Dendritic Cells Responses in Shigella

Author Block:

C. Narayan, BR Thapa, JK Mahajan, Vishal Kant, Balvinder Mohan, Neeam Taneja; PGIMER, Chandigarh, India

Abstract Body:

Background: Dendritic cells (DC) are key regulators of immune response with the ability to affect both the innate and adaptive immune responses and are abundant in the gut mucosa. The severity of shigellosis varies with the serotype involved with *S. dysenteriae* (SD) producing the severest infections and complications with *S. sonnei* (SS) being at other end of spectrum usually causing mild self-limiting diarrhea .While shigellae are known to induce the apoptosis of mature DCs, there is no information in cytokine milieu of DCs incubated with different serotypes of *Shigellae***Methods:** Monocyte derived dendritic cells (MoDCs) were developed from healthy human PBMC after 8 days of culture. They were characterized by four-color flow cytometry technique using Becton Dickinson FACS ARIA III, equipped with 488 nm and 630 nm argon laser and analysed by FACS Diva 6.1.2 Software on the basis of CD11c positive, HLA-DR positive and CD3 negative. DCs were infected with different Shigella serotypes. After 24 hour post infection, relative expression of cytokines IL-1β, IL-6, IL-8, TNF-α, IL-12p70, IL-17, IL-22 and IL-23 was studied by Real Time PCR and data was analysed by Graph prism5. **Results:** IL-8, IL-17A, IL-22A and IL-23 expressions were highest in MoDCs stimulated with *S. dysenteriae* serotype1 and significant serotypic differences were noted between SD & SF and between SD & SS. The transcription levels of IL-23 were down regulated in *S. flexneri* & *S. sonnei* in comparison to normal MoDCs. IL-8 appears to be a major molecule orchestrating mucosal inflammation in shigellosis. It is the primary cytokine which induces neutrophil chemotaxis. SD1 induces more Th17 response which displays pro-inflammatory functions. IL23 is responsible for the expansion of Th17 previously differentiated.IL-23 promotes the development and expansion of activated CD4+ T cells. **Conclusions:** DCs are critical sentinel cells that relay microbial presence either directly or indirectly to naive T cells .In this study we found that Shigella dysenteriae caused maximum release of IL-8. Similarly SD also caused highest release of IL-17A and IL-22A. It was the only serotype which increased IL-23. These findings could explain more severity of SD as compared to SF and SS.

Author Disclosure Block:

C. Narayan: None.

Poster Board Number:

SATURDAY-555

Publishing Title:

Common Correlates of Protection Against *Francisella tularensis* Identified Using Mice and Rats

Author Block:

R. De Pascalis[1], A. Hahn[2], L. Mittereder[1], N. Donart[2], B. Frey[1], H. Brook[1], T. Wu[2], K. Elkins[1]; [1]Food and Drug Admin., Siver Spring, MD, [2]Univ. of New Mexico Hlth.Sci. Ctr., Alburquerque, NM

Abstract Body:

The Live Vaccine Strain (LVS) against tularemia, caused by *Francisella tularensis (Ft)*, is not licensed in US and has uncertain protection against the virulent Type A *Ft*. Thus, better prophylactic vaccines are needed, as well as methods to test their efficacy. Unlike more common human infections, for which vaccine efficacy can be evaluated by field trials, evaluating efficacy against sporadic diseases like tularemia requires alternative strategies. Measuring correlates of protection may complement or replace efficacy large-scale clinical trials, and facilitate studies via the "Animal Rule." Recently, we demonstrated that combining *in vitro* stimulation of murine *Ft* LVS-immune cells with subsequent genomic characterization discriminates *Ft* vaccines of different efficacy, and thus we identified potential correlates of protection. To further explore correlates, we extended our studies to a different animal model. Here, Fischer 344 rats were vaccinated with a panel of LVS-derived vaccines and subsequently given a respiratory challenge with lethal Type A *Ft*. The *in vivo* efficacy of different vaccines was compared with the ability of PBLs from vaccinated rats to control *in vitro* functions and with relative gene expression. Overall the *in vitro* functions, including control of LVS intramacrophage replication, IFN-gamma secretion, and NO production, reflected the hierarchy of protection. In addition, the expression of a panel of genes was more up-regulated in cells from the groups of rats administered the better vaccines. In contrast, genes in cells from naive or poorly protected groups exhibited lower expression or down-regulation. Most importantly, selected potential correlates that were previously identified using murine LVS-immune PBLs were also differentially expressed in rat PBLs. These included IFN-gamma, IL-21, Nos2, LTA, T-bet, IL-12rbeta2, CCL5, CXCL11, GzmB. This method therefore may allow the identification of common potential correlates of protection in multiple animal species, using a clinically accessible cell source, and it has potential for screening of new vaccines candidates.

Author Disclosure Block:

R. De Pascalis: None. **A. Hahn:** None. **L. Mittereder:** None. **N. Donart:** None. **B. Frey:** None. **H. Brook:** None. **T. Wu:** None. **K. Elkins:** None.

Poster Board Number:

SATURDAY-557

Publishing Title:

Roles of Interleukin-17 and Neutrophil-Mediated Host Responses in Murine *Clostridium difficile* Colitis

Author Block:

H. Konishi, T. Yamaguchi, T. Nakagawa, C. Kajiwara, S. Kimura, Y. Ishii, K. Tateda; Toho Univ., Tokyo, Japan

Abstract Body:

Background: Increased neutrophil number in the blood is a well-known hallmark of severe cases of *C. difficile* colitis (CDC). A recent study has reported that IL-23, a major IL-17 inducer, play a role in CDC in animals and humans. However, roles of IL-17, especially neutrophil-mediated host responses, in CDC remained controversial. In the present study, we investigated roles of IL-17 and neutrophil-mediated host responses in mice model of CDC. **Methods:** BALB/c background of IL-17A and F deficient mice (IL-17 KO) were pretreated with an antimicrobial mixture, and challenged orally with *C. difficile* BI/NAP1/027 ($1\text{-}3\times10^6$ cfu/mouse) for survival and host responses. After the challenge, mice were sacrificed at 2 days post infection. Blood and intestine samples (cecum/colon) were examined for cellular infiltration (flow cytometry) and cytokine productions (ELISA and RT-PCR array).**Results:** Significantly lower mortality was observed in IL-17 KO mice (10%), comparing to the control (70%). Neutrophil number in blood was significantly higher in control mice than IL-17KO mice. In accordance with blood results, the number of neutrophils in the intestine was higher in control mice than IL-17 KO mice. In ELISA analysis of intestinal samples, lower productions of inflammatory cytokines in IL-17 KO mice were demonstrated in CXCL1, G-CSF and IL-1β, but not CXCL2, IL-4, IL12p70 and TGF-β. RT-PCR array in intestinal samples demonstrated higher expressions of IL-6 and IL-12b and lower levels of lactoferrin in IL-17 KO mice, whereas no difference was observed in other factors, such as MPO, Slpi, Myd88 and Nod1.**Conclusions:** Our data demonstrated that IL-17 KO mice were more resistant to CDC in mice. The survival benefit was well associated with less neutrophil accumulation and weak inflammatory host responses. Pathogenic roles of neutrophils, especially IL-17-mediated host responses, are warranted for future's investigation.

Author Disclosure Block:

H. Konishi: None. **T. Yamaguchi:** None. **T. Nakagawa:** None. **C. Kajiwara:** None. **S. Kimura:** None. **Y. Ishii:** None. **K. Tateda:** None.

Poster Board Number:

SATURDAY-558

Publishing Title:

***Cryptosporidium parvum* Induces an Intestinal Epithelial Innate Immune Response Involving Type III Interferon**

Author Block:

S. H. Ferguson[1], D. M. Foster[1], B. Sherry[1], S. T. Magness[2], J. L. Gookin[1]; [1]North Carolina State Univ., Raleigh, NC, [2]Univ. of North Carolina, Chapel Hill, NC

Abstract Body:

Cryptosporidium spp., a zoonotic intestinal epithelial parasite, is the second leading cause of infectious diarrheal death in children under 5 in developing countries, and lethal in immunocompromised individuals worldwide. The host immune response is critical for defense against *Cryptosporidium parvum* (*Cp*) however the cellular mechanisms are poorly understood. To gain insight into the host strategy for defense against *Cp*, we examined the transcriptional response of the intestinal epithelium (IECs) to *Cp* in experimentally infected piglets. Upregulated genes were dominated (39%) by targets of interferon (IFN) signaling with ISG15 being the most highly induced (21 fold). We hypothesized that *Cp* induces a strong IFN-mediated host immune response that may be attributed to a recently discovered, epithelial derived, Type III interferon IFN-λ.The identity, origin, and cellular target(s) of IFN in *Cp* infection were investigated using a well-established neonatal piglet model and primary porcine IECs (IPEC-J2). Orogastrically infected and age-matched control piglet ileal IECs and lamina propria (LP) were collected for qRT-PCR and immunoblotting to identify and localize expression of IFN-α, -β, -γ, -λ1, and -λ3, IFN-λR1, and ISG15. To determine if *Cp* infection of IECs alone is sufficient to induce IFN-λ and IFN-λ-dependent gene expression, IPEC-J2 cells were immunoblotted to confirm expression of IFN-λR1 and treated with either *Cp* or rIFN-λ and ISG15 expression examined by qRT-PCR.Infection of piglets with *Cp* resulted in a ~100 fold increase in ileal mucosal expression of IFN-λ3 (p= 0.05). Constitutive and *Cp*-induced expression of IFN-λR1 was identified in both the epithelial crypts and LP. Corresponding with localization of IFN-λR1, ISG15 was significantly upregulated (~59 fold, p=0.008) in the crypt-enriched LP fractions of *Cp* infected piglet ileum. In IPEC-J2 cells, *Cp* infection was sufficient to induce expression of IFN-λ3 and rIFN-λ3 induced ISG15 in a dose-dependent manner.This study identifies expression of IFN-λ as a key response of IEC to *Cp* infection and suggests an important role for IFN-λ in host defense. These observations come from a powerful *in vivo* model of *Cp* infection in which the function of IFN in innate immune defense can be explored.

Author Disclosure Block:

S.H. Ferguson: None. **D.M. Foster:** None. **B. Sherry:** None. **S.T. Magness:** None. **J.L. Gookin:** None.

Poster Board Number:

SATURDAY-559

Publishing Title:

Prelp Enhances Host Innate Immunity against Respiratory Tract Pathogens

Author Block:

G. Liu, D. Ermert, Y-C. Su, B. Singh, M. Johansson, K. Riesbeck, A. M. Blom; Lund Univ., Malmö, Sweden

Abstract Body:

Respiratory tract infections are the leading cause of deaths globally. Increasing antibiotic resistance and low coverage of available vaccines requires alternative therapeutics to treat respiratory tract infections. Here we report that proline/arginine-rich end leucine-rich repeat protein (PRELP) could be a potential therapeutic candidate. PRELP is present in alveolar fluid, resident macrophages/monocytes, myofibroblasts, and adventitia of blood vessels of lung tissue. PRELP specifically binds respiratory tract pathogens *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis*, but not other tested bacterial pathogens. PRELP binds the majority of clinical isolates of *M. catarrhalis* (n = 49), and interacts directly with the ubiquitous surface protein (Usp) A2/A2H of *M. catarrhalis*. PRELP increases C3b deposition and membrane attack complex formation on *M. catarrhalis*, thereby enhancing the killing of *M. catarrhalis* by human serum. Moreover, PRELP competitively inhibits binding of complement inhibitor C4BP on *M. catarrhalis*. Taken together, PRELP enhances the killing of *M. catarrhalis* by human serum via inhibition of C4BP binding. Thus, PRELP might be used as a therapy to modulate the innate immune responses toward respiratory tract pathogens, particularly *M. catarrhalis*.

Author Disclosure Block:

G. Liu: None. **D. Ermert:** None. **Y. Su:** None. **B. Singh:** None. **M. Johansson:** None. **K. Riesbeck:** None. **A.M. Blom:** None.

Poster Board Number:

SATURDAY-560

Publishing Title:

An Analysis of the Impacts of Green Tea on Bacterial Growth and Phagocytosis by Macrophages

Author Block:

A. K. Meyers, C. Thompson; Loyola Univ. Maryland, Baltimore, MD

Abstract Body:

The emergence of significant drug resistance in bacteria has pushed many researchers to develop and evaluate methods to fight this growing problem. One important example of such methods are the use complementary and alternative medicines (CAMs). CAMs are a group of medicines and/or practices that are used in addition to or as an alternative to typical Western medicinal practices. Research has shown that this group is comprised of potentially valuable therapeutic agents with some being purported to improve immune system function. The objective of this proposal is to evaluate the efficacy and mechanism of action for green and black teas, CAMs that has been suggested to improve our body's natural antimicrobial responses- in essence, treating from within. Specifically, the focus of this project was to analyze the effects of green and black teas on phagocytosis: the mechanism by which certain white blood cells "eat" and kill bacteria. Both black and green teas were brewed and added at different concentrations to bacterial broth. As expected, the growth of *Staphylococcus aureus* in the presence of these teas showed no significant differences in growth or survival as compared to controls. Next, RAW 264.7 cells were treated with a simulated human digest of black or green teas and allowed to phagocytose GFP-expressing *Staphylococcus aureus*. Using confocal microscopy, images were taken depicting several time points of phagocytosis that were then compared visually to control cells. These experiments indicated that phagocytosis proceeded faster in the presence of green tea, but not black tea, at all four stages of phagocytosis (attachment, entry, maturation, and degradation). Currently, we are using fluorescence-based assays (in microplates and via confocal) to assess the differences in calcium signaling (speed and quantity) as a potential mechanism that is governing this increased phagocytic speed. Together, these data suggest that green tea (and, potentially, black tea) are able to enhance the microbicidal activity of our innate immune system without directly impacting bacterial growth. Further research is necessary to determine whether these teas and other CAMs have merit as therapeutic agents to enhance the immune response to bacterial infection, but, if the data are encouraging, this could represent a novel, inexpensive, and easy mechanism for improving health and combatting antibiotic resistance.

Author Disclosure Block:

A.K. Meyers: None. **C. Thompson:** None.

Poster Board Number:

SATURDAY-561

Publishing Title:

Activation of the Non-Canonical NF-kB Pathway Following Internalization of Bacteria Expressing a Functional Type III Secretion System

Author Block:

M. C. Duncan[1], **N. G. Herrera**[1], K. S. Johnson[1], J. N. Engel[2], V. Auerbuch[1]; [1]Univ. of California, Santa Cruz, Santa Cruz, CA, [2]Univ. of California, San Francisco, San Francisco, CA

Abstract Body:

The type III secretion system (T3SS) is a bacterial nano-machine utilized by dozens of pathogens to inject effector proteins into mammalian cells, thereby manipulating host defense mechanisms such as phagocytosis. In turn, the mammalian innate immune system has evolved the ability to recognize bacterial deployment of a functional T3SS, triggering activation of several host-signaling pathways. Previously, we showed the *Yersinia pseudotuberculosis* T3SS activates host NF-kappaB, a proinflammatory transcription factor. In this study, we sought to better characterize NF-kappaB induction by the *Yersinia* T3SS. Utilizing both confocal fluorescence microscopy and RNA interference, we found that wildtype *Yersinia*, which remains extracellular by inhibiting phagocytosis through the actin targeting T3SS effectors YopEHO, triggered NF-kappaB activation independently of the non-canonical NF-kappaB kinase NIK. In contrast, *Yersinia* lacking actin-targeting effectors induced a NIK-dependent NF-kappaB response and processing of the non-canonical NF-kappaB subunit p100 to p52 was assessed via western blot analysis. Furthermore, blocking actin polymerization and bacterial uptake using cytochalasin D prevented this NIK dependency. *Yersinia* lacking any T3SS function triggered very low levels of either canonical or non-canonical NF-kappaB. We observed similar results using *Pseudomonas aeruginosa*, which expresses a related T3SS and the actin-targeting effector ExoT. These data indicate that internalized bacteria expressing a functional T3SS trigger NIK-mediated p100/p52 processing. Non-canonical NF-kappaB is primarily important in lymphocyte development and bone metabolism, but has also been associated with the innate immune response to viruses and certain bacteria. Our results suggest that non-canonical NF-kappaB may also play a role in the innate immune response to the bacterial T3SS, and that mammalian cells may be able to distinguish between intra- and extracellular T3SS.

Author Disclosure Block:

M.C. Duncan: None. **N.G. Herrera:** None. **K.S. Johnson:** None. **J.N. Engel:** None. **V. Auerbuch:** None.

Poster Board Number:

SATURDAY-562

Publishing Title:

Cytoplasmic Activation of an Alternative Flagellar System in *Burkholderia pseudomallei*: Implications for the Innate Immune Response to Infection

Author Block:

J. Maloy, J. La, J. Miller; Univ. of California, Los Angeles, Los Angeles, CA

Abstract Body:

Burkholderia pseudomallei (*Bp*) is a facultative intracellular pathogen endemic to Southeast Asia and Northern Australia and is the causative agent of melioidosis. *Bp* isolates have been segregated into genetically distinct Southeast Asian or Northern Australian clades with variations in clinical manifestations corresponding to geographical source. Northern Australian isolates carry an ancestral *Burkholderia thailandensis*-like flagellar and chemotaxis cluster (*BTFC*), while Southeast Asian isolates carry a *Yersinia*-like fimbral cluster (*YLF*) in its place. The *BTFC* locus plays a role in pathogenesis of Northern Australian *Bp* strains by promoting intracellular motility. While *Bp* extracellular swimming motility requires a polar flagellum encoded by the *fla1* locus, motility in the cytoplasm of host cells requires either *bimA*-induced actin polymerization or flagellar motility encoded by the *BTFC* locus. The *BTFC* locus activates intracellular expression of lateral flagella in response to an unidentified cytoplasmic signal. Innate immune mechanisms, particularly NLRC4-driven pyroptosis and production of IL-1β, are critical for host response to *Bp* infection. While previously attributed to type-3 secretion system (T3SS) components, we show that the NLRC4 response is partially dependent on the *BTFC* locus flagellin encoding gene *(fliC2)* in *Bt* and Australian strains of *Bp.* Deletion of *fliC2* results in a decrease in IL-1β production in *wild-type* (*wt*) murine macrophages, without affecting IL-1β release in macrophages deficient in *nlrc4*. Additionally, reconstitution of the NLRC4 inflammasome in 293T cells renders them susceptible to rapid cell death in response to infection with *wt*, but not *ΔfliC2 Burkholderia.* These data suggest that the *BTFC* locus plays an important role in intracellular pathogenesis of *Burkholderia*, and may be an important factor influencing the host innate immune response to infection with Australian strains of *Bp*.

Author Disclosure Block:

J. Maloy: None. **J. La:** None. **J. Miller:** None.

Poster Board Number:

SATURDAY-563

Publishing Title:

TLR3 Is a HSV-1 Sensor That Rapidly Protects CNS Neurons from Infection

Author Block:

O. Ewaleifoh[1], B. Zimmer[2], S. Zhang[3], L. Notarangelo[4], J. L. Casonava[3], G. Smith[1]; [1]Northwestern Univ., Chicago, IL, [2]Mem. Sloan Kettering, NY, NY, [3]Rockefeller Univ., New York, NY, [4]Harvard Univ., Boston, MA

Abstract Body:

Introduction: In born errors in Toll Like Receptor 3 (TLR3), results in increased susceptibility to Herpes Simplex Encephalitis (HSE) in humans. How TLR3 limits HSV-1 infection remains unclear. Induced pluripotent stem cell (iPSC) generated from patient skin cells have made it possible to investigate central nervous system (CNS) resistance to HSV-1 infection in a culture model. **Methods:** Using human iPSC-derived neurons from healthy control and HSE patient's deficient in TLR3, we examined the mechanism of TLR3 protection following infection with recombinant HSV-1.**Results:** Our studies indicate that the anti-HSV activity of TLR3 is unique to CNS neurons and absent from the peripheral nervous system (PNS). Data will be presented supporting that TLR3 senses RNA anchored on the virion surface. The response was rapid and STAT1-independent, with HSV-1 entry and retrograde transport in axons blocked.**Conclusion:** Our results indicate an unusual TLR3-dependent block to infection is operational in CNS neurons that is rapid and interferon independent.

Author Disclosure Block:

O. Ewaleifoh: None. **B. Zimmer:** None. **S. Zhang:** None. **L. Notarangelo:** None. **J.L. Casonava:** None. **G. Smith:** None.

Poster Board Number:

SATURDAY-564

Publishing Title:

Molecular Basis of Brain Infection by *Listeria monocytogenes*

Author Block:

P. Ghosh, E. M. Halvorsen, D. E. Higgins; Harvard Med. Sch., Boston, MA

Abstract Body:

Listeria monocytogenes is an intracellular bacterial pathogen that is frequently associated with foodborne infection. Of particular concern is the ability of *L. monocytogenes* to breach the blood-brain barrier (BBB) to invade the central nervous system (CNS), leading to life-threatening meningitis and encephalitis. The identity of factors necessary for *L. monocytogenes* invasion of the brain has remained elusive. To further our understanding of *L. monocytogenes* infection of the brain, we evaluated the contribution of InlF, a member of the internalin family of surface proteins previously shown to be important for host cell binding and entry. Mice were infected with wild-type or *ΔinlF* bacteria to determine the effect of InlF on *L. monocytogenes* virulence. These studies showed that InlF plays a critical role for successful *L. monocytogenes* colonization of the brain in mice. Subsequent affinity chromatography and mass spectrometry experiments identified host cell Vimentin as a binding partner for InlF. Host cell invasion assays were performed following blocking of surface Vimentin with anti-Vimentin antibody or in the presence of Withaferin A, a chemical inhibitor that leads to cleavage and reorganization of Vimentin. These studies resulted in inhibition of *L. monocytogenes* host cell invasion suggesting that cell surface Vimentin is involved in bacterial internalization by host cells. Further confocal microscopy experiments revealed that *L. monocytogenes* is able to bind cell surface Vimentin and that InlF facilitates *L. monocytogenes*-Vimentin interaction. The requirement of Vimentin for host cell invasion was further confirmed using infection studies with host cells lacking Vimentin. More importantly, *L. monocytogenes* infection experiments with Vimentin normal (Vim+) and knockout (Vim−) mice confirmed the importance of Vimentin for *L. monocytogenes* colonization of the brain. Taken together, these studies for the first time demonstrate an interaction between a host cell receptor (Vimentin) and a bacterial surface protein (InlF) with specific relevance to *L. monocytogenes* brain infection *in vivo*.

Author Disclosure Block:

P. Ghosh: None. **E.M. Halvorsen:** None. **D.E. Higgins:** None.

Poster Board Number:

SATURDAY-565

Publishing Title:

Inhibitory Effects of Oligosaccharides on Enteric Pathogen Association with the Gastrointestinal Barrier

Author Block:

P. Chen, N. Kong, B. C. Weimer; Univ. of California Davis, Davis, CA

Abstract Body:

Bacterial enteric pathogens cause millions of cases of food poisoning in humans and livestock each year and result in billions of dollars in medical care costs and lost revenue. The three leading causes of foodborne, bacterial gastroenteritis are *Salmonella* Typhimurium (*S.* Typhimurium), *Escherichia coli* (*E. coli)*, and *Listeria monocytogenes* (*L. monocytogenes*). In lieu of antibiotics, non-digestible, dietary oligosaccharides, such as mannan and fructo-oligosaccharides, are commonly used as feed additives for livestock to prevent enteric pathogen association with the host. Additionally, human milk oligosaccharides (HMO) are suspected to play a role in minimizing enteric pathogen colonization. In this study it was hypothesized that the presence of oligosaccharides would decrease enteric pathogen association in a dose-dependent manner. Efficacies of HMO and commonly used feed additives, mannan-oligosaccharides and fructo-oligosaccharides, in blocking pathogen association with differentiated colonic epithelial cells (Caco-2) were evaluated using modified gentamicin protection assays in the presence of each oligosaccharide. Results from this study demonstrated that *S.* Typhimurium, *E. coli*, and *L. monocytogenes* each responded differently to specific oligosaccharide treatments. Treatment with mannan-oligosaccharides resulted in increased S. Typhimurium host association as oligosaccharide concentration increased. Treatment with fructo-oligosaccharides resulted in dose-dependent increases in host association in all three pathogens. Pre-blocking of Caco-2 monolayers with HMO was the only treatment that resulted in a dose-dependent decrease in host association across all three pathogens. Pre-blocking with HMO was the only treatment that resulted in decreased host association in *L. monocytogenes* whereas pre-blocking with mannan and fructo-oligosaccharides caused an increase in *L. monocytogenes* host association. These results illustrate the independent effects of different oligosaccharides on enteric pathogens, emphasizing the effects of diet changes on pathogen susceptibility and persistence.

Author Disclosure Block:

P. Chen: None. **N. Kong:** None. **B.C. Weimer:** None.

Poster Board Number:

SATURDAY-566

Publishing Title:

Role of the Extracellular Membrane Sensing Loop of Gras in Resistance to Distinct Cationic Antimicrobial Peptides in *Staphylococcus aureus*

Author Block:

A. Cheung[1], A. Bayer[2], M. Yeaman[2], Y. Xiong[2], N. Donegan[1], **S. Yang**[3]; [1]Geisel Sch. of Med. at Dartmouth, Hanover, NH, [2]Los Angeles BioMed. Res. Inst., Torrance, CA, [3]Chung Ang Univ., Ansung, Korea, Republic of

Abstract Body:

BACKGROUND: The *Staphylococcus aureus* two-component regulatory system, GraRS, is involved in resistance to killing by distinct host defense cationic antimicrobial peptides (HD-CAPs) by regulating downstream target genes such as *mprF* and *dltABCD.* However, the detailed mechanisms by which the extracullular sensing loop (EL) of the GraS senses specific HD-CAPs in not well defined. **Methods: Strains:** Isogenic set of *S. aureus* strains: **i**) COL parent; **ii**) its Δ*graS* knockout; and **iii**) six plasmid-complemented Δ*graS* strains expressing distinct point-mutations within the GraS sensing loop (P38H, F38G, F38A/P39A, F38G/P39G, D35/37/41K, D35/37/41G). These mutations introduced specific changes in charge, bulk and/or polar angles within the sensing loop.**MICs to Daptomycin (DAP) or Polymyxin B:** standard E-test or broth microdilution assays.**HDP susceptibility assays:** standard 2h killing assays using 5×10^3 *S. aureus* CFU vs. LL-37 (PMNs) and RP-1 (platelets).**Surface charge:** cytochrome c binding assay.**Gene expression:** standard qRT-PCR.**Animal virulence model:** A catheter-induced aortic valve infective endocarditis (IE) model in rabbits.**RESULTS:** The COL Δ*graS* mutant was unable to induce *mprF* and *dltA* transcription and, in turn, exhibited significantly increased susceptibilities to DAP, RP-1 and LL-37. Further, the six Δ*graS* mutants expressing point-mutated GraS ELs displayed both moderate increases in PMB and HD-CAPsusceptibility, as well as reductions of *mprF* and *dltA* induction by PMB. Reductions in net positive surface charge were observed in both triple mutants, D35/37/41K and D35/37/41G strains, correlating with the gene expression data. *In vivo*, the Δ*graS* knockout strain displayed significant reductions in bacterial counts achieved in all target tissues in the rabbit IE model ($\geq$ 4-6 $\log_{10}$cfu/g reductions).**CONCLUSIONS:** These results demonstrate that GraS sensor plays an important role in sensing HD-CAPs to induce adaptive survival responses to these molecules in *S. aureus*.

Author Disclosure Block:

A. Cheung: None. **A. Bayer:** A. Board Member; Self; ContraFect Corp.. **M. Yeaman:** None. **Y. Xiong:** None. **N. Donegan:** None. **S. Yang:** None.

Poster Board Number:

SATURDAY-567

Publishing Title:

Comparative Analysis of *Escherichia coli* O157 Growth and Protein-expression, *In Vitro* & *In Vivo*, in Rumen Fluid of Cattle

Author Block:

I. T. Kudva, T. Casey, J. D. Lippolis, J. Trachsel, H. K. Allen; Natl. Animal Disease Ctr., USDA/ARS, Ames, IA

Abstract Body:

Cattle are the primary reservoirs for *Escherichia coli* O157 (O157), a Shiga toxin-producing *E. coli*, with potential for serious extraintestinal sequelae in humans. In a recent study (Kudva IT *et al*. BMC Microbiol. 2014; 14:48), we reported that when cultured in rumen fluid from dairy cattle on the maintenance diet (high in fiber), O157 expresses proteins involved in survival rather than those contributing to virulence. This observation was in contrast to the well-established expression of virulence factors by O157 during colonization of the human host. In the current study, we evaluated if similar results would be obtained when different O157 strains are grown, (i) *in vitro* in rumen fluid from animals on other diets usually provided to dairy cattle, and (ii) *in vivo* within the rumen of fistulated animals on the same diets, using a new method that not only allows such *in vivo* studies in the cattle rumen but also permits reuse of animals. Rumen-fistulated, dairy cattle were fed either the maintenance diet as reported previously, or a lactation diet (high in protein). Three different strains of O157 were studied: EDL 933, 86-24 and a super shed isolate SS-17. As expected, the two diets had differing influences on the ruminal pH and volatile fatty acid (VFA) profiles. The ruminal pH ranged from 6.2 - 7.0, with total VFA concentrations of 109 - 141 μM/ml, among animals fed the maintenance diet. On the other hand, animals fed the lactation diet had ruminal pH ranging between 5.14 - 6.0, and total VFA of 125 - 241 μM/ml. O157 strains demonstrated different growth patterns in these rumen fluids, after 48 h at 39^{o}C, in both the *in vitro* and *in vivo* assays. A greater reduction in O157 viable counts was observed in rumen fluid from, or rumen of, cattle fed the lactation diet compared to the maintenance diet. We are presently profiling the proteomes of O157 isolates recovered from the *in vitro* and *in vivo* assays, to obtain insights into mechanisms adapted by these O157 strains in rumen fluid with different compositions. Such data will be helpful in formulating effective therapeutic and/or diagnostic modalities to curtail O157 in cattle.

Author Disclosure Block:

I.T. Kudva: None. **T. Casey:** None. **J.D. Lippolis:** None. **J. Trachsel:** None. **H.K. Allen:** None.

Poster Board Number:

SATURDAY-568

Publishing Title:

A Previously Undescribed *Pneumocystis jirovecii* Cytochrome B Mutation Associated with Atovaquone Exposure

Author Block:

S. Le Gal[1], N. Argy[2], J. Clain[3], W. Vindrios[2], M. Virmaux[4], R. Dorent[2], J. Lucet[2], Y. Yazdanpanah[2], M. Wolff[2], S. Houzé[2], **G. Nevez**[1]; [1]Brest Univ. Hosp., Brest, France, [2]Bichat Univ. Hosp., Paris, France, [3]IRD, Paris Descartes Univ., Paris, France, [4]Brest Univ., Brest, France

Abstract Body:

Background: Several mutations at the cytochrome b gene of *Pneumocystis jirovecii* (*P.jirovecii*) associated with atovaquone exposure have already been reported. *Pneumocystis* pneumonia (PCP) prophylaxis at the heart transplantation unit of Bichat University Hospital (Paris, France) is essentially based on atovaquone use. In this context, our objective was to genotype at the cytochrome b gene *P.jirovecii* isolates obtained from heart transplant recipients monitored at this hospital. **Methods:** Twenty-two *P. jirovecii* DNA isolates obtained from the 11 heart transplant recipients and 11 unlinked control patients who were contemporaneously diagnosed with *Pneumocystis* infections were examined for genotyping. The cytochrome b gene was amplified and sequenced from both strands. Sequences were aligned and compared with the reference sequence. A medical chart survey was also performed to determine whether the patients had past history of atovaquone prophylaxis or treatment.**Results:** *P.jirovecii* genotyping was successful in 10 out of the 11 heart transplant recipients and in 9 out of the 11 unlinked patients. All heart transplant recipients and 1 out of the 11 patients of second group had past history of atovaquone exposure respectively (11/11vs. 1/11, p <0.01). Type CYB2 harboring a previously undescribed mutation on position 350 (C350T) was identified in 9 out of the 10 heart transplant recipients whereas it was not identified in the second patient group (9/10 vs. 0/11, p <0.01). This transition C350T is associated with an amino acid change Ala 144 Val at the Qo site, which is the target of atovaquone. These results may be explained by *i)* atovaquone selection pressure of the mutant CYB2 type, ii) *P.jirovecii* circulation within the heart transplant recipient population.**Conclusions:** This newly described non-synonymous mutation C350T at the cytochrome b gene of *P.jirovecii* may be associated with atovaquone exposure and its detection may represent a circulation marker of the fungus among patient populations.

Author Disclosure Block:

S. Le Gal: None. **N. Argy:** None. **J. Clain:** None. **W. Vindrios:** None. **M. Virmaux:** None. **R. Dorent:** None. **J. Lucet:** None. **Y. Yazdanpanah:** None. **M. Wolff:** None. **S. Houzé:** None. **G. Nevez:** None.

Poster Board Number:

SATURDAY-569

Publishing Title:

Effects of *Pseudomonas aeruginosa* Biofilms on Wound Healing in a Diabetic Murine Model

Author Block:

C. L. Larrivee, J. A. Gibson, A. M. A. Hunt, S. Navitskaya, S. O'Reilly, J. V. Busik, C. M. Waters; Michigan State Univ., East Lansing, MI

Abstract Body:

Diabetes and obesity have reached epidemic proportions in the United States. According to 2011 CDC reports diabetes affects 25.8 million people, or 8.3% of the U.S. population. Due to the high blood glucose, neuropathy is a common occurrence in diabetic patients which can lead to poor detection of any wounds on the body. Wounds that go undiagnosed have a high chance of becoming infected, and bacterial biofilms have recently been implicated as an underlying cause of chronic non-healing wounds. Using a diabetic murine model, we examined the impact of biofilm formation on the rate of wound healing in diabetic versus non-diabetic mice. C57/BL6 non-diabetic and C57/BL6 streptozotocin induced diabetic mice were wounded. Two surgical wounds 4mm in diameter were created on the backs of each mouse, 5mm splints were sutured around each wound to prevent contraction and dressings were applied to prevent scab formation. While one wound served as control the other was inoculated 24 hours post-surgery with 3 day-old biofilms of a bioluminescent strain of *P. aeruginosa* derived from PAO1 (Xen 41, Perkin Elmer) and in another experiment with a high biofilm forming mutant (*wspF* Xen41). The rate of healing and biofilm viability were then monitored via *in vivo* imaging system (IVIS) and by microscopy until complete recovery. This study established that diabetic mice not infected with biofilms do present a delay in healing compared to non-diabetics. Infection by Xen 41 postponed closure of wounds in the non-diabetic mice but retarded healing even further in diabetic ones. In addition, IVIS imaging showed a rapid decrease in biofilm viability in the non-diabetic mice in contrast to a general initial increase in biofilm in the diabetics. Infection of wounds by biofilms of *wspF* Xen 41 had even more impacting results: infected diabetics were incapable of combating the infection which resulted in death of over 40 percent of the cohort. In conclusion the presence of biofilms impacts wound healing even further than diabetes alone. Furthermore the biofilm forming properties of the infecting strain may decrease clearance by the immune system resulting in higher mortality. Future experiments will investigate the molecular mechanism that contribute to diabetic chronic wounds.

Author Disclosure Block:

C.L. Larrivee: None. **J.A. Gibson:** None. **A.M.A. Hunt:** None. **S. Navitskaya:** None. **S. O'Reilly:** None. **J.V. Busik:** None. **C.M. Waters:** None.

Poster Board Number:

SATURDAY-570

Publishing Title:

A *Salmonella SdiA* Mutant Shows Decreased Ability to Colonize the Inflamed Mouse Gut

Author Block:

K. A. Miller, M. C. Swearingen, A. Sabag-Daigle, B. M. M. Ahmer; Ohio State Univ., Columbus, OH

Abstract Body:

Quorum sensing in bacteria is a way to regulate genes in response to population density. The best characterized quorum sensing system is LuxR/LuxI in *Vibrio fischeri*. LuxI is an acyl homoserine lactone (AHL) synthase. These AHLs bind to LuxR which induces expression of specific host interaction genes. Although many bacteria Proteobacteria contain LuxR/LuxI type systems, there are some that only encode a LuxR homolog and no LuxI. These LuxR homologs that lack a LuxI interaction partner are called solo LuxRs. SdiA is a solo LuxR homolog that is conserved among the *Escherichia, Salmonella, Enterobacter, Cronobacter, Citrobacter,* and *Klebsiella.* Most of these organisms that encode SdiA lack a LuxI homolog and do not produce their own AHLs. Instead, SdiA is used to detect the AHLs synthesized by other bacteria. The SdiA regulon appears to be unique in each genus. In *Salmonella*, the regulon consists of two loci and a total of seven genes. The first locus is the *rck* operon, which is encoded on the virulence plasmid and includes six genes (*pefI, srgD, srgA, srgB, rck,* and *srgC).* The function of most of these genes is unknown, but it has been shown that Rck is responsible for resistance to complement killing and promotes adherence and invasion of epithelial cells. The *srgA* and *pefI* genes affect the *pef* operon, SPI2, and motility. The second locus is a single gene, *srgE,* which encodes an effector of the SPI2-encoded type III secretion system. Here, we have determined that SdiA provides a fitness advantage to *Salmonella* in the CBA/J model of *Salmonella*-induced inflammation. This phenotype is observed during the time that the mouse gastrointestinal tract becomes inflamed. Surprisingly, this fitness phenotype is not observed during a competitive infection in which the wild-type and the *sdiA* mutant are inoculated together. Thus, it appears that the mutant can be complemented in trans by the wild-type. Further experiments are underway to determine if *srgE* or the *rck* operon are involved in this fitness phenotype.

Author Disclosure Block:

K.A. Miller: None. **M.C. Swearingen:** None. **A. Sabag-Daigle:** None. **B.M.M. Ahmer:** None.

Poster Board Number:

SATURDAY-571

Publishing Title:

Limited Maximal Activity without Marked Loss of Potency of Antibiotics against Intracellular Forms of *Staphylococcus aureus* and *Pseudomonas aeruginosa*: An Analysis with 13 Bactericidal Antibiotics from 7 Different Pharmacological Classes in a Pharmacodynamic Model of Human THP-1 Infected Monocytes

Author Block:

F. Van Bambeke, **P. M. Tulkens**; Université catholique de Louvain, Bruxelles, Belgium

Abstract Body:

Background: Uptake, survival, and secondary release of bacteria from phagocytes may explain the relapsing and recurrent character of many infections. We have developed a pharmacodynamic model to measure the potency and maximal activity of antibiotics against *S. aureus* and *P. aeruginosa* phagocytized by human THP-1 monocytes. In brief, infected cells are exposed for 24h to drugs concentrations ranging from 0.01 to 100 x the MIC to obtain full concentration-dependent responses and calculate pertinent pharmacodynamic parameters based on Hill equation [sigmoidal dose-response] (see general reference). **Methods:** Data obtained with fully susceptible *S. aureus* (ATCC25923) or *P. aeruginosa* (PAO1) strains and 13 antibiotics markedly bactericidal in broth were reviewed. Potency (C_s) was defined as the extracellular concentration causing a static effect (no apparent intracellular bacterial growth; expressed in multiples of the MIC as measured in broth [CLSI method]), and maximal activity (E_{max}) as the decrease of the intracellular CFUs over post-phagocytosis level as extrapolated for an infinitely large extracellular drug concentration. **Results:** The Table illustrates that while all antibiotics showed a C_s (potency) against intracellular bacteria similar or only slightly larger than their MIC in broth (no marked loss of potency), none, except oritavancin, achieved the CLSI-defined bactericidal effect (≥ 3 $\log_{10}$ CFU decrease), (marked loss of maximal efficacy [E_{max}]). Bacteria collected from cells and regrown in broth showed unaltered MICs (no selection of resistant subpopulations). **Conclusion:** Across the 7 classes of bactericidal antibiotic examined, all molecules but one failed to be bactericidal intracellularly although largely maintaining their potency. If also taking place in vivo, this may limit the overall efficacy of antibiotic treatments.

Antibiotic Class	Molecule * (typical ref.**)	C_s (x MIC)	E_{max} ($\log_{10}$CFU)
S. aureus			
beta-lactams	oxacillin (1)	2.1	-1.6
	ceftobiprole (2)	0.60	-1.0
	ceftaroline (3)	1.4	-0.56

lipopeptides	daptomycin (4)	2.0	-0.80
fluoroquinolones	moxifloxacin (5)	2.3	-2.0
	ciprofloxacin (6)	2.1	-1.6
pyrrolocytosines	RX-P873 (7)	1.7	-0.72
peptides (defensins)	NZ2114 (8)	0.8	-1.5
deformylase inhibitors	GSK1322322 (4)	7.2	-0.45
lipoglycopeptides	oritavancin (1)	4.8	-3.1
P.aeruginosa			
beta-lactams	meropenem (9) *	0.53	-1.5
fluoroquinolones	ciprofloxacin (9)	2.5	-2.7
polymyxins	colistin (9)	2.5	-1.0
pyrrolocytosines	RX-P873 (4)	1.8	-2.6
* all molecules are highly bactericidal in broth (Emax = - 4 $\log_{10}$ CFU or lower) ** References (AAC=Antimicrob Agents Chemother; JAC=J Antimicrob. Chemother; IJAA: Intern. J Antimicrob Agents): (1) AAC (2006) 50:841-851; (2) AAC (2009) 53:2289-2297; (3) JAC (2013) 68: 648–658; (4) AAC (2015) 59:5747-5760; (5) JAC (2011) 66:596-607; (6) IJAA (2011) 38:52-59; (7) AAAC (2015) 59:4750-4758; (8) JAC (2010) 65:1720-1724; (9) AAC (2013) 57:2310-2318 [* similar results for doripenem and imipenem]			

Author Disclosure Block:

F. Van Bambeke: E. Grant Investigator; Self; Melinta, GSK, The Medicine Company. **P.M. Tulkens:** E. Grant Investigator; Self; Johnson & Jonhson, AstraZeneca, Bayer, GSK. J. Scientific Advisor (Review Panel or Advisory Committee); Self; Bayer. L. Speaker's Bureau; Self; Bayer, GSK.

Poster Board Number:

SATURDAY-572

Publishing Title:

An *In Vitro* Study of Invasion and Virulence of *Francisella tularensis* Strains in Macrophage Cells

Author Block:

S. Chubinidze[1], T. Shutkova[1], M. Grdzelidze[1], E. Nalbandishvili[1], S. Tsanava[1], R. Arner[2], G. Chanturia[1]; [1]Natl. Ctr. for Disease Control and Publ. Hlth., Tbilisi, Georgia, [2]Metabotia, San Francisco, CA

Abstract Body:

Francisella tularensis, the causative agent of tularemia, was detected in Georgia over seven decades ago. Recently, the Repository of Bacteria and Viruses of the National Center for Disease Control and Public Health (NCDC) collected more than one hundred *F. tularensis* strains through surveillance funded by the Defense Threat Reduction Agency (DTRA).*Francisella tularensis* is a facultative intracellular bacterium, able to survive/replicate within macrophages and various non-phagocytic cells. Cell invasion assays using J774 mouse macrophage cells are used to model this. In the DTRA funded project "Epidemiology and Ecology of Tularemia in Georgia", a cell invasion assay using J774 cells infected with five strains (different SNP genotypes) *of F. tularensis* as well as LVS was conducted in triplicate in BSL-3 containment at NCDC.Macrophage cells ($3x10^5$ cells, total volume of 1 mL/well) were added to 12-well plates. *F. tularensis* ($3x10^7$cfu/100µL) was added to each well (100 bacteria per macrophage). The precise concentration of bacterial culture was determined after plating serial dilutions on CHAB and counting colonies.The invasion, survival, and replication ability of the strains was determined by lysing macrophages at different incubation time points (0, 24, 48, and 72 hours) and plating serial dilutions (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5}) of the lysate on CHAB plates; cells were washed three times with DPBS prior to lysis. The isolated colonies were counted and the number of bacterial doublings between time points calculated using the formula: (log of 24; 48; 72 hour counts - log 0; 24; 48 hour counts) x 3.32. An increase in the number of colonies from the 0 hour time point to other time points indicates replication within the macrophages.Wild *F. tularensis* strains showed a higher ability to invade macrophages and grow intracellularly than LVS. Increases in colony numbers were observed across incubation time points. Differences in the invasion, survival, and replication ability among wild strains was observed. Further testing of additional strains from the NCDC collection is ongoing. This research represents common/standard microbiological methods for determining virulence, which is an important part of bacterial characterization.

Author Disclosure Block:

S. Chubinidze: None. **T. Shutkova:** None. **M. Grdzelidze:** None. **E. Nalbandishvili:** None. **S. Tsanava:** None. **R. Arner:** None. **G. Chanturia:** None.

Poster Board Number:

SATURDAY-573

Publishing Title:

Host Responses to Oral Challenge with *Porphyromonas gingivalis* and Nicotine in HLA-DRβ1-Bearing Humanized Mice

Author Block:

I. Sandal[1], D. D. Brand[1], J. Luo[2], A. Prislovsky[1], K. Whittington[1], C. Dong[3], E. F. Rosloniec[1], A. Karydis[2]; [1]Dept. of VA, Memphis, TN, [2]Univ. of Tennessee Hlth.Sci. Ctr., Memphis, TN, [3]Tsinghua Univ., Beijing, China

Abstract Body:

Background: Periodontal disease (PD) is a chronic pro-inflammatory response to oral pathogens such as *Porphyromonas gingivalis* which culminates in alveolar bone loss and loss of dental structure and function. Like rheumatoid arthritis, periodontal disease is influenced strongly by lifestyle factors such as tobacco use, but also greatly influenced by genetic components, such as the "shared epitope," which refers to a conserved linear sequence of amino acids in the DRβ1 chain of the HLA-DRα/β heterodimer between amino acids 67 and 74. Aggressive PD (the most destructive form of PD) is also now thought to be associated with specific HLA haplotypes including DRβ1.**Methods**: Using IL-17F^{rfp} and Foxp3^{gfp} reporters in I-A° C57BL/6 mice expressing chimeric mouse/human HLA-DRβ1 (B6.DR1 mice), we find gingival brushing with *P. gingivalis* results in rapid transient Th17 responses in the peripheral blood and cervical lymph nodes.**Results**: Evidence of a chronic infection can be detected in these nodes three months after oral inoculation. In addition to Th17 responses, we also find a concomitant increase in Th17-related cytokine production that parallels the Th17 activity. Furthermore, we measured a brisk production of antibodies directed against *P. gingivalis* and also against cyclic citrullinated peptides (these ACPAs are widely used as the principle early diagnostic for RA) as well as a loss in trabecular bone that can be detected in peri-articular bone structures, e.g. distal to the alveolar bone structures normally measured in PD models. We also find that trans-dermal nicotine treatment decreases the percentage of $CD4^+$ T cells expressing the Th17 phenotype in the cervical lymph nodes, whether this results in greater numbers in the gingival tissues or simply fewer Th17 cells overall has not yet been determined.**Conclusions**: Our findings suggest that periodontal disease can have far-reaching effects in bone morphology which may be driven by Th17/cytokine/ACPA activity even in the absence of other pathologies.

Author Disclosure Block:

I. Sandal: None. **D.D. Brand:** None. **J. Luo:** None. **A. Prislovsky:** None. **K. Whittington:** None. **C. Dong:** None. **E.F. Rosloniec:** None. **A. Karydis:** None.

Poster Board Number:

SATURDAY-574

Publishing Title:

Single Population Bottlenecks in the Pathogenesis of *Streptococcus pneumoniae*

Author Block:

M. Kono, M. A. Zafar, S. Hamaguchi, J. Weiser; New York Univ. Langone Med. Ctr., New York, NY

Abstract Body:

Streptococcus pneumoniae, a commensal resident of the human nasopharynx, is one of the leading pathogens among children. Especially in the setting of influenza virus co-infection, pneumococcal diseases tend to be more severe and transmission from host-to-host more common. During the multiple steps of infection, the pathogen may have to pass through 'bottlenecks' where its population and diversity is severely restricted, potentially resulting in weak points for intervention and prevention of diseases. In this study, we utilized an infant mouse model for evaluating pneumococcal colonization, disease and transmission among siblings with or without co-infection with influenza. Influenza virus increased the bacterial load of colonization, risk of otitis media and transmission rate. To assess bacterial population bottlenecks, three isogenic mutants, each tagged with a different antibiotic resistance marker were constructed. The bottlenecks and the impact of influenza co-infection were evaluated by examining the distribution of isogenic mutants passing through each stage in pathogenesis following IN challenge and the establishment of colonization. A mathematical model revealed single cell population bottlenecks during bacteremia and transmission among influenza co-infected mice. The number of shed bacteria correlated with the size of the bottleneck in transmission. Treatment with a TLR 3 agonist recapitulated the effect of influenza in driving increased bacterial shedding. The bottleneck in bacteremia was tighter in the setting of influenza and likely due to events before accessing the bloodstream because no bottleneck was observed following IP challenge. Within-host genetic adaptation did not appear to contribute to passage through the bottleneck, because a strain that previously passed through the bottleneck was no more likely to cause bacteremia or transmission when rechallenged compared to strains that had not passed through the bottleneck. We concluded that host innate immune response is the major factor in determining the size of the population bottleneck both in transmission and bacteremia.

Author Disclosure Block:

M. Kono: None. **M.A. Zafar:** None. **S. Hamaguchi:** None. **J. Weiser:** None.

Poster Board Number:

SATURDAY-575

Publishing Title:

Comparisons of Phenotypic and Monosaccharide-Binding Characteristics of Cf-Adapted *Pseudomonas aeruginosa* for Predictors of Binding

Author Block:

D. L. Chance, W. Wang, T. P. Mawhinney; Univ. of Missouri, Columbia, MO

Abstract Body:

Frequent, and eventually chronic, infections with the opportunist *Pseudomonas aeruginosa* (PA) are common for patients with cystic fibrosis (CF). Toward informing new anti-infective treatment strategies, ongoing studies are profiling carbohydrate-binding patterns of CF host-adapted PA. Quantitative binding heterogeneity has been observed between CF isolates with respect to glycopolymers with α-galactose (α-Gal) or β-N-acetylgalactosamine (β-GalNAc) as the pendant sugar. To assess whether specific bacterial features could be predictive of the category of enhanced monosaccharide binding, clinical isolates and laboratory strains shown to bind α-Gal and β-GalNAc glycopolymers were surveyed for phenotypic, enzymatic, and structural characteristics. Monosaccharide binding was determined microscopically with enumeration of fluorescent bacteria following exposure to fluorescent glycopolymers possessing pendant α-Gal or β-GalNAc, and provided quantitative differences among those reactive species. Phenotypic analyses employed rich and selective media plate culture and illustrated signature heterogeneity of PA characteristics of motility, mucoidy, pyocyanin production, colony morphology, texture, color, diffusible pigments, and hemolytic tendencies. Enzymatic profiling via automated microbial identification system provided anticipated similarities in activities defining the species and revealed intraspecies metabolic flexibility. TEM imaging of negatively stained specimens gave evidence of flagella for both motile and nonmotile species, and pili for a subset of isolates. Evaluation for trends and correlations of binding with phenotype, metabolic characteristics, and/or structural features, focused on α-Gal and β-GalNAc high and moderate reactivity, provided no reliable predictors of enhanced binding. Minor trends: nonmotile isolate binding was more frequently in higher category than motile strains; throat isolates tended toward higher category binding for β-GalNAc. Paired nonmucoid/mucoid CF isolates showed no uniform binding trends or enzymatic correlations. To conclude, this survey yielded no single feature or constellation of characteristics which appears to serve as a surrogate predictor of enhanced binding to the monosaccharides α-Gal and β-GalNAc for CF host-adapted *P. aeruginosa*.

Author Disclosure Block:

D.L. Chance: None. **W. Wang:** None. **T.P. Mawhinney:** None.

Poster Board Number:

SATURDAY-576

Publishing Title:

Identification of a Novel Virulence Factor Associated with Multi-drug Resistant *Acinetobacter baumannii*

Author Block:

P. M. Ketter[1], J-J. Yu[2], M. Guentzel[2], J. Seshu[2], K. Klose[2], A. Cap[1], B. Arulanandam[2]; [1]US Army Inst. for Surgical Res., JBSA-Fort Sam Houston, TX, [2]Univ. of Texas at San Antonio, San Antonio, TX

Abstract Body:

Background: Multi-drug resistant *Acinetobacter baumannii* (MDR-Ab) is an emerging infectious disease associated with wound and respiratory infections. While ubiquitous in the environment, MDR-Ab is primarily encountered as a hospital acquired infection (HAI) resulting in significant costs to both patient and hospital. Although MDR-Ab gastrointestinal (GI) tract colonization is common, no studies have assessed mechanism(s) contributing to this phenomenon. Similarly the role of SIgA in either GI tract colonization or respiratory infection is not understood.**Objective:** Assess the protective role of SIgA MDR-Ab GI tract colonization.**Methods:** Wild-type (WT) or SIgA deficient mice were challenged orally with MDR-Ab to assess survival and colonization. Sections of small intestine were collected from infant mice for *ex vivo* attachment assays. Supernatants from MDR-Ab cultures incubated with SIgA were assessed by non-reducing SDS-PAGE and Western blot. RNAseq analysis was performed on mRNA extracted from bacterial pellets. MDR-Ab mutants were generated by homologous recombination. Mutant strain virulence and colonization was assessed by intraperitoneal (i.p.), and oral challenge, respectively.**Results:** SIgA deficient mice exhibited significantly ($p < 0.05$) increase survival and bacterial clearance following MDR-Ab oral challenge. MDR-Ab intestinal attachment was also significantly ($p < 0.0005$) decreased in the absence of SIgA. Breakdown of SIgA by MDR-Ab was observed via Western blot, however, the banding pattern suggested a reductive mechanism. Use of disulfide reductase inhibitors dithionitrobenzoic acid (DTNB) and PX-12 confirmed these findings. Significant ($p < 0.05$) up-regulation of thioredoxin-A (*trxA*) expression was observed by RNAseq. Deletion of *trxA* ablated secreted reductase activity, reduced mortality by 100-fold, and impaired GI tract colonization.**Conclusion:** MDR-Ab GI tract colonization requires SIgA and is mediated by secreted TrxA. Furthermore, inhibition or elimination of *trxA* expression reduces GI tract colonization indicating it may be a novel target for vaccine and chemotherapies.

Author Disclosure Block:

P.M. Ketter: None. **J. Yu:** None. **M. Guentzel:** None. **J. Seshu:** None. **K. Klose:** None. **A. Cap:** None. **B. Arulanandam:** None.

Poster Board Number:

SATURDAY-577

Publishing Title:

Investigating the Role of the LytR Response Regulator in Group B Streptococcal Colonization and Disease

Author Block:

L. Deng[1], R. Mu[1], T. Weston[1], R. Liles[2], K. Doran[1]; [1]San Diego State Univ., San Diego, CA, [2]Louisiana State Univ. at Alexandria, ., LA

Abstract Body:

Streptococcus agalactiae (Group B *Streptococcus*, GBS) is typically a commensal bacterium that colonizes the vaginal tract of healthy women. However, in immune compromised individuals, such as pregnant women, the elderly, and newborns, GBS may transition to an invasive pathogen, resulting in pneumonia, sepsis, urinary tract infections, and meningitis. Despite currently recommended intrapartum antibiotic prophylaxis for GBS-positive mothers, GBS remains the leading cause of neonatal sepsis and meningitis. Little is known about what triggers GBS to switch from asymptomatic colonization to causing invasive disease. One way that bacteria respond to environmental changes is through two-component systems (TCS). TCS typically consist of a membrane-associated stimulus sensor and a cytoplasmic response regulator that alters gene expression. We hypothesize that the LytSR TCS in GBS may play a role in the bacteria's ability to persist in the host and to cause disease. We have tested a mutant GBS strain that lacks the LytR gene in our murine meningitis model and histological studies reveal that it induces increased inflammation of the tissue surrounding the brain as compared to wild type GBS. Additionally, using RT-qPCR, we have seen that human brain microvascular endothelial cells secrete more inflammatory cytokines such as IL-6, IL-8, CXCL1, and IL-1 when infected with the LytR mutant and we confirmed IL-6 and IL-8 protein expression through ELISA. Interestingly, the LytR mutant is cleared rapidly in our murine colonization model while wild type bacteria are able to persist in the mouse vaginal tract. We found that most mice clear the LytR mutant by six days post-inoculation while mice inoculated with wild type GBS remain stably colonized. Further characterizing the action of the LytR regulator during GBS infection can provide insight into how the bacteria stimulates the host inflammatory response. Ongoing studies are underway to identify downstream gene targets of the LytR response regulator in order to better understand the changes in gene expression that occur in the bacteria during the transition from asymptomatic colonization to inflammatory disease. Ultimately, uncovering how LytR signaling affects bacterial colonization and virulence may lead to the development of more effective therapies to prevent GBS infections.

Author Disclosure Block:

L. Deng: None. **R. Mu:** None. **T. Weston:** None. **R. Liles:** None. **K. Doran:** None.

Poster Board Number:

SATURDAY-578

Publishing Title:

Chemotactic Motility of *V.vulnificus* Is Required for Lethality in Mice

Author Block:

K. Yamazaki, T. Kashimoto, T. Kado, S. Ueno; Kitasato Univercity, Towada, Japan

Abstract Body:

Background: *V.vulnificus* is highly motile bacteria with single polar flagellum that is capable of rotating at a high-speed, and known as a causative agent for severe sepsis in immunocompromised individuals who is suffering underlying diseases. Flagellar deletion mutants of *V.vulnificus* are less virulent than that of parental strain in animal models, indicating that the flagellum is crucial to the infection process. The direction of flagellar rotation is based on chemotaxis which is response to environmental factors to access their beneficial conditions. Flagella are complex extracellular structures that require large amounts of energy for operation, so that lacking flagella affect secretory proteins and bacterial growth. To know whether the bacterial motility and chemotaxis were required for virulence in vivo or not, we generated the motility (Δ*pomA*) and the chemotaxis (Δ*cheY*) mutants that retained the complete flagellum, and then the pathogenesis of these two mutants were compared with that of wild-type (WT) in several assays.**Methods:** Generation of mutants; The ΔpomA and ΔcheY were generated by homologous recombination. PomA is required for generation of torque for flagellar rotation, therefore the ΔpomA lost motility but retains the flagellum. CheY is a key regulator of direction of flagella rotation, therefore the lacking of this gene lead to continue swimming straight in bacteria. Cytotoxicity assay; the cytotoxic effect on the HeLa cells were evaluated by Lactate dehydrogenase (LDH) release assay.In vitro swarming assay; Swarming assay was performed by using 0.3% agar containing Luria-Bertani broth. Determination of 50% lethal dose (LD_{50}); LD_{50} values were calculated according to the method of Reed and Muench (Reed and Muench, 1938).**Results:** There were not significant differences in the cytotoxicity against HeLa cells between wild-type, Δ*pomA* and Δ*cheY*. The in vitro swarming motility of the Δ*pomA* and the Δ*cheY* were completely abolished. The LD_{50} of the Δ*pomA* and the Δ*cheY* increased 5- and 2.5-fold, respectively, in comparison with that of the WT.**Conclusions:** Our data indicated that the absence of motility and chemotaxis would not affect the protein secretion, and cytotoxicity in HeLa cells. However, the increase in the lethality for mice of the Δ*pomA* and the Δ*cheY* suggested that chemotactic motility of *V.vulnificus* is required for systemic infection.

Author Disclosure Block:

K. Yamazaki: None. **T. Kashimoto:** None. **T. Kado:** None. **S. Ueno:** None.

Poster Board Number:

SATURDAY-579

Publishing Title:

***Pseudomonas aeruginosa* Outer Membrane Vesicles Triggered by Human Mucosal Fluid and Lysozyme Can Prime Host Tissue Surfaces for Bacterial Adhesion**

Author Block:

M. M. E. Metruccio[1], D. J. Evans[2], M. M. Gabriel[3], J. L. Kadurugamuwa[4], S. M. J. Fleiszig[1]; [1]UC Berkely, Berkeley, CA, [2]Touro Univ., Vallejo, CA, [3]Alcon Res., Ltd., Duluth, GA, [4]Alcon Res., Ltd., Fort Worth, TX

Abstract Body:

Indwelling medical devices, including contact lenses, are risk factors for *P. aeruginosa* infection. *P. aeruginosa* is among those bacteria that can release Outer Membrane Vesicles (OMVs) capable of fusing with host cells to alter their function. Here, we tested the hypothesis that mucosal (tear) fluid triggers OMV release to compromise an epithelial barrier. *P. aeruginosa* strain PAO1 was treated with human tear fluid or its constituents before OMV purification (differential centrifugation, filtration, and ultracentrifugation). Cytotoxicity of OMVs and their impact on barrier function against *P. aeruginosa* were tested using human telomerase immortalized corneal epithelial cells (LDH release assay, CFU counts of traversed bacteria) and mouse corneas (Live/Dead staining, GFP, confocal imaging). Whole mounted OMV treated mouse corneas were also labeled with Ly6G/C specific antibody, a myeloid cell marker. By 1 h, human tear fluid and the tear component lysozyme each greatly enhanced OMV release compared to PBS controls (~100 fold). TEM and SDS-PAGE showed these OMVs were similar in size and protein composition, but differed from biofilm-harvested OMVs. Lysozyme-induced OMVs were cytotoxic to human corneal epithelial cells *in vitro* and murine corneal epithelium *in viv*o. OMV exposure *in vivo* primed the cornea for bacterial adhesion (~4-fold, $P < 0.01$), and enhanced Ly6G/C expression at the corneal surface suggesting neutrophil recruitment. Sonication of OMVs spared cytotoxic activity, but reduced capacity to promote adhesion suggesting the latter required OMV-mediated events beyond cell killing. These data suggest that mucosal fluid induced OMVs could contribute to loss of epithelial barrier function during medical device-related *P. aeruginosa* infections.

Author Disclosure Block:

M.M.E. Metruccio: None. **D.J. Evans:** None. **M.M. Gabriel:** D. Employee; Self; Alcon Laboratories, Inc. **J.L. Kadurugamuwa:** D. Employee; Self; Alcon Laboratories, Inc.. **S.M.J. Fleiszig:** None.

Poster Board Number:

SATURDAY-580

Publishing Title:

LIC13059 and LIC10879 Coding Sequences are Novel Adhesins from *Leptospira interrogans*

Author Block:

P. R. M. Pereira[1], L. G. Fernandes[1], G. O. Souza[2], S. A. Vasconcellos[2], A. L. T. O. Nascimento[1]; [1]Inst. Butantan, São Paulo, Brazil, [2]Univ.e de São Paulo, São Paulo, Brazil

Abstract Body:

Background: Leptospirosis is the most widespread zoonosis and also a major cause of economic loss in animal production worldwide. It has been considered a major emerging infectious disease in the last ten years and it is included in the list of Neglected Tropical Diseases, according to the World Health Organization. The study of new surface antigens, not yet described in *Leptospira interrogans* literature, is intriguing and may indicate an unprecedented knowledge in the initial pathogen-host interaction and a new option in the search for prophylactic targets or serodiagnosis. We set out to clone and express the protein encoding by LIC13059 and LIC10879 to investigate their roles in the interaction with human host components. **Methods:** PCR products were obtained from *L. interrogans* serovar Copenhageni genomic DNA amplification with specific primers. The DNA inserts subcloned in pGEM-T vector were cloned into the expression vector pAE. Strains of *E. coli* Star pLysS were used for expression. The recombinant proteins were purified by metal affinity chromatography. Circular dichroism was carried out on spectropolarimeter. Extracts of different species and serovars of *Leptospira* were prepared in SDS-PAGE, transferred to nitrocellulose membranes and probed with antisera to recognize the native protein. The interaction of recombinant proteins with tissue and plasma components of the host was evaluated by ELISA. **Results:** The genes were amplified without the signal peptide and the recombinant proteins were expressed in form of inclusion bodies. After refolding and purification, circular dichroism analyzes showed that the proteins are structured, mainly in β-sheet. The proteins encoded by the genes are shown to be conserved in the pathogenic strains and absent in saprophytic one. The two recombinant proteins interact with plasminogen, fibrinogen and laminin, and all the interactions were dose-dependent and saturable. rLIC13059 bound to fibrinogen was capable of inhibiting the formation of fibrin clot. The plasminogen captured by both recombinant proteins could be converted into plasmin, a mechanism that could help bacterial penetration in the host. **Conclusions:** Our data suggest that these novel proteins may participate in *Leptospira*-host interactions.

Author Disclosure Block:

P.R.M. Pereira: None. **L.G. Fernandes:** None. **G.O. Souza:** None. **S.A. Vasconcellos:** None. **A.L.T.O. Nascimento:** None.

Poster Board Number:

SATURDAY-581

Publishing Title:

***Burkholderia cenocepacia* Infects Macrophages Trough Induction of Macropinocytosis**

Author Block:

R. Rosales-Reyes[1], D. Calderón-Campos[1], D. Alcántar-Curiel[1], C. Sánchez-Gómez[2], M. A. Valvano[3], J. I. Santos-Preciado[1]; [1]UNAM, Mexico, Mexico, [2]HIMFG, Mexico, Mexico, [3]Queens Univ., Belfast, United Kingdom

Abstract Body:

Background: *Burkholderia cenocepacia* is an opportunistic pathogen that causes respiratory tract infections in patients with cystic fibrosis. Infection can lead to rapid decline of lung function and in some cases to the fatal cepacia syndrome. This bacterium survives within a membrane vacuole in macrophages. The *B. cenocepacia*-containing vacuole shows maturation arrest manifested by a significant delay in acidification and fusion with the lysosome. The mechanism of bacterial invasion to macrophages has not been described.**Methods:** Bone marrow derived macrophages and IC21 macrophage cell line were infected with *B. cenocepacia*. The interactions between *B. cenocepacia* and macrophages were evaluated by light-video-microscopy, immunofluorescence microscopy, and by Gentamicin protection assays. The macropinocytosis induction was evaluated by Flow cytometry and by scanning electron microscopy. The macrophage cell death was determined by LDH quantification and the TNFα release of infected macrophages was assessed by ELISA.**Results:** With the use of light-video-microscopy and confirmed by scanning electron microscopy we identified that *B. cenocepacia* infects macrophages through macropinocytosis induction on the contact site. The invasion of this bacterium into macrophages was inhibited with the pretreatment with Wortmannin and LY294002. With the use of a mutant in the type III secretion system (dT3SS) we determined that the efficiency of bacterial invasion was reduced by 50%, suggesting that *B. cenocepacia* can infect macrophages using another pathway. In addition, the infection of macrophages with *B. cenocepacia wt* or dT3SS does not affect their ability to release of TNFa and the induction of cell death.**Conclusions:** *B. cenocepacia* infects macrophages through the induction of macropinocytosis.

Author Disclosure Block:

R. Rosales-Reyes: None. **D. Calderón-Campos:** None. **D. Alcántar-Curiel:** None. **C. Sánchez-Gómez:** None. **M.A. Valvano:** None. **J.I. Santos-Preciado:** None.

Poster Board Number:

SATURDAY-582

Publishing Title:

Intestinal Invasion By Neonatal *escherichia Coli* Clinical Isolates Is Enhanced By Bacterial Growth At Low Ph

Author Block:

B. K. Cole, M. Ilikj, **S. Chavez-Bueno**; Univ. of Oklahoma HSC, Oklahoma City, OK

Abstract Body:

Background: *E. coli* ingestion causes septicemia in newborns. Bacteria must survive the stomach acid prior to intestinal translocation into the bloodstream. Two neonatal *E. coli* septicemia isolates, RS218 and SCB34, differ in their ability to cause bacteremia in newborn rats. RS218 causes bacteremia when inoculated either intraperitoneally (IP) or orally, whereas SCB34 is virulent only when given orally but not IP. Whether their intestinal invasiveness increases by low pH exposure is unknown. Our main objective was to compare growth, and intestinal invasion of RS218 and SCB34 exposed to different pH. In addition, we assessed transcription of the acid resistance regulator *gadE*. **Methods:** Growth was tested in tissue culture medium (TCM) made with DMEM/Ham's F12 plus fetal calf serum, at pH 7.8 or pH 5. Optical density at 600 nm was measured with the Bioscreen C Reader System. Invasion of T84 intestinal cells was assessed by a modified gentamicin protection assay. RS218 or SCB34 were grown in TCM at pH 7.8 or 5, immediately prior to infecting T84 cells (MOI=10). After 1 hr incubation, the T84 cells were washed and amikacin (in lieu of gentamicin due to SCB34 resistance) was added for 2 hrs before lysis. The recovered intracellular bacteria were counted to calculate percent invasion relative to the inoculum. Expression of *gadE* was assessed by real-time PCR at either pH. Data were normalized to *gapA*; the $2^{-\Delta\Delta CT}$ method was used for analysis. **Results:** Growth of RS218 and SCB34 in mid-log phase was no different at either pH. During stationary phase, growth of each strain was impaired at pH 5 *vs* pH 7.8, but less markedly for SCB34 compared to RS218 ($p<.001$). Invasion of intestinal cells was significantly greater after each strain was grown at pH 5 compared to pH 7.8, with respective values for SCB34 of .47% *vs* .23% ($p<.02$), and for RS218 of .36% *vs* .21% ($p<.04$). Transcription of *gadE* was significantly greater at pH 5 than at pH 7.8 in both strains during stationary phase but not in mid-log phase. **Conclusions:** SCB34 showed better growth than RS218 at low pH. Invasiveness of both strains was enhanced by low pH exposure, particularly in SCB34. Upregulation of *gadE* was observed in both strains at low pH. Further investigation of neonatal *E. coli* virulence factors that respond to acid stress is crucial to develop strategies against neonatal sepsis.

Author Disclosure Block:

B.K. Cole: None. **M. Ilikj:** None. **S. Chavez-Bueno:** None.

Poster Board Number:

SATURDAY-583

Publishing Title:

Uropathogenic *Eschericia coli* Mechanisms of Intracellular Invasion

Author Block:

A. Shea, W-J. Kim, D. Jimenez, Y. Daaka; Univ. of Florida, Gainesville, FL

Abstract Body:

Urinary Tract Infections (UTIs) are the source of 7 million clinic visits and $2 billion in medical costs each year in the United States. Uropathogenic *Escherichia coli* (UPEC) are the causative organism in over 80% of confirmed infection cases. Furthermore, nearly 50% of women worldwide will develop an UTI at some point during their lifetime, and many of these patients will experience recurrent infections. The ability of UPEC to invade host epithelium is hypothesized to be a major source of disease recurrence through providing a quiescent reservoir of bacteria for subsequent infection. This project aims to clarify the mechanisms of bacterial invasion into host epithelial cells of the urinary tract, specifically the kidney and bladder epithelium. A preliminary screening experiment assessed the ability of different UPEC strains to invade human kidney and bladder epithelial cells. An *in vitro* gentamicin protection assay was used to quantify the relative number of invasive bacteria for each strain. While many strains were able to invade human kidney epithelial HK2 cells (ATCC CRL-2190) and bladder epithelial cells (ATCC HTB-9), this invasive phenotype was highly variable. This project aims to expose the molecular mechanisms involved during UPEC invasion of host epithelial cells. The goals are to (1) identify bacterial virulence factors promoting UPEC invasion, (2) determine which host cell membrane components are essential for bacterial entry, and (3) elucidate the intracellular signaling cascade mechanisms manipulated by the bacteria in order to promote entry. Understanding bacterial invasion of human urinary epithelial cells on a molecular level allows for the development of preventative measures which can be applied to the clinic, affecting nearly half of women worldwide.

Author Disclosure Block:

A. Shea: None. **W. Kim:** None. **D. Jimenez:** None. **Y. Daaka:** None.

Poster Board Number:

SATURDAY-584

Publishing Title:

The Gliding Phenotype of *Mycoplasma pneumoniae* on Glycan Receptor Populations

Author Block:

C. Reeves, E. Arnold, D. Leman, L. Chen, J. Locklin, D. C. Krause; Univ. of Georgia, Athens, GA

Abstract Body:

Mycoplasma pneumoniae, best known as the etiologic agent of "walking pneumoniae", is a common cause of human respiratory tract infections. Two receptor populations are believed to be recognized in airway colonization: α2-3 sialoglycoproteins (SGP) and sulfated glycoplipids (SGL). SGP receptor binding is specific to the α2-3 linked sialic acid residue, removal of which results in a loss of attachment. SGL binding is specific for Gal($3SO_4$)β1 residues and is partially inhibited by the presence of dextran sulfate. In the current study we examined *M. pneumoniae* interactions with glass surfaces coated with the SGP laminin or the SGL sulfatide to explore differences in adherence and gliding motility. Analysis of receptor-coated surfaces by Fourier Transform - Infrared Spectroscopy confirmed the presence of the receptor moieties, while ellipsometry showed an increase in coating thickness with higher concentrations for SGL and suggested the presence of a uniform monolayer for SGP. *M. pneumoniae* binding to the coated surfaces was assessed by light microscopy. We observed approximately a three-fold increase in binding to SGL over a concentration range of 1-10 μg, as expected. Likewise, binding to SGP increased approximately 2.5-fold over a concentration range from 0.2-10 μg. *M. pneumoniae* cells on SGP were motile, as expected, and as SGP concentration and thus receptor density increased, the number of motile cells and average gliding velocity likewise increased. Surprisingly, mycoplasmas bound to SGL remained static at all concentrations tested. These results indicate that the nature and density of receptors can influence the *M. pneumoniae* gliding phenotype, which might impact pathogenesis, persistence, and infection outcome.

Author Disclosure Block:

C. Reeves: None. **E. Arnold:** None. **D. Leman:** None. **L. Chen:** None. **J. Locklin:** None. **D.C. Krause:** None.

Poster Board Number:

SATURDAY-585

Publishing Title:

Mechanisms of St258 *Klebsiella pneumoniae* Invasion across the Airway Epithelium

Author Block:

D. Ahn, M. Wickersham, Z. Wang, T. Cohen, A. Uhlemann, A. S. Prince; Columbia Univ., New York, NY

Abstract Body:

The carbapenem resistant ST258 *K. pneumoniae* frequently cause respiratory infections and bacteremia. We postulated that ST258 strains have acquired mutations that facilitate persistence in the lung and lead to invasive infection. The clearance of KP35, a local ST258 strain and a KPRR1 control strain from the murine lung and blood were compared, along with changes in tight junction proteins. KP35 was poorly cleared with $>10^4$ cfus in the lung at 96 h and $>10^3$cfus recovered from the spleen at 48h. Proteomics of airway fluid revealed that KP35 induced changes in adherens junctional proteins. Transient increases in ezrin and occludin were confirmed by qRT-PCR. Confocal imaging and immunoblots illustrated increased occludin at the epithelial junctions at 4 hours, followed by a marked decrease at 24h. KP35 transmigration across polarized airway epithelial cells was over 2 logs greater than the KPPR1 control. Sequence comparison indicated acquisition of 26 proteinases among the 150 novel ortholog groups in KP35 not present in KPPR1. These findings suggest KP35 adaptation to the airway and targeting of epithelial junctional proteins to cause invasive infection.

Author Disclosure Block:

D. Ahn: None. **M. Wickersham:** None. **Z. Wang:** None. **T. Cohen:** None. **A. Uhlemann:** None. **A.S. Prince:** None.

Poster Board Number:

SATURDAY-586

Publishing Title:

Host Glycan Remodeling - Sweet Cross Talk between the Host and the Microbe

Author Block:

N. Arabyan[1], D. Park[1], H. Yu[1], S. Foutouhi[1], C. C. Williams[1], P. Desai[2], J. Shah[3], N. Kong[1], R. Jeannotte[1], X. Chen[1], C. B. Lebrilla[1], B. C. Weimer[1]; [1]Univ. of California, Davis, Davis, CA, [2]Univ. of California, Irvine, Irvine, CA, [3]Univ. of Minnesota, St. Paul, MN

Abstract Body:

The host gut epithelial membrane is shielded by complex glycans to protect the cell. Gastroenteritis begins when invasive pathogens bind and degrade the glyans at the epithelial barrier to gain access of host membrane. While glycan degradation is crucial for infection, this process is poorly understood. We hypothesized that *Salmonella* deploys its glycosyl hydrolases (GHs) to target and degrade host glycans leading to altered infection and glycan remodeling. *Salmonella* was grown in defined medium containing 18 different synthetic glycans modeled after human glycans as the sole carbon source. Significantly differentially expressed ($q<0.05$) GHs were identified and genetically deleted to determine the specific effect on adhesion and invasion *in vitro* with differentiated colonic epithelial cells (Caco-2). HPLC-Chip-TOF MS was used to determine the glycan composition and structure with specific set of enzymes used during infection. Growth analysis showed that *Salmonella* is able to digest and metabolize synthetic glycans. GHs recognized terminal monosaccharides and significantly ($p<0.05$) altered invasion *in vitro*. Sialic acid depletion reduced adherence of *Salmonella* during infection. *Salmonella* used its two GHs *nanH* and *malS* for internalization. Host glycans were altered during *Salmonella* association via the induction of N-glycan biosynthesis pathways leading to host glycan remodeling by increasing fucosylation, mannosylation, and hybrid glycan content, while decreasing sialylation. *Salmonella* increased invasion in Caco-2 cells lacking high-mannose and hybrid glycans which could suggest that high-mannose and hybrid glycans provide protection to the host. Gene expression analysis indicated that the host cell responded by regulating more than 50 genes showing that remodeled glycans are in response to *Salmonella* infection. Our study established the glycan structures on colonic epithelial cells, determined that *Salmonella* required two GHs for internalization, the host cell remodeled the glycan during infection, and host glycan landscape influences the host-microbe interaction. Microbial GHs are understudied and unrecognized virulence factors that may be new therapeutic targets.

Author Disclosure Block:

N. Arabyan: None. **D. Park:** None. **H. Yu:** None. **S. Foutouhi:** None. **C.C. Williams:** None. **P. Desai:** None. **J. Shah:** None. **N. Kong:** None. **R. Jeannotte:** None. **X. Chen:** None. **C.B. Lebrilla:** None. **B.C. Weimer:** None.

Poster Board Number:

SATURDAY-587

Publishing Title:

Effects of Subinhibitory Carvacrol Levels on *Bacillus cereus* Virulence During Endophthalmitis in Retinal Pigmented Epithelial Cells (Arpe-19)

Author Block:

N. Rajabli, S. McDowell, J. L. McKillip; Ball State Univ., Muncie, IN

Abstract Body:

Background: *Bacillus cereus* is one of the major pathogens causing posttraumatic endophthalmitis in humans. *B. cereus* can result in permanent eye damage within 24 hours after initial infection *via* production of multiple extracelluar virulence factors. Carvacrol, an antimicrobial essential oil, may be a promising treatment for *B. cereus* endophthalmitis. Specific events of transcriptional regulation of virulence genes in *B. cereus* during infection of human retinal pigment epithelial (RPE) cells are not well understood. Our aim is to measure relative levels of regulator and virulence gene expression in SIC carvacrol-stressed *B. cereus* during infection of laboratory-cultured ARPE-19 cells. Results of this work will elucidate mechanisms of virulence regulation in this increasingly important food and clinical pathogen.**Methods:** ARPE-19 cells were treated with 10^5 *B. cereus* alone, and *B. cereus* + SIC (1mM) of carvacrol. Relative differences in expression of *plcR*, *nprC*, *hblC*, and *nheA* regulator and virulence gene expression in *B. cereus* treated with SIC of carvacrol, and untreated *B. cereus* was quantified by mean cycle threshold (C_T) value comparisons using real-time PCR, and statistically analyzed using one-way ANOVA in Minitab17. Furthermore, *plcR* gene expression was measured through detection of the green fluorescent protein (GFP) production using transformed *B. cereus* ATCC 14579 with a promoter trapping expression vector (pBAD:*plcR*:GFP) treated with and without the SIC of carvacrol (1mM). The bacteria were inoculated in ARPE-19 cells and visualized using fluorescence microscopy and results quantified by enumeration of fluorescing bacteria within ARPE-19 cells.**Results:** Results reveal that Untreated *B. cereus* invaded ARPE-19 cells significantly less compared to SIC carvacrol-treated bacteria, which revealed full internalization within 48h. GFP(*plcR*)-expressing *B. cereus* were detected as fluorescing significantly more under carvacrol conditions compared to control replicates.**Conclusions:** These data indicate that sublethal chemical stress by carvacrol significantly increases potential virulence of this pathogen, *via* an increase in the global regulator (PlcR) production.

Author Disclosure Block:

N. Rajabli: None. **S. McDowell:** None. **J.L. McKillip:** None.

Poster Board Number:

SATURDAY-589

Publishing Title:

The Role of Flagella Frequency and Influence of Spatial Organization on the Attachment Force of Giardia Lamblia

Author Block:

M. Barefoot, H. Elmendorf, T. Picou; Georgetown Univ., Washington, DC

Abstract Body:

Giardia lamblia is the intestinal pathogen responsible for giardiasis, a diarrheal disease that affects more than 1.2 million people worldwide. An overall objective of the laboratory's research is to understand how *Giardia* attaches to the luminal wall of the small intestine. We hypothesize that the beating of *Giardia's* ventral flagella generates fluid movement, establishing a negative pressure differential, which is primarily responsible for the force of attachment. While evidence exists to support this model, a causal link between flagella beating and attachment has yet to be explicitly demonstrated. I am conducting experiments plating *Giardia* cells on polyacrylamide gels of different concentrations to model attachment under varying fluid flow conditions. Gels with increasing concentration of polyacrylamide are more porous and allow for more fluid flow. *Giardia* on these gels are then imaged by confocal microscopy to determine the corresponding flagella frequency. A potential correlation between flagella frequency and fluid flow would help to causally link flagellar motion to the attachment mechanism. In addition, the spatial arrangement of *Giardia* may also influence attachment by altering fluid flow rates, similar to what has been seen for cooperative flow in ciliate feeding mechanisms. Potentially, fluid flow could similarly be optimized by the spatial arrangement of *Giardia* to maximize attachment force. I have found that the flagella frequency of *Giardia* in pairs is significantly different between the top and bottom parasite (P=5.919e-7). I am performing Particle Image Velocimetry (PIV) to analyze the radius of the flow field out the back end of the parasite and determine the spatial distance between *Giardia* that still can influence the flow. Using this distance to define clustered and paired *Giardia* arrangements, the flagella frequency will be determined and compared to the flagella frequency of singly isolated *Giardia*.

Author Disclosure Block:

M. Barefoot: None. **H. Elmendorf:** None. **T. Picou:** None.

Poster Board Number:

SATURDAY-590

Publishing Title:

Impact of *Escherichia coli* Colonization on Susceptibility to Gastrointestinal Inflammation

Author Block:

H. Kittana; Univ. of Nebraska-Lincoln, Lincoln, NE

Abstract Body:

Several researchers have reported a significant increase in the abundance of specific bacterial species during intestinal inflammation, especially *Escherichia coli,* in a subset of Crohn's disease patients. We sought to determine how different commensal *E. coli* strains influence disease outcome during acute gut inflammation. Using four *E. coli* strains isolated from healthy mice, we observed that two isolates (SWW33 and CEC-1) enhanced intestinal inflammation triggered by treatment with a subpathological dose of dextran sulfate sodium (DSS) in C3H mice harboring a benign gut microbiota known as the altered Schaedler flora (ASF) that is devoid of any *Escherichia* species. In contrast, two commensal *E. coli* strains (CEC-7 and CEC-8) failed to exacerbate disease severity in ASF mice following the moderate-dose DSS treatment. Disease progression was found to be independent of bacterial burden in the gut as colonization levels for each *E. coli* strain did not change significantly following DSS treatment. We also found that SWW33 and CEC-1 colonized mice produced higher levels of the pro inflammatory cytokines IL-6, IFN-gamma and the macrophage recruiting chemokine GM-CSF in the cecum following DSS treatment as compared to control mice or mice colonized with either CEC-7 or CEC-8. Together, these finding suggest that CEC-1 and SWW33 elicit a different and potentially more pathogenic immune response compared to CEC-7 and CEC-8, resulting in increased inflammation and disease severity. Further experiments to determine the specific cell types producing the IL-6 as well as determining the requirement for IL-6 in disease onset are ongoing.

Author Disclosure Block:

H. Kittana: None.

Poster Board Number:

SATURDAY-592

Publishing Title:

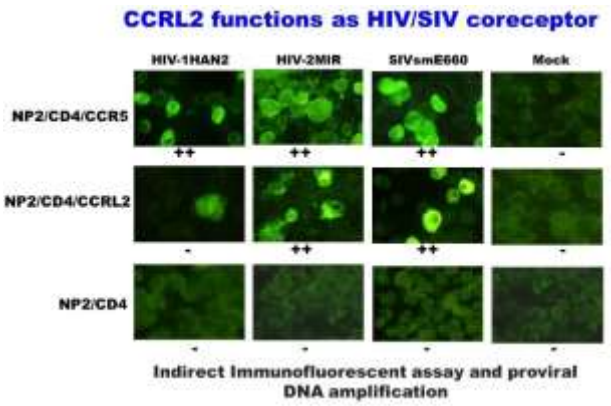
Coreceptor Role of CCRL2 for Human and Simian Immunodeficiency Viruses

Author Block:

S. Islam[1], N. Shimizu[2], A. Jinno-Oue[2], H. Hoshino[2]; [1]Jahangirnagar Univ., Dhaka, Bangladesh, [2]Gunma Univ., Maebashi, Japan

Abstract Body:

Background: Most of the typical chemokine receptors (CKRs) have been identified as coreceptors for various human and simian immunodeficiency viruses (HIVs and SIVs). Several atypical CKRs (ACRs) have also been reported as functional coreceptors. We first time evaluated and described the possible HIV/SIV coreceptor-role of an ACR member, CC-type chemokine receptor like-2 (CCRL2) in this study. **Methods:** Human glioma cell line, NP-2 is normally resistant to all HIV and SIV. The cell was transduced with amplified CD4 as receptor and CCR5, CXCR4 and CCRL2 as coreceptor-candidates to produce indicator NP-2/CD4/Coreceptor cells. Co-culture infection was conducted with C8166/CCR5 cells at 0.1 multiplicity of infection (MOI). Infections were detected by indirect immunefluorescence assay (IFA). Multinucleated giant cells (MGC) in syncytia were quantified after staining with Giemsa. Proviral DNA was detected by PCR and reverse transcriptase (RT) activity was examined.**Results:** Primary isolates, HIV-1HAN2, HIV-2MIR and SIVsmE660 utilized CCRL2 as a new coreceptor. IFA detected viral antigens in infected cells. The results were confirmed by detection of proviral DNA and measurement of reverse transcriptase (RT) in the spent cell-supernatants. MGC were detected in HIV-2MIR and SIVsmE660 infected NP-2/CD4/CCCRL2 cells. HIV-2MIR and SIVsmE660 were found more potent users of CCRL2 than HIV-1HAN2.**Conclusions:** In vitro experiments showed CCRL2 to function as a new coreceptor for some limited primary HIV/SIV isolates. The findings may contribute new insights into HIV/SIV transmission and pathogenesis; however, its in-vivo relevance should be evaluated. Suitable ligands of CCRL2 or its antagonist could be potential HIV entry-inhibitor drug targets.



Author Disclosure Block:

S. Islam: None. **N. Shimizu:** None. **A. Jinno-Oue:** None. **H. Hoshino:** None.

Poster Board Number:

SATURDAY-593

Publishing Title:

Determining the Adhesion and Invasion Potential of *Bacteroides vulgatus* and *Veillonella parvula* on Human Colonic Epithelial Cells

Author Block:

A. Blocker, A. Johnson, E. Norcross; Mississippi Coll., Clinton, MS

Abstract Body:

Background: Inflammatory Bowel Diseases (IBD), including Crohn's Disease and Ulcerative Colitis, are diseases involving the gastrointestinal tract. Recent research suggests that the microbiota of the intestine plays a role in the initiation and aggravation of both diseases. Current theories propose the overabundance of opportunistic pathogens, alterations in the commensal bacteria and dysbiosis as potential roles of intestinal microbiota in the pathogenesis of IBD. *Veillonella parvula* and *Bacteroides vulgatus* are two putative pathogenic microbes involved in IBD, but their specific roles have yet to be elucidated. **Methods:** The adhesion and invasion potential of *V. parvula* and *B. vulgatus* were examined using human colonic epithelial cell line C2BBe1. For adhesion assays, bacterial inoculums were incubated aerobically for 30 minutes with C2BBe1 cells prior to trypsinization. Cells were serially diluted and colony forming units (CFUs) were determined anaerobically. For invasion assays, bacterial inoculums were incubated with C2BBe1 cells aerobically for 2 hours, washed and incubated with antibiotic media for an additional hour. Cells were trypsinized then lysed prior to CFU determination.**Results:** Both bacterial strains yielded similar recovery of adherent bacteria. *B. vulgatus* had adherence of 1.86 x 10^4, 3.27 x 10^4, and 3.99 x 10^5 CFU/mL following bacterial inoculums of 10^7, 10^8, and 10^9 CFU/mL, respectively. *V. parvula* had adherence of 5.11 x 10^3, 8.28 x 10^4, and 3.09 x 10^5 CFU/mL following bacterial inoculum 10^7, 10^8, and 10^9 CFU/mL, respectively. *B. vulgatus* had invasion of 2.83 x 10^3, 1.82 x 10^4, and 5.91 x 10^4, CFU/mL following inoculum 10^7, 10^8, and 10^9 CFU/mL, respectively. Interestingly, *V. parvula* failed to invade C2BBe1 cells.**Conclusion:** The differences in the adhesion and invasion potential of each bacterium may play a role in the pathogenesis of IBD. Further research is needed to confirm the importance of bacterial invasion of gut epithelial cells in IBD and determine if the adhesion and invasion of these bacterial species leads to significant bacteria-induced inflammation.

Author Disclosure Block:

A. Blocker: None. **A. Johnson:** None. **E. Norcross:** None.

Poster Board Number:

SATURDAY-594

Publishing Title:

Exploring Gut Microbial Diversity in Indian Healthy Individuals, Inflammatory Bowel Disease and Colon Cancer Patients Using Metagenomics

Author Block:

V. Bamola[1], R. Chaudhry[1], R. Kapardar[2], B. Lal[2]; [1]All India Inst. of Med. Sci., New Delhi, India, [2]The Energy and Resources Inst., New Delhi, India

Abstract Body:

Background: Gastrointestinal tract (GI) of human is one of the common niches for microflora. These microbes play an important role in intestinal homeostasis in human. GI microbial imbalance can lead to pathological conditions including inflammatory bowel disease (IBD) and colon cancer. Since the dietary habits of Indian population are different from that of the Western world, therefore, findings of studies on gut microbial diversity in various sets of Western population cannot be extrapolated to their Indian counterparts. We have initiated a study to assess gut microbial diversity in Indian population with reference to health and disease.**Methods:** Stool samples have been collected from specific groups including vegetarian (N=13) non-vegetarian (N=12), colon cancer (N=12) and IBD (N=13). Metabolic profiling of gut bacteria was done by fatty-acid methyl ester (FAME) analysis. Amplified ribosomal DNA restriction analysis (ARDRA) using *HaeIII* has been carried out in samples and 16S rDNA library of these were prepared. The taxonomic distinction between the data sets was revealed by comparing and mapping of sequences using Ribosomal data base project (RDP).**Results:** Results indicate that microflora of a healthy adult showed high number of *Bacteroidetes* (84%) and less of *Firmicutes* (4%). When compared with age and sex match patient of colon cancer, there was a remarkable decrease in *Bacteriodetes* (52%) and increase in *Firmicutes* (29%). There was difference in the class Clostridia which was 2 % in healthy individual and 24 % in colon cancer patient. The IBD patients had higher percentage of Bacteroidetes than Firmicutes but lower Bacteroidetes: Firmicutes ratio than healthy non-vegetarian adult. Sequencing was able to indicate the presence and absence of anaerobes even when conventional culture failed to grow. FAME analysis showed significantly lower levels of butyric acid in IBD patients compared to healthy controls.**Conclusions:** RDP profiling shows remarkable differences with unique diversity attributed to unique Indian diet. Results are indicative of role of gut microflora in colonic health and provide insight into microbial community in health and disease and will help to establish certain microbial biomarkers.

Author Disclosure Block:

V. Bamola: None. **R. Chaudhry:** None. **R. Kapardar:** None. **B. Lal:** None.

Poster Board Number:

SATURDAY-595

Publishing Title:

Disentangling Diet And Phylogeny Reveals Both Horizontal And Vertical Evolution Of Microbiomes

Author Block:

M. Groussin[1], F. Mazel[2], J. G. Sanders[3], C. S. Smillie[1], S. Lavergne[2], W. Thuiller[2], E. J. Alm[1]; [1]Massachusetts Inst. of Technology, Cambridge, MA, [2]Univ. Grenoble Alpes, Laboratoire d'Ecologie Alpine, Grenoble, France, [3]Harvard Univ., Cambridge, MA

Abstract Body:

Background: In mammals, host evolutionary history (phylogeny) and diet primarily drive compositional differences of gut microbiota between host species. However, these factors are so deeply confounded, it may be impossible to fully understand their respective impact on the evolution of gut microbiota.**Methods:**Here, we develop a new phylogenetic framework to analyze compositional diversities between communities and disentangle diet and phylogeny. Our approach allows us to precisely characterize the bacterial taxonomic levels at which these two factors primarily shape community compositions. We then use probabilistic approaches to quantify to what extent horizontal and vertical inheritance of symbionts impact gut microbiota evolution with respect to diet and phylogeny.**Results:**Our study shows that host phylogeny and diet drive community assembly at different temporal scales of bacterial evolution. Host diet selects deep microbial lineages, consistent with the conservation of diet-related functions at large bacterial taxonomic scales. Host phylogeny, however, finely discriminates among recently diverged bacterial lineages, consistent with intimate interactions between bacteria and host genetics. Dietary shifts during mammalian evolution are associated with a non-random process of horizontal acquisitions of bacteria, resulting in nested gut microbiota compositions that reflect the nested structure of diets. These shifts did not erase the signal of vertical inheritance of microbiota along host phylogeny: both young and ancient (up to 80 Myr) mammalian clades harbor specific compositions and the proportion of bacteria showing signs of co-speciation (56% of OTUs) is much higher than chance would predict.**Conclusion:**Together, our results shed light on the underlying complexity of horizontal and vertical processes driving the evolution of mammal:microbiota symbiotic systems and reconcile previous, seemingly inconsistent empirical observations on the relative contribution of host phylogeny and diet. Our results open new avenues to further understand the functional and evolutionary drivers of animal:microbiota systems.

Author Disclosure Block:

M. Groussin: None. **F. Mazel:** None. **J.G. Sanders:** None. **C.S. Smillie:** None. **S. Lavergne:** None. **W. Thuiller:** None. **E.J. Alm:** None.

Poster Board Number:

SATURDAY-596

Publishing Title:

Diet-Linked Dynamics of the Human Gut Microbiome

Author Block:

G. ABU-ALI[1], J. Lloyd-Price[2], R. S. Mehta[3], K. L. Ivey[1], J. Izard[4], E. Rimm[1], A. T. Chan[3], C. Huttenhower[1]; [1]Harvard Chan Sch. of Publ. Hlth., Boston, MA, [2]Broad Inst., Boston, MA, [3]Massachusetts Gen. Hosp., Boston, MA, [4]Forsyth Inst., Cambridge, MA

Abstract Body:

Background: We report on the gut microbiomes of 307 participants from the Health Professionals Follow-Up Study, a prospective cohort designed to relate nutrition to health outcomes and, here, to link the microbiome to dietary patterns.**Methods:** The gut metagenome of each participant was surveyed at four timepoints with short (1-3 days) and long (6 month) intervals. Metatranscriptomes were also generated for 96 participants. These data are complemented with seven-day dietary recalls and long-term dietary histories from food frequency questionnaires. Taxonomic profiling and metabolic reconstruction were performed using MetaPhlAn2 and HUMAnN2, respectively.**Results:** In addition to echoing the microbial population structure of similar Western adult cohorts, functional profiling showed that several pathways had bimodal distributions across timepoints, implying a stable substructure possibly related to differences in diet and lifestyle among a subset of ~20 participants. Of the 730 transcriptionally active metabolic pathways, 225 were significantly and stably over-transcribed with RNA/DNA ratios >2. Diversity analysis echoed the structure of other healthy populations and, consistent with previous reports, indicated that taxonomic composition was stable over time. The most abundant taxa were not necessarily the most prevalent (e.g. Akkermansia muciniphila and Ruminococcus bromii), showing greater divergence from an ecological neutral model than has been observed in other cohorts. Lastly, strain-resolved analysis lent support to consistent and stable inter-individual differences, with both genomic variants and functional content differing within species among individuals and over time.**Conclusions:** Deeper insight into the structure and diet-linked variability of the microbiome will guide future efforts in personalized preventative interventions and, eventually, lifestyle-based health maintenance.

Author Disclosure Block:

G. Abu-ali: None. **J. Lloyd-Price:** None. **R.S. Mehta:** None. **K.L. Ivey:** None. **J. Izard:** None. **E. Rimm:** None. **A.T. Chan:** None. **C. Huttenhower:** None.

Poster Board Number:

SATURDAY-597

Publishing Title:

Machine Learning Reveals Microbiome Differences in Exercise Mouse Models

Author Block:

E. Lamoureux, S. Grandy, M. Langille; Dalhousie Univ., Halifax, NS, Canada

Abstract Body:

Background: The human microbiome is known to have a complex, yet vital relationship with human health. While both exercise and the gut microbiome have been shown to be important contributors to health, the direct effects of exercise on the intestinal microbiota in a controlled setting remains understudied. The purpose of this study was to characterize changes in the gut microbiome in response to varying forms of exercise using a mouse model with a controlled environment and diet. **Methods:** Mice were randomly assigned into one of three exercise treatments: voluntary wheel running, forced exercise through the use of a treadmill, and a control group with no access to exercise equipment. Food intake, weight, and body mass composition were monitored over the experimental period. Bacterial 16S rRNA gene sequencing from fecal samples was conducted by the CGEB-IMR using an Illumina MiSeq. Data was analyzed and microbiome differences between treatment groups were evaluated using the standard operating procedures from Microbiome Helper. Further analysis was explored using machine-learning techniques, including Random Forests.**Results:** Mice within the voluntary exercise group were found to intake more food and weigh significantly more than control mice and mice undergoing forced exercise. Microbiome analysis revealed on average 1094 operational taxonomic units per sample, but did not initially identify any major differences between exercise treatments. Machine-learning methods trained with microbiome data could reliably predict the correct treatment for each sample 81% of the time, suggesting a modest but measurable shift in the gut microbiome in response to exercise.**Conclusions:** Our study shows that exercise has an identifiable but limited effect on the gut microbiome and questions the impact of exercise on the human microbiome.

Author Disclosure Block:

E. Lamoureux: None. **S. Grandy:** None. **M. Langille:** None.

Poster Board Number:

SATURDAY-598

Publishing Title:

Rapid Urbanization in China is Associated with Increased Abundance of Commonly Observed Western Gut Microbes

Author Block:

K. Winglee[1], A. G. Howard[2], W. Sha[3], R. Gharaibeh[3], J. Liu[4], D. Jin[4], A. A. Fodor[1], P. Gordon-Larsen[2]; [1]Univ. of North Carolina at Charlotte, Charlotte, NC, [2]Univ. of North Carolina at Chapel Hill, Chapel Hill, NC, [3]Univ. of North Carolina at Charlotte, Kannapolis, NC, [4]Hunan Ctr. for Disease Control and Prevention, Changsha, China

Abstract Body:

Background: The composition of the human microbiome, the set of microorganisms found on or in a host, is correlated with a number of diseases, including obesity, diabetes and cancer. These diseases are also associated with urbanization, and although it has been shown that microbial community composition varies across continents and in traditional versus Westernized societies, few studies have examined urban-rural differences in neighboring communities within a single country undergoing rapid urbanization.**Methods**: Using next generation sequencing of the 16S rRNA gene, we assessed differences in the microbial community of rural versus recently urban subjects from the Hunan province of China. In addition, we analyzed the microbiota-related plasma metabolites.**Results**: There were significant differences in both the microbial and metabolite composition between rural and urban participants at the lower taxonomic levels, although there was no significant difference above the family level. Furthermore, when we compared the taxa in our study to the American cohort from the Human Microbiome Project (HMP), microbes with higher relative abundance in Chinese urban samples were substantially more prevalent in the HMP.**Conclusions**: The microbial changes linked to urbanization in our study have been shown to be associated with diseases in other studies. We hypothesize that selection pressure from urbanization rapidly affects the relative abundance of microbes at more derived levels of the phylogenetic tree, although these changes have not propagated to higher levels. This suggests that urbanization has resulted in convergent evolution in American and urban Chinese microbial composition, causing similar patterns of abundant microbes through similar lifestyles on different continents.

Author Disclosure Block:

K. Winglee: None. **A.G. Howard:** None. **W. Sha:** None. **R. Gharaibeh:** None. **J. Liu:** None. **D. Jin:** None. **A.A. Fodor:** None. **P. Gordon-Larsen:** None.

Poster Board Number:

SATURDAY-599

Publishing Title:

Calorie Restriction Significantly Alters the Intestinal Microbiota of Mice

Author Block:

S. A. Matyi[1], A. Unnikrishnan[1], K. Garrett[1], A. Ericsson[2], A. Richardson[1]; [1]Univ. of Oklahoma Hlth.Sci. Ctr., Oklahoma City, OK, [2]Univ. of Missouri Metagenomics Ctr., Columbia, MO

Abstract Body:

Calorie restriction (CR) as a dietary intervention has been shown to not only extend the lifespan of a variety of animals but can also delay the onset of age-related diseases and improve physiological functions that decline with age. Therefore, it is generally accepted that CR delays aging. Because intestinal microbiota has been shown to play an important role in host health and disease such as obesity and diabetes and because diet is a major influence that can change the composition and function of the intestinal microbiota, we studied the effect of life-long CR on the composition of the intestinal (cecum and fecal) microbiota of mice. Young and old male mice were housed in a specific pathogen-free animal facility and fed *ad libitum* or given a 40% calorie restriction diet. The microbiota of both the feces and cecum were characterized by high-throughput sequencing the V4 region of the bacterial 16S rRNA gene. Here we show that 40% CR significantly changed the overall composition of the intestinal microbiota of old mice. The microbiota of the old CR mice were enriched for similar phylotypes found in young mice, while the old animals fed *ad libitum* were comprised of different phylotypes. In addition, we found that the diversity of the intestinal microbiota declined with age and was preserved by CR. We have also determined if changes in the microbiota are correlated to changes in the transcriptome of colon mucosa. Our data suggest that the preservation of the young intestinal microbiota profile found within old CR mice may play a role in the prevention or delay of age-related diseases as well as the extension in lifespan seen with CR.

Author Disclosure Block:

S.A. Matyi: None. **A. Unnikrishnan:** None. **K. Garrett:** None. **A. Ericsson:** None. **A. Richardson:** None.

Poster Board Number:

SATURDAY-600

Publishing Title:

Gut Microbiota and Cytomegalovirus (Cmv) Infection Interact to Influence Development of the Immune System in Rhesus Macaques

Author Block:

N. R. Narayan, G. Méndez-Lagares, A. Ardeshir, D. Lu, D. Hartigan-O'Connor; Univ. of California, Davis, Davis, CA

Abstract Body:

Background: Environmental factors such as the gut microbiota and chronic latent viral infections have profound impacts on the immune system. Cytomegalovirus (CMV), for example, is a common asymptomatic infection that causes significant changes in the immune system of the host. While a great deal of work has elucidated the effects of individual factors such as CMV, however, little work has been devoted to assessing interactions between viral infections and the gut microbiota.**Methods:** We examined 71 healthy rhesus macaque juveniles (43 CMV-negative, 28 CMV-positive), collecting rectal swabs and peripheral blood. Peripheral blood mononuclear cells (PBMCs) were isolated and we used flow cytometry to assess a comprehensive set of immune markers, including those expressed by subsets of B cells, T cells, and innate immune cells. The microbiota associated with the lumen was assessed by 16S rRNA sequencing of rectal swab samples.**Results:** Both CMV infection and many members of the gut microbiota independently associated with features of the immune system. While CMV infection did not associate with significant shifts in gut microbiota composition, abundances of *Faecalibacterium* and *Bifidobacterium* were decreased in CMV-positive macaques. Linear mixed-effects modeling revealed that CMV infection shifted the relationship between some bacteria and immune subsets. For example, *Prevotella* abundance was significantly associated with various activated monocyte subsets, but only in CMV-negative animals.**Conclusions:** The gut microbiota and CMV influence the immune system individually and in their interactions. Further work will focus on the sources of this interaction, which may be due to altered host innate cell signaling.

Author Disclosure Block:

N.R. Narayan: None. **G. Méndez-Lagares:** None. **A. Ardeshir:** None. **D. Lu:** None. **D. Hartigan-O'Connor:** None.

Poster Board Number:

SATURDAY-601

Publishing Title:

An Ecophylogenetic Approach to Determine the Evolutionary History of the Mammalian Gut Microbiome

Author Block:

C. A. Gaulke, **T. J. Sharpton**; Oregon State Univ., Corvallis, OR

Abstract Body:

Identifying those gut microbes that co-evolve with mammals is important to our understanding of the mechanisms and health implications of host-microbiome interactions. For example, microbiota that are conserved across mammalian species may express a trait that has been subject to selection throughout the evolution of these mammals, possibly because it is critical to health. While advances in environmental DNA sequencing have transformed our understanding of how enteric microbes are distributed across mammalian species, these data are frequently analyzed using phylogenetically agnostic approaches. Such approaches can obscure the detection of diverged groups of bacteria that have been conserved across mammalian species. To provide enhanced resolution into evolutionary associations between gut microbiota and mammals, we innovated a high-throughput ecophylogenetic method, known as ClaatTU (Cladal Taxonomic Units). ClaaTU analyzes phylogenies assembled from environmental DNA sequences collected from a set of microbial communities and profiles the presence and abundance of each monophyletic clade in each community. As a result, it enables the identification of specific microbial clades that are distributed across host communities in a manner indicative of being associated with mammalian evolution. To demonstrate this, we first evaluated the accuracy of this method using statistical simulations. We then applied ClaaTU to several mammalian microbiome datasets spanning varying evolutionary distances and identified clades of gut bacteria that are (1) conserved across groups of mammals, (2) unique to specific mammalian lineages, and (3) putatively co-diversifying with their hosts. Our findings indicate that some mammalian gut microbiota were anciently acquired and subsequently retained in extant lineages, indicating that they may play an important role in mediating host-microbiome interactions and maintaining host health.

Author Disclosure Block:

C.A. Gaulke: None. **T.J. Sharpton:** None.

Poster Board Number:

SATURDAY-602

Publishing Title:

Gut Microbiome of Animal Model Systems: A Comparison of Macaque, Vervet, Mouse and Human

Author Block:

E. J. Bautista[1], K. Mihindukulasuriya[2], Y. Zhou[1], J. R. Kaplan[3], Y. Nobel[4], A. J. Jasinska[5], M. J. Blaser[6], N. B. Freimer[7], E. Sodergren[1], G. M. Weinstock[1]; [1]The Jackson Lab., Farmington, CT, [2]Washington Univ. Sch. of Med., St. Louis, MO, [3]Wake Forest Univ., Winston-Salem, NC, [4]Columbia Univ. Med. Ctr., New York, NY, [5]Univ. of California, Los Angeles, CA, [6]THe New York Univ., New York, NY, [7]Univ. of California-Los Angeles, Los Angeles, CA

Abstract Body:

Animal model systems are important tools to study behavior, metabolic disorders, host-gut microbe interactions, immunity and obesity. Obesity is a growing problem worldwide, leading to increases in diabetes, hypertension, heart disease, stroke, arthritis, high cholesterol and some cancers. Adoption of a Western diet, coupled with a more sedentary lifestyle is key factors in the increase in childhood and adult obesity, both in the Western and developing worlds. Further, diet helps to mold the gut microbiome.Here, we use both macaques and vervets as models to study the effect of diet on the gut microbiome. Macaques were under high fat diet with soy or casein as their protein source. Meanwhile, vervets on standard monkey chow diet were change to a typical American Diet (TAD). We also compare the microbiome of vervet and macaque to the microbiome of mice and humans. Samples were processing following standard protocols for DNA extraction and 16S targeted sequencing. Our pipelines for 16S rRNA were used to determine the taxonomic composition and the metabolic functional potential associated with it.A preliminary comparison analysis show macaques on a casein-based diet have a larger proportion of Prevotella than macaques on a soy-based diet. While Prevotella dominates all vervet stool samples, the amount of Prevotella present was reduced following the introduction of TAD.The gut diversity of macaques was higher with soy, rather than casein, as a protein source. Vervets on a chow diet have higher gut diversity compare to the ones on TAD diet. Currently, we are working on analyzing the metabolic functional potential of the samples, to further understand the impacts of the change in diet on the microbiome.Finally, macaques showed the greatest diversity, followed by vervets, mice and humans. Vervet and macaque gut microbiomes are dominated by Prevotella, humans by bacteriodes and mice by Akkermansia.

Author Disclosure Block:

E.J. Bautista: None. **K. Mihindukulasuriya:** None. **Y. Zhou:** None. **J.R. Kaplan:** None. **Y. Nobel:** None. **A.J. Jasinska:** None. **M.J. Blaser:** None. **N.B. Freimer:** None. **E. Sodergren:** None. **G.M. Weinstock:** None.

Poster Board Number:

SATURDAY-603

Publishing Title:

Assembly of the *Bacteroides thetaiotaomicron* Starch Utilization System (SUS)

Author Block:

M. Foley; Univ. of Michigan, Ann Arbor, MI

Abstract Body:

The human gut microbiota plays an essential role in maintaining optimal health, yet little is known about how many of these organisms harvest nutrients from their environment. Dietary glycans are a controllable variable that shape the composition of the human gut microbiota, and microorganisms that colonize this niche have strategies to efficiently compete for these nutrients. A dominant phylum of the gut, the Gram-negative Bacteroidetes, can utilize numerous dietary and host-derived glycans. Many gut Bacteroidetes devote significant portions of their genome towards polysaccharide utilization loci (PULs) that each encode several proteins that bind, degrade, and import different sugars. Each PUL is individually regulated and encodes for proteins that target a unique glycan structure. Conserved across all known PULs are homologs of the prototypical starch utilization system (Sus) proteins SusC, a TonB-dependent transporter (TBDT), and SusD, a starch binding protein, originally described in the model species Bacteroides thetaiotaomicron (Bt). In the Sus, SusCDEFG operate on the cell surface to capture, degrade and import starch into the cell. SusC and SusD are both required for Bt growth on starch and interact, although the nature of this interaction and other proteins present in this complex are not described. My data demonstrate that SusE, like SusD, can provide a starch-binding site for glycan transport through SusC, which highlights a new feature of the SusE-like family of proteins within the Bacteroidetes. Furthermore, using quantitative proteomics I demonstrate that SusCDE interact in the membrane, and that the stoichiometric ratio of interacting proteins is affected by formaldehyde crosslinking. In hopes of understanding the relationship between the SusCDE complex and the remaining proteins SusFG, I employ single molecule imaging to visualize SusE, SusF, and SusG dynamics in the membrane of live cells. Imaging shows that, unlike the highly mobile SusG, SusE and SusF are static, although the reason for this is unclear. The goal of my research is to understand how these Sus protein interactions facilitate glycan utilization. Because homologs of these proteins are central to all PULs, understanding how they work together to import starch will inform a general model of the Sus-like paradigm used by the Bacteroidetes for glycan uptake.

Author Disclosure Block:

M. Foley: None.

Poster Board Number:

SATURDAY-604

Publishing Title:

Mannosides: An Antibiotic-Sparing Treatment For The Eradication Of Upec Reservoirs From The Host

Author Block:

C. N. Spaulding, A. Kau, Z. Cusumano, J. Janetka, J. I. Gordon, S. J. Hultgren; Washington Univ. in St. Louis, St. Louis, MO

Abstract Body:

Urinary tract infections (UTI) represent a disease which: i) affects ~60% of women; ii) displays a high rate of recurrence; iii) is associated with significant morbidity and has a sizeable economic impact and; iv) is increasingly being caused by multidrug resistant bacteria. The most common causative agent of UTI is uropathogenic *E. coli* (UPEC). UPEC reside in the gut microbiota, are shed in the feces and can be introduced into the bladder. The role of the gut as a primary source of UPEC that lead to UTI is widely accepted, yet little is known about the basis of UPEC gut colonization. To colonize a host, UPEC express a family of adhesive fibers called pili. In mice, acute bladder colonization requires the adhesin protein present at the tip of the type 1 pilus, FimH, to bind to mannosylated proteins expressed on the surface of bladder epithelial cells. Once the bacteria bind the host surface, they can invade the luminal epithelial cells where they can replicate to high levels while protected from many host defenses; thus promoting ongoing infection. We have developed anti-adhesive mannosides that specifically block UPEC's ability to colonize the bladder by binding to FimH with several orders of magnitude higher affinity than the natural receptor. We have shown that mannosides are capable of treating and/or preventing UTI in the mouse model. Here we show that type 1 pili also enhance UPEC intestinal colonization. Interestingly, in mice colonized with human UPEC isolates, treatment with the mannoside results in decreasing colonization of the uropathogen in the gut with minimal other effects on gut microbial ecology, including no significant effects on the abundance of normal resident *E. coli* strains in the microbiota. Eliminating the UPEC reservoir in the gut may help to reduce the rate of recurrent UTI. Thus, this type of therapeutic provides an antibiotic-sparing method, which if translated to the clinic may have the potential to: i) treat patients with UTI and/or; ii) reduce the rate of recurrences by eliminating the UPEC reservoir from the gut. Additionally, a number of studies show that disrupting the gut microbiota with antibiotics may affect its functional properties in ways that are deleterious to the host. Therefore, a compound that can target UPEC, without damaging the remainder of the gut microbiota holds promise as an antibiotic-sparing therapy.

Author Disclosure Block:

C.N. Spaulding: None. **A. Kau:** None. **Z. Cusumano:** None. **J. Janetka:** A. Board Member; Self; Fimbrion Therapeutics. **J.I. Gordon:** None. **S.J. Hultgren:** A. Board Member; Self; Fimbrion Therapeutics.

Poster Board Number:

SATURDAY-605

Publishing Title:

Pancreatic Ductal Adenocarcinoma and Intestinal Microbiome Diversity

Author Block:

S. Pushalkar[1], D. Daley[2], M. Usyk[1], C. Zambrinis[2], G. Miller[2], D. Saxena[1]; [1]New York Univ. Coll. of Dentistry, New York, NY, [2]New York Univ. Langone Med. Ctr., New York, NY

Abstract Body:

Inflammation induced pancreatic ductal adenocarcinoma (PDAC) accounts for 85% of the pancreatic cancers. Gut bacteria play a functional role in maintaining the homeostasis. Accumulating evidence suggests the role of bacterial infections in the manifestation of several malignancies. We hypothesized that intestinal microbial communities may influence PDAC progression. Microbial DNA was extracted and illumina MiSeq sequencing of 16S gene amplicons was used to characterize the gut microbiota in the patients with pancreatic ductal adenocarcinoma (PDA, n=10) and non-PDAC controls (NML, n=10). Bacterial community analysis using Quantitative Insights Into Microbial Ecology (QIIME) and R revealed high abundance of phyla *Firmicutes* (51.8-57.3%) and *Bacteroidetes* (27.0-30.4%) in PDA and NML cohorts. Approximately 4% increase in the levels of *Proteobacteria* ($p>0.1$) and *Fusobacteria* ($p=0.052$), though not significant was observed in the PDAC patients as compared to non-PDAC controls. Taxa, *Veillonella* ($p<0.001$), unclassified *Veillonellaceae* ($p<0.001$), *Fusobacterium* ($p<0.01$), and *Klebsiella* ($p<0.01$) were significantly associated with PDAC patients. Principal coordinate analysis showed two distinct clusters of non-cancer controls and PDAC patient population in response to variation in the host microenvironment. The results suggest dysbiosis in microbial membership and structure and presence of certain microbial signatures associated with pancreatic carcinogenesis. These findings and future studies with larger patient population could facilitate better insights in elucidating the role of gut microbiota in the pancreatic tumor microenvironment that could be of clinical and therapeutic significance.

Author Disclosure Block:

S. Pushalkar: None. **D. Daley:** None. **M. Usyk:** None. **C. Zambrinis:** None. **G. Miller:** None. **D. Saxena:** None.

Poster Board Number:

SATURDAY-606

Publishing Title:

Relationships between Mosquito Intestinal Bacteria and Mosquito-Borne Virus Infection

Author Block:

C. M. Bonavita, J. Anderson; Radford Univeristy, Radford, VA

Abstract Body:

Abstract**Background:**Dengue viruses are endemic to the tropics and affect roughly 390 million people annually. Symptoms include high fever, joint pain, hallucinations, and in some severe cases hemorrhaging. Currently there are no effective preventative or treatment methods for dengue virus. Previous research suggests that intestinal bacteria may hold properties that can manipulate disease transmission in mosquitoes.**Methods:**For my experiment, my goal was to collect dengue vectors from the Madre de Dios region of eastern Peru and to compare the bacteria resident in infected vs. uninfected mosquitoes, as well as differences between the in-forest, near-forest, and in urban area microbiome of the mosquitoes.**Results:**We collected 52 mosquito samples, of which only 10 were potential dengue vectors. Since it is unlikely that we would find the virus in that small a sample pool, we also began examining variations in bacterial communities in mosquitoes from various locations in Virginia using 16S rRNA sequencing or SSCP analysis of asymmetric PCR. *Aedes albopictus* mosquitoes from three locations in Virginia could be distinguished based on their bacterial communities.**Conclusions:**We are currently investigating the relationship of bacteria to Eastern equine encephalitis virus infection in *Culiseta melanura* mosquitoes from the Great Dismal Swamp in Virginia. Much is still unknown about the bacteria that colonize mosquito vectors of disease and how they interact with the pathogens. Our work adds to the growing understanding of those bacteria, and how they might be used to interfere with pathogen transmission.

Author Disclosure Block:

C.M. Bonavita: None. **J. Anderson:** None.

Poster Board Number:

SATURDAY-607

Publishing Title:

***Helicobacter pylori* γ-Glutamyl Transpeptidase is a Potentiator of Vaca-dependent Vacuolation in Gastric Cells**

Author Block:

B. Ho, **S. Ling**; Natl. Univ. of Singapore, Singapore, Singapore

Abstract Body:

H. pylori, the etiological agent of gastroduodenal diseases possesses vacuolating cytotoxin (VacA) that causes cellular vacuolation in host cells in the presence of permeant weak bases, ammonia. We report here the role of γ-glutamyl transpeptidase (GGT), a secretory enzyme of *H. pylori*, in enhancing VacA-dependent vacuolation formation in *H. pylori*-infected gastric epithelial cells. The process involves GGT hydrolysing glutamine and releasing ammonia that accentuates the VacA-induced vacuolation. In contrast, *H. pylori ggt* isogenic mutant (*Δggt*) induced significantly less vacuolation in AGS and primary gastric epithelial cells as compared to the parental strain ($P<0.05$) as shown using semi-quantitative neutral red uptake assay, indicating that GGT potentiates the vacuolating effect of VacA. Not surprisingly, vacuolation was significantly reduced ($P<0.05$) in either the absence of GGT substrate (glutamine) or in the presence of a competitive GGT inhibitor, serine-borate complex. Notably, the addition of purified recombinant GGT (rGGT) *to Δggt*-infected cells markedly restored the vacuolating ability of the mutant, but, rGGT itself did not induce vacuolation independently. Similarly, the addition of exogenous ammonium chloride as a source of ammonia also rescued the ability of *Δggt* to induce vacuolation. Real-time phase-contrast microscopy lends support to the vacuolation events. Taken together, generation of ammonia through glutamine hydrolysis by *H. pylori* GGT is the cause in potentiating VacA-dependent vacuolation. Our findings illustrate GGT as a vital virulence factor in *H. pylori*-associated gastric diseases.

Author Disclosure Block:

B. Ho: None. **S. Ling:** None.

Poster Board Number:

SATURDAY-608

Publishing Title:

The Role of Immunity on *Streptococcus pneumoniae* Shedding and Transmission in an Infant Mouse Model

Author Block:

T. Zangari, Y. Wang, J. N. Weiser; New York Univ. Sch. of Med., New York, NY

Abstract Body:

Epidemiological studies on the effects of the *S. pneumoniae* (Spn) conjugate vaccine in childhood show that its major benefit is the ability to block the establishment of nasopharyngeal colonization and to reduce Spn transmission in the population. In this study, we sought to understand how anti-*S. pneumoniae* immunity affects transmission from host to host. We hypothesized that a critical factor in transmission is the number of Spn shed from the nasopharynx. Using an infant mouse transmission model, we examined the role of immunity on shedding of Spn by pups infected at 4 days-of-life.To study the role of immunity in infant mice, it was necessary to induce immunity in the mother. Adult, female C57BL/6J mice were either infected intranasally (IN) with ~3000 CFU of streptomycin (str)-resistant type 23F or type 4 Spn isolates, or were mock infected. The females were then bred and their pups infected IN with the same strain. Nasal shedding was monitored for 5 days post-infection by tapping the nose of the pups on str plates and enumerating colonies. On day 13 of life the pups were euthanized. Serum was collected and assessed by whole cell ELISA for IgG to the Spn strain with which the mice were infected. Bacteria were collected from the upper respiratory tract by tracheal lavage and colonization was measured by quantitative culture.ELISA showed that sera of pups from infected mothers had a significantly higher anti-*S. pneumoniae* titer compared to pups from mock infected mothers ($p < 0.0001$). There was, however, no significant difference in Spn colonization between immune and non-immune pups. Despite similar levels of colonization, pups with a high anti-type 23F IgG titer shed significantly less Spn than pups from mock-infected mothers ($p < 0.0001$). Similarly, pups with a high anti-type 4 IgG titer shed significantly fewer bacteria than pups from mock-infected mothers ($p = 0.0054$). The effect of maternally-derived immunity of infected pups on transmission of Spn to uninfected littermates will be discussed. Our findings provide a possible mechanistic explanation for the reduced levels of Spn transmission among immunized children.

Author Disclosure Block:

T. Zangari: None. **Y. Wang:** None. **J.N. Weiser:** None.

Poster Board Number:

SATURDAY-609

Publishing Title:

Investigation of Potential Virulence Factors in a Novel *Pseudomonas* Species

Author Block:

E. Klempic, M. Brockett, J. E. Zvornicanin, B. L. Blocher, P. L. Lawrence, J. H. Shinn-Thomas, L. R. Aaronson; Utica Coll., Utica, NY

Abstract Body:

Pseudomonas sp. UC17F4 is a novel species that was isolated from the skin of red-backed salamanders collected in Central New York State. UC17F4 is a unique bacterial species that produces both extracellular pyomelanin and intracellular eumelanin. We have explored the possibility that bacterial melanins are a virulence factor utilizing the microscopic nematode *Caenorhabditis elegans* as a model host organism. *C. elegans* larvae were transferred to lawns of UC17F4 under conditions where the bacteria produced eumelanin. We observed that L1 and L2 stage larvae did not survive in the presence of this bacterium, while L3, L4, and adult worms did. In the present study, UC17F4 was grown on Lawrence minimal media (LMM), a citrate-enriched chemically-defined medium, that allows melanization. On LMM, UC17F4 caused complete loss of L1 stage worm viability between 12-24 hrs. When LMM was supplemented with 0.25% peptone (LMMP) to emulate the composition of Nematode Growth Medium and enhance melanogenesis, L1 worms died within 4 hours after transfer to bacterial lawns. We hypothesized that the rapid loss of viability on LMMP was due to the production of proteases, which could degrade the thin cuticle of larval worms, while the delayed loss of viability on LMM media was due to toxic effects of eumelanin as the worms consumed the bacteria. To test this hypothesis, protease production by UC17F4 biofilms was measured using a fluorometric enzyme assay. Three media formulations were used: LMM, LMMP, and citrate-free LMMP. All biofilm studies showed that the bacteria produced protease only in the citrate-free LMMP, suggesting that citrate represses protease production through carbon catabolite repression. These data also refute our hypothesis that proteases are responsible for the rapid death of worms on LMMP, because bacteria grown on citrate-free LMMP do not kill C. elegans. We also observed that UC17F4 produces higher levels of extracellular pyomelanin on LLMP than on the other media, which could contribute to the rapid loss of viability in the worms. Our findings indicate that protease is not a potential virulence factor in UC17F4, and we are continuing to identify other factors that may be responsible for quick death in juvenile nematodes.

Author Disclosure Block:

E. Klempic: None. **M. Brockett:** None. **J.E. Zvornicanin:** None. **B.L. Blocher:** None. **P.L. Lawrence:** None. **J.H. Shinn-Thomas:** None. **L.R. Aaronson:** None.

Poster Board Number:

SATURDAY-610

Publishing Title:

Bacterial Adrenergic Sensors Regulare Virulence of Enteric Pathogens in the Gut

Author Block:

R. M. Russell, V. Sperandio; Univ. of Texas Southwestern Med. Ctr., Dallas, TX

Abstract Body:

Enteric pathogens such as enterohemorrhacig *E. coli* (EHEC) and *Citrobacter rodentium*, which is largely used as a surrogate EHEC model for murine infections, are exposed to several host neurotransmitters in the gut. Epinephrine and norepinephrine have been extensively reported to increase virulence gene expression in EHEC, acting through two bacterial adrenergic sensors: QseC and QseE. However, EHEC is unable to establish itself and cause its hallmark lesions, attaching and effacing (AE) lesions, on murine enterocytes. To address the role of these neurotransmitters during enteric infection, we employed *C. rodentium*. Both EHEC and *C. rodentium* harbor the locus of enterocyte effacement (LEE) that is necessary for AE lesion formation within the colon. Here we show that expression of the LEE ,as well as other virulence genes is activated by epinephrine and/or norepinephrine, similarly to previous reports in EHEC. Both QseC and QseE are required for LEE gene activation in *C. rodentium*, and the qseC and *qseE* mutants are attenuated for murine infection. *C. rodentium* has decreased ability to colonize and cause disease in dopamine hydroxylase (Dbh -/-) knockout mice, which do not produce epinephrine and norepinephrine. Both adrenergic sensors, QseC and QseE are required for C. rodentium to sense epinephrine and norepinephrine during murine infection. Expression of the LEE genes is decreased in Dbh-/- animals compared to WT, and are mediated through QseC and QseE during murine infection. These data indicate that epinephrine and norepinephrine are sensed by bacterial adrenergic receptors during enteric infection to promote activation of their virulence repertoire.

Author Disclosure Block:

R.M. Russell: None. **V. Sperandio:** None.

Poster Board Number:

SATURDAY-611

Publishing Title:

Trefoil Factor Family Member 2 Regulates Susceptibility to *Escherichia coli* K1 Infection in Neonatal Rats

Author Block:

A. J. McCarthy[1], A. Redin Alonso[2], G. M. H. Birchenough[3], P. W. Taylor[1]; [1]Univ. Coll. London, London, United Kingdom, [2]London Sch. of Hygiene & Tropical Med., London, United Kingdom, [3]Univ. of Gothenburg, Gothenburg, Sweden

Abstract Body:

Background: Neonatal rat pups are susceptible in an age-dependent manner to systemic fatal infection following intestinal colonization of *Escherichia coli* K1. The incomplete development of gastrointestinal (GI) defences, including the mucus barrier, may facilitate translocation of colonizing bacteria into the bloodstream. Thus, two-day-old (P2), but not nine-day-old (P9), rat pups are highly susceptible to *E. coli* K1 infections. This is associated with suppressed expression of trefoil factor family member 2 (TFF2), a small peptide that stabilizes mucin and protects the GI epithelial layer. **Objective:** To investigate the role of trefoil factors in protection against *E. coli* K1 infection by characterizing the spatial expression of *Tff1*, *Tff2* and *Tff3* and mucin maturation during neonatal development, and by determining of the role of TFF2 in regulating infection susceptibility. **Methods:** Temporal changes in *Tff1*, *Tff2* and *Tff3* gene expression and mucin quality were assessed using qRT-PCR and immunohistochemistry in GI sections (P2, P4, P6 and P9), respectively. We compared the ability of *E. coli* K1 to colonize, translocate and cause systemic infection in wildtype and *Tff2*-/- rat P9 pups. **Results** *Tff1* and *Tff2*, but not *Tff3*, increased in expression in the proximal-, middle-, distal- small intestine and colon over the P2 to P6 developmental period, then decreased from P6 to P9. The mucin layer underwent substantial maturation from P2 to P9. The absence of TFF2 in rats did not impact *E. coli* K1 colonization of the GI tract, but did significantly alter the progression to a bacteremic state over 7-days (wildtype 0 %, *Tff2*-/- 100%). A proportion of *Tff2*-/- (22%), but not wildtype (0%), animals exhibited signs of severe systemic infection within 25 days. Two-dimensional bioluminescent imaging confirmed the presence of systemic infection in *Tff2*-/- rats. **Conclusions:** Maturation of the neonatal GI tract over P2 to P9 is accompanied by regulation of trefoil factor expression; in particular, TFF2 appears to play a key role in determining the susceptibility of neonatal rats to *E. coli* K1 infection.

Author Disclosure Block:

A.J. McCarthy: None. **A. Redin Alonso:** None. **G.M.H. Birchenough:** None. **P.W. Taylor:** None.

Poster Board Number:

SATURDAY-612

Publishing Title:

Pseudomonas aeruginosa* GroEL-Mediated Production of PTX3 Enhances the Phagocytic Clearance of *Staphylococcus aureus

Author Block:

H. Shin, **J. Jeon**, U. Ha; Korea Univ., Sejong, Korea, Republic of

Abstract Body:

Background: Infections are often not caused by a colonization of *Pseudomonas aeruginosa* alone but by a consortium of other bacteria. However, little is known about the impact of *P. aeruginosa* on the growth of other bacteria upon coinfection. **Methods**: We measured mRNA and protein levels of PTX3 by applying real-time Q-PCR and ELISA. The effect of PTX3 for the clearance of *Staphylococcus aureus* was examined by a phagocytosis analysis. **Results**: We found that *P. aeruginosa* was capable of inducing the expression of PTX3, and the induction was mediated by secreted proteins present in culture supernatants obtained from *P. aeruginosa*. By fractionating and analyzing the culture supernatants, it was found that the responsible protein was GroEL encoded by PA4385 of *P. aeruginosa*. Site-directed mutated GroEL lost its ability to increase the expression of PTX3 compared to its intact GroEL. Since PTX3 plays an important role in increasing the phagocytosis of microbes, we determined that *P. aeruginosa* GroEL-mediated production of PTX3 indeed enhances the phagocytic clearance of *S. aureus*. **Conclusions**: *P. aeruginosa*-derived GroEL is able to increase the production of PTX3, contributing to increasing the clearance of competitive *S. aureus* infection against same host during coinfection.This research was supported by Basic Science Research Program (NRF-2013R1A1A2059846) and the BK21 plus program of the Ministry of Education, Korea.

Author Disclosure Block:

H. Shin: None. **J. Jeon:** None. **U. Ha:** None.

Poster Board Number:

SATURDAY-613

Publishing Title:

Efflux Pump of Siderophore Staphyloferrin A in *Staphylococcus aureus* Contributes to Bacterial Fitness in Abscesses and Epithelial Cells

Author Block:

H. Nakaminami[1], Q. C. Truong-Bolduc[2], C. Chen[2], E. S. Kim[3], Y. Wang[2], D. C. Hooper[2]; [1]Tokyo Univ. of Pharmacy and Life Sci., Tokyo, Japan, [2]Massachusetts Gen. Hosp., Boston, MA, [3]Seoul Natl. Univ. Bundang Hosp., Gyeonggi-do, Korea, Republic of

Abstract Body:

Recently, two major facilitator superfamily (MFS)-type efflux pumps SfaA and SbnD which are involved in the secretion of the *Staphylococcus aureus* siderophores staphyloferrin A and staphyloferrin B, respectively, were characterized. We have already demonstrated that some staphylococcal MFS-type efflux pumps contribute to bacterial fitness in the murine abscess model and epithelial cells. Furthermore, we found the expression of *sfaA* and *sbnD* genes increased 31- and 4.1-fold in the abscesses, respectively. To assess the further function of SfaA and SbnD in *S. aureus* fitness, we tested its effect on the murine abscess model and intracellular replication in A549 epithelial cell line.We used *S. aureus* RN6390, its Δ*sfaA*, and Δ*sbnD* mutants. Susceptibilities of antimicrobial agents, fatty acids, and polyamines for parent strain RN6390, Δ*sfaA*, and Δ*sbnD* mutants were compared. Swiss Webster male mice that were 4 to 6 weeks old were used for the abscess model. Intracellular replication experiments for parent strain RN6390, Δ*sfaA*, and Δ*sbnD* mutants were performed using the ATCC CCL-185 human lung adenocarcinoma cell line A549.The knockout of *sfaA* and *sbnD* genes did not affect the susceptibilities of the tested chemicals. In competition assays of the murine abscess model using equal inocula of a Δ*sfaA* or Δ*sbnD* mutant and parent strain RN6390, the Δ*sfaA* mutant exhibited growth defects of 2.2-fold. Additionally, intracellular replication was decreased 2.8-fold in the Δ*sfaA* mutant. In complementation experiments, the Δ*sfaA* mutant carrying plasmid-encoded *sfaA* restored the growth fitness in abscesses and epithelial cells. However, Δ*sbnD* mutant showed no growth defect both in abscesses and epithelial cells.Our findings indicate that the efflux pump of the siderophore staphyloferrin A contributes to the ability of *S. aureus* replicate in abscesses and within epithelial cells.

Author Disclosure Block:

H. Nakaminami: None. **Q.C. Truong-Bolduc:** None. **C. Chen:** None. **E.S. Kim:** None. **Y. Wang:** None. **D.C. Hooper:** None.

Poster Board Number:

SATURDAY-614

Publishing Title:

Roles Of The Putative Type Iv-Like Secretion System Key Component Vird4 In Pathogenesis Of *streptococcus Suis* Type 2

Author Block:

X. Jiang, Y. Yang, J. Zhou, W. Fang; Zhejiang Univ., Hangzhou, China

Abstract Body:

Streptococcus suis type 2 (SS2) is a zoontic pathogen causing septic infection, meningitis, bronchopneumonia in pigs and humans. There were two major outbreaks in China in 1998 and 2005 with the infected persons exhibiting streptococcal toxic shock syndrome probably due to excessive release of inflammatory cytokines. However, the major bacterial factors that triggerd the cytokine storms remain unclear. The *S. suis* type 2 genome contains a small cluster of genes including virD4 encoding the putative type IV secretion component. To explore the functions of virD4 in SS2 pathogenesis, a virD4 in-frame deletion mutant was created and tested for virulence-related phenotypes in cultured cell lines and mouse model. We found that virD4 contributed to virulence as shown by about 2-fold increase of LD_{50} and easy clearance in mice with the delta-virD4 mutant, as compared with its parent strain. Deletion of virD4 rendered the bacteria more easily phagocytosed and readily killed in the whole blood, indicating that virD4 might be an anti-phagocytic factor. The delta-virD4 mutant showed reduced level of IL-6 and IL-1beta in the mouse model as well as in macrophage lines RAW264.7 (murine) and 3D4/2 (swine). To mimic the oxidative stress from respiratory burst, both the virD4 mutant and parent strains were subjected to hydrogen peroxide stress to examine differentially expressed proteins in the culture supernatants by MALDI-TOF/MS. Of these numerous proteins involved in DNA damage repair, nucleotide biosynthesis, carbohydrate metabolism and stress modulation, two conserved proteins attracted our attention: Peptidyl-prolyl isomerase (prsA) involved in protein secretion and folding was nearly 9-fold lower in the culture supernatant of the virD4 deletion mutant than its parent strain, while tagatose 1,6-diphosphate aldolase (LacD.1) involved in lactose/galactose metabolism and in regulation of virulence factors was high in the virD4 deletion mutant. Their relationship to virD4 and antiphagocytic activities as well as other functions in SS2 pathogenesis are being explored.

Author Disclosure Block:

X. Jiang: None. **Y. Yang:** None. **J. Zhou:** None. **W. Fang:** None.

Poster Board Number:

SATURDAY-615

Publishing Title:

Influence of Sae and Agr Regulated Factors on the Escape of *Staphylococcus aureus* from Human Macrophages

Author Block:

L. Münzenmayer[1], T. Wolz[1], B. Schulte[1], S. E. Autenrieth[1], M. Fraunholz[2], **C. Wolz**[1]; [1]Univ. Tübingen, Tübingen, Germany, [2]Biozentrum Würzburg, Würzburg, Germany

Abstract Body:

Background: *Staphylococcus aureus* is not a classical intracellular pathogen, but can survive within phagocytes and many other cell types. The pathogen is also able to escape from cells by mechanisms that are only partially understood.**Methods:** We constructed and analysed a series of isogenic *S. aureus* mutants of the USA300 derivative JE2 for their capacity to destroy human macrophages from within. The fate of bacteria was monitored by cell counting, fluorescence microscopy and image stream analysis.**Results**: Intracellular *S. aureus* JE2 caused severe cell damage in human macrophages, and could efficiently escape from within the cells. Damage of macrophages induced by intracellular bacteria was neither linked to activation of apoptosis related caspases 3, 7 or 8 nor to NLRP3 dependent inflammasome activation. To obtain this full escape phenotype including an intermittent residency in the cytoplasm the combined action of the regulatory systems Sae and Agr were required. Mutants in Sae or mutants deficient in the Sae target genes *lukAB* and *pvl* remained in high numbers within the macrophages causing reduced cell damage. Mutants in the regulatory system Agr or in the Agr target gene *psmα* were largely similar to wild type bacteria concerning cell damage and escape efficiency. However, these strains were rarely detectable in the cytoplasm emphasizing the role of PSMs for phagosomal escape.**Conclusion**: Sae regulated toxins largely determine damage and escape from within macrophages, whereas PSMs are mainly responsible for the escape from the phagosome into the cytoplasm.

Author Disclosure Block:

L. Münzenmayer: None. **T. Wolz:** None. **B. Schulte:** None. **S.E. Autenrieth:** None. **M. Fraunholz:** None. **C. Wolz:** None.

Poster Board Number:

SATURDAY-616

Publishing Title:

***In Vivo* Infections with Isogenic 'Phase-Locked' Mutants Illustrate Differences in Colonization Characteristics of Two Major Expression Variants of a Mycoplasma Multigene Family Encoding Variable Surface Lipoproteins**

Author Block:

R. Chopra-Dewasthaly, S. Hegde, W. Jechlinger (Deceased), J. Spergser, R. Rosengarten; Inst. of Microbiol., Univ. of Vet. Med., Vienna, Austria

Abstract Body:

Though implicated to play important roles in mycoplasma-host interactions, the biological significance of large multigene families causing phase variation of immunodominant surface antigens in mycoplasmas has never been directly proven. Using *M. agalactiae* and its high-frequency variable system of Vpma surface lipoproteins as a model, we investigated the *in vivo* significance of such a variable system by comparing the infection characteristics of two major expression variants (VpmaY and VpmaU) of type strain PG2. 'Phase-Locked Mutants' (PLMs), namely PLMU and PLMY, served as ideal tools as they steadily expressed a single Vpma product without further switching. PLMU was found to be outcompeted by PLMY during co-challenge experiments using the sheep intramammary as well as the conjunctival infection model. Quantitative and qualitative mycoplasma testing was performed on various clinical samples collected during infection and necropsy. Vpma phenotypes in recovered mycoplasma populations were assessed via colony immunoblots using monospecific anti-Vpma rabbit hyperimmune antisera. Results indicated an almost complete dominance of PLMY in local infection sites (udders/milk samples, eye/nasal swabs) and repression of the isogenic mutant PLMU in early stages of infection. Apart from these co-infection experiments, sheep were also infected individually with PLMU and PLMY exclusively via the intramammary route. Most interestingly, during the first 24 h p.i., milk obtained from sheep infected with PLMU showed a rapid decline in mycoplasma counts, clearly demonstrating a defect in the host colonization potential of PLMU. In contrast, the CFU counts for PLMY started increasing in milk after 2 h p.i. Results indicate that mycoplasmas expressing VpmaY show a better colonization and survival in the host than those expressing VpmaU. These results lead to the interesting hypothesis that *M. agalactiae* can shift from a local stage to a more systemic stage under certain conditions by switching the expression to VpmaU. This is the first report where mycoplasma PLMs are used in infection studies to prove the *in vivo* significance and differential roles of phase-variable lipoproteins

Author Disclosure Block:

R. Chopra-Dewasthaly: None. **S. Hegde:** None. **W. Jechlinger (Deceased):** None. **J. Spergser:** None. **R. Rosengarten:** None.

Poster Board Number:

SATURDAY-617

Publishing Title:

Mycoplasma agalactiae* Induces Cytopathic Effects in Infected Cells Cultured *In Vitro

Author Block:

S. Hegde, S. M. Hegde, R. Rosengarten, **R. Chopra-Dewasthaly**; Inst. of Microbiol., Univ. of Vet. Med., Vienna, Austria

Abstract Body:

Virulence determinants of mycoplasmas are complex and largely unknown. The disease pathology and the severity of symptoms are usually attributed to the immune responsiveness of the host rather than to the direct effect of the pathogen. This study concentrates on *Mycoplasma agalactiae* that causes Contagious Agalactia in sheep and goats, mainly characterized by mastitis, keratoconjuncitivitis and arthritis. Although the typical pathological features of tissue damage in cases of mastitis are often correlated with host inflammatory cells, the direct role of *M. agalactiae* on host cell damage has never been studied. Here, we investigated the effect of *M. agalactiae* infection on cultured cells *in vitro* using HeLa cells as model system. Compared to the uninfected controls, the presence of *M. agalactiae* was shown to alter the cell morphology, observed 36 h after infection as considerable cell elongation, cytoplasm shrinkage and plasma membrane blebbing. Chromatin condensation and increased caspase-3 cleavage in infected cells after 48 h of infection suggests an apoptosis-like phenomenon. Additionally, *M. agalactiae* infection led to growth retardation and lysis of HeLa cells. Measurement of the amount of LDH released after *M. agalactiae* infection revealed a time- and dose-dependent increase in cell lysis. A significant decrease in LDH release after gentamicin treatment of infected cells confirmed the direct role of cytadhering mycoplasmas in inducing cell lysis. This is the first study demonstrating the ability of *M. agalactiae* to induce irreversible cytopathic effects in infected cells which likely play a role during *in vivo* infection and disease.

Author Disclosure Block:

S. Hegde: None. **S.M. Hegde:** None. **R. Rosengarten:** None. **R. Chopra-Dewasthaly:** None.

Poster Board Number:

SATURDAY-618

Publishing Title:

Functional Characterisation of Meningococcal Neisseria Autotransporter Lipoprotein (NALP)

Author Block:

O. Dufailu[1], N. J. Oldfield[1], A. Aslam[2], J. Mahdavi[1], D. A. A. Ala 'Aldeen[1], K. G. Wooldridge[1]; [1]Univ. of Nottingham, Nottingham, United Kingdom, [2]Umm al-Qura Univ., Mecca, Saudi Arabia

Abstract Body:

Neisseria meningitidis is normally a human nasopharyngeal commensal but is also capable of causing life-threatening septicaemia and meningitis. Autotransporter (or type V-secreted) proteins are an important class of virulence factors found in many Gram-negative pathogens including Neisseria species. Eight autotransporters have been identified in meningococci: IgA1 protease, NhhA, AutA, AutB, NadA, App, MspA and NalP (also known as AspA). NalP is a phase-variably expressed serine protease which cleaves a number of cell surface proteins including itself, MspA, App, IgA1 protease, Lactoferrin-binding protein B (LbpB) and Neisserial heparin-binding protein A (NhbA). The consequences of this proteolytic activity on meningococcal pathogenesis are yet to be fully determined, but have already been shown to influence the sensitivity of meningococci to killing by human whole blood and the ability of meningococci biofilm formation. To enhance our understanding of the role of NalP during meningococcal pathogenesis, we purified functional recombinant NalP passenger domain under non-denaturing conditions using immobilized nickel chromatography. We demonstrated host cellular uptake of the purified recombinant NalP passenger domain using confocal microscopy. Furthermore, NalP was shown to be proteolytically active in *in vitro* assays, and to cleave a number of host proteins likely to play important roles in host-pathogen interactions.

Author Disclosure Block:

O. Dufailu: None. **N.J. Oldfield:** None. **A. Aslam:** None. **J. Mahdavi:** None. **D.A.A. Ala 'Aldeen:** None. **K.G. Wooldridge:** None.

Poster Board Number:

SATURDAY-619

Publishing Title:

***pseudomonas Aeruginosa* Isolates From Chronically Infected Patients With Cystic Fibrosis Show Host Tolerance By *ex Vivo* Viability Studies**

Author Block:

S. Yuan, P. Macpherson, Q. Xuan; Seattle Children's Hosp., Seattle, WA

Abstract Body:

Background: *Pseudomonas aeruginosa* (*Pa*) frequently colonizes the respiratory tract of patients with cystic fibrosis (CF) and is associated with increased severity and mortality in these patients. However, it is known that CF airway adapted *Pa* never invades host blood stream. This study is aimed at comparing bacterial host tolerance by examining *ex vivo* viability of wild-type and CF airway-adapted *Pa* in cultures containing human whole blood.**Methods:** Two strains of *Pa* were used for *ex vivo* bacterial viability studies using 1 ml aerobic cultures containing human whole blood from a healthy adult. While PA14 was used as a wild-type control, a PA-C3 strain was a methionine auxotrophic variant from a CF patient with chronic *Pa* infection. Bacterial viability was examined by serial dilutions of cultures every 8 hours. Logarithmic transformed colony counts (logCC) were used for analysis. Culture materials were also smeared by Gram and Wright stains for observation of bacterial and blood cell morphology.**Results:** Significantly different bacterial *ex vivo* viabilities were detected between PA14 and PA-C3 in cultures containing human whole blood. With the inoculating bacterial concentration of logCC 5.96 for PA14 and logCC 5.78 for PA-C3 respectively, the viability of PA14 rapidly declined to be undetectable with logCC of <2.00 while PA-C3 logCC of 4.45 at 8 hours. Although PA14 and PA-C3 both showed decline in viability, their respective logCCs within the first 24 hours remained significantly different shown by Δlog range of 1.00-2.45 between the two strains. Staining microscopy showed a similar range based on the presence of bacteria, but host cell disintegration started visually recognizable at 24 hours and rapidly thereafter.**Conclusion:** PA-C3 is more tolerant to host immunity shown by its less affected viability *ex vivo* in cultures containing human whole blood materials in the first 24 hours. In contrast to CF *Pa*, wild type PA14 can be rapidly cleared by host blood materials in the first 24h when host cellular materials remain relatively intact. The characteristic tolerance to host clearance of auxotrophic PA-C3 is reflective of symbiotic changes selected long-term within a localized host environment.

Author Disclosure Block:

S. Yuan: None. **P. Macpherson:** None. **Q. Xuan:** None.

Poster Board Number:

SATURDAY-620

Publishing Title:

The Therapeutic Potential of Dnase I in the Treatment of Acute Infective Endocarditis Caused by *Staphylococcus aureus*

Author Block:

H. Chi-Chieh, Chiau-Jing Jung, Jean-San Chia; Oral Biology, Taipei, Taiwan

Abstract Body:

Background: *Staphylococcus aureus* is the most common etiologic agent of acute infectious endocarditis (IE), which is a bacterial infection on the damaged or prosthetic heart valves . Pathologically, IE is featured by the presence of vegetation on the injured heart valve, which is composed of bacteria biofilm and platelet-fibrin complex. Our previous results showed that neutrophil extracellular traps (NETs) induced by bacteria-platelet interaction promote the vegetation formation and digestion of NETs with DNase I prevents vegetation expansion in the streptococcal experimental infectious endocarditis rat model. However the effect of DNase I in *S. aureus*-induced acute IE is unknown. **Method and results:** In *S. aureus*-induced experimental IE rat model, we found microaggregates of bacteria form biofilms with NETs, platelets and fibrins inside the vegetation, which is distinct from what we observed in streptococcal experimental IE rat model. However, DNase I could also significantly decrease bacteremia and the spread of bacteria, and prevent vegetation formation in the rat with acute experimental IE caused by *S. aureus*. **Conclusion:** The underlying mechanism of the pathogenesis of acute IE caused by *S. aureus* is quite different with chronic IE caused by oral streptococci, and DNase I is a potentially new therapeutic approach in the controlling of IE.

Author Disclosure Block:

H. Chi-Chieh: None.

Poster Board Number:

SATURDAY-621

Publishing Title:

Variable Virulence in Mice of O26:H11 *Escherichia coli* Strains That Encode Stx2d and Intimin

Author Block:

E. Trojnar[1], C. Petro[1], P. Mariani-Kurkdjian[2], A. O'Brien[1], **A. R. Melton-Celsa**[1]; [1]USUHS, Bethesda, MD, [2]Hôpital Robert Debré, Paris, France

Abstract Body:

Background: Shiga toxin (Stx)-producing *E. coli* (STEC) strains can cause diarrhea-associated disease outbreaks and, potentially, the life-threatening sequela of infection called the hemolytic uremic syndrome (HUS). There are two major types of Stx, Stx1 and Stx2, which are typically encoded within inducible lysogenic bacteriophages. In a recent publicaion, several HUS-associated O26:H11 STEC isolates from France were characterized. Some of those strains were reported to encode an intestinal mucus-activated Stx2 subtype called Stx2d and the adhesin intimin. In this study, we evaluated the virulence in mice of six of those clinical isolates.**Methods:** The activation capacity or inducubility of the toxin produced by the strains was measured on Vero cells after incubation with mouse intestinal mucus at a concentration of 1 mg/ml or with 0-15 ng/mL ciprofloxacin (Cip). *In vivo* studies were done in streptomycin (Str)- or Str- and Cip-treated male BALB/c mice.**Results:** *In vitro* the Stx2d from all strains showed similar levels of cytotoxicity and degrees of activatability. However, the capacity of the toxin to be induced by Cip from the different O26:H11 isolates ranged from 0- to 600-fold. Although all of the clinical isolates came from patients with the HUS, we observed significant differences in mouse virulence: two strains caused lethality with or without Cip treatment of the mice, two caused disease only when the animals were treated with Cip, and finally, for two isolates, we only observed slight weight loss or mortality regardless of whether the mice were treated with Cip or not.**Conclusion:** Our study highlights the difference in the mouse pathogenicity of six O26:H11 STEC clinical isolates that make Stx2d. We hypothesize that the virulence differences among the strains might be associated with variable toxin induction by Cip and *in vivo* Stx2d levels. The fact that some of these strains only caused mortality when induced by Cip underscores the importance of not treating STEC-associated diarrhea with an antibiotic that can induce toxin expression.

Author Disclosure Block:

E. Trojnar: None. **C. Petro:** None. **P. Mariani-Kurkdjian:** None. **A. O'Brien:** None. **A.R. Melton-Celsa:** None.

Poster Board Number:

SATURDAY-622

Publishing Title:

Novel Importer Proteins AliC and AliD Enhance Virulence of Nonencapsulated *Streptococcus pneumoniae*

Author Block:

J. L. Bradshaw, H. R. Pipkins, L. E. Keller, L. S. McDaniel; Univ. of Mississippi Med. Ctr., Jackson, MS

Abstract Body:

Nonencapsulated *Streptococcus pneumoniae* (NESp) is an emerging pathogen that colonizes the human nasopharynx and has been associated with noninvasive disease such as otitis media (OM) and pneumonia. Expression of the polysaccharide capsule is thought to be required to establish invasive bacteremia and meningitis; thus, current vaccines target the capsule of invasive serotypes. Surprisingly, recent surveillance of invasive pneumococcal disease (IPD) isolates has correlated NESp expressing oligopeptide permeases AliC and AliD with IPD cases. Our study investigates the impact of AliC and AliD on virulence of emerging NESp. MNZ85 and MNZ41 are NESp carriage isolates that express AliC and AliD. Isogenic deletion mutants of *aliC* and *aliD* were created in MNZ85 and MNZ41. Pneumococcal adhesion and invasion of human epithelial cells was assessed utilizing Detroit 562 pharyngeal and A549 pulmonary cells. In vitro assays examining biofilm production and pneumolysin expression were also performed. Pneumococcal colonization was examined in a mouse model following intranasal challenge. Additionally, virulence during an OM infection was investigated in a chinchilla model, and bacterial survival within chinchilla whole blood was quantified in an in vitro assay. The deposition of host immune complement factor, C3b, on the bacterial surface was also measured. Significant differences in epithelial cell adhesion and invasion varied among bacterial strains and epithelial cell lines. Biofilm viability and production increased in deletion mutants, but pneumolysin expression significantly decreased when AliC or AliD was absent. The expression of AliC and AliD enhanced murine nasopharyngeal colonization and was required for OM in a chinchilla model. Furthermore, a deletion of AliC or AliD drastically reduced NESp survival in chinchilla whole blood. Depostion of C3b on the bacterial surface diminished when AliC and AliD were present. Altogether, our data demonstrates that virulence of NESp is enhanced by the presence of AliC and AliD and gives insight as to how relatively avirulent NESp are associated with IPD. Further characterization of NESp virulence mechanisms is becoming more essential as vaccine efforts continue to result in rising NESp isolations.

Author Disclosure Block:

J.L. Bradshaw: None. **H.R. Pipkins:** None. **L.E. Keller:** None. **L.S. McDaniel:** None.

Poster Board Number:

SATURDAY-623

Publishing Title:

Expression of the Nonencapsulated Virulence Protein PspK in Encapsulated *Streptococcus pneumoniae*

Author Block:

H. R. Pipkins, L. E. Keller, J. L. Bradshaw, L. S. McDaniel; Univ. of Mississippi Med. Ctr., Jackson, MS

Abstract Body:

Streptococcus pneumoniae colonizes the human nasopharynx and can cause a wide range of diseases. Most pneumococcal infections are associated with encapsulated pneumococci, making the capsular polysaccharide the target of licensed pneumococcal vaccines. This selective pressure is causing an increased distribution of non-vaccine serotypes, including nonencapsulated *S. pneumoniae* (NESp). A subset of NESp express pneumococcal surface protein K (PspK). We have shown that PspK increases nasopharyngeal colonization, which is required for disease to occur. Due to the natural competence of pneumococci, we predict encapsulated strains could obtain NESp virulence genes. Acquisition of these genes could produce novel, more virulent strains, thus leading to an increase in pneumococcal disease. Therefore, the purpose of our study was to assess the effects of PspK expression in encapsulated pneumococci. An ectopic plasmid expressing PspK was used to generate $PspK^+$ strains of encapsulated pneumococci D39, EF3030, WU2, and TIGR4, serotypes 2, 19F, 3, and 4, respectively. We also developed capsule deletion mutants (Δcap) of each strain via allelic replacement and transformed those mutants with the $PspK^+$ plasmid. We then examined the adhesion and invasion of these strains to human pharyngeal or lung epithelial cells. To determine effects in vivo, we used a mouse model of nasopharyngeal colonization and a chinchilla model of otitis media (OM). PspK expression significantly increased EF3030 and D39 adhesion and invasion of lung cells. WU2 adhesion to pharyngeal cells was also increased, while TIGR4 and D39 pharyngeal adhesion was not significantly altered by PspK expression. In vitro adhesion/invasion results for the Δcap mutants varied. PspK expression did increase murine colonization of the Δcap mutants, but did not alter murine colonization of the encapsulated strains. Expression of PspK increased CFU recovered from our TIGR4 OM model as well. Although PspK expression does increase cellular adhesion of encapsulated pneumococci in vitro, we conclude that it does not increase murine nasopharyngeal colonization in these strains. However, PspK expression does in part compensate for loss of virulence by Δcap mutants in a mouse model. Thus, NESp virulence factor PspK may have a strain dependent impact on pneumococcal virulence.

Author Disclosure Block:

H.R. Pipkins: None. **L.E. Keller:** None. **J.L. Bradshaw:** None. **L.S. McDaniel:** None.

Poster Board Number:

SATURDAY-624

Publishing Title:

Copper at Host-pathogen Interface During Urinary Tract Infection

Author Block:

S. Subashchandrabose; Wake Forest Sch. of Med., Winston-Salem, NC

Abstract Body:

Urinary tract infection (UTI) caused by uropathogenic *Escherichia coli* (UPEC) is one of the most common bacterial infection in humans. Recently, we established the transcriptional profile of UPEC clinical isolates *in vivo* (UPEC RNA isolated from UTI urine sample) and *in vitro* (cultured in urine from healthy volunteers and in LB), using RNA-seq. UPEC genes involved in copper detoxification (*cop*, *cus*, and *cue*) were found to be specifically up-regulated during human UTI. We further demonstrated that: (i) urine Cu content in patients with UTI is higher than healthy subjects; (ii) UPEC undergoes Cu stress during colonization of the human urinary tract; (iii) In mice, UPEC mutants lacking a Cu efflux system are attenuated compared to the parental strain; and (iv) oral Cu supplementation reduces bladder colonization by UPEC in mice. However, the mechanism of copper mobilization to urine remains unknown and is the major question addressed here. Ceruloplasmin is the predominant copper-binding protein in mammals. Therefore, we hypothesized that ceruloplasmin is the source of copper in urine. Urine samples from UTI patients and healthy volunteers were collected and analyzed by Western blotting to detect the presence of ceruloplasmin. Our results indicate that ceruloplasmin is present only in UTI urine samples but not in urine from healthy volunteers. Addition of copper or purified ceruloplasmin to urine from healthy volunteers results in up-regulation of UPEC copper efflux genes, similar to that observed during human UTI. This observation indicates that increased Cu efflux gene expression in UPEC during UTI is a specific response to the presence of copper and ceruloplasmin. Currently, we are investigating the role of UPEC *cop* copper efflux system on the pathogenesis of UTI in a mouse model of infection. In summary, we present evidence for the molecular source of copper found in urine during UTI. Our findings shed new light on the host-pathogen interaction during bacterial UTI.

Author Disclosure Block:

S. Subashchandrabose: None.

Poster Board Number:

SATURDAY-625

Publishing Title:

Study of the Virulence Induced by T3ss and T6ss in *Bordetella bronchiseptica*

Author Block:

M. Cartelle Gestal, K. Dewan, E. Harvill; Univ. of Georgia, Athens, GA

Abstract Body:

Background:*Bordetella* spp. Include important emerging pathogens which infect humans, as well as domestic and wild animals. *B. bronchiseptica* has the widest host range providing researchers a reliable model for the study of *Bordetella spp*. Previous studies have described virulence factors in *Bordetella* spp. but knowledge of their regulation and contributions to virulence remain limited.In order to understand the virulence factors and their regulations, the aim of this study is to explore the role of T3SS and T6SS secretion systems in interactions with the host.**Methods:**The strains used in this study are; RB50 (wild type *B. bronchiseptica*), RB50ΔbscN (a T3SS knock out mutant), RB50ΔclpV (a T6SS knock out mutant) and RB50ΔbscNΔclpV (a double knock out mutant missing both T3SS and T6SS). Mutants were created by allelic exchange. Secreted proteins were precipitated with 21% ammonium sulphate. Cytotoxicity assays were performed using mouse macrophages (264.7 Raw cells).**Results:**Significant differences in the secreted proteins were observed between the wild-type and the three mutant strains. Proteins well expressed in the wild-type were found to be under-expressed in the knock-out mutants (figure 1). These results indicate that T3SS and T6SS play a major role in the protein expression profile in *B. bronchiseptica*.Cytotoxicity assays revealed that the single mutants RB50ΔbscN and RB50ΔclpV showed decreased cytotoxicity in comparison to the wild-type strain RB50 while cytotoxicity of the T3SS/T6SS double mutant was considerably increased.**Conclusions:**The greater cytotoxicity of the double mutant, relative to single mutants and wild type, suggests that with the removal of both secretion systems an alternative virulence pathway is activated in this strain.

Author Disclosure Block:

M. Cartelle Gestal: None. **K. Dewan:** None. **E. Harvill:** None.

Poster Board Number:

SATURDAY-626

Publishing Title:

***Leptospira interrogans* Promotes a Decrease in Antithrombin III and Prothrombin Serum Levels**

Author Block:

L. G. Fernandes[1], A. F. Filho[2], G. Souza[2], S. Vasconcellos[2], E. Romero[3], A. L. Nascimento[1]; [1]Inst. Butantan, São Paulo, Brazil, [2]Univ. of São Paulo, São Paulo, Brazil, [3]Inst. Adolfo Lutz, São Paulo, Brazil

Abstract Body:

Background: Pathogenic bacteria of the genus *Leptospira* are the causative agent of leptospirosis, an emergent infectious disease that affects human and animals worldwide. Severe forms of the disease in humans include jaundice, multiple organ failure and intense hemorrhage. Up to now, mechanisms associated with the hemorrhage foci are yet poorly understood. The relevance of the interaction between coagulation and inflammation in response to severe infection is becoming increasingly clear. Acute inflammation due to a severe infection or trauma results in a systemic activation of coagulation. **Methods:** Thrombin inhibitors and prothrombin levels in human serum samples were evaluated by clotting assays and western blotting. The binding of different leptospiral strains to antithrombin was performed by western blotting. The activation of coagulation (consumption of inhibitors and factors) was validated in hamster model of infection. **Results:** Despite the low levels of antithrombin III in convalescent human serum samples, virulent, culture-attenuated and saprophyte strains of *Leptospira*, are unable to bind and/or degrade this thrombin inhibitor, suggesting an indirect mechanism of pathogenesis. Lower levels of prothrombin were found in serum samples at the onset and convalescent phase of the disease when compared to normal human sera. The concomitant decrease level of antithrombin III and prothrombin suggests a process of stimulated coagulation, which is corroborated by the increase of prothrombin fragment F1+2in the serum samples. The data obtained with hamsters experimentally infected strongly point out that hemorrhage is correlated with decreased levels of thrombin inhibitors and prothrombin. **Conclusion:** Inflammation elicited by leptospires could induce an activation of coagulation, which leads to a diffuse and systemic clot formation and exhaustion of coagulation factors, and subsequently hemorrhage.

Author Disclosure Block:

L.G. Fernandes: None. **A.F. Filho:** None. **G. Souza:** None. **S. Vasconcellos:** None. **E. Romero:** None. **A.L. Nascimento:** None.

Poster Board Number:

SATURDAY-627

Publishing Title:

Establishment of Pathogenic *Leptospira* Infection via One of the Natural Modes of Transmission, the Transdermal Route

Author Block:

N. NAIR[1], L. Richer[2], M. Gomes-Solecki[1]; [1]Univ. of Tennessee Hlth.Sci. Ctr., Memphis, TN, [2]Immuno Technologies Inc., Memphis, TN

Abstract Body:

Leptospirosis is caused by spirochetal bacteria of the genus *Leptospira* and is a major zoonotic disease infecting humans through contaminated water, soil and food sources. Rodents are the major reservoir for this bacterium, which colonizes the kidney and is shed in urine. According to WHO and CDC, direct contact of the contaminated source with mucosal membranes of the body, or abrasions on the skin, are the entry ports for infection. Hence understanding the natural mode of transmission is critical. In this study we aim to address this issue by studying one of the natural modes of transmission, the transdermal route.We experimentally infected C3H/HeJ mice with 10^8 CFU of *Leptospira interrogans* serovar Copenhageni by generating a transdermal wound on the dorsal skin of the mouse. Sterile 1xPBS was applied to the wound control group. Untreated mice were included as naïve controls. Body temperature and weight were monitored for 15 days post infection (dpi). Urine and blood samples were collected to determine bacterial load using quantitative PCR (qPCR). Upon euthanasia, kidney, lung and liver were excised to determine cytokine induction. Histopathology of kidney was performed using PAS staining. Antibody production (total as well as *Leptospira* specific) was determined from sera.There was significant hypothermia and loss of weight observed from 12 to 15 dpi. Data from blood qPCR revealed dissemination of *Leptospira* ($4x10^5$ bacterium/microliter blood) from day 6 until day 12 post-infection. Urine qPCR data indicated high *Leptospira* shedding, ($1x10^6$ spirochete/microliter) from day 10 onwards. Histopathological analysis revealed extensive damage to renal corpuscles, which is consistent with the amount of *Leptospira* detected in the kidney. There was significant induction of *Leptospira* specific IgM and IgG and of TNFa, IFNg, IL1b, MIP-2, RANTES, KC, and IL10 cytokines, suggesting pathogen induced inflammation.Our study shows that transdermal route of *Leptospira* infection is an efficient model for studying a natural mode of disease transmission and establishment of infection.

Author Disclosure Block:

N. Nair: None. **L. Richer:** None. **M. Gomes-Solecki:** N. Other; Self; Immuno Technologies Inc..

Poster Board Number:

SATURDAY-628

Publishing Title:

Polyamine Transport in Pneumococci: A Proteomics Perspective

Author Block:

A. N. Rai[1], L. A. Shack[1], E. Swiatlo[2], B. Nanduri[1]; [1]Mississippi Stat Univ., Mississippi State, MS, [2]Univ. of Mississippi Med. Ctr., Jackson, MS

Abstract Body:

Background: *Streptococcus pneumoniae* is the most common bacterial etiology of community-acquired pneumonia, bacterial meningitis and otitis media worldwide. Genomic plasticity, antibiotic resistance and extreme capsular antigenic variation complicates the design of effective therapeutic strategies. Polyamines such as putrescine, spermidine and cadaverine are essential for pneumococcal virulence. Stringent regulation of intracellular concentration of polyamines in bacteria is controlled by transport and biosynthesis. In pneumococcus, the polyamine transport operon, *potABCD* is highly conserved across different serotypes. We previously showed that a polyamine transporter mutant (*ΔpotABCD*) of *S.pneumoniae* TIGR4 could not survive in the lung tissue in a mouse model of pneumonia. Since polyamines have profound effects on protein expression, we performed expression proteomics to delineate the pneumococcal virulence factors regulated by polyamines. **Methods:** Here, we compared protein expression between the wild-type and *ΔpotABCD* in TIGR4 strain by 1D LC ESI MS/MS. Expression proteomics was validated by western analysis. **Results:** Our results show that the expression of pneumococcal proteins such as choline binding protein A (PcpA), choline binding protein E (CbpE), oligopeptide transport system permease protein (AmiC) and glycosyltransferase (Gtf1) increased in response to genetic deletion of polyamine transport system. In addition, capsular polysaccharide biosynthesis protein (Cps4F), pneumolysin (Ply), iron-compound ABC transporter (Sp_1032), oligopeptide-binding protein (AmiA), and aspartate-semialdehyde dehydrogenase (Asd) showed reduction in expression in response to deletion of transport operon. **Conclusion:** Here, we conclude that the failure of *ΔpotABCD* to establish infection in mice is due to reduced expression of critical pneumococcal virulence factors such as Ply and Cps4F to name a few. A thorough functional characterization of proteins responsive to altered polyamine metabolism can help develop intervention strategies against pneumococci.

Author Disclosure Block:

A.N. Rai: None. **L.A. Shack:** None. **E. Swiatlo:** None. **B. Nanduri:** None.

Poster Board Number:

SATURDAY-629

Publishing Title:

Identification and Regulatory Characteristics of *Vibrio vulnificus plp* Encoding a Phospholipase Essential for Pathogenesis

Author Block:

K. Jang, Z-W. Lee, S. Choi; Natl. Res. Lab. of Molecular Microbiol. and Toxicology, Dept. of Agricultural Biotechnology, Ctr. for Food Safety and Toxicology, and Res. Inst. for Agriculture and Life Sci., Seoul Natl. Univ., 08826, Seoul, Korea, Republic of

Abstract Body:

Mucin glycoprotein is a major component of mucus layer that is the major site of entry for enteric pathogens including *Vibrio vulnificus* and serves as the initiation surfaces for host-microbe interactions. To identify *V. vulnificus* genes induced by mucin, transcriptomes of *V. vulnificus* cells grown with mucin-containing media or exposed to the mucin-secreting HT-29 MTX cells were analyzed using RNA-seq. Among the genes specifically induced by exposure to mucin and mucin-secreting host cells, a gene, annotated as *plp* encoding a putative phospholipase Plp, was identified and further characterized. The amino acid sequences of *V. vulnificus* Plp (*Vv*Plp) were 67% identical to those of *Vibrio anguillarum* phospholipase (*Va*Plp). To examine the role of *Vv*Plp, a mutant with disruption of the *plp* gene was constructed by allelic exchanges, and its virulence was evaluated. Compared with the wild type, the *plp* mutant showed a low level of cytotoxicity toward the HT-29 MTX cells and reduced virulence in mice. The *plp* mutant exhibited significantly lower phospholipase activity that the wild type in the egg yolk emulsion plate, implying that *Vv*Plp contributes to the lipolytic activity of the pathogen and thereby is essential for the pathogenesis. Examination of global regulatory proteins on the expression of *plp* revealed that the transcription activator HlyU and cyclic AMP receptor protein (CRP) upregulate the *plp* expression. The cellular levels of HlyU and CRP were not significantly affected by one another, indicating that the regulator proteins function cooperatively to activate *plp* rather than sequentially in a regulatory cascade. The regulatory proteins directly bind to the upstream of the *plp* promoter P_{plp}. DNase I protection assays, together with the deletion analyses of P_{plp}, demonstrated that HlyU binds to three specific sequences centered at -174, -141.5, and -109.5, and CRP binds specifically to the sequences centered at -68. Consequently, the combined results indicated that *V. vulnificus plp* encodes a phospholipase essential for virulence and is cooperatively regulated by HlyU and CRP.

Author Disclosure Block:

K. Jang: None. **Z. Lee:** None. **S. Choi:** None.

Poster Board Number:

SATURDAY-630

Publishing Title:

Genome-wide Identification of Genes Required During Bloodstream Infection in Multi-drug Resistant *Acinetobacter baumannii*

Author Block:

S. Crepin, S. Smith, H. L. T. Mobley; Univ. of Michigan Med. Sch., Ann Arbor, MI

Abstract Body:

Acinetobacter baumannii, an opportunistic gram-negative bacterium, is rapidly emerging as a leading nosocomial pathogen, particularly among immunocompromised individuals and for patients in intensive care units. *A. baumannii* can infect a wide range of anatomic sites including the respiratory tract, the bloodstream, and wounds. Its long-term persistence on abiotic surfaces and resistance to disinfectants and antibiotics exacerbate the potential of this bacterium as a nosocomial pathogen. However, despite its clinical importance, relatively little is known about the molecular basis of *A. baumannii* pathogenesis. To address this, we constructed a transposon library of 50,000 mutants in the multi-drug resistant strain AB0057 and we performed transposon-directed insertion-site sequencing (TraDIS) experiments to identify mutants with fitness defects in the neutropenic mouse model of bloodstream infection. By comparing relative abundance of insertion sites within the genome in the inoculum and post-infection libraries, we identified a total of 1428 genes and 29 intergenic regions showing a fitness defect of at least 2-fold. By performing a Clusters of Orthologous Groups (COGs) analysis of the fitness factors, the most representative categories were amino acids transport and metabolism, transcription factors and cell wall, cell membrane, and envelope biogenesis categories. To confirm the TraDIS results, genetically defined mutants were constructed. Mutants in fimbriae, secretion systems, c-di-GMP metabolism genes and lytic murein transglycosylases exhibited a fitness defect in the murine spleens and livers. Work is in progress to define the molecular mechanisms of pathogenesis that involve these genes. In summary, using high-throughput screen and targeted mutations, we identified the complete set of genes required for *A. baumannii* infection of the bloodstream. These results represent a major step towards understanding the pathogenesis of *A. baumannii* and will allow us to formulate strategies to manage or prevent *A. baumannii* infections.

Author Disclosure Block:

S. Crepin: None. **S. Smith:** None. **H.L.T. Mobley:** None.

Poster Board Number:

SATURDAY-631

Publishing Title:

Iscr-Mediated Control Of The Hmurstuv Hemin Uptake System In *yersinia Pseudotuberculosis*

Author Block:

L. Schwiesow, H. Miller, 95060, J. Dreiling, N. Herrera, D. Balderas, V. Auerbuch; Univ. of California, Santa Cruz, Santa Cruz, CA

Abstract Body:

The HmuRSTUV hemin uptake system allows *Yersinia* species to uptake and utilize hemoproteins as a source of iron. However, excess heme is toxic and several bacterial heme tolerance mechanisms have been discovered. HmuR is a TonB-dependent outer membrane receptor for heme and hemoproteins. HmuS is thought to be involved in heme tolerance, while HmuTUV comprise a hemin ABC transporter, which transports heme and hemoproteins from the periplasmic space into the bacterial cytoplasm. Our lab previously demonstrated that the human gut pathogen *Yersinia pseudotuberculosis* requires the iron-sulfur cluster-containing global transcriptional regulator IscR to cause disease. Our initial transcriptome analysis revealed that IscR was important for controlling expression of *hmuSTUV*, but not *hmuR*. We hypothesized that IscR controls *hmuSTUV* independently of *hmuR*, perhaps to mitigate heme toxicity under certain conditions *in vivo*. Through use of transcriptional reporters, we show that IscR drives expression of *hmuSTUV* from an intergenic region between *hmuR* and *hmuS*, but cannot drive expression of the promoter upstream of *hmuR*. In addition, we demonstrate that a *Y. pseudotuberculosis* Δ*iscR* mutant has a survival defect when incubated in whole blood, but that overexpression of *hmuSTUV* in the Δ*iscR* genetic background restores this defect. Furthermore, overexpression of *hmuR* in the context of WT *iscR* decreases survival in whole blood. Considering that hemoproteins are a main source of iron in blood, these results suggest that IscR controls expression of *hmuSTUV* to allow for the detoxification of heme brought into the cell by HmuR and indicates that IscR may be important for *Yersinia* growth in blood during disseminated infection.

Author Disclosure Block:

L. Schwiesow: None. **H. Miller:** None. **J. Dreiling:** None. **N. Herrera:** None. **D. Balderas:** None. **V. Auerbuch:** None.

Poster Board Number:

SATURDAY-632

Publishing Title:

Co-Regulation of *Xanthomonas campestris* Iron Homeostasis, Chemotaxis, and Motility by Accessible Iron and a Novel Iron-Responsive Transcriptional Regulator XibR

Author Block:

S. S. Pandey, P. K. Patnana, S. Chatterjee; Ctr. for DNA Fingerprinting and Diagnostics, Hyderabad, India

Abstract Body:

Cellular iron homeostasis of pathogenic bacteria is crucial for survival, growth and proliferation inside the hosts to develop infections (1). Regulation of iron uptake/metabolism associated factors is poorly understood in plant bacterial pathogens. In *Xanthomonas*, Fur (ferric uptake regulator) is the sole known regulator for siderophore biosynthesis and iron uptake system (2). For the first time, we identified the involvement of a novel iron-responsive NtrC family transcriptional regulator (XibR) in the regulation of iron homeostasis associated functions in *Xanthomonas campestris* pv. *campestris* (Xcc) through a genetic screen for altered siderophore production. Our study revealed that XibR regulates the expression of genes involved in iron uptake/metabolism such as siderophore biosynthesis, iron storage, and ferric iron uptake genes. We found that XibR promotes chemotaxis and motility under the low iron condition in Xcc. In this study, we also reported that XibR-ferric iron complex directly binds to the promoters of *xss* (*Xanthomonas* siderophores synthesis), *mot*, and *flg* operons and regulates the expression differentially depending on availability of iron. We showed that XibR is required for optimal biofilm formation and virulence. XibR positively regulates the expression of biofilm and virulence-associated genes such as pili assembly chaperones, type-II/type-III secretion systems and effectors, *hrp* genes, and certain virulence-associated factors. We also identified the role of Xcc siderophore in infection establishment by promoting survival and growth of bacteria under the low iron environment of the host.

Author Disclosure Block:

S.S. Pandey: None. **P.K. Patnana:** None. **S. Chatterjee:** None.

Poster Board Number:

SATURDAY-633

Publishing Title:

Mechanisms of Differential Virulence between *Streptococcus pneumoniae* and *Streptococcus mitis*

Author Block:

H. E. Marshall[1], F. C. Petersen[2], J. S. Brown[1]; [1]Univ. Coll. London, London, United Kingdom, [2]Univ. of Oslo, Oslo, Norway

Abstract Body:

Streptococcus pneumoniae and *Streptococcus mitis* are naso-oropharyngeal commensals that are genetically similar. However, *S. pneumoniae* is highly pathogenic and a common cause of pneumonia and septicaemia, whereas *S. mitis* rarely causes disease. We hypothesise that differences in sensitivity to innate immunity may underlie these differences in virulence phenotype. We compared sensitivity of *S. pneumoniae* and *S. mitis* to neutrophil killing. After opsonisation with serum but not with heat-treated serum or PBS, *S. mitis* was markedly more sensitive to neutrophil killing compared to *S. pneumoniae*. These differences suggested *S. mitis* was relatively complement sensitive, and flow cytometry assays of C3b/iC3b deposition confirmed there was increased complement opsonisation of *S. mitis* compared to *S. pneumoniae*. *S. pneumoniae* resistance to complement is partially dependent on binding of the immune regulator Factor H by the surface protein, PspC. We investigated Factor H binding to *S. mitis* using flow cytometry. The results demonstrated that there was no significant factor H binding to *S. mitis*. By inserting *pspC* of *S. pneumoniae* into *S. mitis*, we demonstrate that expression of PspC enabled *S. mitis* to then bind Factor H. Investigation of C3b/iC3b confirmed a decrease in opsonisation. Furthermore, survival in whole human blood of this modified strain showed an increase, when compared to the wild-type strain. These results suggest that an inability to bind factor H might underpin *S. mitis* sensitivity to opsonisation with complement and neutrophil killing compared to *S. pneumoniae*, and therefore contribute to the differences in virulence between these two commensal species.

Author Disclosure Block:

H.E. Marshall: None. **F.C. Petersen:** None. **J.S. Brown:** None.

Poster Board Number:

SATURDAY-634

Publishing Title:

Production of Shiga Toxin Type 1 (Stx1a) Attenuates the Virulence in a Mouse Model of an O26:H11 *Escherichia coli* Strain That Makes Stx1a and Stx2a

Author Block:

C. D. Petro, E. Trojnar, A. Melton-Celsa, A. O'Brien; Uniformed Services Univ. of the Hlth.Sci., Bethesda, MD

Abstract Body:

Background: Stx-producing *E. coli* (STEC) cause food-borne outbreaks of diarrhea. The two major types of Stx produced by STEC are Stx1a and Stx2a. Although Stx1a is more toxic than Stx2a to Vero cells, Stx2a is more potent than Stx1a in mice. In addition, STEC strains that produce only Stx2a are more commonly linked to a serious sequela of disease, the hemolytic uremic syndrome (HUS), than isolates that make Stx1a or both Stx1a and Stx2a. In this study, we evaluated the virulence of an O26:H11 isolate that produces both Stx1a and Stx2a in a mouse model and determined the effect of passive immunization of the mice with neutralizing antibodies to either or both toxins.**Methods:** A clinical isolate of O26:H11 (stx_{1a}+, stx_{2a}+) was used for these studies. The level of cytotoxicity and degree of inducibility of toxin expression by the strain was measured on Vero cells without or with 15 ng/mL ciprofloxacin (Cip). *In vivo* studies were done in streptomycin (Str, 5g/L in drinking water)- or Str- and Cip- (5 ug/mouse administered by intraperitoneal injection) treated male BALB/c mice. Passive immunization of mice with antibodies against Stx1 or Stx2 was done by tail-vein or intraperitoneal injection.**Results:** *In vitro* toxin production was induced 10-fold from an overnight culture of bacteria grown in Luria Bertani broth supplemented with Cip. In the mouse model, Cip-treatment was required for the O26:H11 isolate to cause weight loss and mortality. Administration of neutralizing antibody against Stx2 to the Str- and Cip-treated and infected mice was protective. However, unexpectedly, when infected mice treated only with Str were given antibody to Stx1, weight loss and mortality was observed (in the absence of Cip).**Conclusions:** Our study shows that pathogenicity in a mouse model for this clinical O26:H11 isolate required Cip administration, most likely due to induction of toxin expression *in vivo*. However, when Stx1a was neutralized the strain became virulent in the absence of Cip, a finding that suggests Stx1a attenuates pathogenicity of this Stx1a+ Stx2a+ O26:H11 strain in mice. We hypothesize that in strains that produce both Stx1a and Stx2a, Stx1a functions to reduce the toxic effects of Stx2a.

Author Disclosure Block:

C.D. Petro: None. **E. Trojnar:** None. **A. Melton-Celsa:** None. **A. O'Brien:** None.

Poster Board Number:

SATURDAY-635

Publishing Title:

Comparison of Virulence Factors between Mdr and Mds *Pseudomonas aeruginosa* Strains

Author Block:

K. Suzuki[1], Y. Mano[1], Y. Nozawa[1], C. Otsuka[1], S. Ishikawa[1], S. Ohtani[1], Y. Shiotani[1], H. Takoi[2], K. Fujita[2], Y. Saito[2], A. Gemma[2], N. Furuya[1]; [1]Bunkyo Gakuin Univ., Tokyo, Japan, [2]Nippon Med. Sch., Tokyo, Japan

Abstract Body:

Background: *Pseudomonas aeruginosa* is a major source of nosocomial infections, and the emergence of multidrug-resistant (MDR) *P. aeruginosa* is a serious problem in many countries. *P. aeruginosa* synthesizes several extracellular products which can act as virulence factors, possesses the flagellum and pili which mediate bacterial surface translocations known as motilities. In order to further clarify the association between magnitude of multidrug-resistance and its effect on *P. aeruginosa* virulence factors, we here compared the production of extracellular products, the bacterial motilities, between MDR *P. aeruginosa* and multidrug-sensitive (MDS) *P. aeruginosa*.**Methods:** Fourteen clinical *P. aeruginosa* strains were used in this study; 7 strains were MDR *P. aeruginosa* and 7 were MDS *P. aeruginosa*. Strains resistant to imipenem (MIC≧16μg/mL), amikacin (MIC≧32μg/mL), and ciprofloxacin (MIC≧4μg/mL) were defined as MDR *P. aeruginosa*, and strains sensitive to all these drugs were defined as MDS *P. aeruginosa*. Elastase and total protease activities, representatives of virulence factors, were determined using the elastin Congo red (Sigma) assay and the Remazol Brilliant Blue-Hide (Sigma) assay, respectively. Motilities of swimming, swarming and twitching were assessed using plate-based assay.**Results:** All mean values for production of elastase and total protease in MDR *P. aeruginosa* strains were significantly smaller than those in MDS *P. aeruginosa*. Mean spreading zones for swimming, swarming and twitching motilities in MDR *P. aeruginosa* strains were significantly smaller than those in MDS *P. aeruginosa* strains. Particularly significant difference was observed in twitching. The average (19.2mm) and median values (14.0mm) for the amounts of twitching in MDR *P. aeruginosa* strains were significantly greater than those (average 31.2mm; median 35.0mm) in MDS *P. aeruginosa* strains ($P<0.005$).**Conclusions:** Overall, this study demonstrates that the extent of multi-resistance in *P. aeruginosa* strains relates to the increase in amounts of the decrease in bacterial motilities, and the decrease in production of elastase and total protease.

Author Disclosure Block:

K. Suzuki: None. **Y. Mano:** None. **Y. Nozawa:** None. **C. Otsuka:** None. **S. Ishikawa:** None. **S. Ohtani:** None. **Y. Shiotani:** None. **H. Takoi:** None. **K. Fujita:** None. **Y. Saito:** None. **A. Gemma:** None. **N. Furuya:** None.

Poster Board Number:

SATURDAY-636

Publishing Title:

***Borrelia burgdorferi* Bb059 Is a Virulence Factor Important for the Development of Lyme Arthritis**

Author Block:

x. xiang, T. Lin, S. Carrasco; Indianapolis Univ. Sch. of Med., Indianapolis, IN

Abstract Body:

Lyme disease is the most commonly reported arthropod-borne infectious disease in the United States and Europe. The causative agent, *Borrelia burgdorferi*, infects humans via tick bites. Lyme arthritis is the major clinical symptoms infected with *B. burgdorferi* in North America. Little is known about the factors in *B. burgdorferi* contributing to the development of Lyme arthritis. In effort to identify virulence factors of *B. burgdorferi*, we have been focusing on putative hemolysin genes predicted in the *B. burgdorferi* genome. One such gene is BB0059, which has homology to hemolysin C of other bacteria. To study the function of BB059 in the infectious cycle of *B. burgdorferi*, we generated a *bb0059* mutant (*bb0059mut*). We found that *bb0059mut* had reduced infectivity in C3H/HeN mice. Furthermore, *bb0059mut* could establish infection in immunocompromised SCID mice, but it could not trigger Lyme arthritis. These data suggest that BB0059 is a new *B. burgdorferi* virulence factor important for mammalian infection and for the development of Lyme arthritis. Further investigation will focus on elucidation of the mechanism of action of BB0059 that leads to the defect in infection and Lyme arthritis.

Author Disclosure Block:

X. xiang: None. **T. Lin:** None. **S. Carrasco:** None.

Poster Board Number:

SATURDAY-637

Publishing Title:

Detection Of The Spi-2 Type Iii Secretion System Needle And Rod Proteins By Mouse Naips

Author Block:

C. Vientos, E. A. Miao; Univ. of North Carolina at Chapel Hill, Chapel Hill, NC

Abstract Body:

Type III secretion systems (T3SSs) are hollow, multiprotein molecular syringes that function to inject effector proteins, orchestrators of host signal modulation, into eukaryotic cells. Many Gram-negative bacterial pathogens encode a set of conserved, virulence genes that collaborate to construct this apparatus; however, *Salmonella typhimurium* possesses two pathogenicity islands (SPI-1 and SPI-2) that produce similar machines used at different points of infection. Host cells are equipped with Nod-like receptors (NLRs), as a defense mechanism, to survey the cytosol, and upon recognition of their cognate ligand, activate an innate immune pathway involving NLRC4 and caspase-1. Accidental injection of SPI-1 components, needle (PrgI) and rod (PrgJ) monomers, have been shown to activate NAIP 1 and 2. Here we further characterize the detection of the SPI-1 and SPI-2 rod and needle proteins by the NAIPs. We use a retroviral lethality screen with bone marrow derived macrophages of varying genotypes and transfections of purified protein. Our results will help lineate the complex nature of detection and evasion of inflammasomes by the *Salmonella* T3SSs.

Author Disclosure Block:

C. Vientos: None. **E.A. Miao:** None.

Poster Board Number:

SATURDAY-638

Publishing Title:

Studying the Response of Alveolar Epithelial Cells in a Three-Dimensional Tissue-Equivalent Respiratory Model to Influenza A Virus Infections

Author Block:

R. Bhowmick, H. Gappa-Fahlenkamp; Oklahoma State Univ., Stillwater, OK

Abstract Body:

Influenza A virus (IAV) is the major agent for primary viral pneumonia and claims approximately 250,000 to 500,000 lives annually worldwide. Pathogenic IAV strains dysregulate cytokines in the lung alveoli, leading to a "cytokine storm", which results in a robust macrophage recruitment into the alveolar spaces. Currently, there are only a few *in vitro* models available for the study of alveolar immune responses during IAV infections. Monolayers of cultured cells (two-dimensional cell culture) is the most commonly used tool, however, this system does not recapitulate the *in vivo* pulmonary physiology, which requires intercellular communication occurring within a three-dimensional (3D) environment. Therefore, to study the immune responses of infected alveolar epithelial cells (AECs), macrophages and the transmigrating antigen presenting cell (APC) precursors, we have developed a novel tissue-equivalent respiratory model (TERM). The TERM is comprised of a 3D collagen-chitosan porous scaffold, seeded with human small alveolar epithelial cells (hSAECs). The hSAECs were maintained at an air-liquid interface (ALI) and were infected with either the highly pathogenic H1N1 or the mildly pathogenic H3N2 strains of IAV. Uninfected cells were included as controls. After infection, cytokine content in the conditioned media and the marker protein expression in the infected cells were measured. Our results show that H1N1 infections lead to a massive increase in cytokine expression of the hSAECs in the TERM. H1N1 infections also lead to a significant change in marker proteins in the hSAECs, while H3N2 infections did not result in a marked change in such marker proteins. The infected phenotype of hSAECs cultured in the TERM more closely resembled *in vivo* observations. Future work includes using the TERM to identify the key mechanisms associated with the ability of pathogenic strains of the influenza virus to attract and differentiate lung cells to a highly inflammatory phenotype. These mechanisms will provide new targets for preventative and therapeutic interventions of influenza infection that will be tested in the TERM.

Author Disclosure Block:

R. Bhowmick: None. **H. Gappa-Fahlenkamp:** None.

Poster Board Number:

SATURDAY-639

Publishing Title:

Cytomegalovirus Blocks the Development of Inflammation Anergy in Intestinal Macrophages by Enhancing Myd88-Dependent Nf-κB Signal Transduction

Author Block:

E. A. Dennis, L. Smythies, M. Shimamura, W. Britt, P. Smith; Univ. of Alabama at Birmingham, Birmingham, AL

Abstract Body:

Background: Healthy intestinal lamina propria macrophages (LpMs) are profoundly down-regulated for cytokine production (inflammation anergy) which is the consequence of near-absent MyD88 expression and dysregulated down-stream NF-κB signaling. Paradoxically, in the setting of cytomegalovirus (CMV) mucosal infection, LpMs are markedly pro-inflammatory, most notably in persons with compromised immune function. **Methods:** We investigated the mechanism whereby macrophages are non-inflammatory in normal mucosa but pro-inflammatory in CMV mucosal disease using primary human LpMs, peripheral blood monocytes, and a clinical isolate of human CMV (TR). CMV infection and replication was assessed in cells by confocal and RT-PCR. Inflammation anergy in macrophages was determined by stimulating cells with TLR4/5 ligands, then measuring cytokine (IL-6, TNF-α, IL-8 or IL-1β) production with ELISA and/or RT-PCR. NF-κB signal pathway intermediates (MyD88, TRAF6, p-IκB-α, p-NF-κB) were measured by ELISA, Western Blot and/or RT-PCR. **Results:** CMV-infection of primary LpMs did not enhance cytokine production in response to TLR4/5 ligation compared to mock-infected LpMs, suggesting that CMV infection does not reverse inflammation anergy in differentiated LpMs. To investigate whether CMV infection prevents the development of inflammation anergy, we used an *in vitro* model of monocyte-to-LpM differentiation, in which monocytes cultured in de-cellularized normal intestinal stroma differentiate into macrophages nearly identical to primary LpMs. Monocytes infected with CMV prior to differentiation were significantly up-regulated for TLR4/5 ligand-induced cytokine production, resistant to stroma-driven development of inflammation anergy, resistant to stroma-driven MyD88 proteosomal degradation, and were significantly enhanced for NF-κB activation compared to mock-infected monocytes.**Conclusions:** Systemic infection of monocytes by CMV before recruitment into the intestinal mucosa strategically promotes the development of pro-inflammatory rather than inflammation anergic LpMs that are primed by CMV to interact with bacteria through TLR4/5, leading to enhanced MyD88-dependent NF-κB signal transduction and increasing the potential for exacerbated mucosal inflammation.

Author Disclosure Block:

E.A. Dennis: None. **L. Smythies:** None. **M. Shimamura:** None. **W. Britt:** None. **P. Smith:** None.

Poster Board Number:

SATURDAY-640

Publishing Title:

Human Cytomegalovirus Induces And Sustains Akt Activity Through A Unique Modulation Of Its Regulators To Mediate Monocyte Survival

Author Block:

O. Cojohari, M. A. Peppenelli, G. C. Chan; SUNY Upstate Med. Univ., Syracuse, NY

Abstract Body:

In a primary human cytomegalovirus (HCMV) infection, monocytes are responsible for spreading the virus systemically. However, monocytes have a short lifespan of ~48 hours (h) in the circulation, after which, in the absence of survival stimuli, they naturally undergo apoptosis. We previously showed HCMV manipulates monocytes to survive past this "48h-viability gate". We found that HCMV entry into monocytes induced a rapid activation of Akt (a major pro-survival protein) within 15 minutes post infection (mpi). Pre-treatment with an Akt inhibitor prevented the virus-induced cell survival, suggesting the rapid activation of Akt leads to induction of early monocyte survival. Moreover, inhibition of Akt 24 hpi also resulted in cell death, indicating that sustained Akt activity is required to maintain the viability of infected cells. However, the mechanisms by which HCMV induces and sustains Akt activity remain unknown. We found the virus activated Akt to a greater extent and with different kinetics compared to M-CSF, another myeloid survival factor, suggesting HCMV uses a unique mechanism of regulating Akt. We previously showed that HCMV stimulates PI3K, Akt's main positive regulator. We now identified that of the three PI3K isoforms, HCMV preferentially uses the p110β isoform to both induce and maintain monocyte survival, in contrast to non-infected cells, which use p110δ. However, to maintain high Akt activity, we hypothesized, the virus not only induces Akt's positive regulator, but also must modulate Akt's negative regulators, PTEN and SHIP-1. Indeed, we found HCMV rapidly inactivates PTEN through phosphorylation by 24hpi. Moreover, SHIP-1 inhibition resulted in a decrease in Akt activity rather than an increase, which was restored upon add-back of SHIP-1's product, PIP2, suggesting HCMV somehow uses SHIP-1 as a positive and not a negative regulator of Akt. Overall, our findings uncover a unique mechanism exploited by HCMV to induce and maintain an enhanced activation level of Akt: by inducing PI3K, inhibiting the activity of the negative regulator PTEN, and hijacking the biological function of SHIP-1, reversing it into a positive regulator, which allows monocytes to survive past the critical "48h-viability gate" and effectively disseminate the virus in the body.

Author Disclosure Block:

O. Cojohari: None. **M.A. Peppenelli:** None. **G.C. Chan:** None.

Poster Board Number:

SATURDAY-641

Publishing Title:

Coxsackievirus B3 Containing Muscle-Specific MicroRNA Targets Induces Alleviative Inflammatory Cytokines Expression and T Cell Activation in Mice

Author Block:

F. He, Z. Xiao, Z. Liu, H. Yao, D. Cao, M. Feng, S. OuYang, Y. Li, Z. Liu; Capital Inst. of Pediatrics, Bei Jing, China

Abstract Body:

Background: Incorporation of cognate miRNA target sequences into viral genomes is an effective approach to attenuate viral tropism that has been applied to target several different viruses. In our previous study, CVB3 engineered to contain muscle specific miRNA targets were proved to have an attenuated replication capacity in the heart of mice, and high titer of neutralizing antibody were also induced. However, cellular immunity of mice immunized with engineered CVB3 were not known.**Methods:** In this study, mice were immunized with 10^4 PFU of engineered CVB3, wild-type and UV-inactivated CVB3, mice serum, heart and splenocytes on days 3,5,7,14 post immunization were collected , cytokines expression were detected by using Cytometric Bead Array method, T-cell activation and T-cell subset were detected by using Flow cytometry, histological analysis and T cell infiltration of the heart were also detected.**Results:** IFN-γ and TNF-α were found significantly decreased in the engineered CVB3 group than wild-type group on day 7(1.48±0.20 vs 8.03±4.70 for IFN-γ, 17.41±5.90 vs 40.31±11.05 for TNF-α, $p<0.05$), both cytokines were higher than the UV-inactivated group on day 5 (4.27±2.05 vs 1.36±0.94 for IFN-γ, 30.96±13.78 vs 7.49±5.23 for TNF-α). CD4+T and CD8+T cells in the splenocytes were detected to have a higher percentage than UV-inactivated CVB3 group on days 3(23.95±1.87 vs 17.59±3.53 for CD4+T and 11.25±1.84 vs 8.70±1.34 for CD48+T ,$p<0.05$), activated CD4+CD69+T and CD8+ CD69+T cells were lower than that of CVB3 wild-type group on day 5 (1.15±0.52 vs 3.02±0.70 for CD4+CD69+T and 0.93±0.49 vs 2.05±0.68 for CD8+CD69+T, $p<0.05$). Th1 and Th17 have a lower percentage than CVB3 wild-type group. We also detected histological and CD4+T and CD8+T cells infiltration in the heart of mice, and found that engineered CVB3 induced only minor dropsy and hemorrhage, and CD4+T and CD8+T cells infiltration were also decreased compared with CVB3 wild-type group.**Conclusions:** Overall, our results indicated that engineered CVB3 could induce a mild immune response that may help against the CVB3 infection in the early stage/acute stage, and further support the rationale for using miRNA-targeting approach to develop live attenuate virus vaccines.

Author Disclosure Block:

F. He: None. **Z. Xiao:** None. **Z. Liu:** None. **H. Yao:** None. **D. Cao:** None. **M. Feng:** None. **S. OuYang:** None. **Y. Li:** None. **Z. Liu:** None.

Poster Board Number:

SATURDAY-642

Publishing Title:

Inflammasome Activation By Human Parainfluenza Virus Type 3

Author Block:

N. K. SHIL, S. M. Pokharel, S. Bose; Washington State Univ., Pullman, WA

Abstract Body:

Background: Human parainfluenza virus type 3 (HPIV3) is a RNA virus belonging to the paramyxovirus family that causes airway inflammation resulting in respiratory illness (croup, bronchiolitis and pneumonia) in children, elderly and immune-compromised individuals. Inflammasome activation results in maturation and production of pro-inflammatory cytokine interleukin-1β (IL-1β). Inflammasome is a oligomeric protein platform that cleaves pro-caspase-1 to yield enzymatically active caspase-1. Caspase-1 is involved in cleavage of pro-IL-1β into mature IL-1β. Although several RNA viruses are known to activate inflammasome, its activation status during HPIV3 infection is still unknown. In the current study we investigated whether HPIV3 activates inflammasome following infection of human macrophages. **Methods:** Human macrophage cell line THP-1 and ASC deficient THP-1 cells were infected with HPIV3. THP-1 cells treated with either caspase-1 inhibitor (Ac-YVAD-CHO), reactive oxygen species (ROS) inhibitor (Diphenyleneiodonium Chloride, DPI), and potassium efflux inhibitor (Glibenclamide) were also infected with HPIV3. Inflammasome activation was measured by - a) analyzing IL-1β levels (by ELISA) in the medium supernatant of HPIV3 infected cells, b) detecting cleaved IL-1β (p17 representing mature IL-1β) by Western blotting and c) examining cleaved portion of caspase-1 (p10 representing active caspase-1) by Western blotting. **Results:** Our studies demonstrated that HPIV3 activates ASC-inflammasome. We observed inflammasome activation in THP-1 cells infected with HPIV3. However, drastic reduction in inflammasome activation was noted in ASC deficient THP-1 cells. Inhibition of caspase-1 activation in THP-1 cells also led to loss of inflammasome activation. Inhibiting ROS production by DPI and blocking potassium efflux with Glibenclamide resulted in diminished inflammasome activation following HPIV3 infection. **Conclusion:** Our studies showed that HPIV3 activates ASC inflammasome in human macrophages. Furthermore, ROS and potassium efflux play an important role during HPIV3 mediated inflammasome activation. .

Author Disclosure Block:

N.K. Shil: None. **S.M. Pokharel:** None. **S. Bose:** None.

Poster Board Number:

SATURDAY-643

Publishing Title:

Respiratory Syncytial Virus Activates Tgf-β Signaling In Macrophages And Tgf-β Signaling Is Regulated By S100a9 Protein

Author Block:

S. M. Pokharel, S. Tsai, N. K. Shil, S. Bose; Washington State Univ., Pullman, WA

Abstract Body:

Background: Human respiratory syncytial virus (RSV) is the leading cause of lower respiratory tract infection and infant hospitalization. RSV infection manifest into two inflammatory diseases, pneumonia and bronchiolitis. Transforming growth factor-β (TGF-β) is an important cytokine regulating inflammation. RSV infectivity is regulated by TGF-β in epithelial cells. However, role of TGF-β during RSV infection of immune cells like macrophages is still unknown. Furthermore, activation of TGF-β pathway (i.e. SMAD2/3 pathway) has not been documented during RSV infection. In the current study we investigated whether - a) RSV triggers TGF-β production from macrophages, and b) RSV activates SMAD2/3 pathway in macrophages. Mechanism regulating TGF-β signaling during RSV infection is yet unknown. S100A9 is known to interact with TGF-β. Therefore, we also studied the role of S100A9 if any in regulating TGF-β production and TGF-β signaling following RSV infection. **Methods:** Mouse bone marrow derived macrophages (BMDMs) were infected with RSV. Active TGF-β and S100A9 protein production from infected cells was measured in medium supernatant by using ELISA assay. Cell lysates were used to assess activation of TGF-β pathway by Western blot analysis of phosphorylated SMAD2 (phospho-SMAD2). BMDMs were also treated with S100A9 blocking antibody (S100A9 Ab blocks extracellular S100A9 activity) during RSV infection. TGF-β production and TGF-β pathway activation in these cells were monitored as described above. **Results:** RSV infection of macrophages led to production of TGF-β and S100A9 proteins. RSV activated TGF-β pathway since we detected phospho-SMAD2 in infected cells. Although blocking S100A9 activity did not affect TGF-β production, S100A9 Ab diminished TGF-β signaling during RSV infection. Reduced levels of phospho-SMAD2 was detected in S100A9 Ab treated macrophages. **Conclusions:** Our studies demonstrated that RSV triggers TGF-β production from myeloid cells like macrophages. We also demonstrate for the first time that RSV activates TGF-β signaling (SMAD2/3 signaling) during infection. In addition, we have identified extracellular S100A9 protein as a positive regulator of TGF-β signaling during RSV infection.

Author Disclosure Block:

S.M. Pokharel: None. **S. Tsai:** None. **N.K. Shil:** None. **S. Bose:** None.

Poster Board Number:

SATURDAY-644

Publishing Title:

P2X Purinergic Receptors Are Key Regulators of HIV-1 Infection and Inflammation

Author Block:

T. H. SWARTZ, M. O'Brien, N. Durham, N. Bhardwaj, B. K. Chen; Icahn Sch. of Med. at Mount Sinai, NEW YORK, NY

Abstract Body:

Background: HIV-1 infection is incurable and causes a chronic inflammation which causes multiple comorbidities, even with virologic suppression. The mechanism of this inflammation is not clearly understood. Purinergic receptors are known to be mediators of inflammatory responses and can contribute to pro-inflammatory cytokine production and lymphocyte cell death. Purinergic receptor signaling is important for HIV-1 infection and we have recently found that inhibition of the P2X subtype purinergic receptors potently blocks HIV-1 productive infection at the level of membrane fusion. This study examines whether virus-induced purinergic signaling is responsible for inflammatory cytokine release during HIV-1 infection.**Methods:** We use fluorescent constructs of HIV-1 to evaluate productive infection by flow cytometry in CD4 T cell lines and primary cells in the presence or absence of purinergic inhibitors. Infected supernatants can be subjected to multiplex bead capture assays to test for an array of human cytokines. We have tested the effect of HIV-1 infection on peripheral blood mononuclear cells and observed levels of pro-inflammatory cytokine production. **Results:** We observe that HIV-1 productive infection in CD4 T lymphocytes is potently blocked by P2X selective inhibitors. Exposure of peripheral blood mononuclear cells to HIV-1 results in induction of pro-inflammatory cytokines and levels are reduced with P2X inhibition. This suggests that P2X inhibitors can block both HIV-1 productive infection and associated inflammation.**Conclusions:** Our findings distinguish purinergic receptors, specifically P2X, as key signaling mediators of HIV-1 infection and inflammation. We believe that these drugs could be used as adjunctive antiretroviral therapy that could serve to reduce the morbidity and mortality associated with HIV-1 chronic inflammation.

Author Disclosure Block:

T.H. Swartz: None. **M. O'Brien:** None. **N. Durham:** None. **N. Bhardwaj:** None. **B.K. Chen:** None.

Poster Board Number:

SATURDAY-645

Publishing Title:

Definition of the Hiv-1 Signalosome on Entry to Dendritic Cells

Author Block:

E. Khatamzas; Oxford Univ., Oxford, United Kingdom

Abstract Body:

Background: Scientific efforts to develop an effective HIV-1 vaccine have been hampered by the unprecedented genetic variability of the virus and its numerous strategies to subvert the function of immune cells, such as dendritic cells (DCs), allowing it to evade immune recognition, and persist in latent reservoirs. Here, DCs play a pivotal role in the initiation of immune responses and are one of the first cells to encounter HIV at mucosal surfaces. The mechanisms by which HIV avoids pattern recognition receptor mediated recognition in endosomes is unclear. **Methods:** Monocyte-derived DCs were infected with HIV-1 or mock for 10 minutes before purification of phosphoproteins. Phophoproteomics was used to identify proteins differentially phosphorylated by HIV-1. A secondary RNAi library was designed to assess the functional effect of these host proteins in transfer of HIV-1 from DCs to CD4+ T cells.**Results:** 340 proteins were differentially phosphorylated by HIV-1 within 10 minutes. These include proteins usually implicated in autophagy, neural synapse formation and trafficking. We identify a trafficking complex containing the biogenesis of lysosomal organelles 1, or BLOC-1, proteins that most strongly affect transfer of HIV-1 to CD4+ target cells. Mutations in BLOC-1 are responsible for Hermansky-Pudlak-Syndrome, a genetically heterogeneous disorder of intracellular vesicle biogenesis. We show that inhibition of Snapin, a component of BLOC-1, results in enhanced localization of HIV-1 in early endosomal (EEA1+) vesicles with TLR8 competent for cytokine and interferon responses. This effect of Snapin was not limited to HIV-1 sensing as removal of Snapin lead to enhanced TLR8 signaling and EEA-1/MyD88 localization following exposure to imidazoquinolones or single stranded RNA. Thus Snapin dissociates TLR8 from EAA1/MyD88 in a pathway utilized by HIV-1.**Conclusions:** Altogether, this suggests a mechanism by which HIV-1 virions hijack a physiological secretory pathway to evade lysosomal degradation and facilitate transfer to CD4+ T cells. Our findings reveal the complexity of the interaction of HIV-1 with the host cell machinery and identify novel mechanisms of the immune evasion tactics usurped by HIV-1 in DCs.

Author Disclosure Block:

E. Khatamzas: None.

Poster Board Number:

SATURDAY-646

Publishing Title:

Impact of Antiretroviral Initiation on Cytokine Levels and During Immune Reconstitution Syndrome in Hiv Patients

Author Block:

Y. A. Vargas Infante, MD, J. I. Lezama Mora, P. M. Del Rio Estrada, V. H. Ahumada Topete, A. Y. Rivero Arrieta, C. E. Ormsby, Y. A. Luna Villalobos, G. Reyes Terán; Inst. Natl. de Enfermedades Respiratorias, Mexico, Mexico

Abstract Body:

Background: The aim of this study was to describe the levels of cytokines at baseline, after start antiretroviral treatment (ART), and at the time of inflammatory reconstitution syndrome (IRIS) in a cohort of HIV-infected patients. **Methods:** HIV-infected adults, ART-naïve, with CD4 count <200 cell/mm^3, who agreed to participate were evaluated at baseline, and after ART initiation on weeks 7, 12, 24. In case of IRIS, an additional sample was obtained. Subjects were recruited from Jul-12 to Jun-14. Demographic and clinical data were collected. Multiplex assays were performed on plasma to determine the serum levels of inflammatory cytokines. We analyzed the relationship between the cytokines, according to the time of exposure to ART, and the occurrence of IRIS. **Results:** Twenty-seven patients were included in the analysis, 21 (78%) were men with median age of 36 (IQR 30, 41) yrs-old. Baseline CD4-count was 37 (IQR 11, 77) cell/mm^3. Sixteen (59%) developed IRIS after a median of 9 [IQR 7,15] weeks of ART. Shingles was the main cause associated to IRIS (n=9/16, 56%). After ART initiation, levels of MIG, and IP-10 were higher in patients of the IRIS group compared to those in the non-IRIS group (Table 1). During the IRIS event, the levels of HGF, IFN gamma, EGF, IL-12 and IL-15 increased. These levels were higher to those observed at the entire evolution of patients within the non-IRIS group. **Conclusions:** Patients who developed IRIS showed higher levels of MIG, and IP-10 after ART initiation, compared to the non-IRIS group. Interestingly, significant differences in levels of HGF, IFN gamma, EGF, IL-12 and IL-15 only emerged during the IRIS episode.

Table 1. Median cytokine levels at baseline and during the follow-up, comparison between the IRIS and non-IRIS groups.

Cytokine	Baseline[1]			First follow-up[2]			Second follow-up[3]			Third follow-up[4]				
	IRIS	Non-IRIS	P*	IRIS	Non-IRIS	P*	IRIS	Non-IRIS	P*	IRIS	Non-IRIS	P*	P** IRIS	P** Non-IRIS
MIG	688.46	452.8	0.42	487.05	329.09	0.09	352.44	158.64	0.027	310.16	124.81	0.02	0.11	0.051
MCP-1	611.56	445.9	0.95	398.98	376.66	0.36	348.19	312.54	0.81	298.96	267.05	0.97	0.001	0.32
IL-1Ra	180.73	131.6	0.01	63.35	80.74	0.25	54.23	63.51	0.67	46.79	52.84	0.47	0.0001	0.002
IL-2R	960.59	676.5	0.31	754.71	503.64	0.06	585.29	393	0.07	446.02	287.84	0.02	0.30	0.04
IP-10	219.91	126.06	0.06	206.89	67.13	0.002	116.05	57.97	0.004	64.5	38.34	0.005	0.004	0.08
HGF	96.05	76.5	0.39	118.14	96.06	0.18	101.44	81.20	0.18	63.22	56.87	0.26	0.041	0.04
MIP-1α	38.05	10.04	0.23	33.04	27.30	0.44	23.77	15.51	0.148	15.51	15.51	0.96	0.000	0.12
IFN-α	39.2	30.56	0.31	25.5	29.08	0.87	20.24	14.95	0.84	14.95	14.95	0.65	0.0001	0.20
IFN-γ	4.77	1.95	0.21	4.89	1.95	0.15	2.18	1.72	0.98	1.72	1.72	0.86	0.048	0.77
IL-13	19.8	11.4	0.04	14.38	8.81	0.09	7.53	8.18	0.07	8.79	8.35	0.86	0.0001	0.03
IL-6	9.08	6.64	0.30	4.65	4.56	0.76	3.03	2.79	0.2	2.11	1.8	0.54	0.09	0.02
EGF	3.51	2.83	0.34	6.15	2.85	0.5	1.5	1.11	0.24	1.3	1.5	0.06	0.025	0.11
IL-5	3.68	0.77	0.17	4.06	2.65	0.79	0.99	0.77	0.33	0.777	0.77	0.54	0.009	0.16
TNF-α	6.51	3.61	0.54	5.84	4.16	0.44	3.58	1.25	0.08	3.51	2.95	0.84	0.007	0.38

IQR, interquartile range; MIG, monokine induced by gamma interferon; MCP, monocyte chemoattractant protein; IL-1Ra, IL-1 receptor antagonist; IP-10, interferon gamma inducible protein 10; HGF, hepatocyte growth factor; MIP, macrophage inflammatory protein; IFN, interferon; EGF, epidermal growth factor; TNF, tumor necrosis factor. * Mann-Whitney **Kruskal-Wallis. [1] Baseline: median of 0.2 (IQR -1.0, 0) weeks before starting ART. [2] First, [3] second and [4] third follow-up were obtained after a median of 7.3 (IQR 4.42, 9.82), 12.1 (IQR 10.4, 15.03) and 24 (IQR 20.6, 25.4) weeks after starting ART, respectively.

Author Disclosure Block:

Y.A. Vargas Infante: None. **J.I. Lezama Mora:** None. **P.M. Del Rio Estrada:** None. **V.H. Ahumada Topete:** None. **A.Y. Rivero Arrieta:** None. **C.E. Ormsby:** None. **Y.A. Luna Villalobos:** None. **G. Reyes Terán:** None.

Poster Board Number:

SATURDAY-647

Publishing Title:

Porcine Circovirus Type 2 Activates Cammkβ to Initiate Autophagy in Pk-15 Cells by Increasing Cytosolic Calcium

Author Block:

Y. Gu, B. Qi, Y. Zhou, X. Jiang, X. Li, W. Fang; Zhejiang Univ., Hangzhou, China

Abstract Body:

Porcine circovirus type 2 (PCV2) has emerged as one of the most important pathogens in pigs since its initial recognition in 1998. It causes porcine circovirus-associated diseases (PCVAD) such as porcine dermatitis and nephropathy syndrome (PDNS), porcine respiratory disease complex (PRDC). PCV2 has a circular single-stranded DNA genome of about 1.7 kb that contains two major ORFs, ORF1 encoding the replicase protein (Rep and Rep') essential for transcription and ORF2 encoding the capsid protein (Cap) for genome packing. The pathogenic mechanisms underlying PCV2 infection remain largely unknown. Our early study shows that PCV2 induces autophagy via the AMPK/ERK/TSC2/ mTOR pathway in PK-15 cells and the autophagic response enhances viral replication. However, the mechanisms of AMPK activation in autophagy induction remain unknown. We used specific inhibitors and RNA interference of target signaling molecules in PCV2-infected cells in combination with Western blotting and flowcytometric and fluoresent calcium measurements. We provide clear evidence that that PCV2 infection upregulated CaMKKβ by increasing cytosolic Ca2+ via IP3R. Elevation of cytosolic Ca2+ did not seem to involve IP3 release from PIP2 by PLC-γ. The N-terminal two-thirds of PCV2 capsid protein played the major part in elevation of cytosolic Ca2+ and upregulation of CaMKKβ. CaMKKβ then activated both AMPK and CaMKI. PCV2 employed CaMKI and WIPI1 as another pathway additional to AMPK signaling in autophagy initiation. Our findings could help better understanding of the signaling pathways of autophagy induction as part of PCV2 pathogenesis. Further research is warranted to study if PCV2 capsid protein interacts directly with IP3R or indirectly with the molecules that antagonize IP3R activity responsible for increased cytosolic Ca2+.

Author Disclosure Block:

Y. Gu: None. **B. Qi:** None. **Y. Zhou:** None. **X. Jiang:** None. **X. Li:** None. **W. Fang:** None.

Poster Board Number:

SATURDAY-648

Publishing Title:

Anti-Viral Immune Response in the Central Nervous System Decreases Proliferation of Neural Stem/Progenitor Cells

Author Block:

A. Kulkarni, T. Scully, L. O'Donnell; Duquesne Univ., Pittsburgh, PA

Abstract Body:

Viral infections in the central nervous system (CNS) are characterized by lymphocyte infiltration into the brain parenchyma and release of pro-inflammatory cytokines. Interferon-gamma (IFN) is a key anti-viral cytokine that is required for non-cytolytic clearance of many viruses from the CNS. Our lab has demonstrated that IFN induces cell type-specific signaling cascades that dictate neural cell survival and proliferation. Here, we address the effects of IFN signaling on the proliferation and differentiation of neural stem/progenitor cells (NSPCs). We hypothesized that IFN would inhibit NSPC proliferation and induce glial differentiation via the Janus activated kinase (Jak)/Signal Transducers and Activators of transcription (STAT) signaling pathways. Using primary wildtype and STAT1-KO NSPCs, we show that IFN inhibits NSPC proliferation in a STAT1-dependent manner as measured through neurosphere growth and BrdU incorporation. IFN exerted a cytostatic effect on NSPCs by blocking cell cycle progression at the late G1/S checkpoint, which was mediated by dephosphorylation of the retinoblastoma protein (pRb) at serine 795 and decreased cyclin E/cdk2 expression. In addition, IFN increased astrocytic differentiation and reduced neurogenesis in vitro. During a neuron-restricted measles virus infection in neonatal mice, IFN protected the NSPC pool, but could not protect young neurons or preserve neurogenesis. Through these studies, we defined the role of IFN-mediated inflammatory signaling in modulating NSPC activity. These studies will also identify potential new targets that could salvage the developing CNS from disruptive effects of an anti-viral immune response. Funding for this work is provided by the NIH (1R15NS087606-01A1) and Duquesne University’s Mylan School of Pharmacy.

Author Disclosure Block:

A. Kulkarni: None. **T. Scully:** None. **L. O'Donnell:** None.

Poster Board Number:

SATURDAY-649

Publishing Title:

Roles of Nipah Virus Attachment, Fusion, and Matrix Proteins on Viral Assembly and Budding

Author Block:

K. Matz, G. Johnston, H. C. Aguilar; Washington State Univ., Pullman, WA

Abstract Body:

Nipah Virus (NiV), in the family *Paramyxoviridae* and genus Henipavirus, is a bio-safety level 4 agent with a mortality rate of 40-90% in humans. Transmission is zoonotic and extends to many mammals. Symptoms of human infection include encephalitis and pneumonia caused by the formation of large multi-nucleated cells termed syncytia. NiV uses two transmembrane glycoproteins to induce viral-cell membrane fusion and viral entry: the attachment (NiV-G) and fusion (NiV-F) glycoproteins. Additionally, the matrix (NiV-M) protein promotes assembly of viral proteins to bud virus-like particles (VLPs). We hypothesized that these three proteins would interact and affect incorporation of each other into VLPs. Previous research indicated that NiV-F buds autonomously, thus we also hypothesized that the cytoplasmic tail (CT) of NiV-F would interact with host cell factors to modulate VLP budding. To test these hypotheses we used a viral budding assay to quantify the production of VLPs after human embryonic kidney 293T cells were transfected with different combinations of NiV proteins including NiV-F CT mutants. Following transfection, VLPs were purified from cell supernatants using sucrose-based ultracentrifugation and analyzed using SDS-PAGE. Flow cytometry and SDS-PAGE densitometry were used to compare the protein expression present in VLPs relative to that on cell surfaces and within cell lysates. A budding index was calculated from the ratio of relative viral protein levels between VLP isolates and cell lysates. Results showed NiV-G incorporation into VLPs increased significantly from single-expression of NiV-G (7%) as compared to co-expression of NiV-F and NiV-G (27%) or NiV-M and NiV-G (35%). Additionally, mutations in the NiV-F CT decreased budding by 60-90% as compared to wild-type NiV-F levels. Lastly, in the presence of mutant cellular factors thought to interact with the NiV-F CT, budding was substantially decreased along with syncytial formation. In conclusion, NiV-M and NiV-F increase NiV-G's incorporation into VLPs, and we uncovered a role for the NiV-F CT in viral particle budding. A greater understanding about characteristics of NiV budding and infection may aid in the development of novel targets for antiviral treatments.

Author Disclosure Block:

K. Matz: None. **G. Johnston:** None. **H.C. Aguilar:** None.

Poster Board Number:

SATURDAY-650

Publishing Title:

Understanding Systems-Level Responses in *Clostridium ljungdahlii* through a Metabolic and Gene-Expression Model

Author Block:

J. Liu, A. Ebrahim, C. Lloyd, J-N. Kim, M. Al-Bassam, K. Zengler; Univ. of California, San Diego, La Jolla, CA

Abstract Body:

As an acetogen, *Clostridium ljungdahlii* can metabolize carbon monoxide (CO), carbon dioxide (CO_2), and hydrogen (H_2) (i.e. syngas) to produce multi-carbon organics. This ability, in addition to other attractive features such as its diverse substrate utilization, makes *C. ljungdahlii* an attractive organism for strain designed chemical production from cheap, renewable sources. To advance towards this goal, a constraint-based modelling method was used to systematize the biochemical, genetic and genomic knowledge of *C. ljungdahlii* into a mathematical framework, enabling a mechanistic description of metabolic physiology. This metabolic and gene expression model (ME-model) accounts for 961 ORFs that not only are responsible for the metabolic network, but also enable the production of transcriptional units, functional RNAs (i.e., tRNAs, rRNAs, etc.), prosthetic groups, and cofactors, as well as the formation and translocation of protein complexes. With this ME-model, we can now compute the molecular constitution of *C. ljungdahlii* as a function of genetic and environmental parameters. For instance, though low CO_2 uptake rate (i.e. low CO_2 partial pressure) may lead to a severe decrease in acetate production, the functional proteomic composition of *C. ljungdahlii* under high and low CO_2 partial pressure remains remarkably similar (Pearson $r = 0.98$, $P < 0.001$). In contrast, low and high fructose uptake rates lead to a more variable composition (Pearson $r = 0.80$, $P < 0.001$). Indeed, the ME-model provides a foundation for predicting and understanding the phenotype of *C. ljungdahlii*, which is vital for effective strain design.

Author Disclosure Block:

J. Liu: None. **A. Ebrahim:** None. **C. Lloyd:** None. **J. Kim:** None. **M. Al-Bassam:** None. **K. Zengler:** None.

Poster Board Number:

SATURDAY-651

Publishing Title:

Extensive Comparative Genomics Analysis of Epidemic and Reference Fish Isolates of *Aeromonas hydrophila S*trains

Author Block:

H. C. Tekedar, S. Kumru, A. Karsi, M. L. Lawrence; Mississippi State Univ., Mississippi State, MS

Abstract Body:

Aeromonas hydrophila is a Gram-negative, mesophilic bacterium that infects aquatic poikilothermic animals, mammals, and humans. Historically, *A. hydrophila* is an opportunistic pathogen of freshwater fish, but since 2009 the U.S. channel catfish industry has been affected by epidemics where *A. hydrophila* is a primary pathogen. To determine the genetic basis for this, we conducted genome sequencing of five *A. hydrophila* strains isolated from catfish pond epidemics (epidemic *A. hydrophila* or EAh) and five historical *A. hydophila* strains (reference *A. hydrophila* or RAh) using a Genome Analyzer IIx (Illumina, Inc., San Diego, CA) at the USDA ARS Catfish Genetics Unit. We recently reported completed genome sequencing of one EAh strain, ML09-119, which was isolated from diseased channel catfish during an outbreak of *A. hydrophila* in a commercial channel catfish pond. We also completed the sequence of one RAh strain, AL06-06, which was isolated from a goldfish in 2006 from the Auburn University Southeastern cooperative fish disease Laboratory in Greensboro, Alabama. We also improved the draft genomes from the other EAh and RAh strains. Results of genome comparisons indicated that only one of the RAh strains carries a full operon of the Type VI secretion system. None of the EAh strains carry plasmids, but three of the RAh strains carry plasmids. Moreover, EAh strains have a complete inositol catabolism pathway, while only one of the RAh strains has this pathway. Our findings will help elucidate the genetic mechanism for increased virulence of EAh strains compared to historical *A. hydrophila* isolates, which could enable improved diagnostics to distinguish EAh and vaccine development.

Author Disclosure Block:

H.C. Tekedar: None. **S. Kumru:** None. **A. Karsi:** None. **M.L. Lawrence:** None.

Poster Board Number:

SATURDAY-652

Publishing Title:

Transcriptomics-Based Genome Annotation of Penicillia

Author Block:

K. K. Pennerman, J. Akinsanya, P. Kurup, G. Yin, J. W. Bennett; Rutgers, The State Univ. of New Jersey, New Brunswick, NJ

Abstract Body:

Compared to plants, animals and bacteria, well-annotated genomic description of filamentous fungi is lacking. This dearth of knowledge limits our ability to understand fungal biology, even in well-known genera such as *Penicillium.* Collectively, the penicillia are fungi of major economic importance including plant pathogens, an opportunistic human pathogen, producers of antibiotics and contributors to a number of food fermentations. We used a bioinformatics approach to improve genome annotation of *Penicillium chrysogenum* (the species used for industrial production of penicillin) and *Penicillium digitatum* (a post-harvest pathogen of citrus fruits). We retrieved reference genomes and gene annotations from NCBI (http://www.ncbi.nlm.nih.gov/genome/) and JGI (http://genome.jgi.doe.gov/). *P. chrysogenum* RNA-seq data were obtained by our collaborators and published *P. digitatum* RNA-seq data were retrieved from the SRA database (http://www.ncbi.nlm.nih.gov/sra). Using gene prediction programs and other bioinformatics tools, we found over 300 previously unannotated, predicted gene sequences of many diverse functions for each genome. These predicted genes were found across the length of the genomes. Our results not only provide new candidates for genetic research, but also highlight the need for more evidence-based gene annotation.

Author Disclosure Block:

K.K. Pennerman: None. **J. Akinsanya:** None. **P. Kurup:** None. **G. Yin:** None. **J.W. Bennett:** None.

Poster Board Number:

SATURDAY-653

Publishing Title:

VRprofile: A Web-Based Tool for *In silico* Profiling of Virulence and Antibiotic Resistance Traits Encoded within Genome Sequences of Pathogenic Bacteria

Author Block:

H. OU, J. Li; Shanghai Jiaotong Univ., Shanghai, China

Abstract Body:

Background: Throughout the entire bacterial domain, a vast repertoire of antibiotic resistance and virulence-associated traits has spread via horizontal gene transfer of various mobile genetic elements. Several bioinformatics tools and databases have been developed to mine the WGS data for the virulence and/or resistance-related genes. However, users frequently have to manually examine the broader context of these sequences to confidently define the gene clusters coding for the entire genetic elements. Here we report a user-friendly web server, called 'VRprofile', as a public resource for performing rapid homology searches and comparisons of virulence and antibiotic resistance-related gene clusters in bacterial genome sequences of various pathogens.**Methods:** The utilized back-end database MobilomeDB was built upon sets of known gene cluster loci of bacterial mobile genetic elements, including prophages, integrative and conjugative elements, class I integrons, IS elements, pathogenicity/antibiotic resistance islands, and type III/IV/VI secretion systems. With discovery of homologous gene cluster based on sequence similarity and gene order, VRprofile is able to recognize most known bacterial virulence factors, antibiotic resistance determinants, and MobilomeDB-archived transfer-related gene clusters in the genomic sequence of a bacteria pathogen within 15 minutes. The server also includes other individual tools with complementary functionality, including CDSeasy that rapidly identifies and functionally annotates protein-coding genes in a raw input contigs/scaffolds/complete sequence, and CGCfinder that quickly performs homologous gene cluster discovery across a user-defined search database of bacterial genomes.**Results:** VRprofile is freely available to all users without any login requirement at http://bioinfo-mml.sjtu.edu.cn/VRprofile.**Conclusions:** By ensuring ease of flexible input options, VRprofile may contribute to meet the increasing demands of bacterial variable region analysis, and aid in real-time definition of disease-relevant gene clusters in pathogenic bacteria of interest.

Author Disclosure Block:

H. Ou: None. **J. Li:** None.

Poster Board Number:

SATURDAY-654

Publishing Title:

Identifying Functionally Important, Uncharacterized Genes in Microbial Communities

Author Block:

E. A. Franzosa[1], A. Shafquat[1], G. Rahnavard[1], E. P. Balskus[2], C. Huttenhower[1]; [1]Harvard T. H. Chan Sch. of Publ. Hlth., Boston, MA, [2]Harvard Univ., Cambridge, MA

Abstract Body:

Background: The prioritization of uncharacterized genes for further study is a challenging gap in microbial research. With thousands of microbial isolate genomes and metagenomes now sequenced each year, automated characterization is a must, isolate homology alone leaves many genes unannotated, and microbial community profiles provide a rich new resource for assigning putative functions for experimental follow-up. **Methods**: In this work, we present a computational method (PPANINI) for prioritizing genes for functional assignment based on their occurrence and properties within microbial communities, including population prevalence and abundance distributions. Our method complements existing approaches for prioritizing genes in the context of single isolate genomes, thus expediting microbial gene characterization efforts. **Results**: We applied PPANINI to collections of human (skin, vaginal, gut, and oral) and environmental (soil) metagenomes to 1) validate the method's performance and 2) identify important uncharacterized genes in these microbial communities. PPANINI correctly prioritized known essential genes as functionally important, while simultaneously downweighting putative lower-priority genes (e.g. non-biological ORFs and pseudogenes). In addition, PPANINI identified large numbers of putatively important but functionally uncharacterized genes, many of which were not prioritized by earlier methods (e.g. phylogenetic profiling). From among the prioritized genes we isolated 26 putative members of the glycyl radical enzyme (GRE) family, which catalyzes a range of important biochemical reactions in anaerobic metabolism. **Conclusions**: We are currently experimentally validating the GREs prioritized by PPANINI, thus demonstrating our method's utility in translating large volumes of gene sequence data to testable hypotheses. The open-source PPANINI software and documentation are available for download at http://huttenhower.sph.harvard.edu/ppanini.

Author Disclosure Block:

E.A. Franzosa: None. **A. Shafquat:** None. **G. Rahnavard:** None. **E.P. Balskus:** None. **C. Huttenhower:** None.

Poster Board Number:

SATURDAY-655

Publishing Title:

Systematic Bioinformatics Analysis of Virulence Associated Factors of *Cryptococcus neoformans* Revisits Different Function of Osmolarity Pathway in Serotypes A and D

Author Block:

A. N. Malachowski, M. Yosri, Y. He, M. A. Olszewski; Univ. of Michigan, Ann Arbor, MI

Abstract Body:

C. neoformans (C.neo) infection originates in the lungs from which it disseminates to CNS causing lethal pathologies. The cryptococcal survival in the lungs and its ability to disseminate into CNS is promoted by a large group of virulence associated factors (VAFs). To enhance comprehensive understanding of VAF roles and their possible relationships, we have gathered all of 284 reported VAFs of *C. neo.* from the scientific literature in a single database. Their relationships have been analyzed using protein sequence analysis tools and other bioinformatics resources: transmembrane localization software (TMHMM v 2.0), gene network mapping software (STRING), a gene pathway database (KEGG), intra-protein motif analysis software (GenomeNet) and the Broad Institute H99 database.Our study is the first to point out that Sho1, an osmosensor in the High Osmolarity Glycerol pathway (HOG), is present in *C. neo.* serotype A but not in serotype D. Analysis of KEGG diagrams and literature reveals that several proteins downstream from Sho1 were characterized in serotype D, suggesting that they may play different roles in either serotype. Furthermore, our network analysis indicates that the center of *C. neo.* virulence network is enriched in regulatory genes and especially kinases, consistent with their high potential for regulation. Finally, using the intra-protein motif analysis we confirmed the high frequency of kinase domains among the VAFs, and found other frequent domains such as the Major Facilitator Superfamily.Our analysis also revealed that the total genome contains 19.4% transmembrane proteins, while our VAF database 19%. While there was no enrichment in the proportion of transmembrane proteins, our results indicate VAFs tend to contain more transmembrane domains per protein than non-VAFs, suggesting larger and more complex proteins.In summary, this study generated a comprehensive database of *C. neo.* virulence factors and uncovered new information on the HOG pathway. It also revealed that *C. neo.* is a prime candidate for further bioinformatics studies, that will enhance understanding of virulence factor network in this and other pathogenic organisms.

Author Disclosure Block:

A.N. Malachowski: None. **M. Yosri:** None. **Y. He:** None. **M.A. Olszewski:** None.

Poster Board Number:

SATURDAY-656

Publishing Title:

Improving the Prediction and the Comparison of Genomic Islands in Draft and Complete Genomes

Author Block:

C. Bertelli, B. K. Dhillon, J. A. Shay, A. C. Lim, M. R. Laird, G. L. Winsor, F. S. L. Brinkman; Simon Fraser Univ., Burnaby, BC, Canada

Abstract Body:

Background: In bacterial and archaeal genomes, genomic islands (GIs) are commonly defined as clusters of genes with probable horizontal origins. They disproportionately encode medically important adaptations, including antimicrobial resistance (AMR) and virulence. Microbial genome sequencing has become rapid and inexpensive, but current computational methods for GI analysis are not amenable for rapid, accurate, user-friendly, and scalable analysis of the thousands of genomes being sequenced.**Material and Methods:** To improve the prediction of GIs, we have developed a new version of IslandPath-DIMOB, part of the IslandViewer suite of GI analysis tools, by implementing (i) a better score of dinucleotide bias to increase sensitivity, (ii) new HMM profiles to search for mobility genes, (iii) a better handling of pseudogenes and some bug fixes, and (iv) the concept of regions of GIs by considering closely positioned GIs as a single region. The performance of IslandPath-DIMOB was assessed against a dataset of GIs identified by comparative genomics. We have initiated the development of IslandViewer version 4 that facilitates expanded comparative visualization and analysis of a large number of genomes.**Results and Conclusion:** The new version of IslandPath-DIMOB has a notable increase in recall of greater than 10% for most genomes, while maintaining similar accuracy and precision. A novel IslandViewer interface extends circular and linear visualization of the GIs using interactive GenomeD3Plot. This web-based interface enables a smooth browsing of genome content and GI predictions, with overlaying annotation of genes and highlighted AMR and virulence factors. Genome analyses provided new insight into the evolution of AMR genes. The new features of IslandPath-DIMOB and IslandViewer will enable faster prediction and easier comparison of GIs in draft and complete genomes, facilitating direct use by biologists and clinicians.

Author Disclosure Block:

C. Bertelli: None. **B.K. Dhillon:** None. **J.A. Shay:** None. **A.C. Lim:** None. **M.R. Laird:** None. **G.L. Winsor:** None. **F.S.L. Brinkman:** None.

Poster Board Number:

SATURDAY-657

Publishing Title:

Prokaryotic Sequence Analysis Resources In The Analysis Engine

Author Block:

S. Nadendla, S. C. Daugherty, R. S. Adkins, J. Crabtree, S. Agrawal, A. Shetty, O. White, A. A. Mahurkar, M. Giglio; Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract Body:

It is now relatively inexpensive and easy to acquire the genome sequence of a prokaryotic species. However, effective analysis of that sequence data is still a challenge for many researchers. To address this need, the Institute for Genome Sciences (IGS) has developed the Analysis Engine (http://ae.igs.umaryland.edu), a series of pipelines installed on publicly accessible cloud resources or as stand alone downloadable virtual machines.Here we describe ongoing work to add additional pipelines and tools to the resource as well as to increase the scalability and efficiency of existing pipelines. The analysis resources currently available in the Analysis Engine include: 1) automated structural and functional annotation of single genomes using the IGS Prokaryotic Annotation Pipeline; 2) comparative genomic analysis for up to 100 genomes at a time; 3) transcriptome analysis providing differential expression results; and 4) assembly of genomes. We provide the tool Manatee for visualization and curation of genome data, and Sybil for visualization of comparative analysis results that include ortholog groups and syntenic regions. We have recently created a Manatee virtual machine (VM) that allows users the option to have complete control of their genome data at their local site, allowing them to easily engage in continuous curation independent of IGS infrastructure. In ongoing work we will continue to incorporate additional pipelines and tools into the service and will strive for further integration between our tools and other resources. Finally, the Analysis Engine has a significant outreach component and provides training through workshops held both at relevant conferences and on-site at the Institute for Genome Sciences at the University of Maryland School of Medicine in Baltimore Maryland.

Author Disclosure Block:

S. Nadendla: None. **S.C. Daugherty:** None. **R.S. Adkins:** None. **J. Crabtree:** None. **S. Agrawal:** None. **A. Shetty:** None. **O. White:** None. **A.A. Mahurkar:** None. **M. Giglio:** None.

Poster Board Number:

SATURDAY-658

Publishing Title:

Statistical Evidence for a Relationship between Phage Isolation Location and Shared Gene Families

Author Block:

B. Q. Dang, D. Jacobs-Sera, W. H. Pope, D. A. Russell, G. F. Hatfull; Univ. of Pittsburgh, Pittsburgh, PA

Abstract Body:

Mycobacteriophages are viruses that infect mycobacteria, including the causative agent of tuberculosis, *Mycobacterium tuberculosis*. As such, research in characterizing mycobacteriophage genomes may lead to insight for novel techniques for treatment of tuberculosis. From a data set of 767 sequenced and annotated mycobacteriophages that included GPS coordinates of where the phages were isolated, we investigated whether there was a relationship between geographical isolation location and gene content. The density-based spatial clustering of applications with noise (DBSCAN) algorithm was used to organize closely-isolated phages into groups, with a 1 mile search radius and a 3 neighboring-points requirement. After establishing these "geogroups" of phages isolated in close proximity, each geogroup's CLASP index was calculated. The CLASP index stands for Cluster Average Shared Phams, where phams refers to genes belonging to the same gene family. The CLASP index is a representation of how closely related a group of genomes are, and is calculated by averaging the percent of shared phams between all possible pairwise combinations within a cluster or group. The genomes and coordinates were then randomly shuffled, and a statistical t-test revealed that the average of all CLASP indices were significantly (pvalue: 0.00152) different than the average of all CLASP indices of the original, unshuffled data set. Being that the t-test identifies the data sets as significantly different, it implies that CLASP indices are nonrandom and are attributed to the specific, closely-isolated mycobacteriophages for a given geogroup.

Author Disclosure Block:

B.Q. Dang: None. **D. Jacobs-Sera:** None. **W.H. Pope:** None. **D.A. Russell:** None. **G.F. Hatfull:** None.

Poster Board Number:

SATURDAY-659

Publishing Title:

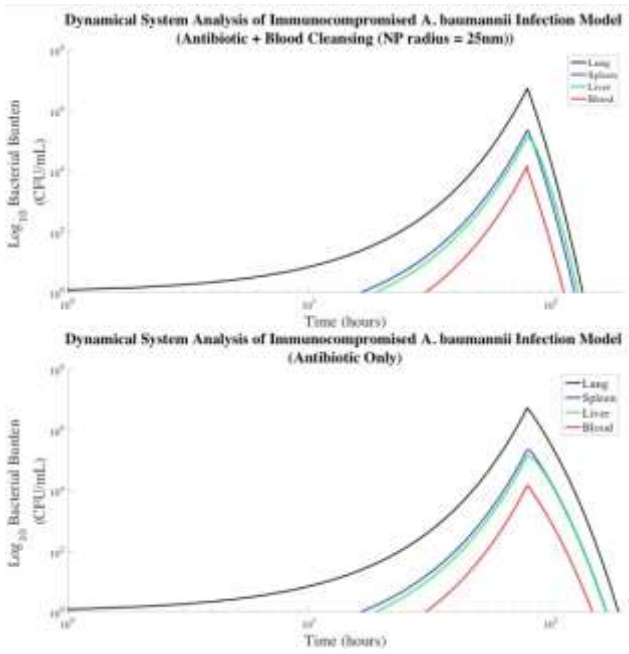
Mathematically Modeling the Biodistribution Kinetics of *Acinetobacter baumannii* Infection

Author Block:

S. E. Miller, C. S. Bell, M. McClain, T. Cover, T. D. Giorgio; Vanderbilt Univ., Nashville, TN

Abstract Body:

A multicompartmental pharmacokinetic model was developed to study the biodistribution of *Acinetobacter baumannii* in bacteremia. The model includes parameters to represent physical and immunological interactions, bacterial net growth, transport among tissues (lungs, spleen, liver, blood), antibiotic treatment, and extracorporeal blood cleansing. Five first-order homogenous ordinary differential equations were developed as a model representation of the physiological system. This dynamic systems analysis approach was used to explore the impact of extracorporeal blood cleansing based on magnetophoretic separation of nanoparticle-bacteria complexes from the blood in combination with antibiotic treatment. Previously published empirical data was used to assign values to model parameters. The validity of the model was confirmed through comparison with experimental data from scientific literature. This is the first multicompartmental model of bacteremia that includes extracorporeal elimination of bacteria from the bloodstream and antibiotic treatment. This kinetic model provides an approach to quantitatively assess bacterial clearance in complex living systems. The new addition of an extracorporeal circuit reduces the time of total bacteria clearance from the blood of an immunocompromised mouse model by 35 hours, compared to antibiotic treatment alone. Our results suggest that extracorporeal blood cleansing in combination with antibiotic treatment may significantly reduce *A. baumannii* concentration in blood and reduce the transport of bacteria to distant organs. These factors are critical contributors to disease progression, and thus, this combination treatment has the potential to significantly improve patient outcomes.



Author Disclosure Block:

S.E. Miller: None. **C.S. Bell:** None. **M. McClain:** None. **T. Cover:** None. **T.D. Giorgio:** None.

Poster Board Number:

SATURDAY-660

Publishing Title:

Detection of an Unusual Plasmid in Spm-1 Producing *Pseudomonas aeruginosa*

Author Block:

A. Nascimento[1], **W. Martins**[2], L. Fehlberg[2], M. Munoz[3], N. Lincopan[3], A. Vasconcelos[1], A. Gales[2]; [1]Lab. Natl. de Computação Científica, Petrópolis, Brazil, [2]Univ.e Federal de São Paulo, São Paulo, Brazil, [3]Univ.e de São Paulo, São Paulo, Brazil

Abstract Body:

Background: Plasmids are extra chromosomal DNA, which normally harbor genes involved in bacterial adaptability. However, due to high capacity of P. aeruginosa isolates incorporate into its genome the genes that confer great selective advantage, the detection of plasmids in this species is not common. Our aim was to evaluate if the presence of this unusual plasmid would confer any virulence advantages to the SPM-1-producing P. aeruginosa (SPM-1-PSA) ST277 clone.**Methods:** After studying the genomes of SPM-1-PSA recovered from the Brazilian territory, we identified two isolates harboring an unusual plasmid (pPSABr). Plasmid assembly was performed using Newbler v 3.0. SABIA pipeline was used for gene prediction and automatic annotation. The curing of plasmid using acridine orange and consecutive passages onto LB agar was carried out as previously described. The ability of bacteria to initiate biofilm formation on polystyrene plastic was evaluated. Virulence experiments were performed testing six isogenic SPM-1-PSA isolates previously sequenced including two strains carrying pPSABr. To assess the virulence of was SPM-1-PSA isolates in vivo using the Galleria mellonella model of infection.**Results:** Assembly of the plasmids showed that strains 3448 and 7790 carried an identical plasmid, pPSABr, with 49 Kb size and 58.89% GC content. A total of 61 ORFs were present in pSPM-1. Interestingly, 50 of 61 (≈ 82%) ORFs were annotated as hypothetical proteins without known function. No resistance genes were detected in pPSABr. Genes encoding for secretion systems types III and IV were detected in this plasmid. SPM-1-PSA not harboring pPSABr killed all *G. mellonella* (5 larvae) approximately in sixteen hours, while strains harboring pPSABr did it at 22 hours. Cure of pPSABr failed despite many attempts. All isolates were weakly adherent in the biofilm formation test.**Conclusions:** Despite carrying genes encoding secretion systems, the acquisition of pPSABr seems to decrease the pathogenicity of SPM-1-PSA clone ST277. Although pPSABr codifies proteins of unknown function, they may be important for the P. aeruginosa cell since pSPM-1 was stable. However, more studies are necessary to determine the function of proteins encoded by pSPM-1 genes.

Author Disclosure Block:

A. Nascimento: None. **W. Martins:** None. **L. Fehlberg:** None. **M. Munoz:** None. **N. Lincopan:** None. **A. Vasconcelos:** None. **A. Gales:** None.

Poster Board Number:

SATURDAY-661

Publishing Title:

Bacterial Proteins and Complexes: Evolution, Function, and Perturbations

Author Block:

J. H. Caufield, P. H. Uetz; Virginia Commonwealth Univ., Richmond, VA

Abstract Body:

Bacterial diversity reveals the colossal scale of genetic flux, especially among seemingly well-conserved proteins. The functions of many of these protein components remain unclear. Even the most well-studied *E. coli* strains contain genomes with hundreds of open reading frames of unclear function. Just 1% of published bacterial protein sequences have annotations from experimental results. With the goal of finding novel functional context, we studied thousands of uncharacterized bacterial proteins with a bifurcated strategy: we used more than 300 *E. coli* model protein complexes to predict the presence of complexes across bacteria and combined published interactions into meta-interactomes of orthologous groups (OGs). We have also used bacteriophage proteins to illustrate protein relationships often missing from interactome studies but crucial to protein evolution.Computational analyses of more than 1,000 bacterial species and their protein complexes revealed how nearly all complexes deviate from the *E. coli* model in other species. Only 14 out of 285 model protein complexes are fully conserved across 95% of the ~1,000 genomes investigated. We then used a set of more than 50,000 protein interactions, combined into a meta-interactome, to focus on protein interaction conservation outside protein complexes. Our meta-interactome contains more than 43,000 distinct OG vs. OG interactions involving proteins from more than 250 bacterial species yet only 68 OG-OG interactions have evidence from than two species. This approach provides evidence for interaction predictions beyond what existing experimental data sets provide. The meta-interactome provides evidence for interactions for 951 of 4175 *Bacillus subtilis* proteins and 509 of 843 *Rickettsia prowazekii* proteins, both species without experimental protein interactomes.Together, these methods place proteins of unknown function in the context of well-conserved proteins and their interactions. We have explored the function of selected bacterial proteins, using a variety of studies, including the expression of a bacteriophage protein to perturb the host. We have developed methods to express phage proteins as sources of interactome disturbance and phenotype change. Identifying functions for bacterial proteins, whether they interact with phage proteins or not, may reveal novel physiological activities or potential antibiotic targets.

Author Disclosure Block:

J.H. Caufield: None. **P.H. Uetz:** None.

Poster Board Number:

SATURDAY-662

Publishing Title:

Heat Shock Protein 90 Shapes *Candida albicans* Genome Architecture

Author Block:

K. Dong[1], A. Forche[2], S. Milne[1], L. Alaalm[1], J. Berman[3], S. Diezmann[1]; [1]Univ. of Bath, Bath, United Kingdom, [2]Bowdoin Coll., Brunswick, ME, [3]Tel Aviv Univ., Tel Aviv, Israel

Abstract Body:

Hsp90 regulates the emergence of novel traits in animals, plants and fungi. It does so by stabilizing up to 10% of the eukaryotic proteome. In *Candida albicans*, Hsp90 is a key regulator of virulence and drug resistance. Since *C. albicans* does not engage in canonical meiosis to generate genetic variations, it has evolved a repertoire of alternative routes to diversify its genome. Forche *et al*. (mBio, 2011) demonstrated that Loss-of-Heterozygosity (LoH) provides a mechanism for generating genetic variation aiding in the response to environmental stresses and possibly adaptation. Indeed, stressors such as H_2O_2 or fluconazole altered rates and types of LoH events in *C. albicans*, suggesting that LoH generates genetic diversity thereby facilitating adaptation and ultimately evolution. We hypothesize that Hsp90 affects LoH rates and events in *C. albicans* and thus genome integrity and phenotypic variation. To test this hypothesis, we created reporter strains and conducted fluctuation assays measuring rates of LoH at 9 genomic loci located on 4 chromosomes. We were able to demonstrate that both, pharmacological inhibition and genetic depletion of Hsp90, increase LoH rates. Subsequent SNP-RFLP genotyping analyses at 2 loci indicated that reduced Hsp90 function not only alters LoH rate but more importantly the types of LoH events when compared with standard growth conditions. Pharmacological inhibition of Hsp90 increases local recombination like gene conversion or break-induced recombination events. Growth at high temperature results in increased whole-chromosome homozygosis. The emergence of different types of LoH in response to Hsp90 stress suggests that *C. albicans* may utilize distinct forms of LoH in response to different stressors. To characterize types of LoH with higher granularity than SNP-RFLP, we are currently conducting ddRAD sequencing, which facilitates the identification of karyotypic changes across the entire genome. We furthermore complement our genotyping efforts with fitness assays and measurements of drug resistance to establish a functional relationship between Hsp90, LoH and fitness. This would have theoretical and clinic implications and further our knowledge of how Hsp90 shapes an organism's genome structure with consequences for antifungal drug resistance and fungal virulence.

Author Disclosure Block:

K. Dong: None. **A. Forche:** None. **S. Milne:** None. **L. Alaalm:** None. **J. Berman:** None. **S. Diezmann:** None.

Poster Board Number:

SATURDAY-663

Publishing Title:

Mcrx, A Novel Modification- Dependent Restriction Enzyme In *e.Coli* B

Author Block:

A. Fomenkov[1], D. K. Dila[2], B. P. Anton[1], Z. Sun[1], R. J. Roberts[1], E. A. Raleigh[1]; [1]New England Biolabs, Ipswich, MA, [2]Univ. of Wisconsin-Milwaukee, Sch. of Freshwater Sci., Milwaukee, WI

Abstract Body:

Modification-dependent restriction enzymes (MDE) play two interconnected major roles in prokaryote evolution. First, they protect cells from phage infection, particularly from virulent phage with nonstandard nucleotides such as T4. Second they promote stability of genome lineages by restricting incorporation of genomic DNA from lineages with different DNA modification patterns. At least 10 families of such enzymes have been described, although for many families the mechanism of action is not clear. Understanding the diversity, distribution and mechanisms of action of these remarkable enzymes is critical to understanding microbial populations: how prokaryotic lineages are established, how the breakdown of the protections relates to acquisition of pathogenic potential, and how the interplay of MDE with phage, plasmids and transforming DNA affect microbiome functions.The earliest genetically-defined MDE was identified in *E. coli* B in the 1960s. Initially designated r_6, and later $rglA_B$ or $mcrA_B$, it has remained biochemically uncharacterized. It has been clear for many years that the gene is not related to the $mcrA_K$ ($rglA_K$, $r6_K$) gene found in *E. coli* K-12, and thus is here renamed *mcrX* to avoid confusion.McrX was characterized here genetically and biochemically. To develop expression strains for conventional restriction enzymes, establishment of protective cognate modification methyltransferases is required. The widely-used host BL21(DE3) could not be used for this purpose due to expression of three MDE. In the process of developing a usable derivative, ER2566, we characterized inactivating mutations in *mcrX* that allowed methyltransferase establishment. The genome sequence of ER2566 revealed that the *mcrX* gene is located in a genomic island with variable contents in different wild *E. coli* isolates. *In vitro* characterization of the wild type BL21(DE3) activity followed. As expected from its genetic properties, the enzyme digests DNA with 5-hydroxymethylcytosine (from phage T4) or with 5-methylcytosine in particular sequences. Unlike most MDE, the recognition sequence is fairly well-defined, and the position of cleavage is fixed within the recognition site.

Author Disclosure Block:

A. Fomenkov: None. **D.K. Dila:** None. **B.P. Anton:** None. **Z. Sun:** None. **R.J. Roberts:** None. **E.A. Raleigh:** None.

Poster Board Number:

SATURDAY-664

Publishing Title:

Transcriptomic Study of Currently Circulating Immunogen Deficient *Bordetella pertussis* Strains

Author Block:

C. Lam, Y. Peng, M. Williams, M. Tondella, L. Pawloski; CDC, Atlanta, GA

Abstract Body:

The development of an inactivated whole cell vaccine (wP) against *Bordetella pertussis* was instrumental in reducing mortality against pertussis disease (whooping cough). In the 1990s, acellular vaccines (aP) with fewer adverse side effects, replaced the original wPs. These aPs only contain 3-5 components of the original wP, specifically pertussis toxin (Ptx), pertactin (Prn), and filamentous haemagglutinin (Fha) and in some vaccine formulations, two fimbrial proteins (Fim2/3). Since then, pertussis has steadily been increasing to levels last observed in the 1950s. Previous genomic analysis of the vaccine strains has revealed a very different genome from currently circulating strains. Furthermore, ongoing molecular surveillance of *B. pertussis* has identified isolates which do not express one or more of these vaccine immunogens, and the occurrence frequency of such strains is also increasing steadily across the US. It is imperative to understand the changes occurring at both the genomic and transcriptomic level that may be contributing to the perceived increased fitness of the currently circulating strains. In this study, we performed RNA sequencing on a representative sample of modern isolates to reveal the different transcriptome profiles compared to old vaccine strains. Total RNA was isolated from six strains deficient in Ptx, Prn or Fha. For comparison, RNA was also isolated from two vaccine strains (Tohama I and CS) and two recently isolated wild type strains, respectively. RNA samples were also depleted of ribosomal RNA and DNA before constructing cDNA libraries. Illumina MiSeq was used to sequence the cDNA libraries and expression analysis was performed using CLC Genomic Workbench (8.5). Comparative transcriptomic analysis was between immunogen deficient isolates and wild type strains, as well as recent isolates and legacy vaccine strains; and the strain specific expressing genes were identified. Preliminary analysis showed that immunogen deficient strains produce transcripts of unique genes that were not identified in the vaccine strains. Further investigation of these genes revealed functions involved with protein transport and secretory systems, which may be compensating for the loss of function as a result of antigen non-expression.

Author Disclosure Block:

C. Lam: None. **Y. Peng:** None. **M. Williams:** None. **M. Tondella:** None. **L. Pawloski:** None.

Poster Board Number:

SATURDAY-665

Publishing Title:

Dynamics of *Escherichia coli* from Human Populations in Tanzania

Author Block:

T. S. Richter[1], T. H. Hazen[1], D. Lam[1], C. L. Coles[2], J. C. Seidman[2], Y. You[2], E. K. Silbergeld[3], C. M. Fraser[1], **D. A. Rasko**[1]; [1]Univ. of Maryland Sch. of Med., Baltimore, MD, [2]Dept. of Intl. Hlth., Johns Hopkins Bloomberg Sch. of Publ. Hlth., Baltimore, MD, [3]Dept. of Environmental Hlth., Johns Hopkins Bloomberg Sch. of Publ. Hlth., Baltimore, MD

Abstract Body:

The stability of non-pathogenic bacterial populations in the human gastrointestinal tract is not fully appreciated and represents a significant knowledge gap regarding gastrointestinal community structure, resistance to incoming pathogenic bacterial introductions, as well as antibiotic treatment. The current study examines the genomic content of *Escherichia coli* isolates from individuals in Tanzania at three time points spanning a six month time period with or without antibiotic treatment. The findings in this study highlight the dynamic nature of the *E. coli* population in the human gastrointestinal tract, as during the six-month interval, no one individual contained phylogenomically related isolates at all three time points. Additionally, while the majority of the isolates at any one time point were phylogenomically similar, the majority of individuals did not contain phylogenomically similar isolates in more than two time points. This suggests that at any one time point there is a dominant clone of *E. coli* within the gastrointestinal tract of an individual, but that this dominance was transient. Examination of canonical *E. coli* virulence factors and antimicrobial resistance genes identified similar, but distinct patterns when compared to the phylogenomic analysis. There is a greater variability in the gene presence and absence patterns being observed among the antimicrobial resistance genes. Gene content analyses emphasized the diversity among *E. coli* isolates and identified gene presence/absence differences between isolates from hosts of different age, sex, antibiotic exposure, or gastrointestinal disease state. Interestingly, there was a lack of correlation with genes associated with diarrhea and the clinical presentation of diarrhea. This is the first study of the dynamic nature of the population structure of *E. coli* in the human host.Keywords: *Escherichia coli*, microbial genomics, population dynamics

Author Disclosure Block:

T.S. Richter: None. **T.H. Hazen:** None. **D. Lam:** None. **C.L. Coles:** None. **J.C. Seidman:** None. **Y. You:** None. **E.K. Silbergeld:** None. **C.M. Fraser:** None. **D.A. Rasko:** None.

Poster Board Number:

SATURDAY-666

Publishing Title:

Shared Alleles Among Group-A, -C and -G *Streptococci* Exist Due to Molecular Trans-species Polymorphism Rather Than Asymmetric Horizontal Gene Transfer

Author Block:

V. Bahrambeigi, A. M. Bolivar, H. Du, X. Li, X. Shi, K. Khanna, V. Varghese, P. C. Hu, E. J. Thompson, **A. KALIA**; The UT MD Anderson Cancer Ctr., Houston, TX

Abstract Body:

Background: β-hemolytic isolates of Lancefield group C and group G Streptococci (GCS-GGS) identified as *Streptococcus dysgalactiae* subsp. *equisimilis* can infect humans and other mammals. GCS and GGS now approximate or surpass group A streptococci (GAS) as the predominant cause of invasive β-hemolytic streptococcal infection. The transfer of genes from GAS into GCS-GGS genomes via horizontal gene transfer (HGT) is common. The dynamics of HGT between GAS and GCS-GGS are complicated. Some HGT events are asymmetric (aHGT), which can further be categorized as additive- or replacing-type aHGT. The mechanisms of replacing-type aHGT remain elusive. It is unclear (i) how replacing-type aHGT may shape diversity in the global GCS-GGS gene pool, and (ii) whether aHGT dynamics differ spatially and temporally.**Methods**: Whole genome sequences of 10 GCS-GGS isolates that caused invasive disease using the Illumina MiSeq platform were determined. Draft assemblies were generated via the Seqman NGen or the Velvet assembler. Genome annotation was performed with the RASTtk and Blast2Go. To elaborate on the global extent and nature of aHGT, we performed pairwise comparisons between GCS-GGS genomes (N = 23; 13 genomes were obtained from the NCBI) and 25 complete GAS genomes.**Results**: Estimated genome size of the six sequenced isolates ranged from 2 – 2.2 Mb. Intraspecific analyses revealed extensive heterogeneity in genome content, and strains differed in terms of phage and other mobile element content, presence or absence of CRISPRs and other strain-specific features. Interspecific comparisons with 25 complete GAS genomes representing multiple *emm*-types revealed that *all GCS-GGS genomes* harbored genes that were identical to those in diverse GAS strains. Strikingly, the extent and nature of identical genes shared between GCS-GGS and GAS genomes was largely similar, mostly neutral, and independent of the GAS *emm*-type thereby precluding any single GAS donor.**Conclusion**: Data suggest that identical gene content between GAS and GCS-GGS is not due to aHGT (or replacement HGT). We propose that identical gene content between GAS-GGS-GCS reflects molecular trans-species ancestral polymorphisms indicating very recent speciation.

Author Disclosure Block:

V. Bahrambeigi: None. **A.M. Bolivar:** None. **H. Du:** None. **X. Li:** None. **X. Shi:** None. **K. Khanna:** None. **V. Varghese:** None. **P.C. Hu:** None. **E.J. Thompson:** None. **A. Kalia:** None.

Poster Board Number:

SATURDAY-667

Publishing Title:

Analysis of Intergenic Repeat Sequences of *Clostridium botulinum* and *Clostridium sporogenes*

Author Block:

C. Nasongkla; Cal Poly Pomona, Pomona, CA

Abstract Body:

Clostridium botulinum is a spore-forming, Gram-positive bacteria that belongs to the phylum Firmicutes. It produces botulinum neurotoxin, the most poisonous neurotoxin known to man. The genomes of several *Clostridium botulinum* strains have been sequenced and annotated, which provides a great tool for mining this bacterial species. Comparative analysis of gaps between *Clostridium botulinum* Hall A and ATCC 3502 identified interspersed DNA repeats. Additionally, the repeats have been found in other closely related species, including *Clostridium sporogenes*, a highly homologous genome to *C. botulinum* without the botulinum toxin, providing a surrogate model system. Repeats have been found throughout eukaryotes and prokaryotes. Previously considered "junk DNA", these repeats are thought to have stable, secondary RNA structures suggesting their roles in gene expression. This study focuses on a repeat sequence designated "Intergenic Repeat 1". Analysis of Repeat 1 sequences identified six subgroups of repeats named 1A, 1B, 1D, 1Q, 1T, 1U, and 1V, whose size ranges from 85 bp to 241 bp and frequency of each repeat ranges from 1 to 21 sequences per genome. Repeat sequences run in both directions on the genome of *Clostridium botulinum* ATCC 3502 and Hall A. The genomes of *C. sporogenes* are not sequenced, with *C. sporogenes* 15579 the only strain that is partially sequenced. PCR cloning and sequencing of the repeat sequences in *C. sporogenes* showed some similar and a novel repeat (designated as 1W) when compared to the repeats of *C. botulinum*. RNA isolation and reverse transcription revealed that the repeats are transcribed into RNA in *C. sporogenes* 15579. The sequences of all repeats are flanked by direct repeats of TTTAT, which also occurred in bcr1-bcr3 repeats of *Bacillus cereus*. BLAST analysis of 1A repeat showed various degrees of similarities to sequences in 5 other *Clostridium* species and 9 other strains of Group I *Clostridium botulinum*. Phylogenetic relationships among the organisms based on the repeats are different from the phylogeny based on functional profile indicating the repeats have a slightly different evolutionary relationship as compared to the host genome.

Author Disclosure Block:

C. Nasongkla: None.

Poster Board Number:

SATURDAY-668

Publishing Title:

Comparative Genomics and Evolution of Amylase-Binding Proteins of Oral Streptococci

Author Block:

E. M. Haase[1], Y. Kou[2], A. Sabharwal[1], Y-C. Liao[3], H-H. Lin[3], T. Lan[1], C. Lindqvist[1], F. A. Scannapieco[1]; [1]Univ. at Buffalo, Buffalo, NY, [2]China Med. Univ., Shenyang, China, [3]Univ. at Buffalo, Miaoli County, Taiwan

Abstract Body:

Many oral streptococcal species bind salivary amylase by expressing a diverse range of amylase-binding proteins (ABPs). The aim of this study was to identify ABPs from a collection of streptococcus strains, and use sequence and bioinformatic analyses to explore their evolutionary relationships.Sixty-two strains from 14 oral streptococcus species were grown overnight in TSBY. Cells were collected by centrifugation and DNA was isolated. Concentrated culture supernatants were used for the detection of ABPs by the amylase-ligand binding assay. ABPs of diverse size were selected for N-terminal sequencing from 18 strains. Whole genome sequencing was performed to identify the genes associated with the ABPs. After sequencing, assembly and annotation, AbpA-like sequences were searched for using size and 'amylase binding protein', and confirmed using the N-terminal sequence. AbpB-like sequences were identified using the sequence obtained from primer-walking of *abpB* performed using degenerate primers. Other novel ABPs were identified using the N-terminal sequence. Alignment and phylogenetic analyses ascertained the evolutionary relationships among ABPs of these strains and database homologs.Nineteen strains from 6 oral streptococcal species expressed ABPs. N-terminal sequencing was performed on 36 ABPs. Among them, 14 ABPs matched amylase-binding protein A (AbpA) with identities ranging from 75-100%, and 6 ABPs matched protein B (AbpB) with 75-100% identity. Eleven unique ABPs were identified as peptidoglycan-binding proteins, hypothetical proteins or choline-binding proteins. AbpA-like sequences could be divided into five subgroups based on the N-terminal sequence and shared high similarity in the signal sequence, C-terminal sortase-binding motif and stop codon. While AbpB-like sequences were highly similar to peptidase C69 from several streptococcal species, they were functionally different from the inter-related AbpAs. The sequence of the novel groups also diverged from AbpA and AbpB.ABPs have the ability to bind amylase, but have divergent functions. There is no evidence that one group evolved from another. A combination of sequence alignment and synteny plot analysis suggests random uptake of *abpA* by horizontal gene transfer.

Author Disclosure Block:

E.M. Haase: None. **Y. Kou:** None. **A. Sabharwal:** None. **Y. Liao:** None. **H. Lin:** None. **T. Lan:** None. **C. Lindqvist:** None. **F.A. Scannapieco:** None.

Poster Board Number:

SATURDAY-669

Publishing Title:

Identification of Essential Genes of *Pseudomonas aeruginosa* for It Is Growth in Airway Mucus

Author Block:

m. abdo, **S. Yoon**; Yonsei Univ., Seoul, Korea, Republic of

Abstract Body:

Pseudomonas aeruginosa has been identified as an important causative agent for airway infection mainly in cystic fibrosis. This disease is characterized by defective mucociliary clearance induced in part by mucus hyper-production. Mucin is a major component of airway mucus and is heavily *O*-glycosylated with a protein backbone. Airway infection is known to be established with bacterial adhesion to mucin. However, genes involved in mucin degradation or utilization still remains elusive. In this study, we thought to provide a genetic basis of *P. aeruginosa* airway growth by identifying those genes.First, we compared genome-wide expression profiles of PAO1, a prototype *P. aeruginosa* laboratory strain, grown in M9-mucin (M9M) and M9-glucose (M9G) media by RNASeq analyses. *PA2939* and *PA0122* genes encoding putative aminopeptidase and a hemolysin homologue were among top 5 genes, whose expression were increased >100-fold in M9M-grown PAO1. Second, a PAO1 transposon (Tn) insertion mutant library was screened for mutants defective in growth in M9M media. Six mutants with Tn insertion in *trpC* (*PA0651*), *trpD* (*PA0650*), *trpF* (*PA3113*), *leuA* (*PA3792*), *leuD* (*PA3120*), *purF* (*PA3108*) genes were determined to exhibit faulty growth in M9M. These genes are involved in the synthesis of leucine and tryptophan. Importantly, all of these mutants were incapable of establishing mouse airway infection, suggesting that these gene functions are required for *P. aeruginosa in vivo* infectivity.Our results indicate that *P. aeruginosa* possesses a unique genetic repertoire and regulatory mechanisms for establishing competitive respiratory infection. Further mechanistic dissection of this particular process will reveal new drug targets, inhibition of which could control recalcitrant *P. aeruginosa* infections.

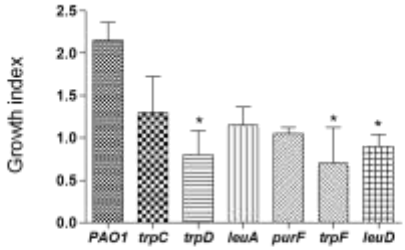


Figure 2: *Pseudomonas aeruginosa* selected mutants in M9Mucin media. PAO1 and mutants in M9M. The cell viability calculated using growth index (growth index= log(CFU $_{\text{after 16 hours}}$/ CFU $_{\text{at 0 time}}$ in M9Mucin media). Three independent experiment were done with mean and ± SD (error bar). P value calculated using ANOVA test.

Author Disclosure Block:

M. abdo: None. **S. Yoon:** None.

Poster Board Number:

SATURDAY-670

Publishing Title:

Genome-Wide Identification Of Genes That Are Essential For Non-Replicating Mycobacterium Tuberculosis

Author Block:

Y. Minato, A. D. Baughn; Univ. of Minnesota, Minneapolis, MN

Abstract Body:

Most anti-tubercular drugs are effective against actively replicating populations of *Mycobacterium tuberculosis* but have limited efficacy against non-replicating populations of bacilli. The presence of such drug tolerant populations of bacilli require protracted treatment times for the eventual clearance of latent and active tuberculosis (TB). To comprehensively identify essential genes for non-replicating *M. tuberculosis*, we utilized a Next-Gen Sequencing (NGS)-based approach, transposon sequencing (Tn-seq). We generated a transposon library of *M. tuberculosis* H37Rv on a custom made defined nutrient rich (TB rich) agar medium. Approximately 3 x 10^6 colonies were collected (TB rich plate sample). A portion of the TB rich plate sample was washed twice with 10 mM Tris-Cl (pH.6.8) and resuspened in the same buffer and then spotted onto 0.22 µm membrane filter. The membrane filter was placed on 10 mM Tris-Cl (pH6.8) agar plate and incubated at 37°C for a month both aerobically and anaerobically. The membrane filters were then transferred onto the TB rich agar plate and incubated at 37°C for at least six generations of bacterial growth (TB starvation - O_2, and TB starvation + O_2 samples). Genomic DNA were purified from the three samples, fragmented, ligated to adaptors for Illumina sequencing, and amplified transposon junction by PCR. The amplified transposon junction region was sequenced by Illumina Hi-seq 2500 at the University of Minnesota Genomics Center. The Tn-seq data was analyzed by ARTIST, a recently developed Matlab-based pipeline for identifying essential genomic regions (1). We identified at least 25 genes that were essential for *M. tuberculosis* survival in starvation - O_2 but not in starvation + O_2. Among these genes, we have confirmed that the mutant strain of *gltB*, the gene encoding the large subunit of the ferredoxin- dependent glutamate synthase, showed a survival defect under anaerobic conditions.

Author Disclosure Block:

Y. Minato: None. **A.D. Baughn:** None.

Poster Board Number:

SATURDAY-671

Publishing Title:

Involvement of Exopolysaccharide in *Stenotrophomonas maltophilia* Biofilm Formation

Author Block:

G. ANDERSON; Indiana Univ. Purdue Univ. Indianapolis, Indianapolis, IN

Abstract Body:

The Gram-negative pathogen *Stenotrophomonas maltophilia* causes numerous serious human infections, including respiratory, urinary tract, skin, bloodstream, soft tissue, and wound infection. Mortality reaches 70% in some cases. Due to innate antibiotic resistance, this microbe is increasing in incidence and is currently the 11th most common isolate from nosocomial infections. However, our understanding of *S. maltophilia* pathogenesis is in its infancy. Several studies have suggested a role for biofilm in infection, but the molecular details of *S. maltophilia* biofilm formation are unknown. Reasoning that polysaccharide secretion is vital for biofilm formation of many microorganisms, we screened a *S. maltophilia* transposon mutant library for strains with reduced binding to the polysaccharide-binding stain Congo red. We found that mutation of the gene *gpmA* resulted in colonies that were paler than wild type on plates with Congo red, suggesting reduced polysaccharide secretion. *gpmA* encodes for phosphoglycerate mutate, a key glycolysis/gluconeogenesis gene, and as such it impacts polysaccharide metabolism. Importantly, *gpmA* mutation also reduced biofilm formation, while growth was unaffected. Using genetic and biochemical assays, we are identifying polysaccharide pathways involved in *gpmA*-mediated polysaccharide production as well as the composition of the secreted biofilm polysaccharide. We are also exploring *gpmA*-mediated biofilm formation on biotic and abiotic surfaces, as well as in an *in vivo* infection model. These experiments reveal one of the first factors known to be involved in *S. maltophilia* biofilm formation. Exploitation of biofilm polysaccharide pathways could lead to new avenues for treatment of this recalcitrant microbe.

Author Disclosure Block:

G. Anderson: None.

Poster Board Number:

SATURDAY-672

Publishing Title:

Interspecies Electron Transfer Enables Photosynthesis of *Prosthecochloris aestuarii*

Author Block:

P. T. Ha, Stephen R. Lindemann, Liang Shi, James K. Fredrickson, Michael Mardigan and Haluk Beyenal; Washington State Univ., Pullman, WA

Abstract Body:

Recent studies have provided evidence suggesting that direct electron transfer (DIET) is one of the mechanism for electron exchange in syntrophic consortia. Many studies have focused on the DIET in methanogenic communities. Although it is known that phototrophs are the major primary energy providers that generate organic substrates via photosynthesis for heterotrophs in the phototrophic community, far less is known if the phototrophic community can also use DIET to exchange electrons. In this study, we report the evidences suggesting phototrophic bacteria also function as the electron accepter that obtain energy, as the form of electrons, directly from heterotrophs for their photosynthesis. Here we show that the phototrophic bacterium *Prosthecochloris aestuarii* can accept electrons directly from a metal reducing bacterium *Geobacter sulfurreducens* for photosynthesis. *P.aestuarii* and *G. sulfureducens* cannot grow individually using acetate as electron donor but can grow in co-culture. We found that *P. aestuarii* can use solid electrode as electron donor for photosynthesis. Both the DIET between *G. sulfurreducens* and *P. aestuarii* and the electron uptake from electrode by *P. aestuarii* were light-dependent processes under which the photosynthesis occurs. As the phototrophic microbial community have big potential application for wastewater treatment, bioremediation and fuel conversion from light and CO_2. This finding contributes the important knowledge for further engineering of synthetic microbial community for our desires.**Keywords:** direct electron transfer, cytochrome c, photosynthesis, *Geobacter sulfurreducens*, *Prosthecochloris aestuarii*

Author Disclosure Block:

P.T. Ha: None.

Poster Board Number:

SATURDAY-673

Publishing Title:

The Dna Damage Response May Regulate Bacterial Multicellularity

Author Block:

K. Gozzi, C. Ching, S. Paruthiyil, I. Lin, V. G. Godoy, Y. Chai; Northeastern Univ., Boston, MA

Abstract Body:

Bacillus subtilis is a gram-positive bacterium that is capable of forming multicellular communities, known as biofilms. Biofilm formation in *B. subtilis* is a well-studied process that incorporates multiple signals and regulatory pathways. Here, we present evidence showing the impact of DNA damage and the SOS response on bacterial biofilm development. In *B. subtilis*, the SOS response is triggered by DNA damage and mediated by RecA, a recombinase that, upon formation of nucleoprotein filaments with single-stranded DNA, acts as a co-protease and induces autocleavage of LexA. LexA is a master repressor for dozens of genes involved in the SOS response. Here we demonstrate that a knockout of *lexA* resulted in significant repression of biofilm formation, indicating a role of DNA damage sensing in biofilm formation. We further identified the ability of the protein product of *sda*, a LexA-controlled gene, to strongly negatively regulate biofilm formation. To elucidate the impact of the SOS response on biofilm formation, static biofilms grown in LB supplemented with glycerol and manganese were examined over time. A *B. subtilis* P_{yneA}-gfp (SOS gene) and P_{yqxM}-mKate2 (matrix gene) dual transcriptional fusion reporter was constructed and gene expression of biofilm cells was tracked over time using single-cell fluorescent microscopy. An inverse relationship between the SOS response and matrix production in cells within the biofilm was observed over time. To further investigate this relationship, *B. subtilis* biofilm cultures were treated with hydrogen peroxide, an endogenous reactive oxygen species and source of DNA damage. Biofilms treated with hydrogen peroxide demonstrated delayed and less robust biofilm formation, suggesting that DNA damage and thus the SOS response negatively regulate biofilm formation. In addition, we show that SlrR, a key regulator for biofilm formation and a LexA-like protein, autocleaves itself when exposed to long filaments of RecA nucleoproteins during the SOS response using *in vitro* cleavage assays. Interestingly however, when exposed to shorter RecA filaments, the autocleavage of SlrR is much less efficient, indicating that SlrR may only cleave itself and thus further inactivate biofilm formation when there are high levels of DNA damage. These findings imply a putative linkage between biofilm formation and DNA damage signals in *B. subtilis*.

Author Disclosure Block:

K. Gozzi: None. **C. Ching:** None. **S. Paruthiyil:** None. **I. Lin:** None. **V.G. Godoy:** None. **Y. Chai:** None.

Poster Board Number:

SATURDAY-674

Publishing Title:

Loss of Type IV Pilus Retractive Force Generates Spatiotemporal Patterns in *Neisseria gonorrhoeae* Microcolonies

Author Block:

K. Eckenrode[1], I. Spielman[1], K. Alzurqa[1], C. A. Weber[2], W. Poenisch[2], V. Zaburdaev[2], N. Biais[1]; [1]Brooklyn Coll. of the City Univ. of New York, Brooklyn, NY, [2]Max-Planck Inst. for the Physics of Complex Systems, Dresden, Germany

Abstract Body:

As antibiotic resistant bacteria steadily increase their global prevalence, new targets to curb bacteria virulence become increasingly important. *Neisseria gonorrhoeae* (GC) produce extracellular protein filaments named type IV pili (tfp), which can possess a variety of functions such as cellular adherence, DNA uptake, electron transfer, and twitching motility. Due to GC's pathogenic nature, it is critical to understand the role of the polymeric appendage during microcolony—a biofilm precursor—formation. When tfp retract from extracellular space into periplasmic space a mechanical force is produced. Pilus retractive forces are fueled by an intracellular ATPase, pilT. When the pilT gene is deleted (ΔpilT), pili are still produced and present in the extracellular space but cannot retract and thereby apply force. A ΔpilT mutant will produce viable microcolonies, even without retractive force, due to the lateral sticky binding of the tfp. The current study aims to understand the role of physical forces during GC tfp bacterial aggregation. Here, we present a set of microcolony formation assays, in which fluorescent ΔpilT GC strains and wild type (WT) GC strains were mixed in equal numbers. Spatiotemporal fluorescent patterns are observed for ΔpilT cells in ~30-50 μm microcolonies at single cell resolution after 3 hours of incubation. Resultant fluorescent microscopy images demonstrate that ΔpilT consistently localized to the outer perimeter of the microcolony when mixed with non-fluorescent WT GC. The experimental results are compared to the results of in silico simulations. These results suggest that upon loss of tfp retractive forces, these retraction-deficient cells, through interactive with WT cells are unable to integrate normally into the microcolony and are subsequently segregated away from the center on the microcolony.

Author Disclosure Block:

K. Eckenrode: None. **I. Spielman:** None. **K. Alzurqa:** None. **C.A. Weber:** None. **W. Poenisch:** None. **V. Zaburdaev:** None. **N. Biais:** None.

Poster Board Number:

SATURDAY-675

Publishing Title:

Investigating the Role of Quorum Sensing in Overall Biofilm Formation During the Epibiotic-parasitic Relationship Between *Actinomyces odontolyticus* (Xh001) and Its Epibiont, a Tm7 Phylotype (Tm7x)

Author Block:

J. Bedree[1], B. Bor[1], J. S. McLean[2], X. He[1], W. Shi[1]; [1]Univ. of California-Los Angeles, Los Angeles, CA, [2]Univ. of Washington, Seattle, WA

Abstract Body:

Background: We observed enhanced biofilm formation ability in the TM7x associated XH001 background compared to XH001 as monospecies. To elucidate the genetic basis of the observed phenotype, meta-transcriptomic profiling was implemented, revealing a set of differentially regulated genes in the TM7x-associated XH001 background versus XH001 as mono-species. The most highly expressed gene, an *lsrB* homologue, encodes a putative periplasmic binding protein for the auto inducer (AI) 2 signaling molecule. The primary focus of this study was to establish a genetic system for XH001 to elucidate the role of AI-2 quorum sensing in biofilm formation dictated by the epibiotic-parasitic relationship between XH001 and TM7x.**Methods:** We established a genetic system in XH001 enabling the successful construction of an XH001*lsrB* defective mutant. This mutant was subjected to phenotypic analyses, including growth kinetics and biofilm formation. Meanwhile, the impact of the *lsrB* mutation on the establishment and maintenance of the epibiotic parasitic interaction between XH001 and TM7x was further investigated. Utilizing a developed TM7x re-attachment method, we monitored this relationship using phase contrast microscopy and Fluorescence *in situ* Hybridization (FISH). The biofilm formation capability of TM7x-associated XH001 and the XH001*lsrB* mutant was investigated. Confocal microscopy was employed to evaluate changes in biofilm formation.**Results:** Under planktonic growth conditions, the XH001*lsrB* mutant did not display noticeable phenotypic variation as mono-culture in comparison to XH001 wild type. Strikingly, phase contrast microscopy and FISH analysis revealed significantly less micro-aggregates in liquid culture as TM7x establishes association with the XH001*lsrB* mutant compared to XH001 wild type. We evaluated this mutation in the context of biofilm formation and observed equal biofilm formation capability between XH001 wild type and XH001*lsrB*. Intriguingly, the *lsrB* mutation greatly reduced duo-species biofilm formation of XH001 and TM7x as they establish physical association.**Conclusions:** AI-2 quorum sensing likely plays a significant role in modulating duo-species biofilm formation as XH001 establishes its epiparasitic relationship with TM7x.

Author Disclosure Block:

J. Bedree: None. **B. Bor:** None. **X. He:** None. **W. Shi:** None.

Poster Board Number:

SATURDAY-676

Publishing Title:

Udp-Galactose - Signal and Toxin in *Bacillus subtilis*

Author Block:

C. W. Habib, Y. Chai; Northeastern Univ., Boston, MA

Abstract Body:

Uridine-diphosphate-galactose (UDP-Gal) is a sugar metabolite generated from glucose by the highly conserved Leloir pathway. Though production of UDP-Gal is a standard cellular function, accumulation of this metabolite causes unexpected toxicity in both prokaryotes and eukaryotes, the molecular mechanism for which is unclear. In the model organism *Bacillus subtilis*, toxicity is presented as rapid cell death, and occurs when a mutation or deficiency is present in the last of the three Leloir pathway genes, *galE*. Herein, using a screen of second-site suppressor mutations and next generation sequencing techniques, we identify several potential targets for the toxicity of UDP-Gal. We additionally identify a catalyst for the production of UDP-Gal, *yhxB*, as well pathways for the production of biofilm and inhibition of secondary sugar uptake mechanisms which serve as rescue mechanisms for the reduction of toxicity. We propose a mechanism for the toxicity in *Bacillus* involving the incorrect incorporation of UDP-Gal in to cell wall synthesis, leading to cell wall stress and structural deformities.Further, we identify the function of UDP-Gal as an intracellular signaling molecule involved in both biofilm production and cell size determination. Biofilms are complex extracellular matrixes consisting of secreted proteins and polysaccharide components, formed in response to environmental stresses as a survival mechanism. We show that the expression of *galE*, and thereby levels of UDP-Gal, is independent of the Leloir pathway and closely regulated with that of the polysaccharide component of the biofilm. We further show that cell size and UDP-Gal production are closely regulated, with formation of UDP-Gal serving to titrate out UDP-Glucose, which has known roles in cell size signaling. As many of these genes and pathways are highly conserved, these findings are wide reaching to both gram positive and negative organisms, with implications possible in the understanding of eukaryotic disease.

Author Disclosure Block:

C.W. Habib: None. **Y. Chai:** None.

Poster Board Number:

SATURDAY-677

Publishing Title:

Degradation of Phage Transcripts by Crispr-Associated Rnases Enables Type III Crispr-Cas Immunity

Author Block:

W. Jiang, P. Samai, G. W. Goldberg, L. A. Marraffini; The Rockefeller Univ., New York, NY

Abstract Body:

Clustered, regularly interspaced, short, palindromic repeats (CRISPR) loci and their associated (*cas*) genes encode an adaptive immune system that protects prokaryotes from viral infection. Immunity is achieved through small-RNA-directed recognition and degradation of the viral genome. CRISPR-Cas systems can be classified into three types based on their *cas* gene content. Type III CRISPR-Cas immunity is achieved through the co-transcriptional cleavage of DNA targets and their transcripts. Whereas DNA cleavage is essential for immunity, the function of RNA targeting is unknown. Here we show that transcription-dependent targeting results in a sharp increase of viral genomes in the host cell when the target is located in a late-expressed phage gene. In this targeting condition, mutations in the active sites of the type III-A RNases Csm3 and Csm6 lead to the accumulation of the target phage mRNA and abrogate immunity. Csm6 is also required to provide defense in the presence of mutated phage targets, when DNA cleavage efficiency is reduced. Our results show that the degradation of phage transcripts by CRISPR-associated RNases ensures robust immunity in situations that lead to a slow clearance of the target DNA.

Author Disclosure Block:

W. Jiang: None. **P. Samai:** None. **G.W. Goldberg:** None. **L.A. Marraffini:** None.

Poster Board Number:

SATURDAY-678

Publishing Title:

Characterization of the Polyamine-Specific NspS-MbaA Signaling Pathway Controlling *Vibrio cholerae* Biofilm Formation from Polyamine Input through to Phenotypic Output

Author Block:

R. Sobe, E. Karatan; Appalachian State Univ., Boone, NC

Abstract Body:

Biofilm formation plays a major role in the *Vibrio cholerae* infectious cycle by enhancing environmental persistence and shielding members of the biofilm from acidic pH of the stomach during infection. Two polyamines, norspermidine and spermidine, have opposite effects on *V. cholerae* biofilm formation. Norspermidine is self-generated and enhances *V. cholerae* biofilm formation. Spermidine is encountered within the human host and diminishes biofilm formation. These effects are abolished in mutants lacking either component of a two-protein signaling system: the periplasmic binding protein, NspS, and the transmembrane c-di-GMP phosphodiesterase, MbaA. Interestingly, mutation of several NspS binding cleft amino acids predicted to play a role in polyamine binding result in severe defects in biofilm formation. The goal of this project was to characterize the NspS-MbaA signal transduction pathway from polyamine input through to biofilm output. Biofilm and Vibrio polysaccharide (*vps*) transcription assays were used to determine how norspermidine and spermidine influence these phenotypes in NspS ligand-binding pocket mutants. Thermal shift assays (TSAs) were used to investigate the capacity of NspS mutants to bind various concentrations of norspermidine and spermidine *in vitro*. Here we show that, in response to norspermidine and spermidine, NspS ligand-binding pocket mutants have altered capacity to influence *vps* gene transcription and biofilm formation *in vivo*. However, purified NspS mutants maintain the ability to bind these polyamines as well as the wild-type protein *in vitro*. Our results suggest that the effect of norspermidine and spermidine on *vps* gene transcription and biofilms is a consequence of an interaction between NspS with MbaA to alter phosphodiesterase activity of the latter rather than polyamine binding to NspS. Altogether, this work provides further characterization of the first polyamine-based signaling system controlling biofilm formation identified in bacteria.

Author Disclosure Block:

R. Sobe: None. **E. Karatan:** None.

Poster Board Number:

SATURDAY-679

Publishing Title:

Single-Base Change by Laboratory-Driven Evolution Eliminates Biofilm Formation in *Desulfovibrio vulgaris* Hildenborough

Author Block:

K. B. De Leon[1], G. M. Zane[1], G. P. Krantz[2], A. P. Arkin[3], P. D. Adams[3], M. W. Fields[2], J. D. Wall[1]; [1]Univ. of Missouri, Columbia, MO, [2]Montana State Univ., Bozeman, MT, [3]Lawrence Berkeley Natl. Lab., Berkeley, CA

Abstract Body:

Desulfovibrio vulgaris Hildenborough (DvH) is a sulfate-reducing bacterium present in heavy-metal and radionuclide contaminated sites, often as a biofilm. Yet, the genetic requirements of DvH biofilm formation have not been determined. Our goal is to determine the mechanisms and genetic requirements of biofilm formation in DvH. In pursuing this goal, inter-laboratory collaboration has led to the discovery that two wild-type DvH strains, both originally from ATCC 29579, have diverged in biofilm formation due to laboratory-driven evolution. Our wild-type DvH (DvH-MO) is partially deficient in biofilm formation as compared to data published for what should have been the same strain used in Matthew Fields' lab at Montana State University (DvH-MT). The genomes were re-sequenced from planktonic cultures of DvH-MT and DvH-MO, and DvH-MO steady-state biofilm. In DvH-MO, a single nucleotide polymorphism (SNP) in the ABC transporter (DVU1017) of a type I secretion system (T1SS) has resulted in an Ala635 to Pro change in a conserved α helix near the ATP-binding site. However, after DvH-MO forms a biofilm, a secondary SNP predominates and results in Leu635. We hypothesized that this Ala to Pro change inhibits protein transport by the T1SS and the secondary SNP resulting in a Leu restores transport. This was confirmed by introduction of the SNPs into DvH-MT. Therefore, protein transport via the T1SS is required for biofilm formation in DvH and a single nucleotide change due to laboratory-driven evolution is sufficient to stop biofilm formation. Proteins encoded in DVU1012 and DVU1545 both contain T1SS export motifs and are abundant in the DvH biofilm matrix. Inhibited transport of these proteins may have caused biofilm deficiency in DvH-MO. The double deletion mutant is deficient in biofilm formation similar to ΔDVU1017. As either DVU1012 or DVU1545 is sufficient to form biofilm, these biofilm structure proteins likely require DVU1017 for export. These findings have led to a proposed mechanism for biofilm formation in DvH and emphasize the importance of monitoring laboratory-driven evolution, especially between collaborating laboratories.

Author Disclosure Block:

K.B. De Leon: None. **G.M. Zane:** None. **G.P. Krantz:** None. **A.P. Arkin:** None. **P.D. Adams:** None. **M.W. Fields:** None. **J.D. Wall:** None.

Poster Board Number:

SATURDAY-680

Publishing Title:

Size Distribution and Predatory Characteristics of *Pseudomonas aeruginosa* Outer Membrane Vesicles Isolated from Biofilms

Author Block:

A. C. Cooke, J. W. Schertzer; Binghamton Univ., Binghamton, NY

Abstract Body:

Gram-Negative bacteria produce Outer Membrane Vesicles (OMVs) in planktonic cultures and in biofilms. Differences between planktonic and biofilm OMVs suggest that OMVs may have different functions in different environments. Previous studies used electron microscopy and proteomic analyses to conclude that *P. aeruginosa* OMVs isolated from biofilms are smaller than planktonic OMVs, and that the two types of OMVs differ in protein content. We characterized planktonic OMVs and biofilm OMVs isolated from *P. aeruginosa* strains PAO1 and PA14. Using single particle tracking technology, we calculated the size of OMVs in solution based on Brownian motion. We also assessed the predatory ability of the OMVs against planktonic *Staphylococcus epidermidis*, as well as *S. epidermidis* grown on solid medium using a filter disk assay. Contrary to previous studies, size distributions between the OMV samples overlapped with one another, and differences between the average diameters of the OMVs tested were not statistically significant. Regardless of how they were tested, PA14 OMVs were more predatory against *S. epidermidis* than the PAO1 OMVs. However, the differences in predation between biofilm OMVs and planktonic OMVs of the same strain were not statistically significant. Our results suggest that the physical differences between biofilm OMVs and planktonic OMVs do not contribute to a difference in OMV predation, and that further investigation is required in order to conclude whether biofilm OMVs and planktonic OMVs differ in size. Future studies should investigate other characteristics to better elucidate how physical differences contribute to functional differences between OMVs in planktonic and biofilm cultures.

Author Disclosure Block:

A.C. Cooke: None. **J.W. Schertzer:** None.

Poster Board Number:

SATURDAY-682

Publishing Title:

Identification and Analysis of Genes Responsible for Penicillin Tolerance in Group A *Streptococcus* Biofilms

Author Block:

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Abstract Body:

Group A *Streptococcus* (GAS) is an important human pathogen that causes a number of diseases with a wide range of severity. The ability of GAS to form biofilms, complex microbial communities that adhere to a surface and secrete an extracellular matrix, has been demonstrated both *in vitro* and *in vivo*. It has been hypothesized that clinical failure of antibiotic treatment of GAS may be due in part to the formation of biofilms. However, little is known about the genes involved in GAS biofilm growth. In order to globally identify those genes and their products, we took a comprehensive approach using both transcriptomic and proteomic analyses to compare *in vitro* grown planktonic and biofilm cultures. Using both high-throughput RNA sequencing and liquid chromatography-tandem mass spectrometry (LC-MS/MS) shotgun proteomics, were able to identify 94 genes and 47 proteins whose expression differed between planktonic and biofilm cultures at multiple time points. Furthermore, the biofilm-upregulated proteins accounted for a majority of the proteins that were subsequently identified through immunoproteomics as being expressed *in vivo* in an animal model of a biofilm-mediated GAS infection.In order to study the role that certain biofilm-upregulated proteins play during biofilm growth, insertion mutants were constructed to abrogate expression of specific genes. Knocking out gene expression in the *arc* operon, which encodes genes important for pH homeostasis, caused a significant increase in susceptibility to penicillin when compared to the parent strain. After 4 hours of penicillin exposure (20 μg/ml), there was a 0.58 log-fold reduction in CFUs in the parent strain biofilm compared to a 1.41 log-fold reduction in the *arc* mutant biofilm ($p<0.01$). This difference was also seen after exposing WT and *arc* mutant biofilms to penicillin for 24 hours (0.82 vs 3.44 log-fold reduction, respectively, $p<0.0005$). This difference in penicillin sensitivity was not seen in planktonic cultures after 4 hours (3.25 vs 3.52, respectively; $p=0.73$) or 24 hours (6.99 vs 4.32, respectively; $p=0.1093$) of antibiotic treatment. This is the first report of a genetic component of penicillin resistance in GAS biofilms, and may lead to a better understanding of how to treat complex Group A *Streptococcus* infections.

Author Disclosure Block:

J.A. Freiberg: None. **Y. Le Breton:** None. **K.S. McIver:** None. **M.E. Shirtliff:** None.

Poster Board Number:

SATURDAY-683

Publishing Title:

Links between Anr and Quorum Sensing in *P.aeruginosa* Biofilms

Author Block:

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Abstract Body:

Pseudomonas aeruginosa (*Pa*) is capable of causing acute and chronic infections at multiple body sites, and persistent *Pa* infection in the airways of cystic fibrosis (CF) patients is correlated with decreasing lung function and increased mortality. In established infections, *Pa* cells reside primarily within multicellular biofilms that are oxygen-depleted environments, and it is not yet well understood how oxygen limitation impacts *Pa* virulence. The cellular response to low oxygen or anoxia in *Pa* is controlled by the transcription factor Anr, and our analyses of *Pa* RNA harvested directly from CF patient sputum or bronchoalveolar lavage have revealed that Anr-regulated transcripts are abundant *in vivo*. Based on our finding that Anr is necessary for normal biofilm development both *in vitro* and on human CFBE cells, as well as for persistence in a model of acute lung infection, we hypothesize that Anr controls biological processes that are important for infection. To gain further insight into the Anr-regulon, we analyzed the transcriptome of two strains and their Δ*anr* derivatives, after growth as biofilms in 1% O_2. The two strains were the laboratory strain strain PAO1 and a clinical isolate with a loss of function mutation in the master regulator of quorum sensing *lasR*, a type of mutant frequently isolated from infections. We found that Anr regulated the expression of multiple pathways known to be involved in virulence, including iron acquisition and storage, CupA fimbriae, type VI secretion, and production of the 4-hydroxy-2-alkylquinolines. Additionally, these data and subsequent analyses found an inverse relationship between LasR-mediated quorum sensing and Anr activity in six pairs of constructed or naturally occurring *lasR* mutants. In a survey of 101 *Pa* clinical isolates from keratitis infections collected during the Steroids for Corneal Ulcers Trial (SCUT), we identified 22 isolates with loss-of-function mutations in *lasR*. We determined that these strains had high-level expression of Anr-regulated pathways, and a retrospective analysis showed that the presence of *lasR* mutants was correlated with poorer patient outcomes in this study.

Author Disclosure Block:

J.H. Hammond: None. **E.F. Dolben:** None. **W.P. Hebert:** None. **K. Ray:** None. **P. Lalitha:** None. **D.A. Hogan:** None. **M.E. Zegans:** None.

Poster Board Number:

SATURDAY-684

Publishing Title:

A Novel Function for Comk in Regulating Differentiation During *Bacillus subtilis* biofilm Development

Author Block:

E. Hunter, Y. Chai; Northeastern Univ., Boston, MA

Abstract Body:

Bacillus subtilis is a Gram-positive, soil-dwelling bacterium that is commonly used to study biofilm development. Under conditions of high cell density, nutrient limitation, and hypoxia, *B. subtilis* will form highly-structured multicellular communities by secreting a self-produced polymeric substance. Biofilm cells differentiate and serve various functions in the community, therefore genetic regulatory networks are necessary to ensure cells fulfill only their designated responsibilities. López et al. (2009) demonstrated that during biofilm development, *B. subtilis* cells differentiate into surfactin- and matrix-producing subpopulations; this illustrates that differentiation into certain cell fates is often mutually exclusive. Environmental stresses can also induce expression of ComK, the master transcription regulator of genetic competence. ComK has positive and negative regulatory functions, and activates genes for DNA uptake and integration. Recently, the consensus sequence for ComK-DNA binding has been identified in the promoter for *sinI*, a key gene in biofilm development. This suggests that ComK binds the *sinI* operator to repress matrix production. In this research, we propose that competent and matrix-producing cells belong to distinct populations in the biofilm community. We demonstrate that ComK overexpression by an IPTG-inducible promoter inhibits biofilm development in *B. subtilis*. Furthermore, beta-galactosidase assays were used to show that ComK overexpression leads to downregulation of matrix genes. This added layer of regulation is a strategy to prevent competent cells from producing biofilm matrix, most likely because the matrix would interfere with DNA uptake.

Author Disclosure Block:

E. Hunter: None. **Y. Chai:** None.

Poster Board Number:

SATURDAY-685

Publishing Title:

Characterization of a New Ap41-Like Pyocin from *Pseudomonas aeruginosa* Isolated from Human Microbiota

Author Block:

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Abstract Body:

Bacteriocins produced by *P. aeruginosa* (pyocins) exhibit bactericidal activity against related species, including multidrug-resistant (MDR) strains. To date, three different types of pyocins have been identified based on their structure (i.e., R-type, F-type and S-type). While R and F-type pyocins are high-molecular-weight protein complexes that resemble phage tails, S-type pyocins are binary protein complexes consisting of a large protein killing and a smaller immunity protein. This study aimed characterize a new pyocin produced by a commensal *P. aeruginosa* strain (named ET02) recovered from a human patient in Brazil. From an initial screening performed on a collection of clinical and environmental *P. aeruginosa* isloates, pyocins produced by *P. aeruginosa* strain ET02 were selected for further investigation due to their wide spectrum activity against multidrug-resistant (MDR) *P. aeruginosa* strains. Antimicrobial activity of pyocins was evaluated by the Gillies and Govan method of pyocin typing. The pyocin synthesis was induced by adding mitomycin C and pyocins molecules were then precipitated by ammonium sulfate. The high-molecular weight R and F-type pyocins were sedimented by ultra-centrifugation while the S-type pyocins remained in supernatant this ultracentrifugation. The fraction containing the S-type pyocins from ET02 strain displayed the highest killing activity against carbapenemase (SPM-1, GIM-1, VIM-1, IMP-1, KPC-2 and GES-5)-producing *P. aeruginosa* strains. Analysis of mass spectrometry this fraction identified the presence of an S-type pyocin belonging the subtype AP41 (GenBank accession number: D12705.1). In this regard, further sequencing of DNA fragments containing the encoded genes of this pyocin (PyoAP41-ImuAP41) revealed a new AP41 variant (92% similarity), which showed differences in the catalytic domain. In summary, we hereby describe a new isoform of the AP41 pyocin presenting a wide spectrum activity against carbapenemase-producing *P. aeruginosa* strains. The clinical therapeutic potential of this new pyocin is worthy of further investigation.

Author Disclosure Block:

H. Turano: None. **F. Gomes:** None. **L. Netto:** None. **N. Lincopan:** None.

Poster Board Number:

SATURDAY-686

Publishing Title:

Axenic Biofilm Formation by the Cyanobacterium *Synechocystis* PCC 6803 Requires Cell Surface Structures and Occurs Under Nutrient Limitation

Author Block:

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Abstract Body:

Phototrophic biofilms are key to nutrient cycling in natural environments and engineered bioreactors. The literature on mixed-species phototrophic biofilms is extensive, but there are few studies describing biofilm formation by a single (axenic) species of phototrophic microbe. The cyanobacterium *Synechocystis* PCC 6803 is a model organism for the study of oxygenic photosynthesis and a promising candidate for microbial biofuel production. We hypothesize that phototrophic bacteria such as *Synechocystis* are able to form axenic biofilms, and use cell surface structures such as pili to attach and adhere to surfaces, similar to other biofilm-forming heterotrophic bacteria. Using microscopy and the crystal violet biofilm assay, we have found that *Synechocystis* forms biofilms of cells and extra-cellular material when shifted to nutrient-depleted medium. Axenic wild-type (WT) *Synechocystis* does not form biofilms in nutrient-replete medium (BG11). *Synechocystis* mutants lacking genes required for synthesis of cell surface structures such as pili and the S-layer do not form biofilms under nutrient limitation. We conclude that pili and the S-layer are necessary but not sufficient for biofilm formation by WT *Synechocystis*: an additional adhesion factor must be induced, such as through nutrient limitation, in order for pili and S-layer to facilitate biofilm formation by WT cells.To further elucidate the molecular mechanisms of adhesion by *Synechocystis*, we compared outer membrane fractions of WT and mutant cultures under induced (nutrient-limited) and uninduced conditions. SDS-PAGE of these OM proteins showed that nutrient-limited WT cultures experience a shift in the S-layer protein band; additionally, a second band is present in induced, aggregated cultures that is not in uninduced, unaggregated cultures. These data support that the molecular mechanism for aggregation by WT *Synechocystis* is synthesis and/or modification of cell surface structures in response to environmental stimuli such as nutrient limitation. We will perform RNA sequencing of *Synechocystis* cultures and also mass spectrometry of OM protein bands to further elucidate the genetic and molecular mechanisms of induction and subsequent aggregation by *Synechocystis*.

Author Disclosure Block:

R.C. Allen: None. **B.E. Rittmann:** None. **W. Vermaas:** None. **R. Curtiss:** None.

Poster Board Number:

SATURDAY-687

Publishing Title:

Gbpa Promotes Resistance of Nt*hi* 86-028np to Antibiotics and Oxidants within Biofilms *In Vitro* and Persistence *In Vivo*

Author Block:

B. Pang, W. Swords; Wake Forest Sch. of Med., Winston Salem, NC

Abstract Body:

Nontypeable *Haemophilus influenzae* (NT*Hi*) is a common human airway commensal that can cause respiratory tract diseases including otitis media (OM). During colonization and infection, NT*Hi* persists within surface-attached biofilms to resist environmental stress and host clearance. Our lab and others have shown that oxidative stress responses are related to persistence of NT*Hi* within biofilms. Recently we found that a chimeric antioxidant enzyme, peroxiredoxin-glutaredoxin (PgdX), was related to NT*Hi* resistance to oxidative stress and host clearance in chronic OM infections. Glutathione (GSH) is the essential intracellular reductant of the PgdX. However NT*Hi* cannot synthesize GSH and has to import it from environment via GSH binding protein (GbpA). In this study, we sought to define the factors of GbpA in stress responses and persistence of NT*Hi* within biofilms.An NT*Hi* 86-028NP *gbpA* null mutant was generated and compared to the parental strain. We measured the GSH level within whole cell lysates and observed that GSH in the *gbpA* mutant was not detected. It confirmed that GbpA was essential for the GSH uptake in NT*Hi* 86-028NP. Using a static biofilm system, we found that the *gbpA* mutant formed thinner and rougher biofilms compared to the parental strain. Using COMSTAT software to quantify biofilm properties, we observed that the thickness and biomass of the *gbpA* mutant biofilm were decreased. Although there was no survival defect in the mature biofilms of the *gbpA* mutant strain within the general condition, the *gbpA* mutant biofilm was more sensitive to multiple antibiotic and oxidative stresses. The role of GbpA in persistence *in vivo* was tested using the chinchilla infection model for OM. Animals were infected with ~1000 CFU of bacteria. Bullae and effusion fluids were harvested for bacterial counts at 7, 14, and 21d post infection. The results showed that the *gbpA* mutant had decreased resistance to clearance compared to the parental strain and the incidence rate of the *gbpA* mutant biofilms observed in the chinchilla upper bulla was significantly less than that of the parental strain at the late time point post infection.We conclude that GSH uptake mediated by GbpA promotes resistance of NT*Hi* 86-028NP to antibiotics and oxidants within biofilms and bacterial persistence in chronic OM infection.

Author Disclosure Block:

B. Pang: None. **W. Swords:** None.

Poster Board Number:

SATURDAY-688

Publishing Title:

Investigation of Surface Sensing and Response Mechanisms by P. Aeruginosa

Author Block:

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Abstract Body:

Biofilms are surface-attached microbial communities. We are interested in understanding how microbes transition from a free-swimming to a surface-associated lifestyle. The gram-negative bacterium *Pseudomonas aeruginosa* displays a number of surface-associated behaviors, including biofilm formation as well as surface motility. The challenge for this microbe is to effectively coordinate these different behaviors to respond appropriately to prevailing surface conditions.Results from our recent studies of early cell-surface interactions indicate that *P. aeruginosa* utilizes a step-wise regulatory cascade involving successive signaling events via two distinct second messengers to control surface interaction events. Initial growth on surfaces leads to an increase in levels of the second messenger cAMP. This burst of cAMP requires a chemotaxis-like protein PilJ with its cognate chemosensory protein (Chp) complex and the type IV pilus (TFP), leading to production of cAMP via the adenylate cyclase, CyaB. cAMP and its receptor protein Vfr, together with the FimS/AlgR two-component system up-regulate expression of the TFP protein, PilY1, upon surface growth. PilY1 is subsequently secreted to the cell surface via the TFP whereby it then signals via the TFP alignment complex (PilMNOP) and the diguanylate cyclase, SadC, to promote production of the second messenger c-di-GMP. Increased levels of c-di-GMP promote biofilm formation and repress surface motility. Thus, this hierarchical regulatory cascade allows *P. aeruginosa* to coordinately regulate its surface behaviors upon surface interaction and growth.Current studies are focused on determining the precise molecular mechanisms governing the early surface stimulation of cAMP. Using the bacterial two-hybrid assay as a first step in assessing protein interactions, we have identified an interaction network between PilJ, CyaB, FimS and PilA, all of which are predicted or have been shown to reside in the inner membrane of *P. aeruginosa*. We are exploring the possibility that these protein-protein interactions are involved in the regulation of cAMP synthesis upon surface interaction.

Author Disclosure Block:

S.L. Kuchma: None. **G. O'Toole:** None.

Poster Board Number:

SATURDAY-689

Publishing Title:

Tfp Mediated Retraction Force Modulates Antibiotic Resistance in *Neisseria gonorrhoeae*, the Causative Agent of Gonorrhea

Author Block:

B. E. Ford, N. Biais; Brooklyn Coll. of the City Univ. of New York, Brooklyn, NY

Abstract Body:

The potential emergence of multi antibiotic-resistant *Neisseria gonorrhoeae* strains is a clear and imminent threat. Previous work has demonstrated that mechanical forces generated by the retraction of Type IV pili (Tfp) borne by *Neisseria* bacteria play a direct role in many aspects of *Neisseria* biology and in particular, microcolony formation. Microcolonies are precursors to biofilm formation and, as biofilms have been implicated in both antibiotic resistance and pathogen persistence in the environment, could be an important step in the gain of antibiotic resistance. Here we aim to assess whether the Tfp generated mechanical force crucial in microcolony formation also plays a role in antibiotic resistance. Wild-type *Neisseria gonorrhoeae*, as well as its derivative ΔpilT (mutant lacking the pilT gene—coding for an ATPase motor protein—and thereby incapable of retractive forces), were grown for 1 h in the presence of either growth media or growth media supplemented with one of six different antibiotics—Erythromycin (Erm; 50 μg/ml), Chloramphenicol (Chl; 250 μg/ml), Nalidixic acid (NA; 30 μg/ml), Kanamycin (Kan; 80 μg/ml), Ciprofloxacin (Ci; 0.12 μg/ml) and Cephalexin (Ce; 50 μg/ml); the first three being bacteriostatic and the latter three bactericidal—at the minimum inhibitory concentration (MIC), either with or without disruption of microcolonies, and subsequently assessed for viability. Preliminary results suggest that the formation of microcolonies is protective against antibiotic action for the wild type bacteria only; for mutants incapable of producing mechanical forces (e.g. the ΔpilT strain), loose microcolony aggregation leads to an increase in cell death under all tested antibiotic selection (save for Kanamycin selection as Kan resistance is also the selective marker for that mutant). These findings suggest that Tfp generated mechanical forces do indeed play an important role in modulating bacterial antibiotic susceptibility.

Author Disclosure Block:

B.E. Ford: None. **N. Biais:** None.

Poster Board Number:

SATURDAY-690

Publishing Title:

Cell Division Regulator Mipz in "Caulobacter Crescentus"

Author Block:

Y. Refes, B. He, M. Thanbichler; Philipps Univ. of Marburg and Max Planck Inst. for Terrestrial Microbiol., Marburg, Germany

Abstract Body:

Caulobacter crescentus is a Gram-negative bacterium that divides asymmetrically and serves as a well-established model to study the bacterial cell cycle. Each division of a *C. crescentus* cell gives rise to two morphologically and physiologically different cells: one motile cell called “swarmer cell”, and a non-motile cell called “stalked cell”. In *C. crescentus*, proper cell division requires the formation of a bipolar gradient of the regulatory ATPase MipZ that ensures the establishment of the cytokinetic FtsZ ring (Z ring) at midcell. Although the basic function of the MipZ system has been worked out, several issues remain to be clarified, including the mode of interaction between MipZ and different interaction partners, such as FtsZ, the origin-bound chromosome partitioning protein ParB, and the nucleoid. An important objective of this project is to identify the FtsZ-, ParB-, and DNA-binding regions of MipZ and thus understand how ATPase-driven changes in the oligomerization state of MipZ affect its interaction pattern. To this end, we systematically exchanged surface-exposed residues of MipZ by alanine-scanning mutagenesis. Analyzing the subcellular distribution of the mutant proteins and their ability to support division site placement, we then identified mutations that likely affect the interaction with FtsZ, DNA or ParB. The different MipZ variants were purified and further characterized *in vitro* to verify the initial, phenotype-based categorization. We found that ParB- and DNA-binding regions are overlapping and that the DNA-binding and FtsZ-binding interfaces of MipZ comprise residues located on opposite sides of the dimer. This result is consistent with the previous finding that the regulatory effect of MipZ is specific for its dimeric form and that only the dimeric form binds DNA and FtsZ. We also found that the DNA-binding region consists mainly of positively charged arginine and lysine residues lining the subunit interface, suggesting that the binding of MipZ to DNA is mainly mediated through interaction of positively charged residues with the negatively charged DNA phosphate backbone. These results provide the first detailed analysis of the interaction determinants of MipZ and, thus, deepen our knowledge of the molecular mechanism underlying the function of this intriguing cell division regulator.

Author Disclosure Block:

Y. Refes: None. **B. He:** None. **M. Thanbichler:** None.

Poster Board Number:

SATURDAY-691

Publishing Title:

Identifying Sites on MinC Important for Cell Division by Mutagenesis and Phenotypic Screening

Author Block:

C. J. LaBreck, J. L. Camberg; Univ. of Rhode Island, Kingston, RI

Abstract Body:

The Min system in *Escherichia coli*, including MinC, MinD, and MinE, controls the spatiotemporal placement of the FtsZ-ring and prevents FtsZ assembly near the cell poles. MinC is the effector of the Min system and antagonizes FtsZ polymerization through interactions at the MinC N- and C-terminal domains. MinC destabilizes FtsZ polymers by an interaction that is stimulated by MinD, which binds to the MinC C-terminal domain. To gain mechanistic insight into how MinC interacts with both FtsZ and MinD and identify the specific interaction sites important for function, we expressed MinC mutant proteins to native levels from the chromosome and performed a phenotypic screen to identify mutants with functional defects. Mutations in MinC were constructed by both random and site-directed mutagenesis of *minC*. Copies of *minC* from the mutant library were reinserted at the native locus by selective recombination into a *minC* deletion strain. We screened isolated recombinants for cell morphology and overall length distribution. Strains deleted for *minC* and those expressing defective MinC mutant proteins form anucleate minicells and short filaments in liquid culture. Using this strategy, we identified several single amino acid substitutions that inactivate MinC and mapped mutations to both the N- and C-domains of MinC. To determine if mutations in *minC* impair direct interactions with FtsZ or MinD, we purified MinC mutant proteins from *E. coli* and analyzed protein interactions in biochemical assays and assessed MinC dimerization in vitro. We tested MinC mutant proteins for inhibition of FtsZ polymerization and identified several mutations that impair the activity of MinC to inhibit GTP-dependent polymerization of FtsZ. We identified two regions on the predicted surface of MinC, one on the MinC N-terminal domain and one on the C-terminal domain, as important for FtsZ interactions. To determine if MinC mutant proteins form a complex with MinD, we used a sedimentation assay to detect ATP-dependent copolymers assembled by MinC and MinD. Several MinC mutant proteins show reduced copolymer formation with MinD, indicating that they are impaired for a direct interaction with MinD. These experiments provide important insight for the identification of surface exposed regions of MinC that mediate interactions with FtsZ and MinD and are important for cell division.

Author Disclosure Block:

C.J. LaBreck: None. **J.L. Camberg:** None.

Poster Board Number:

SATURDAY-692

Publishing Title:

Mraz Is a Transcriptional Repressor of the *ftsz* Cluster in the Model Organism *Mycoplasma genitalium*

Author Block:

C. Martínez-Torró, S. Torres-Puig, M. Huguet-Ramón, C. Muñoz-Navarro, E. Querol, J. Piñol, O. Q. Pich; Univ.t Autònoma de Barcelona, Cerdanyola del Vallès, Spain

Abstract Body:

Cell division in bacteria is a complex process involving the coordinated participation of many proteins. In *Escherichia coli*, the division and cell wall cluster consists of sixteen genes implicated in peptidoglycan biosynthesis and assembly of the cell division apparatus. Genome reduction in mycoplasmas prompted the loss of many genes related to cell division and accordingly, in the cell wall-less bacterium *Mycoplasma genitalium*, the cell division cluster (known as the *ftsZ* cluster) contains only four genes: *mraZ* (MG_221), *mraW* (MG_222), MG_223 and *ftsZ* (MG_224). The MG_225 and MG_226 genes, located immediately downstream and in the same orientation as the *ftsZ* gene cluster, code for two putative amino acid permeases apparently unrelated to cell division. In a previous study, we demonstrated that *ftsZ* was dispensable for *in vitro* growth in *M. genitalium*. This finding highlights the unique nature of *M. genitalium* as the *ftsZ* gene is essential for survival in most bacteria. In the current study, we obtained null mutants of *mraZ*, *mraW* and MG_223 by allelic exchange, demonstrating that the four genes of the *ftsZ* cluster are non-essential in *M. genitalium*. Transcriptional analysis of the *mraZ* mutant revealed a significant upregulation (~12-fold) of the *mraW*, MG_223 and *ftsZ* genes, indicating that MraZ is a transcriptional repressor of the cell division cluster in this model organism. Transcription of the MG_225 gene was also upregulated in the *mraZ* mutant. In contrast, transcription of the *ftsZ* gene cluster was essentially unaltered upon deletion of the *mraW* gene. The repressor role of the MraZ protein is in agreement with recent work in *E. coli* and *Corynebacterium glutamicum*. Remarkably, a putative binding site for MraZ was also identified in the upstream region of the *ftsZ* cluster. On the other hand, we created fluorescent fusions of the four proteins encoded in the *ftsZ* cluster at their respective native loci. We did not observe any fluorescence when these mutants were obtained in a wild-type strain background. However, fluorescent cells were apparent in an *mraZ* mutant as well as in non-adherent mutant backgrounds. This result reinforces the repressor role of MraZ in *M. genitalium* and advocates an important role for the cell division apparatus in non-adhering populations of this bacterium.

Author Disclosure Block:

C. Martínez-Torró: None. **S. Torres-Puig:** None. **M. Huguet-Ramón:** None. **C. Muñoz-Navarro:** None. **E. Querol:** None. **J. Piñol:** None. **O.Q. Pich:** None.

Poster Board Number:

SATURDAY-693

Publishing Title:

Protein Interactions at the Conserved C-Terminus of FtsZ Affect the Dynamic Exchange of Subunits in the Z-Ring During Cell Division in *Escherichia coli*

Author Block:

M. G. Viola, C. J. LaBreck, J. Conti, J. L. Camberg; Univ. of Rhode Island, Kingston, RI

Abstract Body:

The highly coordinated process of bacterial cell division enables the separation of a mother cell into two daughter cells. FtsZ, an essential cell division protein, assembles into a dynamic ring (Z-ring) at the site of constriction. The Z-ring is composed of a bundled network of overlapping filaments, which are stabilized and/or destabilized by many cell division proteins. As the Z-ring assembles, the nucleoid associated protein SlmA binds FtsZ to prevent Z-ring assembly over the nucleoid, while the Min system, including MinC, MinD and MinE, establishes a polar gradient of MinC to inhibit Z-ring assembly at the poles. The ATP-dependent protease ClpXP and the putative ATPase ZapE have also been shown to destabilize FtsZ polymers in vitro, however the in vivo roles for these activities are unclear. To determine if proteins that destabilize FtsZ polymers in vitro also modulate the dynamic exchange of subunits in the Z-ring during division, we bleached a small region of fluorescence from Z-rings containing Gfp-FtsZ and monitored recovery in wild type and deletion strains. Cells deleted for *minC*, *slmA*, *clpX*, *clpP* or *zapE* contain Z-rings that exchange subunits 50-80% more slowly than Z-rings in wild type cells. FtsZ contains a conserved region near the C-terminus that is important for interactions with many division proteins, including MinC, SlmA, and ClpX. Expression of an FtsZ mutant protein with a C-terminal substitution, Gfp-FtsZ(R379E), in wild type cells causes cell filamentation, impaired Z-ring formation and slow Z-ring dynamics, similar to the rate of subunit exchange in cells expressing the FtsZ GTP hydrolysis mutant FtsZ(G105S). Expression of the double mutant Gfp-FtsZ(G105S, R379E) causes aberrant, membrane-associated Z-rings and severe mislocalization defects in a strain containing chromosomal *ftsZ(G105S)* at the permissive temperature, indicating that both GTP hydrolysis and C-terminal protein interactions are critical for Z-ring assembly. Together, these results demonstrate that the fast rate of subunit exchange in the Z-ring is mediated by FtsZ GTP hydrolysis and by proteins that modulate FtsZ polymerization through direct interactions at the FtsZ C-terminus.

Author Disclosure Block:

M.G. Viola: None. **C.J. LaBreck:** None. **J. Conti:** None. **J.L. Camberg:** None.

Poster Board Number:

SATURDAY-694

Publishing Title:

Functional Analysis of the Bacterial Cell Division Atpase Zape

Author Block:

E. DiBiasio, M. G. Viola, A. Velasquez, J. L. Camberg; Univ. of Rhode Island, Kingston, RI

Abstract Body:

When bacterial cells divide, a large cytoskeletal structure called the Z-ring assembles at the division site. The major protein in the Z-ring is FtsZ, a tubulin homolog and GTPase that assembles into polymers and organizes into the Z-ring. In *Escherichia coli*, many cell division proteins interact with FtsZ and direct Z-ring assembly, while others may modulate constriction or direct cell wall insertion and remodeling. Several accessory proteins that interact with FtsZ are called Z-ring associated proteins (ZAPs). The Zaps (ZapA, ZapB, ZapC, ZapD, and ZapE) are recruited to the divisome and may influence Z-ring assembly or stability. ZapE was recently identified to be an ATPase that accumulates during late constriction in Gram-negative bacteria, including *E. coli*, and is important for bacterial growth under low-oxygen conditions and high temperatures (Marteyn, et al., 2014, *mBio*, 5(2):e00022-14). In vitro ZapE destabilizes FtsZ polymers suggesting that it may promote Z-ring disassembly in vivo. To investigate the role of ZapE during division, we constructed a *zapE* deletion strain to determine if the absence of *zapE* leads to a change in average cell length indicating impaired cell division. Cells deleted for *zapE* are similar in length to wild type cells and have normal Z-rings by microscopy using the fluorescent Z-ring reporter Gfp-FtsZ. However, strains deleted for *zapE* and *minC* are extensively filamentous, with the majority of cells longer than 10 μm. Synthetic cell division phenotypes have also been observed when the *minC* gene is deleted in combination with *slmA* and *clpX*. MinC spatially regulates placement of the Z-ring in vivo by preventing Z-ring assembly near the cell poles. These results demonstrate that when division site selection is impaired by deletion of *minC*, other FtsZ-assembly regulators including ZapE are important for promoting division. To further evaluate ZapE function and interactions in vivo, we are using random and site-directed mutagenesis to construct a library of *zapE* mutants. The library will be inserted into the chromosome at the native *zapE* locus by selective recombination, and isolated recombinants will be screened for overall cell length to identify loss of function mutations. These results will provide important insight for understanding the role of ZapE in cell division.

Author Disclosure Block:

E. DiBiasio: None.

Poster Board Number:

SATURDAY-695

Publishing Title:

FtsEX Regulates Divisome Activity by Interacting with FtsA in *E. coli*

Author Block:

S. Du, J. Lutkenhaus; Univ. of Kansas Med. Ctr., Kansas City, KS

Abstract Body:

Background: Cytokinesis in *E. coli* is mediated by a ring-shaped multi-protein complex called the divisome. The divisome is activated by FtsN, the last essential division protein localizing to the division site . FtsN is a bitopic membrane protein and it does this by activating FtsA in the cytoplasm (^{N}FtsN) and relieving the inhibition of peptidoglycan synthesis by the FtsQLB complex in the periplasm (^{E}FtsN) (5, 6) . Interestingly, these ATP lesion mutants of FtsEX allow divisome assembly, suggesting that ATP hydrolysis of FtsEX is necessary for divisome activation.**Methods and Results:** Using one of the ATP lesion mutants of FtsEX (FtsE^{D162N}X), we confirmed that overexpression of FtsE^{D162N}X blocks cell constriction but not assembly of the divisome. Overexpression of FtsN counteracts the division inhibitory activity of FtsE^{D162N}X, suggesting that FtsE^{D162N}X has opposite function of FtsN (blocks divisome activation). Hyperactive mutants of FtsB and FtsL are also resistant to overexpression of FtsE^{D162N}X. However, hyperactive mutants of FtsA become more sensitive to overexpression FtsE^{D162N}X, indicating that FtsE^{D162N}X blocks divisome activation in the cytoplasm. Screening for extragenic mutations that provide resistance to FtsE^{D162N}X revealed mutations in FtsB, FtsL and FtsW as well as mutations in FtsA. Characterization of these mutations suggested that the isolated mutations in FtsB, FtsL and FtsW are similar to the previously isolated hyperactive mutations of FtsB and FtsL, while the mutations on FtsA impair the interaction between FtsA with FtsX. We further identified a region on FtsA that is important for FtsA interaction with FtsX and showed that this interaction is important for cell constriction to occur but not divisome assembly.**Conclusions:** These results together suggest that FtsEX uses the energy of ATP hydrolysis to regulate divisome activity by interacting with FtsA. As FtsEX governs cell wall hydrolysis at the division site, our findings thus provide a mechanism by which cell wall synthesis (divisome activation) and hydrolysis are coupled at the division site in *E. coli*.

Author Disclosure Block:

S. Du: None. **J. Lutkenhaus:** None.

Poster Board Number:

SATURDAY-696

Publishing Title:

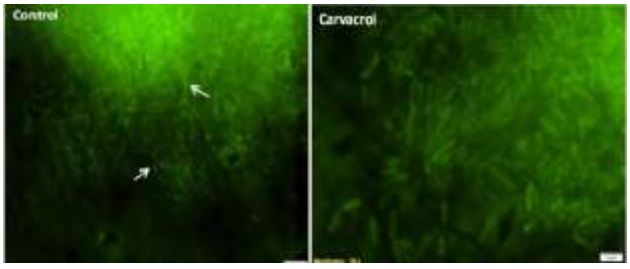
Effect of Essential Oils Compounds on Bacterial Cell Division

Author Block:

M. Albano[1], I. C. da Silva[1], A. Savietto[1], F. C. Bérgamo Alves[1], L. Nunes Barbosa[1], B. F. Murbach Teles Andrade[1], A. Marques Pereira[1], H. Ferreira[2], A. Fernandes Júnior[1]; [1]Univ.e Estadual Paulista "Júlio de Mesquita Filho", Botucatu, Brazil, [2]Univ.e Estadual Paulista "Júlio de Mesquita Filho", Rio Claro, Brazil

Abstract Body:

Background: The rise of drug resistance has prompted pharmaceutical industry to develop new antimicrobial drugs and bacterial cell division is a interesting new target. FtsZ is the central protein of bacterial cell division that forms the Z-ring at midcell, which enables septation. The aim of this study was to investigate plant essential oil compounds as inhibitors of bacterial cell division. **Methods:** Mutant cells of *X. citri* subsp. *citri* expressing GFP-ZapA were cultivated until the OD at 600 nm reached ~0.3 and then subjected to treatments with cinnamaldehyde, eugenol and carvacrol at concentrations corresponding to MIC_{70} (previously determined) for 3h at 30°C and immobilized on agarose-covered slides for observations by fluorescence microscopy. Control treatment was carried out with cells cultivated in the presence of 1% DMSO. Cells were visualized by using an Olympus BX-61 microscope and documented with a monochromatic XM-10 camera. Image processing and analyses were conducted by using Cell-F (Olympus). **Results:** Cinnamaldehyde and carvacrol reduced the frequency of Z-ring occurrence in X. citri subsp. citri and perturbed the Z-ring morphology. In the absence of compounds, the normal pattern of divisional septum was observed in the midcell. Eugenol did not affect the divisional septum.**Conclusions:** Our results suggest that cinnamaldehyde and carvacrol can inhibit bacterial cell division, apparently by perturbing the cytokinetic of Z-ring assembly. This could be an alternative to new treatments of infection diseases.



Author Disclosure Block:

M. Albano: None. **I.C. da Silva:** None. **A. Savietto:** None. **F.C. Bérgamo Alves:** None. **L. Nunes Barbosa:** None. **B.F. Murbach Teles Andrade:** None. **A. Marques Pereira:** None. **H. Ferreira:** None. **A. Fernandes Júnior:** None.

Poster Board Number:

SATURDAY-697

Publishing Title:

Role of Amidase in Cell Morphogenesis: New Insights from C*aulobacter crescentus*

Author Block:

A. DUBEY, R. Priyadarshini; Shiv Nadar Univ., Greater Noida, India

Abstract Body:

Growth and division of bacterial cell is intimately linked to remodeling of the peptidoglycan layer. Separation of daughter cells during bacterial cell division requires hydrolysis of septal peptidoglycan by peptidoglycan hydrolases. Hydrolases are the enzymes capable of cleaving the covalent bonds in peptidoglycan. One such enzyme is *N*-acetylmuramyl -L-alanine amidase that removes the peptide side chain from peptidoglycan and plays crucial role in septum cleavage. While wealth of knowledge is available about the enzymatic activity and structure of amidases, not much is known about their regulation in bacterial cell. *Caulobacter crescentus* is an eminent model system for studying cell morphogenesis. It is a dimorphic bacterium that divides asymmetrically giving rise to two different types of cells; swarmer cells and stalked cells which are genetically identical but morphologically different. Genome analysis reveals that in *C. crescentus* there is one annotated amidase homolog and this enzyme is the focus of our study. To study the localization of amidase, we created C-terminus mCherry fusion and our preliminary data showed that amidase-mCherry is localized at the cell division site and this medial localization is dependent on FtsZ in *C. crescentus*. Covisualization of amidase and FtsN in synchronized cells revealed that amidase is recruited to cell division site after FtsN, suggesting that it is a part of late cell division machinery. To more precisely assess the role of amidase, we determined the effect of amidase over activity by expressing it from plasmid (pJS14) under the control of xylose inducible promoter. In presence of xylose cell exhibited chaining indicating that over-expression of amidase leads to defects in cell division and separation. Based on these results we conclude that amidase plays a crucial role in morphogenesis of *C. crescentus.*

Author Disclosure Block:

A. Dubey: None. **R. Priyadarshini:** None.

Poster Board Number:

SATURDAY-699

Publishing Title:

Variability of Thickness in Individual Cable Bacteria

Author Block:

T. Yang, L. P. Nielsen; Aarhus Univ., Aarhus C., Denmark

Abstract Body:

Cable bacteria stretch their multicellular filaments from the oxic surface sediment, through the suboxic zone and into the sulfidic zone centimeters below. They transport electrons from sulfide to oxygen, presumably via strings in a periplasmic space shared by all the cells. In developing, mixed populations of cable bacteria the average thickness of filaments has been found to increase with time and depth in the sediment. By more detailed examination we found that this was a character of single bacteria, and eventually the sulfide exposed end of a filament could be more than two times thicker and have eight times bigger cell volumes compared to the oxic end. High frequencies of dividing cells, 7% in the thin end and 11% in the thick end, showed that cells in both ends were actively dividing and not in a diseased stage.We propose that the thickness flexibility is an adaption to competition among cable bacteria for more and more distant and depleted electron donors and acceptors: Under some conditions thinning is most advantageous by allowing more efficient substrate uptake and increased filament length relative to biomass. Other conditions makes thickening more advantageous as it enhances strength and electron conductivity provided the number or thickness of periplasmic strings increases accordingly. How the morphological changes are regulated and the role of motility, energy storages, and carbon sources will also be discussed.

Author Disclosure Block:

T. Yang: None. **L.P. Nielsen:** None.

Poster Board Number:

SATURDAY-700

Publishing Title:

Role of Peptidoglycan Remodeling Machinery Components in Regulating Engulfment and Coat Localization During *Clostridium difficile* Spore Formation

Author Block:

J. Ribis, K. Fimlaid, A. Shen; Univ. of Vermont, Burlington, VT

Abstract Body:

Clostridium difficile is a major nosocomial pathogen that is the leading cause of healthcare associated diarrhea worldwide. In order for this obligate anaerobe to transmit infection, it must form metabolically dormant spores through a developmental pathway known as sporulation. A critical step during sporulation is the engulfment of the forespore by the mother cell, which in the model spore former *Bacillus subtilis* depends on the peptidoglycan hydrolases SpoIID and SpoIIP in complex with the transmembrane protein SpoIIM. Disruption of any one of the genes encoding the *B. subtilis* DPM complex prevents engulfment and thus spore formation. While homologs of these proteins exist in *C. difficile*, their role in engulfment has not been characterized. In addition, the *C. difficile* protein, SpoIIQ, likely has endopeptidase activity and is required for engulfment as well as proper coat localization in contrast with *B. subtilis*. In this study, we use allelic exchange to construct deletions of genes encoding putative engulfment-related proteins and are evaluating engulfment and coat localization in the resulting single, double, triple, and quadruple mutants. Deletion of *spoIID, spoIIP,* and *spoIIQ* resulted in engulfment and coat localization defects, with the latter phenotype being distinct from observations in *B. subtilis,* Loss of SpoIIM had minimal impact on *C. difficile* spore formation, suggesting that SpoIIM may not complex with SpoIID and SpoIIP in *C. difficile* unlike in *B. subtilis*. These analyses suggest that diverse mechanisms may control engulfment in spore-forming organisms and could inform the development of strategies for inhibiting *C. difficile* engulfment and thus disease transmission.

Author Disclosure Block:

J. Ribis: None. **K. Fimlaid:** None. **A. Shen:** None.

Poster Board Number:

SATURDAY-701

Publishing Title:

Mreb Regulates Polar Geometry and Stalk Elongation in *Caulobacter crescentus*

Author Block:

M. A. Bamimore[1], B. P. Bratton[2], E. A. Klein[1]; [1]Rutgers Univ.-Camden, Camden, NJ, [2]Princeton Univ., Princeton, NJ

Abstract Body:

The variety of bacterial cell shapes and sizes found in nature are largely determined by the structure and composition of the peptidoglycan cell wall. *Caulobacter crescentus*, a Gram-negative bacterium, synthesizes a long thin stalk appendage at one pole that elongates in response to phosphate limitation. Though we and others have recently uncovered the mechanisms underlying stalk localization and the establishment of stalk diffusion barriers, the mechanism of stalk synthesis remains unknown. The cytoskeletal protein MreB is known to regulate peptidoglycan synthesis in the cell body and is required for stalk synthesis. Furthermore, we have shown that MreB localizes to the stalk pole in response to phosphate starvation. Based on recent reports that the motion of MreB filaments along the cell body mediates cell width, we hypothesize that polar MreB similarly can guide stalk diameter. Using a series of MreB point mutants with unique cell shapes, we have begun to correlate polar curvature with stalk diameter. Using transmission electron microscopy (TEM), we have measured stalk diameters in the mutant collection. When compared to wild-type cells, 7/9 mutants had statistically significant changes in stalk diameter ($p<0.05$) ranging from 88-121% of wild-type.To correlate stalk diameter with polar curvature, we are expressing cytoplasmic GFP in each of the mutants and generating 3-D reconstructions of the cells using confocal microscopy. We are currently developing tools to quantitatively describe polar geometry to determine whether it is linked to stalk diameter. Our findings in this project will help to establish a basis for further characterization of the mechanisms underlying stalk biogenesis.

Author Disclosure Block:

M.A. Bamimore: None. **B.P. Bratton:** None. **E.A. Klein:** None.

Poster Board Number:

SATURDAY-702

Publishing Title:

Characterization of a Novel Lethal Mutation in *E. coli* Gtpase Era

Author Block:

A. Weaver, X. Zhou, N. Costantino, D. Court; Natl. Cancer Inst., Frederick, MD

Abstract Body:

Purpose: The highly conserved *E. coli* GTPase Era has a known role in 30S ribosomal subunit biogenesis but also affects cell division by an unknown mechanism. We have generated an Era mutant with a multi-residue change in the G domain, Era647, that leads to lethal cell filamentation and the inability to form colonies under certain conditions. Once characterized, this mutant may be useful in identifying *E. coli* cell division factors with which Era interacts. **Methods:** To study the effects of expression level and temperature on the lethality of Era647, we have placed the mutant allele on the chromosome under the native Era promoter P_{rnc} or under a temperature inducible version of the strong bacteriophage lambda promoter P_L as well as under the arabinose induced promoter P_{BAD} on a pUC derived plasmid. Viability of these strains, in terms of the ability to form colonies, was determined by growing strains at 32°C in liquid LB media with aeration overnight, then titering on LB agar plates at 32°C and 42°C. Ampicillin and arabinose were added to maintain and induce expression from plasmids. **Results:** Expression of Era647 from the native chromosomal locus does not reduce cell viability when compared to WT Era similarly expressed at 32°C or 42°C. Expression of Era647 from chromosomal P_L or from the fully induced pUC plasmid does reduce viability by roughly 10^4x at 42°C when compared to WT Era similarly expressed while expression of Era647 from the pUC plasmid does not reduce viability of cells at 32°C. **Conclusions:** We have determined that the lethality of Era647 is dependent upon both high temperature and high expression.

Author Disclosure Block:

A. Weaver: None. **X. Zhou:** None. **N. Costantino:** None. **D. Court:** None.

Poster Board Number:

SATURDAY-703

Publishing Title:

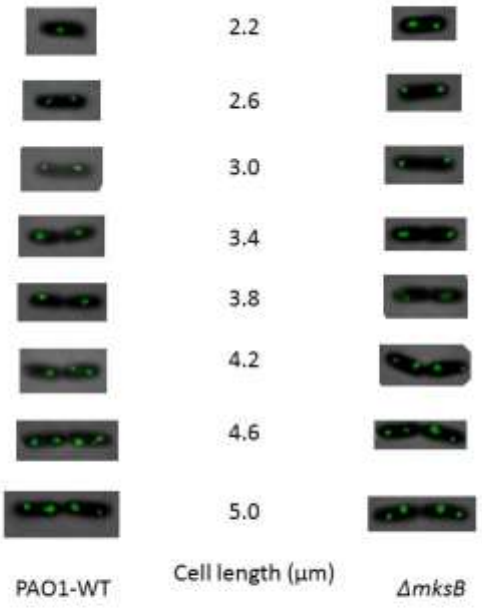
On the Contribution of Mksbef to Chromosome Segregation in *Pseudomonas aeruginosa*

Author Block:

B. K. BHOWMIK, V. V. Rybenkov; Univ. of Oklahoma, Norman, OK

Abstract Body:

Background: Condensins play a crucial role in global chromosome organization in both prokaryotes and eukaryotes. Pseudomonas aeruginosa expresses two condensins, the canonical SMC-ScpAB and the unconventional MksBEF. This study investigates the influence of MksBEF on chromosome segregation in *P. aeruginosa*.**Methods:** PAO1 condensin mutant, *ΔmksB* was constructed through homologous recombination. Several *tetO* repeats were cloned into specific loci of PAO1 chromosome and used as a target for *tetR*-CFP chimera expressed from a plasmid under an IPTG inducible promoter. Chromosomal loci were then observed using fluorescence microscopy to investigate their localization. **Results:**When grown in minimal media (doubling time 1 hr), the *oriC* and *dif* proximal regions were located towards the opposite poles of the newly born cells. The *oriC* region readily relocated towards mid-cell, following replication, splits into two foci, and remained at the cell quarters for the rest of the cell cycle. Separation of the chromosomal arms occurred later, followed by the separation of the *dif* region which splits at the very end of cell cycle and remained close to the newly formed poles of daughter cells. When grown in rich media (doubling time 52 mins) chromosome segregation followed the same pattern. However, the next round of replication began before the completion of cell division. As a result, the daughter cells contain two separate *oriC* foci. Significant differences could be observed between *ΔmksB* and PAO1-WT cells.



Segregation of oriC region in PAO1-WT and ΔmksB cells in grown in minimal media supplemented with citrate

Conclusions: Our findings indicate that the chromosomes are longitudinally organized both in PAO1-WT and *ΔmksB* cells and that MksBEF has marked effect on chromosome segregation. Conditions that support faster growth rates promote faster replication initiation without significant changes in the pattern of chromosome segregation.

Author Disclosure Block:

B.K. Bhowmik: None. **V.V. Rybenkov:** None.

Poster Board Number:

SATURDAY-704

Publishing Title:

Intrinsic Role of *Helicobacter pylori* min Proteins

Author Block:

Y. Nishida, H. Takeuchi, N. Morimoto, M. Kira, A. Okazaki, Y. Mastumura, T. Sugiura; Kochi Med. Sch., Kochi Univ., nankoku city, Japan

Abstract Body:

Background: Bacterial cell division is coordinated by many molecules, including FtsZ and Min proteins. In *Escherichia coli*, FtsZ forms a Z-ring structure at the division site. Min proteins (MinC, D and E) are responsible for the Z-ring formation at midcell. *Helicobacter pylori* conserves *min* genes with less than 50% homology to *E. coli*, thus the mechanisms are still unknown. Our aim is to analyze the function of *H. pylori* Min proteins and the interactions between FtsZ and Min proteins.**Methods:**A wild-type HPK5 and its derivative mutants (ΔMinC, D, E, CD and DE) disrupted by a Km^r and Cm^r cassette, were used. Bacteria were cultured in Brucella Broth supplemented with 10% horse serum with shaking at 37°C under microaerophilic condition (10% CO_2). Cells were examined by the absorbance, CFU, and Gram's stain whenever appropriate times. The cellular localization of FtsZ was detected by immunofluorescent (IF) staining. The interactions between FtsZ and Min proteins were examined by immunoprecipitation (IP)-western blotting (IP-WB) using *H. pylori* specific polyclonal antibodies were prepared with His-fusion proteins.**Results:**Except two strains (ΔMinCD and ΔMinDE), four strains showed no significant difference for cell growth and division. Gram-stain demonstrated that all disruptants became filamentous cell with different degree. The appearance rate of coccoid was obviously decreased in ΔMinE strain, consisting with high CFU count. IF staining showed FtsZ localized out of the center except HPK5 and ΔMinE. Especially in ΔMinD, FtsZ dispersedly distributed irrespective of the nucleoid position. Each Min protein interacted with FtsZ by IP-WB assay.**Conclusion:**Min proteins are involved in morphological shape determination by functional cooperation of FtsZ during cell division. Specifically, MinD plays a key role in the nucleoid occlusion system. MinE affects the morphological conversion to coccoid. Min functions were different between *E. coli* and *H. pylori*. We showed the unique characteristics of *H. pylori* Min proteins.

Author Disclosure Block:

Y. Nishida: None. **H. Takeuchi:** None. **N. Morimoto:** None. **M. Kira:** None. **A. Okazaki:** None. **Y. Mastumura:** None. **T. Sugiura:** None.

Poster Board Number:

SATURDAY-705

Publishing Title:

Role of the Semi-Conserved Histidine Residue in the Light-Sensing Domain of LitR, a MerR-Type Photosensory Transcriptional Regulator

Author Block:

H. Takano; Nihon Univ., Fujisawa, Japan

Abstract Body:

The LitR/CarH protein family transcriptional regulator is a new type of photoreceptor based on the function of adenosyl B_{12} ($AdoB_{12}$) as a light-sensitive ligand. Here, we studied the structure and function of the light-sensing ($AdoB_{12}$-binding) domain at the C-terminus (LitR-CTD) of LitR from a thermophilic gram-negative bacterium, *Thermus thermophilus* HB27. We identified a semi-conserved histidine residue (His^{132}) involved in the function of LitR, and revealed the X-ray structure of the LitR-CTD in complex with hydroxocobalamin (OHB_{12}). The *in vivo* mutation of His^{132} within LitR caused a reduction in the rate of carotenoid production in response to illumination. BIAcore analysis revealed that the illuminated-$LitR_{H132A}$ possesses high DNA-binding activity compared to the wild-type protein. The crystal structure of the OHB_{12}-bound LitR-CTD was determined at 2.52 Å, which was closest to that of monomethylamine corrinoid protein from *Methanosarcina barkeri*. The OHB_{12} bound to LitR was tucked with His^{177}, a conserved residue of the B_{12}-binding motif, and His^{132} from both sides. The ability of $LitR_{H132A}$ to associate with $AdoB_{12}$ was reduced compared with that of the wild-type protein in an equilibration dialysis experiment. Overall, these results suggest that His^{132} of LitR-CTD is involved in the association with $AdoB_{12}$ as well as the light-sensitive DNA-binding activity.

Author Disclosure Block:

H. Takano: None.

Poster Board Number:

SATURDAY-706

Publishing Title:

Molecular Analysis of *glnR* and the *vicRKX* Two-Component System in the pH-Dependent Expression of the *Streptococcus salivarius* 57.I Urease Operon

Author Block:

S-C. Huang, Y-Y. M. Chen; Chang Gung Univ., Taoyuan, Taiwan

Abstract Body:

Ureolysis by *Streptococcus salivarius* is critical for pH homeostasis of dental plaque and prevention of dental caries. The expression of *S. salivarius* urease is enhanced by acidic pH and excess amounts of glucose. The differential expression is regulated mainly at the transcriptional level on the promoter 5' to *ureI*, p_{ureI}. Our previous study demonstrates that CodY directly represses p_{ureI} expression and the repression is more pronounced at pH 7 compared to cells grown at pH 5.5. The expression of p_{ureI} remains sensitive to pH and glucose concentration in the *codY*-deficient background, suggesting that additional regulator is involved in the urease regulation. Recent sequence analysis revealed a putative VicR box and two GlnR boxes 5' to the CodY box. The results of DNA affinity precipitation assay, electrophoretic mobility shift assay (EMSA), and chromatin immunoprecipitation-PCR indicated that both VicR and GlnR interacted with the predicted binding boxes on p_{ureI}. By establishing a p_{ureI}-chloramphenicol acetyltransferase gene (*cat*) fusion system in *S. salivarius*, it was found that mutations in the VicR box upregulate p_{ureI} expression, whereas mutations in the GlnR boxes downregulate p_{ureI} expression. Isogenic mutant strains (*vicRKX*- and *glnR*-null) and complemented strains (harboring *S. salivarius vicRKX* and *glnR*) were generated in the recombinant *Streptococcus gordonii* host harboring a wild-type p_{ureI}-*cat* fusion on *gtfG* to investigate the regulatory function of VicR and GlnR. The recombinant strains were grown in a chemostat under glucose-excess (100 mM) and -limiting (20 mM) conditions, at pH 7 and 5.5. Results of p_{ureI}-*cat* activity indicated that the repression by VicR was more pronounced at pH 7 whereas GlnR was more active at pH 5.5. Furthermore, the results of EMSA and promoter analysis indicated that the C-terminal domain of the RNA polymerase α subunit interacted with the VicR box to enhance p_{ureI} expression in the absence of VicR. The overall regulation by CodY, VicR and GlnR in response to pH ensures optimal expression of urease in *S. salivarius* when the enzyme is most needed.

Author Disclosure Block:

S. Huang: None. **Y.M. Chen:** None.

Poster Board Number:

SATURDAY-707

Publishing Title:

Application of *Pseudomonas aeruginosa* as a Genetic Tool for the Molecular Study of *Corynebacterium glutamicum*

Author Block:

J-H. Lee, Y-J. Kim, H-S. Shin, H-S. Lee, U-H. Ha; Korea Univ., Sejong, Korea, Republic of

Abstract Body:

Background: We previously identified a putative acyltransferase encoded by NCgl0350 of *Corynebacterium glutamicum*, which is induced by cell-free culture fluids obtained from stationary-phase growth of both *C. glutamicum* and *Pseudomonas aeruginosa*, providing evidence for interspecies communication. However, upstream regulators responsible for the autoinduction have not been elucidated yet.**Methods:** A growth-permissive assay and β-galactosidase assay were conducted to determine the transcriptional expressions. To identify the upstream regulators, we used *P. aeruginosa* as a genetic model to substitute for *C. glutamicum*.**Results:** The putative acyltransferase expression was induced by culture fluids obtained from diverse Gram-negative and -positive bacterial strains. A homologous acyltransferase encoded by PA5238 of *P. aeruginosa* was identified and found to be induced by those culture fluids, as observed for NCgl0350 of *C. glutamicum*. Because *C. glutamicum* is difficult to study using transposon mutagenesis approaches, the homologous gene PA5238 was used to identify PA5309 as an upstream regulator of expression. A homologous D-amino acid dehydrogenase encoded by NCgl2909 of *C. glutamicum* was cloned based on amino acid similarity to PA5309, and its role in the regulation of NCgl0350 expression was confirmed.**Conclusions:** We successfully applied *P. aeruginosa* to substitute for the molecular study of *C. glutamicum*, resulting in identifying a D-amino acid dehydrogenase as an upstream regulator of the autoinduction of a putative acyltransferase in *C. glutamicum*.

Author Disclosure Block:

J. Lee: None. **Y. Kim:** None. **H. Shin:** None. **H. Lee:** None. **U. Ha:** None.

Poster Board Number:

SATURDAY-709

Publishing Title:

ampM*, a Member of L2-*ampM* Operon, Involved in the *mrcA* Inactivation-Mediated β-Lactamase Expression in *Stenotrophomonas maltophilia

Author Block:

Y-W. Huang, C-J. Wu, Y. Wang, T-C. Yang; Natl. Yang-Ming Univ., Taipei, Taiwan

Abstract Body:

The L2 β-lactamase gene of *Stenotrophomonas maltophilia* has been shown to be involved in β-lactams resistance and its expression is regulated by the *ampR* gene, which divergently locates upstream of the L2 gene. In this study, L2 and its downstream gene, designated as *ampM* hereinafter, were shown to be an operon, as revealed by Quantitative Real-Time PCR (QRT-PCR), and Reverse Transcriptase PCR (RT-PCT). The expression of the *ampM* gene depended on the *ampR*-regulated L2 promoter (P_{L2}) and its own promoter (P_{ampM}). The *ampM* gene previously annotated as a putative Na^+/H^+ antiporter in the genome project of *S. maltophilia* was found to be less involved in the sodium homeostasis. Since *ampM* and *L2* form an operon, we assessed the involvement of AmpM in the β-lactamase expression. An *ampM* isogenic deletion mutant, *ΔampM*, was constructed, and the basal- and induced- β-lactamase activities between WT and *ΔampM* were evaluated. Inactivation of *ampM* little affected the basal and induced β-lactamase activities. In our previous study, we have demonstrated that the *ampD*$_I$, *mrcA*, and *mltD1* deletion mutants, *ΔampD*$_I$, *ΔmrcA*, and *ΔmltD1*, display a similar phenotype of basal-level β-lactamase upexpression; therefore we further assessed the involvement of AmpM in this phenotype. The *ΔampM* allele was introduced into the chromosomes of *ΔampD*$_I$, *ΔmrcA*, and *ΔmltD1,* respectively, yielding double mutants of *ΔampD*$_I$*ΔampM*, *ΔmrcAΔampM*, and *ΔmltD1ΔampM*. The basal-level β-lactamase activities of these double mutants and their parental strains were comparatively determined. Inactivation of *ampM* partially decreased the basal-level β-lactamase activity of *ΔmrcA* mutant, but hardly affected those of *ΔampD*$_I$ and *ΔmltD1* deletion mutants.

Author Disclosure Block:

Y. Huang: None. **C. Wu:** None. **Y. Wang:** None. **T. Yang:** None.

Poster Board Number:

SATURDAY-710

Publishing Title:

The Global Regulator WetA Governs Fungal Development and Secondary Metabolism

Author Block:

M-y. Wu, J-H. Yu; Univ. of Wisconsin-Madison, Madison, WI

Abstract Body:

Background: The genus *Aspergillus* represents the most common fungi in the environment and can serve as a model system for genetic study of filamentous fungi. The main reproductive mode of aspergilli is the formation of asexual spores called conidia. BrlA, AbaA, and WetA are proposed as the central regulators of asexual development (conidiation) in aspergilli. BrlA and AbaA are transcription factors, yet the WetA-mediated regulatory mechanism is still unknown. Here, we characterized WetA's functions and further investigated the underlying molecular regulatory mechanism.**Methods:** We characterized WetA's functions in *A. nidulans* (FGSC4) and *A. flavus* (NRRL3357) by generating *wetA*-null mutants. For genome-wide expression studies, RNA-seq was carried out using RNA samples extracted from *A. nidulans* WT and *wetA*-null mutant conidia. The library was sequenced (PE100bp) using the Illumina HiSeq2500 generating final of over 10^7 reads/sample. For in vivo WetA-DNA interaction studies, ChIP-PCR was performed using MAGnify Chromatin Immuno-precipitation System (Invitrogen) with anti-WetA antibody. Samples were collected from *A. nidulans* and *A. flavus* WT conidia. The enriched DNA were confirmed by PCR.**Results:** The *wetA*-null mutants form wet and white conidia, which display deficient structure and autolyze in 3 days. Loss of *wetA* leads to decreased levels of conidial trehalose, the major protectant for thecellular integrity. Consistently, the *wetA*-null conidia show reduced viability under normal and stressed conditions. WetA also regulates conidiation timing in a species-specific manner. The growth rate and aflatoxin production data indicated that WetA is not only involved in asexual development, but also in vegetative growth and secondary metabolism. Northern analysis shows that WetA is a negative regulator of its upstream regulators and exerts feed-back control for development in both species. RNA-seq data show that WetA plays a global regulatory role in conidiogenesis. The ChIP-PCR results show that WetA binds to the upstream region of *brlA*, indicating the direct repressive role of WetA on *brlA* expression.**Conclusions:** WetA is an evolutionary conserved central developmental regulator in certain groups of Ascomycetes. This is the first study demonstrating that WetA is a DNA-binding protein, which plays a global regulatory role on asexual development, vegetative growth and secondary metabolism.

Author Disclosure Block:

M. Wu: None. **J. Yu:** None.

Poster Board Number:

SATURDAY-711

Publishing Title:

Determining the Architecture of a Succinate-Mediated Catabolite Repression Controlled Promoter in *Sinorhizobium meliloti*

Author Block:

J. Johnson, C. Mirarchi, M. Arif, L. Ngaba, C. Arango; St. Joseph's Univ., Philadelphia, PA

Abstract Body:

Sinorhizobium meliloti, a Gram negative soil bacterium that fixes nitrogen when in symbiosis with legumes, exhibits succinate-mediated catabolite repression (SMCR), where the presence of succinate controls expression of a number of genes. The regulatory mechanisms of SMCR in *S. meliloti* are not well understood, and, although it is known that a phosphotransferase system is involved, a global regulator has not been identified. Uncovering the architecture of SMCR-regulated promoters may shed light on the presence of such regulator and help identify the members of the SMCR regulon. The purpose of this study is to identify sequences that are critical for regulation of gene expression in the model SMCR-controlled operon *agp-melA*. This operon, induced by raffinose but repressed in the presence of succinate, codes for proteins for raffinose utilization and is under the control of the local activator AgpT. To determine the promoter architecture, a 400 bp region upstream of *agp-melA*, P*melA*, was identified as necessary for proper regulation. This region was used to investigate the binding site of the activator ApgT, using radio-labeled wild-type and mutant DNA, and crude protein *S. meliloti* extracts in an Electrophoresis Mobility Shift Assay. Additionally, a P*melA*:*gfp* reporter plasmid was used to find other regulatory sequences, using two approaches: the promoter was subjected to random point mutations through error-prone PCR, and 3'- and 5'- shortened promoters were constructed. The library of random and shortened mutant P*melA* was screened for altered *gfp* expression in colonies grown on raffinose plates in the presence and absence of succinate using low-magnification fluorescence microscopy. Eighty random P*melA* mutants and four 5'end-shortened promoters that exhibited reduced expression were identified. The phenotypes of a subset were confirmed monitoring fluorescence in liquid culture. Sequencing of two of the random P*melA* mutants, and analysis of the 5' end shortened ones revealed a 26-bp region that is critical for expression. We are conducting EMSA assays to elucidate if this is the binding site of AgpT. Sequencing of the remaining random mutants is ongoing and may reveal the location of other regions critical for SMCR regulation of the *agp-melA* operon.

Author Disclosure Block:

J. Johnson: None. **C. Mirarchi:** None. **M. Arif:** None. **L. Ngaba:** None. **C. Arango:** None.

Poster Board Number:

SATURDAY-712

Publishing Title:

A New Regulatory Cascade Controlling *ompF* Expression with Temperature

Author Block:

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Abstract Body:

In *Escherichia coli*, OmpF is an important outer membrane protein, which serves as a passive diffusion pore for nutrients and antibiotics, and for excretion of toxic products. Regulation of OmpF expression is well documented and is known to involve a network of transcriptional and translational regulators responding to osmolarity, stress and temperature. However, the detailed mechanism leading to increased OmpF levels at ambient temperature (25°C) versus 37°C is still unknown. Using RT-qPCR, we quantified OmpF mRNA levels and showed that two mutations in *lon* (coding for the Lon protease) and in *bluR* (coding for a MerR-like regulator) prevented high expression of *ompF* at ambient temperature. Using a chromosomal fusion of *ompF* promoter region and *lacZ* (*ompFp-lacZ*), we measured similar activity of *ompF* promoter at 37°C for both wild-type and *lon bluR* mutant, while a decreased activity was observed in the *lon bluR* mutant at 25°C. Deletion of the *ycgZ-ymgABC* operon in the *lon bluR* mutant, known to be regulated by BluR, restored wild-type like expression of OmpF at 25°C. The stability of YcgZ, YmgA, YmgB and YmgC proteins was then analyzed in both wild type and *lon* strains using the vector pBAD for expressing the proteins. Our results showed that YcgZ protein was highly unstable in the presence of Lon (wild type background) while a high amount of YcgZ was observed in the *lon* mutant. The quantity of YmgA, YmgB and YmgC was similar in wild type and *lon* strains indicating that these proteins were not degraded by the Lon protease. Furthermore, overexpression of YcgZ at 25°C significantly decreased *ompFp-lacZ* activity in a strain carrying mutations in *lon, bluR and ycgZ-ymgABC*. In summary, our study identified a new regulatory cascade that controls levels of OmpF with temperature. This pathway involves BluR, which controls expression of YcgZ, a repressor of OmpF transcription and a substrate of the Lon protease. As *Enterobacteriaceae* may transit through many different locations encountering shifts to lower temperatures, an understanding of how these microorganisms adaptto temperature changes is particularly important in medical and food industry where the prevention of bacterial contamination is imperative.

Author Disclosure Block:

V. Duval: None. **S.B. Levy:** None.

Poster Board Number:

SATURDAY-713

Publishing Title:

Structure-Function Studies of the *Schizosaccharomyces pombe* Ell-Eaf Transcription Elongation Complex

Author Block:

N. Sharma[1], P. Dabas[1], K. Sweta[1], S. Gopalan[2], K. Jain[1], R. Conaway[2], J. Conaway[2];
[1]G.G.S.Indraprastha Univ., New Delhi, India, [2]Stowers Inst. for Med. Res., Kansas City, MO

Abstract Body:

Transcription is an important step in the control of gene expression in eukaryotic cells. Research over the past few decades has primarily focussed on the preinitiation and initiation stages of transcription. However, recent studies have shown that transcription elongation also constitutes a major step of transcription regulation. A plethora of elongation proteins have been discovered, and elucidating the roles of each of these proteins is a challenge. The ELL (Eleven Nineteen Lysine Rich Leukemia) and EAF (ELL associated factor) family of proteins suppress transient pausing of the RNA polymerase II enzyme along the DNA template in *in vitro* transcription assays. However, the structure and function(s) of these proteins still await a detailed characterization. A single homologue of ELL and EAF proteins is present in *Schizosaccharomyces pombe*. In this study, we have used different approaches to elucidate the functional and structural properties of these proteins in *S. pombe*. Our results show that deletion of either ELL or EAF causes slow growth of cells under optimum growth conditions. Moreover, exposure to DNA damaging agents reduces the viability of ELL and EAF null mutants. In comparison, no growth defect was observed under a variety of environmental stress conditions tested. To determine if both these proteins act in the same pathway, we have also looked at the effect of deleting both these genes on different phenotypes. Truncation mutants have also been constructed to delineate the regions of these proteins important for the observed phenotypes, as well as required for transactivation and transcription elongation. Yeast two-hybrid and co-immunoprecipitation assays have been used to map the domain required for interaction between ELL and EAF proteins. Localization of these proteins has also been studied. Our yeast two hybrid analysis has revealed that the *S. pombe* and human ELL-EAF proteins do not heterodimerize. We have also used CD spectroscopy to gain insights into the structure of the EAF protein. In summary, our results provide novel insights into the structure and functions of these proteins.

Author Disclosure Block:

N. Sharma: None. **P. Dabas:** None. **K. Sweta:** None. **S. Gopalan:** None. **K. Jain:** None. **R. Conaway:** None. **J. Conaway:** None.

Poster Board Number:

SATURDAY-714

Publishing Title:

Protein-Protein Interaction of *Campylobacter jejuni* Post-Transcriptional Regulator CSRA and Putative Flagellar Chaperone Fliw

Author Block:

J. Li[1], **C. A. Fulmer**[1], A. Upchurch[1], L. K. H. Tran[1], P. M. H. Tran[1], J. S. Fiedler[1], G. Gutierrez-Sanchez[2], S. A. Thompson[1]; [1]Augusta Univ., Augusta, GA, [2]Univ. of Georgia, Athens, GA

Abstract Body:

Campylobacter jejuni is a leading causative agent of bacterial gastroenteritis and is attributed to as much as 40-80% of cases of Guillain-Barré syndrome. We showed that the homodimeric post-transcriptional regulator CsrA is involved with biofilm formation, motility, and host cell invasion, each of which require functional flagella. While *Escherichia coli* CsrA activity is regulated by interactions with small non-coding RNAs, we demonstrated that *C. jejuni* CsrA activity is likely regulated by protein-protein interactions between CsrA and FliW, a putative flagellar chaperone. Using pulldown assays, protein-protein crosslinking, and surface plasmon resonance, we demonstrated binding of CsrA to FliW. *C. jejuni* CsrA notably differs from *E. coli* CsrA in the C-terminal region of the protein, both in amino acid sequence and in length. PHYRE[2] analysis of *C. jejuni* CsrA predicts that this region lies on the outside of CsrA homodimers, leading to the hypothesis that this is the site of protein-protein interactions between CsrA and FliW. Deletion analysis of the CsrA C-terminus showed that the region of -16 to -28 amino acids (relative to the CsrA C-terminus) was required for FliW binding. We are currently using site-directed mutagenesis of this region of the CsrA protein to identify amino acids that are required for *C. jejuni* CsrA-FliW protein-protein interaction. These results suggest a model that binding of FliW near the CsrA C-terminus destabilizes the second CsrA RNA binding domain and thereby modulates its regulatory activity, linking flagellar synthesis with CsrA regulation of metabolic and other pathogenesis-related features of *C. jejuni*.

Author Disclosure Block:

J. Li: None. **C.A. Fulmer:** None. **A. Upchurch:** None. **L.K.H. Tran:** None. **P.M.H. Tran:** None. **J.S. Fiedler:** None. **G. Gutierrez-Sanchez:** None. **S.A. Thompson:** None.

Poster Board Number:

SATURDAY-715

Publishing Title:

Lambdoid Phages Modulate Regulation of the Pathogenicity Island Locus of Enterocyte Effacement (Lee) on Pathogenic *Escherichia coli*

Author Block:

J. D. Hernandez-Doria, V. Sperandio; Univ. of Texas Southwestern Med. centre, Dallas, TX

Abstract Body:

Enterohemorrhagic *Escherichia coli* (EHEC) causes bloody diarrhea and hemolytic uremic syndrome (HUS) worldwide. HUS is caused by Shiga toxin (Stx), and the genes encoding this toxin are carried within the late genes of a lambdoid bacteriophage. The stx phage can be either lysogenic or lytic. Another important pathogenicity island is the locus of enterocyte and effacement (LEE) that encodes for a type three secretion system, and adhesin and its receptor that are necessary to form attaching and effacing (AE) lesions on epithelial cells. Here we show that there is regulatory "cross-talk" between the LEE and the stx phage. The phage transcription factor Cro activates expression of the LEE genes and promotes AE lesion formation. Cro directly binds to the regulatory region of *ler*, which encodes the activator of all LEE genes. The Cro protein differs among different Stx-phages, suggesting that modulation of LEE expression may vary among different EHEC strains.

Author Disclosure Block:

J.D. Hernandez-Doria: None. **V. Sperandio:** None.

Poster Board Number:

SATURDAY-716

Publishing Title:

Transcriptional Regulation of Quinate Metabolism in *Listeria monocytogenes*

Author Block:

S. M. Prezioso, D. Christendat; Univ. of Toronto, Toronto, ON, Canada

Abstract Body:

The shikimate pathway is an essential metabolic pathway in bacteria, plants, and fungi, which leads to the synthesis of the three aromatic amino acids among other important aromatic compounds. Quinate is an abundant plant derived compound that can be catabolized by some bacteria through a branch of the shikimate pathway to generate energy, although little is known about the regulation of quinate utilization in bacteria. The genome of *Listeria monocytogenes* includes an operon that contains quinate utilization enzymes (shikimate/quinate dehydrogenase and dehydroquinate dehydratase), and a LysR type transcriptional regulator (named QuiR1) that is upstream and divergently transcribed. Using differential scanning fluorimetry and electrophoretic mobility shift assays, we have shown that QuiR1 binds to shikimate in order to modulate its activity at this divergent promoter. Data from *lacZ*-promoter fusion experiments demonstrate that QuiR1 functions as an activator of quinate operon transcription in the presence of shikimate and an auto-repressor of its own transcription. Additionally, the crystal structure of the QuiR1 effector binding domain was determined to a resolution of 2.5 Å in complex with its effector, shikimate. Since Listeria does not have the familiar downstream enzymes involved in quinate catabolism, the role of quinate metabolism in *L. monocytogenes* is also intriguing.

Author Disclosure Block:

S.M. Prezioso: None. **D. Christendat:** None.

Poster Board Number:

SATURDAY-717

Publishing Title:

Investigating the Effect of the Calr Transcription Factor on Expression of Calcium-regulated Genes *In Vibrio* Parahaemolyticus

Author Block:

R. Manges, L. Post, K. Bies, L. Swanson, J. Enos-Berlage; Luther Coll., Decorah, IA

Abstract Body:

Background: Although calcium is known to influence gene expression in some bacteria, the mechanisms and physiological benefits of this regulation are not well understood. *Vibrio parahaemolyticus* is a ubiquitous marine bacterium and major cause of seafoodborne illness. Notably, this organism experiences varying calcium levels in its ocean, estuary, and human GI tract habitats. Previous isolation of Tn5*lux* gene fusions identified over 40 distinct *V. parahaemolyticus* loci whose expression was influenced by calcium. CalR, a LysR-type transcription factor, was initially found to repress calcium-dependent swarming and Type III secretion in *V. parahaemolyticus*. We hypothesized that CalR might also be involved in the regulation of other calcium-regulated genes. To test this idea, we examined the effect of overexpressing or knocking out *calR* on expression of calcium-regulated genes. **Methods and Results**: A CalR overexpression vector was introduced into the collection of calcium-regulated Tn5*lux* fusions, and twelve loci were identified whose expression was altered by increased levels of CalR. Six of these loci were chosen for further analyses based on their potential to increase understanding of the CalR regulon. A *calR* knockout mutation was introduced into each of these strains, and gene expression was examined in triplicate in both $calR^+$ and $calR^-$ strains grown under varying calcium conditions. Lack of CalR resulted in elevated gene expression in five Tn5*lux* fusions and decreased gene expression in one fusion, with $calR^-/calR^+$ fold changes ranging from three to over 20-fold. Genes whose expression was regulated by CalR had established or predicted functions in motility, amino acid transport, extracellular polysaccharide transport, iron acquisition, hemolysis, and protease activity. For all loci, the phenotypic impact of the *calR* mutation was most pronounced under low calcium conditions. **Conclusion:** These data serve to expand the CalR regulon in *V. parahaemolyticus* and support the idea that CalR is repressing expression of multiple genes under low calcium conditions. The existence of one locus whose expression is enhanced by CalR suggests additional regulatory complexity. Collectively, these data provide useful clues to further our understanding of calcium regulation in bacteria.

Author Disclosure Block:

R. Manges: None. **L. Post:** None. **K. Bies:** None. **L. Swanson:** None. **J. Enos-Berlage:** None.

Poster Board Number:

SATURDAY-718

Publishing Title:

Crystal Structure of Dimerization/Oligomerization Domain of an Mvat Homolog in *Pseudomonads*

Author Block:

C. Suzuki-Minakuchi[1], K. Kawazuma[1], J. Matsuzawa[1], D. Vasileva[1], Z. Fujimoto[2], T. Terada[1], K. Okada[1], H. Nojiri[1]; [1]The Univ. of Tokyo, Tokyo, Japan, [2]Natl. Inst. of Agrobiological Sci., Tsukuba, Japan

Abstract Body:

Background: H-NS family proteins, one of the nucleoid-associated proteins, repress genes acquired through horizontal gene transfer. MvaT homologs in *Pseudomonas* have almost negligible amino acid sequence identity with H-NS but can complement an *hns*-related phenotype of *Escherichia coli*. It remains unknown whether the molecular mechanisms for their function are the same between MvaT and H-NS homologs. Here, we solved the crystal structure of the dimerization/oligomerization domain of TurB (TurB_nt$_{61}$), an MvaT homolog in *Pseudomonas putida* KT2440, to get an insight into functional manner of MvaT homologs.**Methods:** To obtain oligomerization-deficient derivatives of TurB_nt$_{61}$, we introduced the alanine substitutions at seven residues, which were predicted to be involved in homo-oligomerization from the previous study [1]. Among them, TurB_nt$_{61}$-R8A was crystallized using a precipitant solution containing ammonium acetate. Initial structural determination was conducted by the single-wavelength anomalous diffraction using the selenium absorption peak. Crystal structure of the native TurB_nt$_{61}$-R8A was determined by the molecular replacement.**Results:** The structure of TurB_nt$_{61}$-R8A was determined at 2.3 Å resolution. The crystal contained one dimer molecule per asymmetric unit, in which two subunits were interlocked at the C-terminal region. This “dimerization site” was stabilized by a hydrophobic core and hydrogen bonds, and was similar to that of H-NS. N-terminal region of TurB_nt$_{61}$ missing the above “dimerization site” still formed homo-dimers but not homo-oligomers, suggesting that TurB has additional “dimerization site” in its N-terminus. The fact that two dimerization sites are necessary for oligomerization is similar to that of H-NS. But, N-terminal “dimerization site” in TurB_nt$_{61}$ was predicted to be formed by a coiled-coil motif, whereas the corresponding “dimerization site” in H-NS is interlocked by additional two small helices.**Conclusions:** We found that TurB and H-NS have partly similar but different oligomerization mechanisms. The results can shed light on the evolutionary pathway of H-NS family proteins.

Author Disclosure Block:

C. Suzuki-Minakuchi: None. **K. Kawazuma:** None. **J. Matsuzawa:** None. **D. Vasileva:** None. **Z. Fujimoto:** None. **T. Terada:** None. **K. Okada:** None. **H. Nojiri:** None.

Poster Board Number:

SATURDAY-719

Publishing Title:

Uncovering Structural Determinants Associated with Ligand and Dna Binding Specificity in a Conserved Subfamily of Arac Transcription Regulators

Author Block:

G. G. Willsey, E. L. English, M. J. Wargo; Univ. of Vermont, Burlington, VT

Abstract Body:

Catabolism of glycine betaine in *Pseudomonas aeruginosa* is regulated by two transcription factors, SouR and GbdR. GbdR induces genes encoding all steps in the pathway in response to GB and dimethylglycine, while SouR only induces the genes for sarcosine demethylation in response to sarcosine. GbdR and SouR are members of the glutamine amidotransferase1-like subfamily of AraCt transcription regulators (GATRs), and their sequence homology and phylogenetic distribution suggest common ancestry and extensive gene transfer. Despite their widespread distribution among proteobacteria, little is known about these regulators aside from their basic two domain architecture. The close phylogenetic similarities between SouR and GbdR and their partially overlapping regulons provide us with a powerful system to understand the molecular basis of the ligand binding and promoter specifies for the GATR subfamily. To gain insight into the ligand recognition mechanism of GATR's we have devised a gain-of-function genetic screen coupling the random mutagenesis of *souR* with sarcosine anaologs ethylglycine and dimethylglycine as potential inducing ligands with the goal of identifying residues influencing activation specificity. Through this process we have identified numerous mutants exhibiting either sarcosine hyper-responsive, sarcosine-repressive, ethylglycine-responsive, or constitutive phenotypes, and are currently characterizing the genotypic signatures of each. Additionally, we are in the process of performing a second gain of function screen to identify signatures within the promoter region that direct the differential DNA binding specificities of SouR and GbdR. Together, data collected from these screens will provide the first insight into the GATR activation mechanism, and will generate the information necessary to guide future structure-function studies.

Author Disclosure Block:

G.G. Willsey: None. **E.L. English:** None. **M.J. Wargo:** None.

Poster Board Number:

SATURDAY-720

Publishing Title:

Quorum-sensing Gene Regulation by *Vibrio harveyi* luxr Requires an Activity in Addition to Dna Binding

Author Block:

J. A. Healy, **J. C. van Kessel**; Indiana Univ., Bloomington, IN

Abstract Body:

Quorum sensing is a method of cell-cell communication that allows bacteria to sense and respond to changes in population density. Bacteria use quorum sensing to control processes that are more beneficial when performed by groups of cells acting together, such as virulence factor production, biofilm formation, competence, and bioluminescence. In vibrios, quorum-sensing gene expression is controlled by the master transcription factor LuxR, which controls >600 genes, including those that produce bioluminescence. *Vibrio harveyi* LuxR is classified as a member of the TetR superfamily of transcription factors but is unique because it both activates and represses transcription. We isolated three mutants of LuxR that are defective for transcription activation of the bioluminescence genes but retain wild-type transcription repression of other promoters. Each of these mutations lies outside the N-terminal DNA binding domain and instead are located closer to the C-terminus on alpha-helix 7. The LuxR L139P mutant does not show any defects in DNA binding activities at any tested binding site *in vitro*. Thus, we hypothesized that another activity - in addition to DNA binding - is required for transcription activation of the bioluminescence genes. We developed a reporter assay in *Escherichia coli* to monitor LuxR transcription activation and repression simultaneously: activation of the bioluminescence genes (*gfp*) and repression of a hydrolase gene (*mCherry*). Using the LuxR L139P mutant and this reporter, we performed a suppressor screen via EMS mutagenesis to isolate *E. coli* mutants with increased activation of the bioluminescence genes. Several *E. coli* mutants increased activation two- to four-fold, suggesting that another factor in *E. coli* plays a role in transcription activation together with LuxR. Surprisingly, we also isolated several *E. coli* mutants that decreased repression activity. Characterization of these mutants will enable us to identify possible interaction partners or other *trans*-acting elements that regulate transcription activation of quorum-sensing genes with LuxR. Because LuxR proteins are the central regulators of quorum sensing in all vibrios, this research will uncover novel functions and interactions that are required for quorum-sensing gene regulation.

Author Disclosure Block:

J.A. Healy: None. **J.C. van Kessel:** None.

Poster Board Number:

SATURDAY-721

Publishing Title:

The Involvement of Nucleoid-associated Proteins in Luxr-directed Transcription *In Vibrio* Harveyi

Author Block:

R. Chaparian, S. G. Olney, J. C. van Kessel; Indiana Univ., Bloomington, IN

Abstract Body:

Quorum sensing is a cell-cell communication process in which bacterial cells produce, release and respond to extracellular chemical signaling molecules to evaluate local population density. Bacteria coordinate group activities such as bioluminescence, virulence factor production, and biofilm formation via quorum sensing. In *Vibrio harveyi*, the master quorum-sensing transcription factor LuxR activates and represses >600 genes. However, the mechanistic details by which this is achieved are unclear; we have identified 7 LuxR binding sites within the promoter region and will characterize their individual contributions to transcription. Using biochemical and genetic approaches we identified the nucleoid associated proteins IHF, Fis and H-NS as regulators of the *luxCDABE* operon, a model LuxR-dependent promoter. IHF bends the *luxCDABE* promoter DNA ~90° *in vitro*, and binding of IHF *in vivo* is required for precise timing and levels of activation of this operon. Conversely, H-NS functions solely as a repressor of transcription, and we have shown that H-NS inhibits transcription of the *luxCDABE* operon *in vivo*. To gain insight on the mechanistic details regarding LuxR-driven transcription activation, we will utilize *in vitro* transcription assays to monitor the formation of transcriptionally competent nucleoprotein complexes. We performed gel-shift assays in the presence of LuxR and IHF in which we observed synergistic binding. Furthermore, using RNA-Seq we demonstrated that the LuxR and IHF regulons overlap at 18 promoters. Collectively, our data implicate nucleoid-associated proteins in LuxR-dependent gene regulation in the *Vibrio* clade.

Author Disclosure Block:

R. Chaparian: None. **S.G. Olney:** None. **J.C. van Kessel:** None.

Poster Board Number:

SATURDAY-722

Publishing Title:

Irf-3 Regulates Rig-I Expression in Ifn-Independent Manner in Response to Dsrna

Author Block:

R. Hayakari, T. Matsumiya, F. Xing, H. Yoshida, T. Imaizumi; Hirosaki Univ. Grad. Sch. of Med., Hirosaki, Aomori, Japan

Abstract Body:

The innate immune system is the first line of host defense against invading pathogens. Retinoic acid-inducible gene-I (RIG-I) plays an important role in the innate immune responses. Upon viral infection, RIG-I senses viral RNA in cytoplasm triggering the activation of antiviral signaling to produce type I interferon (IFN). In this cascade, IFN regulatory factor-3 (IRF-3) is known to be a crucial molecule. Although RIG-I contributes to the antiviral signaling, RIG-I itself is known to be one of IFN-inducible genes. However, the mechanism wherein viral infection stimulates the expression of RIG-I has not been completely elucidated. We transfected the cells with polyinosinic-polycytidylic acid (poly I:C), a synthetic double-stranded (ds)RNA, to mimic viral infection. Using IFN-α/β receptor-null U5A cells and their parental 2fTGH cells, we previously reported that a part of RIG-I expression is regulated in an IFN-independent manner in response to dsRNA. To elucidate what factor(s) regulates the RIG-I expression, we generated a series of luciferase reporter constructs containing RIG-I promoter and analyzed the promoter activity. Although a number of consensus sequences of transcriptional factors were identified in the RIG-I promoter region by computational analysis, deletion of putative IRF binding site abolished its transcriptional activity. This result suggests that IRF regulates RIG-I gene expression through the activation of the RIG-I promoter. We further tested the influence of IRF-3 on the expression of RIG-I using IRF-3-specific RNAi and found that the knockdown of IRF-3 significantly decreased the RIG-I expression level in poly I:C-transfected U5A and 2fTGH cells. Our results suggest that a part of the expression of RIG-I is regulated directly by IRF-3 in an IFN-independent manner. IRF-3 may be one of the key molecules in antiviral signaling-mediated RIG-I expression.

Author Disclosure Block:

R. Hayakari: None. **T. Matsumiya:** None. **F. Xing:** None. **H. Yoshida:** None. **T. Imaizumi:** None.

Poster Board Number:

SATURDAY-724

Publishing Title:

A Repurposed Repressor: The *Salmonella* typhimurium Counter-silencer, Slya

Author Block:

W. R. Will, S. J. Libby, F. C. Fang; Univ. of Washington, Seattle, WA

Abstract Body:

Counter-silencing is a mechanism of transcription regulation in which the activity of a repressor protein bound in the vicinity of a bacterial promoter is disrupted by additional proteins. Counter-silencing has fewer architectural requirements than canonical transcriptional activation and therefore plays an important role in facilitating the regulatory integration of horizontally-acquired bacterial genes. To examine the features that characterize an effective counter-silencer, we performed a molecular and genetic characterization of the SlyA protein, a MarR-family transcriptional regulator that represses the expression of a drug efflux pump and counters the silencing of multiple virulence genes in Salmonella Typhimurium. Using in vitro transcription and footprinting assays, we have demonstrated that SlyA lacks activation activity and functions as a repressor of its own transcription. Furthermore, SlyA exhibits relatively weak target specificity, binding to high-affinity sites and subsequently spreading outwards to form an oligomeric nucleoprotein complex. Notably, like other MarR regulators, SlyA activity is modulated by aromatic carboxylates such as salicylate. SlyA-mediated repression and counter-silencing are inhibited by aromatic carboxylates, whereas its ability to bind DNA is not, suggesting that salicylate interactions alter the conformation of SlyA. In contrast to Salmonella, SlyA plays a minor regulatory role in Escherichia coli despite a high degree of homology (89.6%) between the two species. Genetic analyses revealed that the E. coli and S. Typhimurium slyA alleles are interchangeable, but the promoter regions exhibit considerable divergence, resulting in substantially higher slyA expression levels in S. Typhimurium. This suggests that mutations altering the Salmonella slyA regulatory circuit have been critical for the evolution of SlyA from a minor transcriptional repressor to a counter-silencer that plays an essential role in virulence.

Author Disclosure Block:

W.R. Will: None. **S.J. Libby:** None. **F.C. Fang:** None.

Poster Board Number:

SATURDAY-725

Publishing Title:

Regulation of *yidC2* in *Streptococcus mutans*

Author Block:

S. Palmer; The Ohio State Univ., Columbus, OH

Abstract Body:

Background: The universally conserved YidC/Oxa/Alb family of integral membrane chaperones-insertases has diverse functions in the assembly and insertion of respiratory chain complexes in bacteria, mitochondria, and chloroplasts, respectively. Enteric bacteria generally have one essential YidC, while streptococci and most Gram-positive bacteria encode two YidC paralogs. In the cariogenic bacterium *Streptococcus mutans*, deletion of *yidC1* has only minor effects, whereas *yidC2* mutants are severely impaired in growth, biofilm, and stress tolerance. Importantly, deletion of either gene results in reduced virulence in a rat caries model. In *Bacillus subtilis yidC2* is differentially expressed in response to YidC1 function, through ribosome stalling on the upstream gene *mifM*. However, streptococci do not have *mifM*, and very little is understood about the regulation of the *yidC* genes. It's been proposed by others (1) that the LiaSR two-component system (TCS), which regulates the cell-envelope stress response, regulates *yidC2* expression in *S. mutans*, however molecular data supporting this theory is limited. **Methods:** 5' RACE was used to map the promoter of the *yidC2* gene from *S. mutans*. Promoter-reporter gene fusions were constructed between the *yidC2* promoter and a chloramphenicol acetyltransferase (*cat*) gene from *Staphylococcus aureus*, and point mutations were introduced into the predicted LiaR binding site. The promoter-reporter constructs were inserted in single copy into the mannitol PTS operon of the wildtype strain, and various LiaFSR mutant backgrounds. Reporter strains were grown under different conditions (pH 5.0, oxygen, 50 μg/ml bacitracin, and various growth domains) and CAT activity measured. **Results:** *YidC2* promoter activity was decreased in stationary phase compared to exponential, and increased in the presence of bacitracin. While deletion of *liaR* had no effect on *yidC2* promoter activity, point mutations in the predicted LiaR binding site reduced activity by 3-fold. Deletion of *liaS* and *liaF* increased *yidC2* promoter activity by 1.5-fold and 3-fold, respectively, while deletion of *liaSR* or *liaFSR* had no effect. **Conclusions:** Collectively, our results indicate the *yidC2* gene is transcribed from a sigma 70 type promoter that is differentially regulated (either directly or indirectly) by the LiaFSR TCS through a predicted LiaR-binding motif located just 5' of the -35 promoter element.

Author Disclosure Block:

S. Palmer: None.

Poster Board Number:

SATURDAY-726

Publishing Title:

Preference of Glucose over Mannitol in *Escherichia coli* Is Independent of Enzyme Iia^{glc}

Author Block:

m. choe, K. Heo, H-Y. Lee, Y-R. Kim, Y-J. Seok; Seoul Natl. Univ., Seoul, Korea, Republic of

Abstract Body:

Carbon catabolite repression (CCR) is generally regarded as a regulatory mechanism to ensure sequential utilization of carbohydrates in various microorganisms. The regulatory mechanisms underlying the preferential use of glucose over other sugars have been intensively studied in *Escherichia coli* since the discovery of the *lac* operon by Jacque Monod. Previous studies on CCR suggest the model based on 'inducer exclusion' and 'induction prevention', especially for the case of glucose and lactose. Both of 'inducer exclusion' and 'induction prevention' are regulated by the phosphorylation state of EIIA^{Glc}. However it is still under debate how CCR is controlled in other cases and preferential utilization among PTS sugars cannot be explained based on inducer exclusion and induction prevention. In this study, we found that the preferential use of glucose over another PTS sugar mannitol is independent of the phosphorylation state of EIIA^{Glc}. Our data suggests that the regulatory mechanism of glucose/mannitol CCR is far different from that of glucose/lactose. Unlike in the glucose/lactose model, EIIA^{Glc} is not the major factor in the regulatory mechanism of the preferential use of glucose over mannitol. Even in the absence of EIIA^{Glc}, glucose is preferred over mannitol. Interestingly, however, preference of glucose is abolished in a mannitol operon repressor 'MtlR' deletion mutant. The mannitol operon is composed of 3 genes, *mtlA*, encoding mannitol PTS permease, *mtlD*, encoding mannitol-1-phosphate dehydrogenaseand *mtlR*, encoding putative mannitol operon repressor, and its expression is known to be negatively regulated by MtlR. In summary, unlike the model proposed for the preferential utilization of glucose over lactose, where the phosphorylation state of EIIA^{Glc} is known to play a central role, MtlR is likely to be the key factor for the preferential utilization of glucose over mannitol as a carbon source.

Author Disclosure Block:

M. choe: None. **K. Heo:** None. **H. Lee:** None. **Y. Kim:** None. **Y. Seok:** None.

Poster Board Number:

SATURDAY-727

Publishing Title:

Specificity of a Sphingosine Induced Protective and Metabolic Cassette of *Pseudomonas aeruginos*a

Author Block:

L. Hinkel, M. J. Wargo, A. E. LaBauve, J. A. Meadows; Univ. of Vermont, Burlington, VT

Abstract Body:

Sphingolipids are abundant in cell membranes and their breakdown products, ceramide and sphingosine, are frequently encountered by bacterial pathogens during infection. Sphingosine is an antimicrobial and it contributes to bacterial growth control on skin and in the lungs. Pseudomonas aeruginosa can detect sphingosine via the AraC-family regulator SphR to induce genes involved in sphingosine resistance (sphA) and metabolism (sphBCD). sphA encodes a ß-barrel protein involved in sphingosine resistance and uptake, while sphBCD encode for a putative periplasmic cytochrome C, putative sphingosine oxidase, and putative sphingosine aldolase, respectively. Here we report on studies aiming at understanding the ligand/substrate specificity of these proteins using genetic and biochemical methods. Using a variety of sphingosine analogs we have established the ligand requirements for SphR as including the primary amine in the head group of sphingosine, while the presence and length of the hydrocarbon tail and trans double bond are not as critical for sphingosine detection by SphR. We are in the process of determining the importance of the hydroxyl groups to SphR ligand specificity. We are using similar techniques to characterize these requirements for the sphingosine resistance (SphA) and metabolism (SphBCD) components. This study adds to current knowledge concerning mechanisms that bacteria use to detect, protect against, and metabolize sphingosine.

Author Disclosure Block:

L. Hinkel: None. **M.J. Wargo:** None. **A.E. LaBauve:** None. **J.A. Meadows:** None.

Poster Board Number:

SATURDAY-728

Publishing Title:

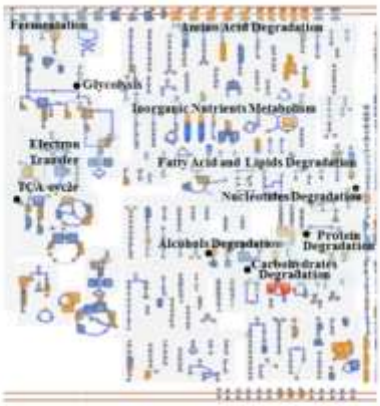
Significance of an 8-Bp Variation in a Global Regulator, *mext, S*equence in *Pseudomonas aeruginosa*

Author Block:

A. Correia[1], J. Malone[2], A. Desbois[3], L. Crossman[1], J. Wain[1]; [1]Univ. of East Anglia, Norwich, United Kingdom, [2]John Innes Ctr., Norwich, United Kingdom, [3]Univ. of Stirling, Stirling, United Kingdom

Abstract Body:

The global transcriptional regulator *mexT*, a mutational hotspot; has two sequence variants that commonly co-exist within the *P. aeruginosa* population: drug susceptible PAO1 and the norfloxacin non-susceptible and reduced motility *nfxC* mutant (selected for on chloramphenicol agar). The conversion between PAO1 and *nfxC* phenotypes is associated with an 8 bp sequence **(CGGCCAGC)** in *mexT*. Using isogenic mutants, we examined the effects of the 8 bp deletion on the *mexT* regulon and central metabolism. This is critical to understanding the response of *P. aeruginosa* in different environments and its adaptive mode of survival e.g. biofilm formation.PAO1 (8 bp, two copies) and it isogenic mutant PAO1*Δ8-bp* (8 bp, one copy) were phenotypically characterised using phenotypic microarrays (Biolog), motility and antibiotic susceptibility tests (broth dilution). Virulence testing was performed in *Galleria* models (University of Stirling) and transcriptome analysis using RNA-seq (Illumina).**Phenotypic microarray tests showed differences in central metabolism between PAO1 and** PAO1*Δ8-bp* (Figure). **PAO1 swarming and swimming behaviour were two and three times greater compared to** PAO1*Δ8-bp*, respectively. PAO1 was significantly more virulent than PAO1*Δ8-bp* in the *Galleria mellonella* infection model. Transcriptome analysis revealed that reads after the *mexT* 8-bp insertion declined across the gene in PAO1 yet 169 genes were upreglated by this strain as opposed to 22 upreglated by PAO1*Δ8-bp*. These data suggest that *mexT* remains active despite the 8 bp duplication and has effects on central metabolism, chemotaxis and cell adhesion. We are currently investigating the molecular mechanism.



Section of the Kegg map showing central metabolism overlaid with RNA transcriptome and phenotypic microarray data

Based on RNA-seq data, differences in metabolism between PAO1 and PAOL*18-bp* are colour coded and graded from 0.1 (blue) -22 (red) Log_{10}. Pathways indicated with ● illustrate differences found using phenotypic microarray data. Analysis of the area under the curve (AUC) showed that growth rates of PAO1 exceeded that of PAOL*18-bp* by two fold, in the presence of specific carbohydrate and nucleotide based substrates. PAOL*18-bp* however was more metabolically active on peptide-nitrogen based sources producing AUCs ≥ 2.

Author Disclosure Block:

A. Correia: None. **J. Malone:** None. **A. Desbois:** None. **L. Crossman:** None. **J. Wain:** None.

Poster Board Number:

SATURDAY-729

Publishing Title:

Acid Induced Overexpression of the *Mycobacterium tuberculosis Rv3488* Gene Effects Pigment Production in *Mycobacterium smegmatis*

Author Block:

B. Saviola; Western Univ. of Hlth.Sci., Pomona, CA

Abstract Body:

Mycobacterium tuberculosis continues to be a threat and difficult to treat. Mycobacterial antibiotic resistance is also on the rise especially in regions of the world such as Africa. During infection, *M. tuberculosis* bacilli invade and enter phagosomes of macrophages where they persist and are exposed to a variety of stressors, including reactive oxygen species and acididty. The *lipF* gene encodes a lipase or esterase within the mycobacterial genome and a promoter upstream of this gene has been shown to be upregulated by acidic stress. The minimal acid inducible promoter region of *lipF* is upregulated by acidity from pH as low as 4.3 to as high as 6.4, pH's that can be encountered within phagosomes of macrophages. We have engineered a plasmid to use the *mpr* promoter to drive expression of proteins at acidic pH, thus creating a tightly controlled expression vector for mycobacteria. *Rv3488* a gene upstream of *lipF* and divergently transcribed is situated close to the minimal acid inducible promoter of *lipF* in the *M. tuberculosis* genome. We used this acid inducible minimal promoter region (*mpr)* to make a construct in which *mpr* drives expression of the *Rv3488* gene, thus making this gene highly acid inducible which does not normally occur in wild type *M. tuberculosis*. It has yet to be established what induces *Rv3488* expression in wild type *M. tuberculosis*. We hypothesized that Rv3488, being a DNA binding protein, may regulate some gene responses, and may also be involved in acid stress responses as it is placed so close to the acid induced *lipF* gene. *Rv3488*, encoding a DNA binding protein, could be tightly regulated by *pMPR-RV3488* with no expression at pH 7.0 and robust expression at pH 4.5. Upon acid induced over expression of *Rv3488* with acid exposure it was determined that in *Mycobacterium smegmatis* acid induced pigment production was inhibited, while acid induced over expression of *gfp* did not result in a similar inhibition of acid induced pigment production. Acid induced pigment production was previously established in *M. smegmatis* and *Rv3488* appears to participate in its stress induced regulation in this mycobacterium.

Author Disclosure Block:

B. Saviola: None.

Poster Board Number:

SATURDAY-730

Publishing Title:

Culturing *Coccidioides:* Optimizing *In Vitro* Culture Media to Reflect Nutrient Availability *In Vivo*

Author Block:

H. L. Mead, B. M. Barker; Translation Genomics Res. Inst.-North, Flagstaff, AZ

Abstract Body:

Background: The pathogenic soil dwelling fungi, *Coccidioides immitis* and *C. posadasii* can be found in arid desert regions and cause an estimated 150,000 cases of coccidioidomycosis, also known as valley fever, in the United States each year[i]. The current media for culturing spherules is not nutritionally representative of a mammalian respiratory system or conducive to *in vitro* immunology experiments. Supplemented RPMI media was used to produce the spherule form in a previous study[ii]. RPMI media is routinely used in cell culture applications making it useful for *in vitro* immunology experiments. **Methods:** Using the supplemented media and an attenuated biosafety level two (BSL2) *Coccidioides,* preliminary trials were conducted to determine which factors, such as cell density, temperature, time, CO_2 and O_2 concentration, would affect spherule development. Potential conditions were identified, reproduced in triplicate, and compared to determine the most successful method of growth.**Results:** The BSL2 strain cultured in the supplemented RPMI media successfully grew spherules at several conditions similar to the host environment. Temperature ranged between 37-39°C and CO_2 concentration between 5-15%. The supplemented RPMI media produced both spherule and mycelial morphology under normoxic conditions. Spherule development between the two media types was relativity equivalent, and the highest percentage of parasitic morphology occurred at 15 % CO_2. Hypoxia impaired the growth of mycelial structures while allowing for growth of spherules. Additionally, hypoxic conditions inhibited growth in the control, Converse media, while only slowing growth in the supplemented RPMI.**Discussion:** The supplemented RPMI media is an excellent candidate for culturing *Coccidioides* spherules and can be applied to future research in the BSL3 laboratory. The components of the media provide the organism with nutrients that are similar to those found in a host respiratory system and the conditions of growth are favorable for mammalian immune cells allowing *in vitro* immunology experiments.

Author Disclosure Block:

H.L. Mead: None. **B.M. Barker:** None.

Poster Board Number:

SATURDAY-731

Publishing Title:

Effects of Pyoverdine for the Growth Suppression of Diverse Microbes

Author Block:

Y. Lee[1], Y-J. Kim[1], J-H. Lee[1], H. Yu[2], K. Lee[1], S. Jin[3], U-H. Ha[1]; [1]Korea Univ., Sejong, Korea, Republic of, [2]Hyundai Pharmaceutical Co., Yongin, Korea, Republic of, [3]Univ. of Florida, Gainesville, FL

Abstract Body:

Background: Interspecies interactions play a role in altering the composition of communities in terms of the course and severity of infections. However, little is known about the mechanism of interspecies interactions in microbial coinfections. **Methods**: We applied a technique of transposon mutagenesis to screen mutants with defects in growth suppression. To identify responsible components for the growth suppression, HPLC was used to analyze secreted metabolites. **Results**: Cell-free culture supernatants obtained from *P. aeruginosa* suppressed the growth of a number of bacterial strains such as *Corynebacterium glutamicum*, *Bacillus subtilis*, *Staphylococcus aureus* and *Agrobacterium tumefaciens*, but had little effect on the growth of *Escherichia coli* and *Salmonella typhimurium*. By performing transposon mutagenesis, TatC encoded by PA5070 of *P. aeruginosa* was identified, and the supernatant of its mutant did not suppress the growth. Molecular analysis of supernatants showed that pyoverdine was a secondary metabolite present in culture supernatants of the wild-type strain, but not in those of the PA5070 mutant. Supplementation of $FeCl_2$ as a source of iron compromised the growth suppression effect of supernatants, indicating that pyoverdine-mediated iron acquisition is responsible for the growth suppression. **Conclusions**: Thus, this study provides the action of TatC-dependent pyoverdine translocation for the growth suppression, and it might aid understanding of the impact of *P. aeruginosa* in the complex community of bacterial species upon coinfection.This research was supported by Basic Science Research Program (NRF-2013R1A1A2059846) and the BK21 plus program of the Ministry of Education, Korea.

Author Disclosure Block:

Y. Lee: None. **Y. Kim:** None. **J. Lee:** None. **H. Yu:** None. **K. Lee:** None. **S. Jin:** None. **U. Ha:** None.

Poster Board Number:

SATURDAY-732

Publishing Title:

Post-translational Modification by Acetylation Regulates *Vibrio cholerae* Virulence in a *Drosophila melanogaster* Model of Infection

Author Block:

K. Liimatta, **A. E. PURDY**; Amherst Coll., Amherst, MA

Abstract Body:

The pathogen *Vibrio cholerae* can infect eukaryotic organisms by deploying toxins, proteases, and other secreted effectors. *V. cholerae* may also affect host physiology and health by altering the metabolic milieu of the host's gastrointestinal (GI) tract. Recent work by Hang and colleagues (2014) demonstrated that *V. cholerae* lethally infects *Drosophila* by manipulating levels of acetate, a shared central metabolite, in the fly GI tract. By expressing acetyl-CoA synthetase (Acs), the key enzyme required for converting acetate to acetyl-CoA, *V. cholerae* causes intestinal steatosis in the fly, contributing to its death. Acs is carefully regulated transcriptionally and post-translationally in *E. coli* and *Salmonella*. However, our understanding of its regulation in *V. cholerae* is incomplete. While transcription of Acs may be controlled by novel signaling pathways in *Vibrio*, post-translational modification by acetylation may be widely conserved. *V. cholerae* carries copies of the *patZ* and *cobB* genes that are required for acetylation and deacetylation of Acs and other proteins, but their role in *V. cholerae* physiology or virulence are unknown. The goal of this project is to test the hypothesis that proper acetylation of central metabolic enzymes, including Acs, is required for (i) utilization of acetate as a carbon source, and (ii) *V. cholerae* virulence in a *Drosophila* model. We have demonstrated that an in-frame deletion of *cobB*, required for activation of Acs by deacetylation, in *V. cholerae* behaves similarly to a strain carrying a deletion in *acs.* The *cobB* mutant is incapable of growing on acetate as a sole carbon source but displays no growth defect in rich media. As expected, virulence towards *Drosophila* is significantly reduced, although not completely abrogated, in the *cobB* mutant. These results suggest, for the first time, that acetylation may be an important regulator of *V. cholerae* physiology and virulence during infection, and we are currently undertaking both biochemical and genetic approaches to test the hypothesis that targeted acetylation of Acs is mediating these phenotypes. Future work will explore mechanisms that regulate activity of the acetylase and deacetylase enzymes, and proteomics approaches will reveal the extent to which acetylation modifies proteins important to *V. cholerae* survival and infection.

Author Disclosure Block:

K. Liimatta: None. **A.E. Purdy:** None.

Poster Board Number:

SATURDAY-733

Publishing Title:

Integration ofCarbon Metabolism and Quorum Sensing Through the Bara/Sira-csra And LsrRegulatory System in *Salmonella typhimurium*

Author Block:

E. V. Gart[1], R. C. Laughlin[2], C. C. Hung[3], S. Myers[1], K. E. McWhinney[1], C. Altier[3], S. D. Lawhon[1]; [1]Texas A&M, College Station, TX, [2]Texas A&M Kingsville, Kingsville, TX, [3]Cornell Univ., Ithaca, NY

Abstract Body:

Salmonella Typhimurium uses autounducer-2 (AI-2) signaling molecule to regulate the *Salmonella* pathogenicity island-1 (SPI-1) Type 3 Secretion System (T3SS), flagellar gene expression, and biofilm formation. AI-2 is sensed and internalized by the *lsrACDB*-encoded transporter. Following intracellular phosphorylation, AI-2 represses the transcriptional repressor protein LsrR, allowing the transcription of *lsr*- and SPI-1 - encoded genes. The BarA/SirA two-component system also controls SPI-1 T3SS through the RNA-binding protein CsrA which affects mRNA stability and translation. However, the interaction between the BarA/SirA-CsrA regulatory cascade and the *lsr* operon is unknown. The goal of this study was to determine whether the BarA/SirA-CsrA regulatory cascade regulates *lsr* expression and AI-2 transport in *S.* Typhimurium. Extracellular AI-2 was measured in mutant (*sirA, barA, csrA, csrBC* and *barA-ackA-pta*) and parent (WT) strains of *S.* Typhimurium using the luminescent response of reporter *V. harveyi* to *Salmonella* AI-2. The *β*-galactosidase activity of *lsrR-lacZ* and *lsrD-lacZ* transcriptional fusions was assayed at early-, mid-, and late-log phase in parent and mutant strains. Mobility shift assays were performed to determine whether CsrA-His binds to *lsrR* and *lsrD* mRNA. Analysis of variance followed by Tukey's test ($P<0.05$) was used to determine the differences in the extracellular AI-2 and *β*-galactosidase activity. The mid-log phase expression of *lsrR* is positively regulated by BarA/SirA and CsrBC. CsrA suppresses *lsrR* expression at the mid-log phase and suppresses *lsrD* expression at the early-, mid- and late-log phase. Additionally, CsrA binds to *lsrR* and *lsrD* mRNA. Our results demonstrate the cross-talk between the BarA/SirA-CsrA and *lsr* regulatory systems in regulating AI-2 quorum sensing in *S.* Typhimurium. Therefore we propose a model where *lsrD* expression, and therefore AI-2 internalization, is constantly suppressed by CsrA and LsrR. This is counteracted at the mid-exponential phase by CsrA binding to the *lsrR* transcript and relieving the repression of the AI-2 transporter.

Author Disclosure Block:

E.V. Gart: None. **R.C. Laughlin:** None. **C.C. Hung:** None. **S. Myers:** None. **K.E. McWhinney:** None. **C. Altier:** None. **S.D. Lawhon:** None.

Poster Board Number:

SATURDAY-734

Publishing Title:

Brnq1 in *Staphylococcus aureus* is a Leu/Val Transporter Required for Determining Branched-chain Membrane Fatty Acid Content

Author Block:

J. C. Kaiser[1], S. Sen[2], B. J. Wilkinson[2], D. E. Heinrichs[1]; [1]Univ. of Western Ontario, London, ON, Canada, [2]Illinois State Univ., Normal, IL

Abstract Body:

The branched-chain amino acids (BCAAs; Ile, Leu, Val) are important nutrients for the metabolism and growth of the human pathogen *Staphylococcus aureus*, as in addition to their role in protein synthesis they are precursors for the branched-chain fatty acids (BCFAs). The BCFAs constitute ~50% of membrane fatty acids and are necessary for environmental adaptation and resistance to membrane stresses. We have found that *S. aureus* represses Leu and Val synthesis, and instead prefers to acquire these nutrients from the extracellular milieu via two BCAA transporters; BrnQ1 and BcaP. We therefore hypothesized that BCAA transporters are necessary to supply the precursors for BCFA synthesis, and that mutation of such transporters would result in altered BCFA content in the cellular membrane. Membrane composition analysis revealed that the membrane of a *brnQ1bcaP* mutant was completely devoid of Leu- and Val-derived iso-BCFAs, corresponding with the role of these genes in Leu and Val transport. The membrane of the *brnQ1* mutant also lacked Leu- and Val-derived iso-BCFAs, implicating BrnQ1 as the predominant Leu and Val transporter. Confirming this, we determined that BrnQ1 is the higher affinity transporter and is essential for the growth of *S. aureus* under BCAA limiting conditions, whereas BcaP plays a secondary role in growth. Paralleling the absence of Leu- and Val-derived BCFAs, we observed an increase of Ile-derived anteiso-BCFAs from 45% to 90% of total membrane fatty acids, which we surmise is dependent on Ile acquisition via the Ile-specific transporter, BrnQ2. Ile-derived anteiso-fatty acids increase membrane fluidity, and we found that *S. aureus* compensates for compromised membrane fluidity by incorporating more staphyloxanthin, a pigmented carotenoid that reduces membrane fluidity. These data demonstrate the importance of BCAA acquisition for supplying the BCAAs for BCFA synthesis, and thus for maintaining the appropriate membrane content for environmental adaptation of *S. aureus*.

Author Disclosure Block:

J.C. Kaiser: None. **S. Sen:** None. **B.J. Wilkinson:** None. **D.E. Heinrichs:** None.

Poster Board Number:

SATURDAY-735

Publishing Title:

Branched-Chain Fatty Acids (Bcfas) In The *staphylococcus Aureus* Membrane And Their Physiological Significance

Author Block:

R. P. Ring[1], S. Sirobhushanam[2], C. Gatto[2], B. Wilkinson[2], V. Singh[1]; [1]A. T. Still Univ. of Hlth. Sci., Kirksville, MO, [2]Illinois State Univ., Normal, IL

Abstract Body:

Background: Maintenance of membrane fluidity is critical for *Staphylococcus aureus* to overcome diverse environmental conditions and to a large extent is governed by BCFA content. While branched-chain α-keto acid dehydrogenase (BKD) is the key enzyme in BCFA synthesis, a *S. aureus* BKD-deficient mutant still produces substantial levels of BCFAs. The pyruvate dehydrogenase (PDH) complex was hypothesized to contribute the BCFA production as PDH and BKD both belong to the α-ketoacid dehydrogenase complex family. This study investigated the role of PDH in conjunction with BKD in BCFA metabolism and its significance in *S. aureus.* **Methods:** This study was carried out using BKD, PDH, and BKD-PDH deficient derivatives of methicillin resistant *S. aureus* (MRSA) strain USA300 (WT). Differences in growth kinetics at 37°C were evaluated spectrophotometrically by measuring OD_{600}. Membrane BCFAs were analyzed using gas chromatography and membrane fluidity by fluorescence polarization. Carotenoid levels were estimated by measuring the A_{465} of methanol extracts from 48h cultures. MIC values were determined for oxacillin (OXA), vancomycin (VAN), daptomycin (DAP), bacitracin (BAC), and D-cycloserine (DCS) by broth microdilution in duplicate.**Results:** The BKD and PDH deficient strains grew slower than WT, while the BKD-PDH double mutant grew much slower than WT. BCFAs made up 50% of membrane lipids in WT, but only 31% in the BKD-deficient strain. In contrast, the BCFA level was ~80% in the PDH-deficient strain and 38% in the BKD-PDH double mutant. Of all strains tested, the BKD mutant showed decreased membrane fluidity. Compared to WT (100±9.1%), the BKD-deficient strain produced more staphyloxanthin (141±7.6%, $p = 0.03$) and the PDH-deficient strain produced lower levels of carotenoids than WT (47±5.6%, $p = 0.007$). In MIC studies, the BKD-deficient strain showed reduced OXA and DAP resistance. There was no difference in resistance to BAC, VAN, or DCS among these strains.**Conclusions:** The BCFA composition of the cell membrane in *S. aureus* seems to significantly impact cell growth, membrane fluidity, and resistance to certain antibiotics. Carotenoid production appears inversely correlated with BCFA content and may be key for *S. aureus* membrane fluidity under BCFA-deficient conditions.

Author Disclosure Block:

R.P. Ring: None. **S. Sirobhushanam:** None. **C. Gatto:** None. **B. Wilkinson:** None. **V. Singh:** None.

Poster Board Number:

SATURDAY-736

Publishing Title:

***Staphylococcus aureus* Uses Proline and Arginine as Primary Carbon Sources When Grown on Defined Media Lacking Glucose**

Author Block:

C. Halsey[1], S. Lei[2], M. K. Lehman[1], V. Eckrich[1], R. Powers[2], P. D. Fey[1]; [1]Univ. of Nebraska Med. Ctr., Omaha, NE, [2]Univ. of Nebraska Lincoln, Lincoln, NE

Abstract Body:

S. aureus has the ability to establish an infection in a wide variety of unique metabolic niches within the human host. However, it is unclear what specific carbon and nitrogen sources are available to *S. aureus* in specific host niches and how these specific growth conditions may affect virulence factor expression and subsequent invasion, metastasis or quiescence. We hypothesize that *S. aureus* encounters niches within the host where proteins, peptides, or free amino acids are the only carbon source available. To assess which amino acids are important for growth in media lacking glucose, *S. aureus* JE2 and mutants were grown aerobically (250 rpm, 37 degrees, 10:1 flask to volume ratio) in complete defined medium lacking glucose (CDM) and particular amino acids. It was first determined that JE2 grown in CDM is auxotrophic for arginine, valine and leucine. Use of mutants that do not have functional pathways generating glutamate from either arginine or proline were unable to grow in this media. Furthermore, a mutation in glutamate dehydrogenase (*gudB*), which generates 2-oxoglutarate from glutamate, abrogated growth in CDM suggesting that glutamate derived from arginine and proline serves as the major carbon source in this media. Nuclear Magnetic Resonance (NMR) studies using uniformly ^{13}C-labeled glutamate, arginine and proline confirmed this observation. Lastly, a mutation in acetate kinase (*ackA*) also abrogated growth of JE2 in CDM suggesting that ATP generation via substrate level phosphorylation from pyruvate generating amino acids (e.g. alanine, serinine, glycine) is also critical for growth. In conclusion, our data suggest that *S. aureus* uses arginine and/or proline as primary carbon sources to generate glutamate when *S. aureus* is grown on media lacking glucose. Furthermore, the glucogenic amino acids that generate pyruvate may serve as a primary energy source to generate ATP via the PTA/AckA system. These data may suggest that pathways important for glutamate catabolism or ATP generation via PTA/AckA are important for growth in niches where glucose is not abundant such as abscesses within skin and soft tissue infections.

Author Disclosure Block:

C. Halsey: None. **S. Lei:** None. **M.K. Lehman:** None. **V. Eckrich:** None. **R. Powers:** None. **P.D. Fey:** None.

Poster Board Number:

SATURDAY-737

Publishing Title:

***Staphylococcus aureus* Nitric Oxide Synthase (SANOS) Modulates Respiratory Metabolism and Cell Physiology**

Author Block:

A. B. Mogen[1], R. K. Carroll[2], J. Culver[3], C. Petucci[3], L. N. Shaw[4], K. C. Rice[1]; [1]Univ. of Florida, Gainesville, FL, [2]Ohio Univ., Athens, OH, [3]Sanford Burnham Prebys Med. Discovery Inst. at Lake Nona, Orlando, FL, [4]Univ. of South Florida, Tampa, FL

Abstract Body:

S. aureus is a notorious human pathogen that can be resistant to multiple antibiotics. A promising target for drug development in this organism is saNOS, as a link between this enzyme's inhibition and increased antimicrobial efficacy has been previously established. Although the mechanism is currently unknown, saNOS contributes to *S. aureus* virulence, as well as protection against exogenous oxidative stress and antimicrobials. However, little is known about the potential effects of this enzyme on *S. aureus* physiology in the absence of exogenous stress. When grown aerobically, endogenous reactive oxygen species (ROS) levels, including the respiratory by-product superoxide, were elevated in a *S. aureus nos* mutant, independent of catalase activity. Multiple transcriptional and metabolic changes were also observed in the *nos* mutant, as assessed by RNAseq and targeted metabolomics analyses, respectively. Specifically, expression of genes associated with oxidative and nitrosative stress responses, anaerobic/lactate metabolism, and cytochrome biosynthesis were increased in the *nos* mutant relative to wild-type, whereas expression of several virulence factor genes was decreased. Metabolites utilized to produce reducing equivalents by the right arm of the TCA cycle were depleted in a *nos* mutant, whereas fumarate and malate levels were increased relative to wild-type. A significant reduction in intracellular lactate levels was also observed in the *nos* mutant. Collectively, these results support a model in which the absence of saNOS results in increased respiration and endogenous ROS accumulation, which may signal a switch to an alternative lactate-based respiratory metabolism. These results build upon previously-published observations that NO can limit cellular respiration in bacteria and mitochondria, and suggests that bacterial NOS is a previously-unappreciated modulator of bacterial respiratory metabolism.

Author Disclosure Block:

A.B. Mogen: None. **R.K. Carroll:** None. **J. Culver:** None. **C. Petucci:** None. **L.N. Shaw:** None. **K.C. Rice:** None.

Poster Board Number:

SATURDAY-738

Publishing Title:

Mapc, but Not Mapa Is Essential to the Viability of *Mycobacterium bovis*, and Is a Potential Drug Target

Author Block:

M. Vanunu, D. Barkan; Koret Sch. of Vet. Med., Rehovot, Israel

Abstract Body:

Background: *Mycobacterium tuberculosis* (Mtb) kills more than 1.5 million people each year, and 1/3 of the global population is estimated to be latently infected. The recent emergence of drug-resistant strains emphasizes the need for new treatments, and thus for new drug targets. The search for new drug targets led us to investigate the Methionine aminopeptidase (MAP) pathway, a post-translation modification in most newly synthesized proteins in all living cells, including mycobacteria. Mtb, and its attenuated strain BCG, possess two variants of the gene - *mapA* (Rv0734) and *mapC* (Rv2861c). The essentiality of these genes was investigated in recent studies but is still unclear. Olyleye et al (2009) suggested by chemical inhibition that *mapA*, but not *mapC*, was essential. On the other hand, analysis of a transposone mutant-library (Griffin 2011) suggested the opposite. A targeted deletion of neither gene was performed, thus leaving the question of their essentiality unanswered. **Methods:** We decided to delete the genes *mapA* and/or *mapC* by specialized transduction, based on TM4 phage. Because these genes may be essential, we first pre-complemented the bacteria with a second copy of the gene at the *attB* site. After obtaining a mutant deleted in the native gene and confirming the deletion using several PCR reactions, we attempted to remove the complementing copy from the *attB* site by exchanging for another *attB*-integrating cassette, carrying a different selection marker. This way, a decisive proof of essentiality for each of these genes can be obtained. **Results:** The first gene we investigated was *mapC*. We pre-complemented BCG with an extra copy of the gene. After deleting the native gene by phage infection we exchanged the second copy with an empty cassette, an extra copy of *mapA*, or another copy of *mapC*. We obtained no colonies where the complementing *mapC* gene was removed, showing *mapC* is essential for *in-vitro* growth. To investigate *mapA*, we created a pre-complemented mutant, and a deletion of the native *mapA* is underway. **Conclusion:** Our results confirm the essentiality of the methionine amino-peptidase pathway to the viability of BCG (and thus, Mtb). Contrary to previous reports, of the two genes *mapC* is the essential one, and is a legitimate target for future drug development. The essentiality of *mapA* will be determined in the next few months.

Author Disclosure Block:

M. Vanunu: None. **D. Barkan:** None.

Poster Board Number:

SATURDAY-739

Publishing Title:

The Developmental Inhibitor Hetn is Produced from an Internal Translational Start Site within the Filamentous Cyanobacterium *Anabaena* sp. Pcc 7120

Author Block:

O. S. Rivers[1], P. Videau[2], S. M. Callahan[1]; [1]Univ. of Hawaii Manoa, Honolulu, HI, [2]Oregon State Univ., Corvallis, OR

Abstract Body:

Formation and maintenance of a periodic pattern of terminally differentiated nitrogen-fixing cells called heterocysts by the filamentous cyanobacterium *Anabaena* sp. PCC 7120 is dependent on the developmental regulators PatS and HetN. HetN is annotated as a 287 aa protein, but the mature HetN-derived signal that is transferred between cells, likely via SepJ-mediated intercellular channels, is yet to be defined. Similar signal ranges produced by a wild-type allele of *hetN* and one encoding a HetN-YFP fusion protein that is not exchanged between cells suggests that the full-length protein is not the mature signal. Mutants containing variants of *hetN* in which the annotated and/or potential internal start methionines were changed to leucine were created to assess the possible use of one or more of these potential translational start sites in creation of the HetN developmental signal. The mutant phenotypes suggest an internal translational start site at position M119 relative to the annotated start site. The data is consistent with the theory that a smaller peptide within the sequence of HetN is the inhibitory signal produced by heterocysts and transported to vegetative cells within the *Anabaena* sp. PCC 7120 developmental system.

Author Disclosure Block:

O.S. Rivers: None. **P. Videau:** None. **S.M. Callahan:** None.

Poster Board Number:

SATURDAY-740

Publishing Title:

CSPA and CSPC are Required in Germination of Spores *Clostridium perfringens* Strain SM101

Author Block:

P. K. Talukdar, M. R. Sarker; Oregon State Univ., Corvallis, OR

Abstract Body:

Clostridium perfringens is a Gram-positive, anaerobic, spore forming bacterium resides in different environmental niches including gastrointestinal tracts of humans and animals. The spores of *C. perfringens* are resistant morphotype and metabolically inactive until it sense signals from molecules termed germinants and going back to its metabolically active vegetative form via germination process. In recent years, a plethora of information has been gathered regarding the germination of *C. perfringens* spores, but still several questions remains unanswered. Different germinant receptors have been identified that sense the signals from germinants and activate other protein functions. Cortex hydrolysis is one of the major events in germination process, as the hydrolysis of cortex allows the spores' core to uptake water and thus quickens the germination process. The major cortex lytic enzyme SleC is being processed by the activation of CspB, a serine protease. Although we know that in *Clostridium difficile*, CspA and CspC have roles in the activation of CspB, but the role of these two proteins in *C. perfringens* is still yet to be determined. In this study, we showed by promoter expression assay that *cspA-cspC* is organized as a bicistronic operon and expressed only during sporulation. To investigate the role of CspA and CspC, we constructed a single Δ*cspA* or Δ*cspC* mutant derivative in *C. perfringens* strain SM101. The germination assay with different germinants showed that both mutants have deficiency in germination. These results are in agreement with the DPA release data, as both Δ*cspA* and Δ*cspC* spores released less DPA than wild-type spores during germination. The outgrowth assay and the colony forming efficiency also confirmed the germination defects in spores of both mutants. The Western blot analysis with SleC antibody showed that during germination, SleC processing was blocked in spores of both mutants. In summary, our study showed that both CspA and CspC serine proteases play major roles in spore germination by directly activating the SleC and/or CspB.

Author Disclosure Block:

P.K. Talukdar: None. **M.R. Sarker:** None.

Poster Board Number:

SATURDAY-741

Publishing Title:

Regulation of Spore Germination in *Clostridium difficile* by Csp Family Proteases

Author Block:

Y. Kevorkian, A. Shen; Univ. of Vermont, Burlington, VT

Abstract Body:

Clostridium difficile Infection (CDI) is a major healthcare-associated disease that is transmitted by *C. difficile*'s metabolically dormant spore form. Upon entering the gut, spores germinate, resuming metabolism and leading to release of toxins. In *C. difficile*, germination depends on the Csp family of subtilisin-like serine proteases and the cortex hydrolase SleC. The pseudoprotease CspC acts as the primary germinant receptor that initiates germination upon binding specific bile salts. CspC activates CspB, which in turn removes an inhibitory pro-peptide from SleC. Active SleC degrades the protective cortex layer, allowing dormant spores to resume metabolism and growth. We previously investigated the role of CspA, a pseudoprotease domain fused to CspB prior to interdomain processing. Mutational analyses revealed that the CspA domain controls CspC germinant receptor levels in mature spores and is required for optimal spore germination, particularly when CspA is fused to the CspB protease. However, study of the individual Csps was complicated by the polar effects caused by the TargeTron mutagenesis method and the requirement for multi-copy plasmid complementation, which may cause experimental artifacts. To overcome these limitations, we now use allele coupled exchange to create individual deletions of the genes encoding CspB, CspA, and CspC, as well as to complement these mutations in single copy on the chromosome. These individual deletion mutants exhibit significant decreases in germination efficiency. Since their germination defects are similar, our preliminary data are consistent with the hypothesis that direct interactions between Csp family proteases are vital for maintaining efficient germination.

Author Disclosure Block:

Y. Kevorkian: None. **A. Shen:** None.

Poster Board Number:

SATURDAY-742

Publishing Title:

Characterization of the Early Events of Germination of *Clostridium difficile* Spores

Author Block:

T. Kochan, P. Hanna; Univ. of Michigan, Ann Arbor, MI

Abstract Body:

Clostridium difficile (*Cd*), a Gram-positive anaerobe, is a leading cause of both hospital- and community- acquired antibiotic associated diarrhea. *Cd* infection (CDI) typically occurs after treatment with broad-spectrum antibiotics disrupts the normal gut microbiota, allowing for *Cd* colonization. While CDI is a toxin-mediated disease, its capacity for transmission and, therefore, disease is dependent on spore formation and germination. *Cd* spores become metabolically active (germinate) when receptors packaged within the spore recognize host-specific molecules (e.g., bile salts, glycine) known as germinants. Currently, the mechanism of germination in *Cd* is not completely understood. Many of the genes required for germination in other spore forming bacteria, including the known germinant receptors, are not encoded by sequenced *Cd* strains. The receptor for the bile salt taurocholate, CspC, has been identified. However, the receptor(s) for the necessary co-germinant, glycine, remains unknown. Although it is known that glycine is required for efficient germination of *Cd* spores, the mechanism controlling this requirement remains to be elucidated. In this work, we provide evidence that glycine plays a role in the release of calcium-dipicolinic acid (Ca-DPA) from the spore core. While both taurocholate (Tc) and glycine are required for efficient germination, supplementation with exogenous Ca-DPA can eliminate the requirement for glycine. Germination was measured by loss of optical density. Tc-Ca-DPA induced germination leads to direct activation of SleC (cortex lytic enzyme) and requires CspC, indicating that Tc-Glycine and Tc-Ca-DPA induced germination function through a similar pathway. We have also identified an outer membrane associated protein that may facilitate access of germinants into the spore. A mutant lacking this protein germinates slowly in response to BHIS+Tc, indicating that germinants may have restricted access within the spore. These data strongly suggest that glycine induces the release of Ca-DPA from the spore core, and Ca-DPA transmits the germination signal to activate cortex hydrolysis via SleC.

Author Disclosure Block:

T. Kochan: None. **P. Hanna:** None.

Poster Board Number:

SATURDAY-743

Publishing Title:

Protein Composition of Infectious Spores Reveals Novel Sexual Development and Germination Factors in *Cryptococcu*s

Author Block:

M. Huang, A. S. Hebert, J. J. Coon, C. M. Hull; Univ. of Wisconsin-Madison, Madison, WI

Abstract Body:

Spores are an essential cell type required for long-term survival across diverse organisms in the tree of life and are a hallmark of fungal reproduction, persistence, and dispersal. Among human fungal pathogens, spores are presumed infectious particles, but relatively little is known about this robust cell type. Here we used the meningitis-causing fungus *Cryptococcus neoformans* to determine the roles of spore-resident proteins in spore biology. Using highly sensitive nanoscale liquid chromatography/mass spectrometry, we compared the proteomes of spores and vegetative cells (yeast) and identified eighteen proteins specifically enriched in spores. The genes encoding these proteins were deleted, and the resulting strains were evaluated for discernable phenotypes. We hypothesized that spore-enriched proteins would be preferentially involved in spore-specific processes such as dormancy, stress resistance, and germination. Surprisingly however, the majority of the mutants harbored defects in sexual development, the process by which spores are formed. One mutant in the cohort was defective in the spore-specific process of germination, showing a delay specifically in the initiation of vegetative growth. Thus, by using this in-depth proteomics approach as a screening tool for cell type-specific proteins and combining it with molecular genetics, we successfully identified the first germination factor in *C. neoformans*. We also identified numerous proteins with previously unknown functions in both sexual development and spore composition. Our findings provide the first insights into the basic protein components of infectious spores and reveal unexpected molecular connections between infectious particle production and spore composition in a pathogenic eukaryote.

Author Disclosure Block:

M. Huang: None. **A.S. Hebert:** None. **J.J. Coon:** None. **C.M. Hull:** None.

Poster Board Number:

SATURDAY-744

Publishing Title:

Role of Petrobactin in *Bacillus anthracis* Outgrowth from Spores

Author Block:

A. Hagan[1], A. Tripathi[2], D. Sherman[2], P. Hanna[1]; [1]Univ. of Michigan Med. Sch., Ann Arbor, MI, [2]Univ. of Michigan Life Sci. Inst., Ann Arbor, MI

Abstract Body:

Bacillus anthracis, a Gram-positive spore forming bacterium, is the causative agent of anthrax. Anthrax, a highly lethal disease, is accompanied by growth to high titers in the blood following germination and outgrowth of the spore in the iron-limited host. Iron is an important nutrient required for multiple cellular processes including DNA replication and ATP generation. As a result, iron acquisition by bacterial pathogens in the iron-limited host environment is critical for pathogenesis. One method of iron acquisition employed by many bacteria is the production of siderophores, small molecules with high binding affinities for iron. Siderophores are secreted into the environment under iron-limited conditions to acquire iron for growth. Siderophore biosynthesis is an energetically costly process involving expression of multiple biosynthetic enzymes when vegetative cells encounter iron-limited conditions. *B. anthracis* has multiple methods of acquiring iron but the siderophore petrobactin (PB) is the only system involved in murine models of anthrax virulence. While much is known about PB biosynthesis and import, little is known about the role of PB in early outgrowth of *B. anthracis* from spores. As *B. anthracis* is capable of infecting the iron-limited host environment from the transcriptionally dormant spore, we sought to identify the role of PB in this process. Outgrowth of spores in iron-depleted medium (IDM) as compared to iron-replete medium (IRM) revealed that wildtype and a mutant deficient for production of the siderophore bacillibactin (Δ*dhb*) achieved outgrowth in both conditions. Spores of PB biosynthesis (Δ*asb*) or import (Δ*fpuA*) mutants outgrew in IRM but failed to achieve detectable levels of outgrowth in IDM. The exogenous supplementation of purified PB to Δ*asb* spores rescued outgrowth in IDM. These data indicate a requirement for PB in early spore outgrowth in iron-limited conditions. As the spore begins in a transcriptionally dormant state, we hypothesized that PB is biosynthesized during sporulation and packaged within the spore, ready for export and iron retrieval upon germination. Mass spectrometry confirms the presence of PB in wildtype but not Δ*asb* spores. Future work will identify the location of PB in the spore and parse the role of pre-packaged PB from PB *de novo* synthesis during *B. anthracis* outgrowth from spores.

Author Disclosure Block:

A. Hagan: None. **A. Tripathi:** None. **D. Sherman:** None. **P. Hanna:** None.

Poster Board Number:

SATURDAY-745

Publishing Title:

Determination of Cyclic Dimeric Gmp Targets in *Streptomyces coelicolor* Using Rna Sequencing

Author Block:

M. A. Stark, J. A. Bennett; Otterbein Univ., Westerville, OH

Abstract Body:

The second messenger molecule cyclic dimeric GMP (c-di-GMP) regulates a myriad of processes in gram-negative bacteria. However, little is known about the roles it plays in gram-positive bacteria. The second messenger molecule has been shown to regulate biofilm formation, motility, cell cycle progression, and virulence. Differential gene expression in two strains of the gram-positive bacterium, *Streptomyces coelicolor*, was studied using RNA sequencing to determine direct and indirect gene targets of c-di-GMP signaling. RNA was collected in triplicate from the double mutant and wild-type strain of *S. coelicolor* for use in the RNA sequencing experiment carried out on an Illumina Hi-Seq instrument. The *rmdA rmdB* (regulator of morphology and development) double mutant was compared to the wild-type strain known as MT1110. It is known that there is increased intracellular pooling of c-di-GMP in the double mutant which means that c-di-GMP is not being broken down. The *rmdA* and *rmdB* genes are needed for the formation of aerial mycelium, an important step in the life cycle of *S. coelicolor*. The RNA sequencing experiment implicated many known developmental genes in *S. coelicolor* as targets of c-di-GMP including the *chaplins*. The eight *chaplin* genes which are known to play a role in the formation of aerial mycelium by coating them in a hydrophobic sheath that enables them to break the surface tension of the substrate were all downregulated in the double mutant compared to the wild-type. Many additional known developmental genes were shown to be differentially regulated compared to the wild-type. Excitingly, many novel genes were also implicated as potential targets of c-di-GMP regulation in this RNA sequencing study. The study gained needed information about c-di-GMP signaling and its effect on various developmental genes in *S. coelicolor*. *S. coelicolor* is a model organism for the group of soil-dwelling bacteria that produce anti-tumor agents, immunosuppressant drugs, and over two-thirds of commercially used antibiotics.

Author Disclosure Block:

M.A. Stark: None. **J.A. Bennett:** None.

Poster Board Number:

SATURDAY-746

Publishing Title:

Characterization of Random Transposon Insertion Mutants Defective for Development in a Filamentous Sporulating Bacterium

Author Block:

S. G. Kirk[1], J. A. Bennett[1], J. R. McCormick[2]; [1]Otterbein Univ., Westerville, OH, [2]Duquesne Univ., Pittsburgh, PA

Abstract Body:

Streptomyces coelicolor is a filamentous, sporulating bacterium commonly found in soil and is a model system to study for microbial cell biology and development. Streptomycetes produce many commonly medically utilized antibiotics. The purpose of this study was to identify new developmental mutants. In this study, ten random transposon insertion mutants with interesting developmental defects were identified by visual analysis on solid media. Preliminary characterization was done by microscopy to further understand the mutant phenotypes. Using Inverse PCR and DNA sequencing, the transposon insertion sites were identified to further analyze the mutants. Insertions were identified in both known and novel developmental genes. One of the identified insertion mutants, with a spore shape defect, has a transposon insertion in a gene that codes for protein of unknown function. Another mutant has a segregation defect as judged by DNA staining using confocal microscopy. This strain has a transposon insertion in a gene that codes for a putative membrane protein. Genetic complementation of the insertion mutations and/or PCR-targeted deletions are currently being performed to determine if the mutant visual and microscopic phenotypes are linked to the insertion mutation.

Author Disclosure Block:

S.G. Kirk: None. **J.A. Bennett:** None. **J.R. McCormick:** None.

Poster Board Number:

SATURDAY-747

Publishing Title:

Signaling and Activating the *Streptomyces coelicolor* Response Regulator Ramr Involved in Cell Differentiation

Author Block:

J. Girouard, T. Perkins, R. S. Greenwell, Jr.; Worcester State Univ., Worcester, MA

Abstract Body:

The soil-dwelling actinomycete bacterium *Streptomyces coelicolor* has been studied for both its complex developmental life cycle and production of secondary metabolites. The life cycle of these filamentous bacteria begins as emergence from spores to form vegetative hyphae as they colonize the nutrient layer of soil, and then eventually breach the surface as aerial hyphae and finally form spores. In order to transition from vegetative to aerial growth, the cells must escape from the colony aqueous environment by secreting biosurfactants such as the small lantibiotic peptide SapB. SapB has previously been shown as an important molecule in aerial hyphae development.Production of SapB is regulated by the response regulator RamR. We are investigating the mechanism by which RamR is activated. Cells expressing RamR proteins with amino acid substitutions generated at potential sites of phosphorylation are being analyzed for mature SapB production. Substitutions at those amino acids important for RamR function will create cells unable to produce mature SapB, detected via Western blot analysis.The signal molecule that stimulates production of SapB transduced through RamR has not been identified. Additionally, the kinase that activates RamR activities has not been isolated, as the *ramR* gene is not located in an operon with an encoded signal kinase. This work seeks to determine the identity of both the signal molecule and the activating kinase. There are 19 orphan kinases in the genome of *S. coelicolor*, of which we have 11 distinct kinase-null mutants that are being tested for mature SapB production via Western Blot. These experiments will provide insight into the function of RamR in production of SapB.

Author Disclosure Block:

J. Girouard: None. **T. Perkins:** None. **R.S. Greenwell:** None.

Poster Board Number:

SATURDAY-748

Publishing Title:

Characterization of a Conserved Multi-gene System Regulating Developmentally-associated Gene Expression in *Streptomyces coelicolor*

Author Block:

J. W. Sallmen, J. R. McCormick; Duquesne Univ., Pittsburgh, PA

Abstract Body:

Streptomyces coelicolor is a Gram positive, filamentous soil dwelling bacterium that exhibits a complex life cycle including the transition from a vegetative mycelium to a sporulating, aerial mycelium. Early genetic studies identified two classes of genes that resulted in developmental blocks, bald and white. The bald (*bld*) phenotype occurs when colonies cannot produce aerial hyphae. White (*whi*) colonies exhibit incomplete sporulation and/or loss of production of the concurrently produced grey pigment. While some of the original mutants have been explored, the developmental functions of many of the identified genes are not well understood. Of particular interest is a multi-gene regulatory system, with multiple homologues of each gene present in *S. coelicolor* chromosome and are well conserved among other streptomycetes and other morphologically complex actinomycetes. Two of these genes encode a predicted helix-turn-helix protein (WhiJ-like proteins) and a small, acidic protein of unknown function (BldB-like proteins). In order to explore the roles of these genes in development, one such system involved in the regulation of the spore-associated protein (*sap*) operon, *sapCED*, was analyzed. *sapR* and *sapS* are *whiJ* and *bldB*-like genes, respectively, and are a multi-gene regulatory system that is divergently transcribed from *sapCED*. In order to investigate the potential roles of this gene system in regulation of this operon, null mutants were isolated by recombineering. Single and double null mutants were isolated and the effects on expression were analyzed by the extraction of spore-associated proteins using a nonlethal detergent wash and were fractionated on a Coomasie Blue stained SDS-PAGE. Analysis shows an increase in the production of the *sapCED* encoded spore-associated proteins in both single mutants and double mutants of *sapR* and *sapS* with no obvious effect on other Saps. This phenotype was confirmed by genetic complementation of the *sapR* and *sapS* mutants. In addition, *sapR* and *sapS* were cloned into the Bacterial Two-Hybrid system based on the reconstitution of adenylate cyclase. Preliminary analysis suggests an interaction between SapR and SapS, and SapS with itself. These results together suggest a role for SapR and SapS in negatively regulating the development-associated *sapCED* operon.

Author Disclosure Block:

J.W. Sallmen: None. **J.R. McCormick:** None.

Poster Board Number:

SATURDAY-749

Publishing Title:

Natural Products Alter the Rates of Physiological Differentiation or Antibiotic Production in *Streptomyces* Species

Author Block:

R. S. Greenwell, Jr.; Worcester State Univ., Worcester, MA

Abstract Body:

Microorganisms coexist in nature by forming complex communities of a wide variety of species actively engaging with one another via various forms of communication. It was recently proposed that natural product compounds, such as antibiotics, may be produced as signals for cellular communication that modulate microbial behavior, morphology, and physiology. The effects of these natural products on microbial communities are of great importance for understanding how these organisms contend with each other in nature.We are examining the capacity of the soil bacterium *Streptomyces coelicolor* and other Streptomycetes to have their complex developmental life cycles altered when exposed to other environmental microbes and natural products. We have identified numerous environmental bacterial isolates that can inhibit or accelerate morphological differentiation of *S. coelicolor* and/or alter the expression of the pigmented antibiotics undecylprodigiosin (Red) or actinorhodin (Blue). Environmental microbes that alter Streptomycete differentiation allow us to monitor interspecies interactions that influence complex developmental processes of actinomycetes and gain perspective on the dynamics of the soil microbiome. The products secreted by these environmental microbes are currently being isolated to determine their mechanism of action.We have also demonstrated that a number of naturally-produced antibiotic compounds can influence *S. coelicolor* development when present in sub-lethal concentrations. Of the natural products tested, we identified that erythromycin and bacitracin can trigger *S. coelicolor* to more rapidly transition to aerial hyphae and form spore chains. These two antibiotics trigger independent pathways that allow *S. coelicolor* to form aerial hyphae, as observed by the ability of these compounds to bypass the morphological defects of various *S. coelicolor* developmental mutants. The ability of antibiotic compounds to trigger differentiation and spore formation in *S. coelicolor* provides evidence that these organisms recognize and contend with these compounds in nature via alternative resistance mechanisms, such as antibiotic indifference.

Author Disclosure Block:

R.S. Greenwell: None.

Poster Board Number:

SATURDAY-751

Publishing Title:

Temperature Regulation of Capsule and Biofilm Development in *Klebsiella pneumoniae*

Author Block:

S. Haxhi, C. O'Connor, R. Girresch; Maryville Univ., St. Louis, Saint Louis, MO

Abstract Body:

Background: *Klebsiella pneumoniae* is a pathogenic bacterium commonly found in clinical settings, as well as the environment. Research has demonstrated that certain factors contribute to this species' relative pathogenicity, two of which are explored in this study: 1) its polysaccharide capsule and 2) its ability to form biofilms on medical instruments. With growing concern over antibiotic resistance, research into the development and transmission of pathogenic organisms is critical. **Methods:** Capsule staining Cells were stained with India ink and observed under fluorescent microscope. Capsule size was quantified at various points in the growth curve (2, 4 and 6 hours incubation) through the program ImageJ.Biofilm formation Bacteria was inoculated for 24 hours and then transferred to the biofilm assay the next day where they were incubated for another 24 hours. Biofilm staining was made using crystal violet and quantification of the biofilm was made using iMark microplate reader at 550nm. (Protocol by US National Library of Medicine National Institutes of Health, 2011, George A. O'Toole.**Results:** No significant difference was found in capsule size at 26 and 37 degrees Celsius. A greater number of capsules were present at 37 degrees than at 26 degrees Celsius agar 24 hrs incubation. Capsule development demonstrates a similar growth curve to that of the standard bacteria growth at 37 degree celsius. No significant difference was found in biofilm formation at 26 or 37 degree celsius **Conclusions:** From the capsule staining and biofilm analysis we found out that the temperature does not have a significant effect on this bacterium's pathogenicity. Future work could be done by altering other effectors including humidity or nutrient availability in different stages of the growth.

Author Disclosure Block:

S. Haxhi: B. Collaborator; Self; Rebecca Girresch, Christopher O'Connor. **C. O'Connor:** None. **R. Girresch:** None.

Poster Board Number:

SATURDAY-752

Publishing Title:

Role of *wzi* Gene in Capsule Development in *Klebisella pneumoniae*

Author Block:

R. Girresch, C. O'Connor; Maryville Univ., St. Louis, MO

Abstract Body:

Klebsiella pneumoniae is a pathogenic bacterium commonly found in clinical settings. Research has demonstrated that certain factors contribute to this species' relative pathogenicity, including its polysaccharide capsule. The literature identifies several genes involved in capsule development in *Klebsiella*, most notably the *wzi* gene. This research sought to quantify *wzi* gene expression levels at critical points in *Klebsiella*'s growth curve. These critical points of capsule development were observed during a 6-hour incubation period of cultured Lennox nutrient broth. Optical density was observed hourly and dramatic increases at 3, 4 and 5 hours of incubation at 37 degrees Celsius. For this reason, these time points in *Klebsiella*'s growth curve were used for study of *wzi* gene expression and its role in capsule development. At each time point (3, 4 and 5 hours incubation), 2 milliliters of cultured broth was centrifuged and cellular DNA was extracted using the Qiagen extraction kit. Samples were stored overnight at -20 degrees Celsius. RNA was extracted from each sample using the Qiagen extraction kit. Samples were then stored at -80 degrees Celsius. Success of extraction was determined by gel electrophoresis. cDNA was synthesized from RNA using the Qiagen kit. Primers for the *wzi* gene were then developed and gene was amplified through PCR, establishing cDNA synthesis was successful. While cDNA synthesis was successful, quantification of *wzi* gene expression was not fully determined because Real-Time PCR was not successful. Each sample demonstrated expression of *wzi*. These results make it difficult to tease out the significance of *wzi* gene in capsule development, specifically at these time points determined to be critical. Future work will include generating a *wzi* gene knockdown strain for comparison to wild-type *Klebsiella* to better elucidate the gene's role in capsule development. Similarly, other genes noted to contribute to capsule development will be included in the assay to better understand the interaction of these genes during the bacterium's growth curve.

Author Disclosure Block:

R. Girresch: None. **C. O'Connor:** None.

Poster Board Number:

SATURDAY-753

Publishing Title:

The *Aspergillus flavus fluP*-Associated Polyketide Metabolite Promotes Production of Sclerotia

Author Block:

P-K. Chang[1], L. L. Scharfenstein[1], K. C. Ehrlich[1], J. D. Di Mavungu[2]; [1]Southern Regional Res. Ctr., Agricultural Res. Service, U. S. Dept. of Agriculture, New Orleans, LA, [2]Faculty of Pharmaceutical Sci., Ghent Univ., Ghent, Belgium

Abstract Body:

Aspergillus flavus is able to produce a variety of polyketide-derived secondary metabolites, including the hepatocarcinogenic aflatoxin. The fungus reproduces and disseminates predominantly by production of conidia. It also produces sclerotia, which are hardened mycelia aggregates used to cope with unfavorable growth conditions. In the present study, we examined the role of the polyketide synthase gene, *fluP*, of secondary metabolite gene cluster 41 on fungal development by gene deletion and overexpression approaches. The *fluP* deletion strain grew and produced aflatoxin normally, but it produced a much lower amount of sclerotia than the wild type. In contrast, the *fluP*-overexpressing strain produced a higher amount of sclerotia than the wild type. When the *fluP*-overexpressing strain was co-cultured with the wild type and the deletion strain, it elevated their sclerotial production at edges of contact. Acetone extracts of the *fluP*-overexpressing strain but not of the deletion strain exhibited the same effect on promoting sclerotia production of the wild type. These results suggest that FluP polyketide synthase is involved in the synthesis of a diffusible metabolite that serves as a signal molecule to regulate sclerotial production.

Author Disclosure Block:

P. Chang: None. **L.L. Scharfenstein:** None. **K.C. Ehrlich:** None. **J.D. Di Mavungu:** None.

Poster Board Number:

SATURDAY-754

Publishing Title:

Tests for Coevolution in a Microbial Mutualism

Author Block:

S. G. Wallen Jr, V. Bravman, I. Stroynyy, S. Rhothisen, A. Macarulay, Y. Demissie, C. Feng, K. Hillesland; Univ. of Washington Bothell, Bothell, WA

Abstract Body:

Coevolution, where one species adapts to the evolutionary changes of another, can increase the rate of evolution, cause species diversification, and can give rise to complex adaptations. It is unclear, however, whether these effects apply to mutually beneficial interactions between species commonly found in microbial communities. We tested whether the bacteria *Desulfovibrio vulgaris* and the archaea *Methanococcus maripaludis* coevolved during 1000 generations of evolution in laboratory conditions that forced them to cooperate with each other to survive. If *D. vulgaris* adapted to the evolving *M. maripaludis* population, then it should have higher fitness when paired with *M. maripaludis* from its past, and lower fitness with *M. maripaludis* that it did not evolve with. To test this, we revived frozen communities of *D. vulgaris* and *M. maripaludis* from generations 0, 300, 780, 1000, 1500, and 2000 of the laboratory evolution experiment, used antibiotics to separate *D. vulgaris* and *M. maripaludis* populations, and then paired *D. vulgaris* from the 1000 generation timepoint (D1000) with *M. maripaludis* populations from each past and future timepoint. To control for overall evolutionary changes in *M. maripaludis*, the growth rate and yield of each mixed-history community was compared to a control pairing of the cognate *M. maripaludis* population and ancestral *D. vulgaris*. All communities containing D1000 grew faster and had higher yield than communities containing only ancestral *D. vulgaris*, regardless of how long the *M. maripaludis* population had previously evolved. This result shows that 1000-generation *D. vulgaris* was compatible with both past and future partners. In addition, the growth rate and yield of D1000 communities with past *M. maripaludis* was not significantly different from those with future *M. maripaludis*. Thus, if *D. vulgaris* coevolved with *M. maripaludis* during 1000 generations in obligate mutualism, the resulting mutations did not impact community growth rate or yield.

Author Disclosure Block:

S.G. Wallen Jr: None. **V. Bravman:** None. **I. Stroynyy:** None. **S. Rhothisen:** None. **A. Macarulay:** None. **Y. Demissie:** None. **C. Feng:** D. Employee; Self; University of Wahington Bothell. **K. Hillesland:** D. Employee; Self; Assistant Professor; University of Washington.

Poster Board Number:

SATURDAY-755

Publishing Title:

The Role of Rpob Mutation 526 in *Mycobacterium tuberculosis* Virulence *In Vitro* & *In Vivo*

Author Block:

V. L. Campodonico, D. Rifat, J. A. Miller, P. C. Karakousis; Johns Hopkins Univ., Baltimore, MD

Abstract Body:

Background: Over 90% of *Mycobacterium tuberculosis* (Mtb) rifampin resistance is due to mutations in the *rpoB* gene encoding the β-subunit of RNA polymerase. Most mutations occur in an 81-bp fragment of the *rpoB* gene spanning codons 507-533, with the majority of clinical isolates containing point mutations in codons 531, 526 and 516. In the current study, we hypothesized that *rpoB* is required for full Mtb virulence. **Methods:** Bacterial strains used included wild-type Mtb CDC1551, an *rpoB* H526D mutant, isolated from Mtb CDC1551 grown on 1µg/ml rifampin-containing plates and confirmed by DNA sequencing, and the *rpoB* complemented strain by reintroduction of the native *rpoB* gene and confirmed by Southern blot. The growth of each strain was studied in nutrient-rich broth, and during nutrient starvation and progressive hypoxia at 37°C. Fixed phosphotungstate negative stains were used to determined bacterial morphology and to measure the longitudinal axis of each strain by transmission electron microscopy. Female BALB/c mice were aerosol-infected with Mtb. On Days 1, 14, 28, 56, 98 and 135 post-infection, the lungs and spleens of mice (n=4-5) were homogenized, diluted and plated on Middlebrook 7H11 agar plates for CFU counts. The upper lobe of the left lung was processed for histological examination. **Results:** No obvious differences in growth and survival were observed between the mutant and the WT strain during nutrient starvation or progressive hypoxia. The mean length of *rpoB* mutant bacilli was shorter during progressive hypoxia relative to that of wild-type and complement bacilli ($p<0.01$), but longer during nutrient starvation ($p=0.04$). Deficiency of *rpoB* was associated with reduced Mtb survival in mouse lungs following infection, as bacillary burden in lungs of mice infected with rpoB mutant was 1.8, 1, 1.4 and 0.7 $\log_{10,}$ lower than that of WT infected mice on days 14, 28, 56 and 98 respectively and with a lower percentage of lung area affected by granuloma (4% in lungs of mice infected with rpoB mutant versus 25% in mice infected with WT strain on day 135) **Conclusions:** Our data suggest that the H526D mutation is relatively dispensable for Mtb survival during various physiologically relevant stress conditions *in vitro*, but is required for full virulence in mouse lungs.

Author Disclosure Block:

V.L. Campodonico: None. **D. Rifat:** None. **J.A. Miller:** None. **P.C. Karakousis:** None.

Poster Board Number:

SATURDAY-756

Publishing Title:

Toxin YafQ Reduces *Escherichia coli* Growth at Low Temperatures

Author Block:

T. K. Wood, Y. Zhao; Pennsylvania State Univ., University Park, PA

Abstract Body:

Toxins of toxin/antitoxin (TA) systems have several roles in cell physiology with reduction in metabolism being the clearest. Under stress, toxin YafQ of the YafQ/DinJ *Escherichia coli* TA system alters metabolism by associating with ribosomes and cleaving transcripts with in-frame 5'-AAA-G/A-3' sites. Antitoxin DinJ is a global regulator that represses its locus as well as influences the level of the stationary sigma factor RpoS. Here, we investigated the influence temperature on activation of toxin YafQ. We found that deletion of the gene for the antitoxin, *dinJ*, resulted in both reduced metabolism and slower growth at 18^{o}C but not at 37^{o}C. The reduction in growth could be complemented by producing DinJ from a plasmid. Using a transposon screen to reverse the effect of the absence of DinJ, two mutations were found that inactivated the toxin YafQ; corroborating this result, a clean deletion of *yafQ* in the Δ*dinJ* ΔKm^{R} strain restored both metabolism and growth at 18^{o}C. In addition, production of YafQ was more toxic at 18^{o}C compared to 37^{o}C. Therefore, YafQ is more effective at reducing metabolism at low temperatures.

Author Disclosure Block:

T.K. Wood: None. **Y. Zhao:** None.

Poster Board Number:

SATURDAY-757

Publishing Title:

Mutational Analysis of the Periplasmic Sensor Domain of the *Porphyromonas gingivalis* Fims Histidine Kinase

Author Block:

K. Nishikawa; Aichi Gakuin Univ., Nagoya, Japan

Abstract Body:

Porphyromonas gingivalis (Pg) is a Gram-negative oral anaerobe closely associated with adult periodontitis. Known virulence factors of this organism include FimA fimbriae which are important for colonization in the gingival sulcus and evasion of the host immune system. The biogenesis of the fimbriae is positively regulated at the transcriptional level by FimS-FimR two-component system (TCS). The FimS histidine kinase comprises a putative periplasmic sensor domain containing multiple TPR motifs, which are often identified in the surfaces responsible for protein-protein interactions. The environmental signal that the TCS responds to is still unknown. In this study, a FimS random mutant library was constructed to identify the critical amino acids within the periplasmic domain for its sensory function. An error-prone PCR method was used to introduce random mutations in the sensor coding region of the wild-type *fimS*. The mutant *fimS* fragments were cloned into a pT-COW-based, novel expression vector, and introduced into a *fimS*-knockout Pg strain by electroporation. More than fifty transformants were found to be complemented with exogenous *fimS* genes, and further analyzed by direct sequencing to identify the mutation sites in the periplasmic domain-coding region. Their phenotypes in terms of the production of FimA were also examined by western blot using an anti-FimA peptide antibody. Interestingly, at least five clones had a single missense mutation within one of the TPR motifs that was sufficient for the FimA-deficient phenotype, indicating that these amino acids in the TPR motifs are critical for the sensory function of FimS. These results support the idea that the unknown environmental signal for FimS-FimR TCS could be a kind of protein/peptide molecule.

Author Disclosure Block:

K. Nishikawa: None.

Poster Board Number:

SATURDAY-758

Publishing Title:

Thioredoxin Protects *Salmonella* Against Oxidative Stress of the Nadph Phagocyte Oxidase Independently of Thiol-Disulfide Oxidoreductase Enzymatic Activity

Author Block:

J-S. Kim, M. Song, L. Liu, M. Husain, A. Vázquez-Torres; Univ. of Colorado, Sch. of Med., Aurora, CO

Abstract Body:

The thiol-disulfide oxidoreductase CXXC catalytic domain of thioredoxin contributes to antioxidant defense in organisms as phylogenetically distant as humans and bacteria. We indicate that the oxidoreductase activity of thioredoxin-1 protects *Salmonella enterica* serovar Typhimurium against the bacteriostatic activity of hydrogen peroxide *in vitro*, but does not seem to add to antioxidant defenses of this enteropathogen *in vivo*. Nonetheless, thioredoxin-1 defends *Salmonella* from the oxidative stress emanating from the NADPH phagocyte oxidase expressed in the innate response of macrophages and mice in a thiol-disulfide oxidoreductase-independent fashion. Thioredoxin-1 binds to and stabilizes the SPI2 master regulator SsrB independently of the canonical CXXC catalytic motif. Thus, thioredoxin-1 activates intracellular SPI2 gene transcription that is needed for resistance of *Salmonella* to both reactive oxygen species generated by the NADPH phagocyte oxidase and oxygen-independent host defenses of lysosomes. These investigations indicate that the horizontally-acquired virulence determinant SsrB is under post-translational regulation by ancestral thioredoxin.

Author Disclosure Block:

J. Kim: None. **M. Song:** None. **L. Liu:** None. **M. Husain:** None. **A. Vázquez-Torres:** None.

Poster Board Number:

SATURDAY-759

Publishing Title:

Redox Status Affects Biofilm Formation and Pyocyanin Synthesis in *Pseudomonas aeruginosa*

Author Block:

T. A. Van Laar, T. J. Birges, J. Thomas, B. Hazen, M. Rawat; California State Univ., Fresno, Fresno, CA

Abstract Body:

Low molecular weight (LMW) thiols are involved in protection against oxidative stress through the detoxification of reactive oxygen species (ROS). One of the most important LMW thiols is glutathione (GSH), which is able to reduce ROS, becoming oxidized to glutathione disulfide (GSSG) in the process. GSSG can be recycled to GSH through the activity of glutathione reductase, a critically important step for the regeneration of GSH and thus protection against ROS. *Pseudomonas aeruginosa* is a Gram-negative bacterium normally found in soil and water that can cause opportunistic infections, particularly in the lungs of cystic fibrosis (CF) patients. Previous work has shown that a mutant in GSH synthetase (*gshB*) has increased sensitivity to antibiotic therapy, indicating that GSH may play a role in tolerance of antibiotic treatment. To further study the role of GSH in *P. aeruginosa*, we obtained mutants in numerous genes responsible for GSH biosynthesis and recycling. We found that the *gshA* mutant does not produce any GSH and has a slight growth delay when compared to wild type. The *gshA* mutant is also defective for biofilm formation, supporting the idea that the redox state of *P. aeruginosa* is important for biofilm formation. We noted that the GSH mutant has reduced levels of swarming motility and pyocyanin (an important secreted virulence factor) production when compared to wild type. Finally, the *gshA* mutant has increased sensitivity to oxidative stressors. Taken together, these data suggest that an imbalance in the redox state of *P. aeruginosa* may decrease its virulence. We are in the process of further characterization of a mutant disrupted in *gor*, a glutathione reductase responsible for recycling GSH, to understand how GSH contributes to the redox balance in *P. aeruginosa*.

Author Disclosure Block:

T.A. Van Laar: None. **T.J. Birges:** None. **J. Thomas:** None. **B. Hazen:** None. **M. Rawat:** None.

Poster Board Number:

SATURDAY-760

Publishing Title:

Glutathione Synthesis Protects *Streptococcus agalactiae* against Hypochlorous Acid and Hydrogen Peroxide Oxidative Stresses

Author Block:

T. George, L. Grant, Y. Dhindsa, A. Patel, P. Desai, M. Schwabe, S. Patel, B. Janowiak; Saint Louis Univ., St. Louis, MO

Abstract Body:

Background: *Streptococcus agalactiae*, or Group B Streptococcus (GBS), is a leading cause of neonatal meningitis. GBS maintains high levels of an antioxidant known as glutathione, which is produced by a gamma-glutamylcysteine synthetase - glutathione synthetase encoded by the *gshAB* gene. The hypothesis was that GBS produces large quantities of glutathione in order to protect itself from the harsh oxidants produced by the host immune system during infections. **Methods:** In order to test the hypothesis, the *gshAB* gene was knocked out and complemented back in three clinically relevant GBS strains, GBS Ia A909, GBS III COHI, and GBS V 2603V/R. The bacteria were exposed to two immunologically relevant stresses, hypochlorous acid (HOCl) and hydrogen peroxide (H_2O_2), and differential killing by HOCl & H_2O_2 were compared between wild type, glutathione-deficient, and *gshAB*-complemented GBS for all three clinically relevant strains. The comparisons were performed in both exponentially-growing bacteria as well as GBS grown to stationary phase.**Results:** For all three clinically relevant strains, the killing by both HOCl and H_2O_2 were statistically similar between the wild type and complemented strains. However, for all three strains, glutathione-deficient GBS were more efficiently killed by HOCl than H_2O_2, when compared to either wild type or complemented GBS. These results were observed for both the exponentially- and stationary- grown GBS.**Conclusion:** Based on these results, it does appear that GBS relies on production of glutathione for the protection against immunologically relevant oxidative stresses such as HOCl and H_2O_2. Future studies will focus on other relevant stresses to GBS such as pH, osmotic, and metal stresses.

Author Disclosure Block:

T. George: None. **L. Grant:** None. **Y. Dhindsa:** None. **A. Patel:** None. **P. Desai:** None. **M. Schwabe:** None. **S. Patel:** None. **B. Janowiak:** None.

Poster Board Number:

SATURDAY-761

Publishing Title:

Persistent *Mycobacterium Bovis*-Bcg is Resistant to Glutathione Induced Reductive Stress Killing

Author Block:

N. Patel, R. Lawrence, **R. Yancey**, E. Gianacopoulos, K. Parker, M. Peteroy-Kelly; Pace Univ., NY, NY

Abstract Body:

The global incidence of tuberculosis has been slowly declining since 2003. Despite this, approximately nine million individuals contracted the disease with mortality rates approaching 20% in 2013. Therefore, there is still a great need to better understand how mycobacteria respond to the environments they may encounter within the host to develop effective therapies and vaccines. This study focuses on the redox stress response in mycobacteria elicited by a host-derived, thiol-based detoxification molecule, glutathione (GSH). Although the growth and viability of *M. bovis*-BCG (BCG) was hampered by exposure to 8 mM GSH, oxygen depleted, persistent BCG (NRP BCG) resisted GSH-mediated killing. Fast growing mycobacteria also resisted GSH-mediated killing. To determine the mechanisms behind these observations, we evaluated the levels of intracellular ATP in both BCG and NRP BCG exposed to 8 mM GSH. Intracellular ATP levels increased from 0.13 to 2.3 uM in BCG upon exposure to GSH. The levels of ATP remained low and unchanged when NRP BCG was exposed to GSH. Using both HPLC and a cell-based thiol detection assay, it was determined that GSH stimulates the production of mycothiol (MSH) by BCG approximately 5.7 fold. The levels of MSH did not change upon exposure of NRP BCG to GSH. MSH is an alternative, thiol-based detoxification molecule employed by mycobacteria. Changes in the cytoplasmic concentrations of this molecule are suggestive of redox imbalances. Together, GSH and MSH may introduce excess reducing equivalents into the mycobacterial cytoplasm; leading to reductive stress. The modulation of NAD^+ levels through alterations in ATP metabolism can enhance the cells ability to bind excess reducing equivalents and serve as a mechanism to restore the cellular redox balance when cells experience reductive stress. These results are corroborated by RNAseq data that suggests that pathways involved in regenerating NAD^+ (such as fatty acid biosynthesis) are activated in BCG exposed to GSH. An evaluation of NRP BCG RNAseq data is ongoing. Taken together, these data suggest that killing of BCG by GSH may result from reductive stress that cannot be controlled. NRP BCG appears to be resistant to GSH-induced reductive stress.

Author Disclosure Block:

N. Patel: None. **R. Lawrence:** None. **R. Yancey:** None. **E. Gianacopoulos:** None. **K. Parker:** None. **M. Peteroy-Kelly:** None.

Poster Board Number:

SATURDAY-764

Publishing Title:

Glutathione Protects *Synechococcus* pcc 7942 Against Environmental Stress

Author Block:

M. Rawat, A. Strankman, M. Abou-Naoum, J. Thomas, B. Hazen, S. Hartanto, T. Johns, T. Brooks, A. Rajkarnikar- Singh; California State Univ.-Fresno, Fresno, CA

Abstract Body:

Glutathione (GSH), a major low molecular weight thiol in cyanobacteria, protects the cell from oxidative stress caused by reactive oxygen species produced as by-products of photosynthesis and aerobic respiration. GSH contributes to the reducing environment within the cell by acting as an electron donor, becoming oxidized to glutathione disulfide (GSSG) in the process. While the functions of GSH are widely investigated in higher plants, not as much is known about the role of GSH in cyanobacteria. To investigate the role of GSH and GSH dependent enzymes in protecting cyanobacteria from oxidative and other environmental stresses, *Synechococcus* PCC 7942 transposon mutants disrupted in *gshB*, coding for the enzyme catalyzing the second step of glutathione synthesis, *gor*, coding for the glutathione reductase that maintains the glutathione in a reduced condition, and *gscR*, coding for the nitrosoglutathione reductase/ formaldehyde reductase, were analyzed. Growth of mutants and the complemented strains was measured under a variety of stresses, including oxidative and nitrosative metal stress. While the growth of the *gshB* mutant was adversely affected under several different stresses, the *gor* mutant behaved like wildtype and still contained substantial GSH. However, activity assays with recombinant Gor validated its function as a glutathione reductase. Intriguingly, RNAseq analysis of the *gor* mutant indicated that the mutation resulted in the expression of phage genes, which was not observed in the *gshB* mutant. Both *gscR* and the *gshB* mutants were more susceptible to nitrosative stress and formaldehydes than wildtype and activity assays with recombinant GscR demonstrated that GscR is able to act as a nitrosothiol reductase and formaldehyde dehydrogenase. Proteomic analysis of *S*-nitrosylated proteins in the wildtype and the *gscR* mutant and *S*-glutathionylated proteins in the wildtype and the *gshB* mutant revealed that multiple processes are affected by nitrosylation and glutathionylation of cysteine residues in *Synechococcus* PCC7942. Our data thus indicates that GSH plays an important role in protection against a number of stresses in *Synechococcus* PCC7942.

Author Disclosure Block:

M. Rawat: None. **A. Strankman:** None. **M. Abou-Naoum:** None. **J. Thomas:** None. **B. Hazen:** None. **S. Hartanto:** None. **T. Johns:** None. **T. Brooks:** None. **A. Rajkarnikar- Singh:** None.

Poster Board Number:

SATURDAY-765

Publishing Title:

Copper Resistance in Acinetobacter Baumannii

Author Block:

C. L. Williams[1], J. J. Gilbreath[1], S. L. Tyner[2], A. C. Jacobs[2], D. V. Zurawski[2], D. S. Merrell[1]; [1]Uniformed Services Univ. of the Hlth.Sci., Bethesda, MD, [2]Walter Reed Army Inst. of Res., Silver Spring, MD

Abstract Body:

Acinetobacter baumannii is an important emerging pathogen that is capable of causing many types of severe infection, especially in immunocompromised hosts. Since *A. baumannii* rapidly acquires antibiotic resistance and infections are on the verge of being untreatable, novel therapies are desperately needed. Recently, the antimicrobial properties of copper against various bacterial pathogens have been described, and clinical studies have shown that copper coated surfaces can reduce microbial burden and rates of infection in the hospital setting. To investigate the utility of copper-based antibacterial strategies against *Acinetobacter* infections, we characterized copper resistance in a diverse panel of *A. baumannii* strains. Exposure to increasing concentrations of copper in liquid culture and on solid surfaces resulted in dose-dependent and strain-dependent effects; levels of copper resistance varied broadly across strains, and were more pronounced in liquid culture. Examination of the growth phase dependent effect of copper on *A. baumannii* revealed that resistance to copper increases dramatically in stationary phase. Moreover, exposure of cultures to sub-inhibitory concentrations of copper allowed the bacteria to adapt to and grow in high concentrations of copper; this 'copper tolerance response' is likely achieved via increased expression of copper resistance mechanisms. Indeed, genomic analysis revealed numerous putative copper resistance proteins that share amino acid homology to known proteins in *Escherichia coli* and *Pseudomonas aeruginosa*. Transcriptional analysis revealed significant up-regulation of these putative copper resistance genes following brief copper exposure. To our knowledge, this is the first investigation into the copper resistance mechanisms of *A. baumannii*. Characterization of copper resistance mechanisms may aid in the search for novel antibiotics against *Acinetobacter* and other highly antibiotic resistant pathogens.

Author Disclosure Block:

C.L. Williams: None. **J.J. Gilbreath:** None. **S.L. Tyner:** None. **A.C. Jacobs:** None. **D.V. Zurawski:** None. **D.S. Merrell:** None.

Poster Board Number:

SATURDAY-766

Publishing Title:

Identification of Reduction-independent, Tellurite Resistance Determinants in Antarctic Bacteria

Author Block:

C. Muñoz, F. Cornejo, M. Figueroa, C. Pinto, E. Morales, F. Arenas, C. Vásquez; Univ. de Santiago de Chile, Santiago, Chile

Abstract Body:

Microorganisms can tolerate metals and others toxics because their genomes encode resistance determinants that are relatively specific and have been described in a variety of bacteria. There are several general metal resistance mechanisms including metal volatilization, metal chelation (intra- and extracellular), increased efflux, decrased uptake, and enzymatic detoxification, among others (Lemire et al., 2013). For the oxyanion tellurite, one of the best described resistance mechanisms is the reduction to elemental tellurium by proteins like flavoproteins (Castro et al., 2008). Furthermore, proteins encoded in the *ter*, *kil*A*Tel*AB, and *teh*AB operons, are implicated in tellurite resistance, although the mechanism of this process remains to be elucidated- and does not seem to be related to tellurite reduction. With the goal of finding novel mechanisms of tellurite resistance, we isolated bacteria from Antarctica, an environment that naturally imposes many types of stress that have been related to the toxicity of tellurite, including ROS. Specifically, we isolated a strain of *Staphylococcus haemolyticus* carrying 3 plasmids, and found that the larger one (pBNF01A) increases the resistance of *E. coli* to tellurite ~ 100 fold. Sequencing of plasmid revealed the presence of ORFs involved in metal resistance, glutaredoxin and hypothetical proteins. The *E. coli* wild type strain carrying or not pBNF01A showed similar physiological responses to tellurite exposure, including no differences in ROS production, activity of antioxidant enzymes, levels of intracellular thiols or in tellurite reductase activity. In addition, we isolated a strain of *Psychrobacter glacincola* with a high resistance to tellurite (MIC 2.3 mM). Sequencing of the genome showed the presence of genes involved in metal resistance, response to oxidative stress and tellurite resistance (*ter* genes). Surprisingly, *E. coli* carrying plasmid pBNF01A as well as *P. glacincola* did not show increased resistance to other metals and/or oxidizing agents, indicating that the resistance determinants are specific for tellurite.

Author Disclosure Block:

C. Muñoz: None. **F. Cornejo:** None. **M. Figueroa:** None. **C. Pinto:** None. **E. Morales:** None. **F. Arenas:** None. **C. Vásquez:** None.

Poster Board Number:

SATURDAY-767

Publishing Title:

Identification of Zinc-Regulated but Zur-Independent Genes in *Francisella*

Author Block:

G. Moreau, B. Mann; Univ. of Virginia, Charlottesville, VA

Abstract Body:

Francisella tularensis has been classified as a Tier 1 select agent by the Centers for Disease Control because it has a low infectious dose, is easily aerosolized, and infection is potentially lethal. Zinc is an essential nutrient for all cells, and one mechanism host cells use to control bacterial growth is to limit pathogen access to zinc. As *Francisella* grows effectively intracellularly, it must have efficient mechanisms of acquiring zinc from host cells, but these mechanisms have not been well characterized. Many bacteria use a zinc-responsive transcriptional regulator, Zur, to control expression of genes required for zinc uptake. We performed RNAseq with a wildtype and Zur transposon mutant of *Francisella novicida* to identify Zur-regulated genes. We identified only four genes that were differentially regulated in the *zur* mutant strain. Of the identified genes, only two are thought to encode functional proteins in the virulent *Francisella tularensis* ssp. *tularensis* Schu S4 strain, and none were predicted to encode for transporter proteins. These results suggest that the virulent Schu S4 strain must have mechanisms of responding to zinc-limiting conditions that are Zur-independent. To identify genes involved in the uptake of zinc in zinc-limiting conditions, we performed RNAseq with both *Francisella novicida* and *Francisella tularensis* Schu S4 that were either untreated or treated with TPEN, a zinc-chelating agent. Our future directions are to identify and characterize genes that are important for zinc acquisition and survival in the zinc-limiting environment of the host.

Author Disclosure Block:

G. Moreau: None. **B. Mann:** None.

Poster Board Number:

SATURDAY-768

Publishing Title:

Control of Antibiotic Resistance and Virulence is Coupled in *Acinetobacter baumannii*

Author Block:

M. J. Gebhardt, H. A. Shuman; Univ. of Chicago, Chicago, IL

Abstract Body:

Acinetobacter baumannii is a serious human health threat because many currently circulating, highly virulent strains have acquired multiple antibiotic resistance determinants. In addition, our understanding of the molecular mechanisms that underlie *A. baumannii* pathogenesis and innate resistance to antibiotics and environmental stresses is incomplete. In particular, the transcriptional networks that govern the expression of genes involved in virulence and antibiotic resistance remain largely uncharacterized. We recently performed a large scale, unbiased forward genetic screen to identify genetic elements required for *A. baumannii* to grow within the larvae of the insect, *Galleria mellonella*, an established infection model for microbial pathogens. The screen identified 300 genes required for survival and/or growth within *G. mellonella* larvae. Interestingly, we also discovered that several genes required for virulence are also required for antibiotic resistance, suggesting a link between virulence and antibiotic resistance in this emerging pathogen.Two genes of particular interest are *gigA* and *gigB* (for *G*rowth *i*n *G*alleria), which show a defect for both growth within and killing of *Galleria* larvae. The *gigA* gene encodes a predicted two-component response regulator protein while *gigB* is predicted to encode a hypothetical protein containing a STAS domain (Sulphate Transporter and Anti-Sigma factor antagonist). Interestingly, mutant strains harboring a deletion of either *gigA* or *gigB* display a survival defect in the presence of several environmental stressors, including low pH, heavy metals and antibiotics. Finally, suppressor analysis identified a connection between *gigA*/*gigB* with the Nitrogen Phosphotransferase system (PTS^{Ntr}). These findings suggest that GigA and GigB, possibly in conjunction with the PTS^{Ntr} system, comprise a signal transduction pathway that controls not only adaptation to environmental stresses, including antibiotic exposure, but also virulence in *A. baumannii*.

Author Disclosure Block:

M.J. Gebhardt: None. **H.A. Shuman:** None.

Poster Board Number:

SATURDAY-769

Publishing Title:

Phenotypic Response Of Bacteria To Antibiotics At Single Minute Time Scales

Author Block:

K. Babcock, C. Schneider, P. Harris, S. Markakis, S. Strenn; Affinity Biosensors, Santa Barbara, CA

Abstract Body:

Clinical tests of the response of bacteria to antibiotics monitor growth over many hours. It is well documented, however, that the stress response in a bacterial cell can occur within minutes after exposure. We tested a method for measuring the phenotypic changes in bacteria at time scales down to one minute, and monitored very short-term changes in bacteria cultures in real time when exposed to antibiotics.We used mechanically resonant microchannels (ref Burg) to measure the masses and concentrations [#/ml] of individual microbes drawn from broth microdilution cultures. Fluidics were designed to maximize throughput, so that up to 1,000 microbes could be measured in one minute. A number of microbe species were tested in the presence of bactericidal and bacteriostatic antibiotics. A typical test protocol grew the microbes from low concentrations ($<1x10^6$/ml) until they reached their growth phase, then added the antibiotic. Throughout this process the culture was sampled once per minute and quantified *via* number concentration and the distribution of individual microbe masses. Bacteriostatic antibiotics could then be diluted away and the culture monitored for resumption of growth.For ampicillin added to *E. coli* K12 (ATCC 10798) at a concentration four times the minimum inhibitory concentration, the mean microbe mass was observed to increase within three minutes. The number concentration began to decrease at the same point, apparently caused by decay into small-mass debris which appear simultaneously in the mass distribution. For tetracycline, replication ceased within two minutes, while there were no significant changes in mass distribution. After diluting the tetracycline 100-fold, replication resumed after 40 minutes. At high enough concentrations of tetracycline, growth did not resume for several hours, if at all, indicating a transition to bactericidal action. This transition can be studied in detail as a function of antibiotic exposure time and concentration. Response time to antibiotic exposure varied by strain, with some showing rapid growth of filamentous forms.The results demonstrate that phenotypical response can be detected down to the one-minute timescale, enabled by the combination of high number throughput and the measurement of individual microbe masses. Among numerous potential applications are antibiotic discovery, dosing, and modes of action.

Author Disclosure Block:

K. Babcock: D. Employee; Self; Affinity Biosensors. K. Shareholder (excluding diversitied mutual funds); Self; Affinity Biosensors. **C. Schneider:** D. Employee; Self; Affinity Biosensors.

D. Employee; Spouse/Life Partner; Affinity Biosensors. K. Shareholder (excluding diversitied mutual funds); Spouse/Life Partner; Affinity Biosensors. **P. Harris:** D. Employee; Self; Affinity Biosensors. **S. Markakis:** D. Employee; Self; Affinity Biosensors. **S. Strenn:** M. Independent Contractor; Self; Affinity Biosensors.

Poster Board Number:

SATURDAY-770

Publishing Title:

Novel Genes Involved in the Generation of Persister Cells in Ampicillin-exposed Cultures of Uropathogenic *Escherichia coli* (Upec)

Author Block:

R. C. Molina-Quiroz[1], D. Lazinski[2], S. B. Levy[1]; [1]Ctr. for Adaptation Genetics and Drug Resistance, Tufts Univ., Sch. of Med., Boston, MA, [2]Tufts Univ., Sch. of Med., Boston, MA

Abstract Body:

Uropathogenic *E. coli* (UPEC) is the main ethiologic agent of urinary tract infections worldwide, generating aproximatelly 80% of the clinic cases every year. Most of these infections are chronic and recurrent and represent a global health threat. Relapsing infections have been associated with the generation of persister cells. This subpopulation is characterized by a transient non-hereditary state with the ability to survive lethal concentrations of different antibiotics. To date it has been shown that these subpopulations are generated by stocastic processes and a role for toxin-antitoxin modules (TA), stringent response among others, has been described. We used a high-throughput genetic screen to identify mutants with an increased fitness after exposure to Ampicillin. To do this, we used a transposon library containing ~360.000 mutants in the CFT073 genetic background and we mapped the insertions in the bacterial chromosome by Illumina-sequencing. This library was exposed to a lethal concentration of Ampicillin during 6 hours and survivors were enriched by overnight growth in LB medium. This approach allowed us to identify 30 mutants under positive selection in Ampicillin-exposed cultures, most of them metabolic and membrane proteins. Construction and evaluation of bacterial persistance of mutants corresponding to *dsbA* (thiol:disulfide interchange protein *dsbA* precursor), *cyaA* (adenylate cyclase) and *udhA* (pyridine nucleotide transhydrogenase) validated our screen. These results suggest a role –non-described to date- of novel metabolic genes associated with persister cell generation.

Author Disclosure Block:

R.C. Molina-Quiroz: None. **D. Lazinski:** None. **S.B. Levy:** None.

Poster Board Number:

SATURDAY-771

Publishing Title:

Daptomycin Exposure Selects for Novel Mutations Resulting in Enhanced Pigmentation in Methicillin-resistant *Staphylococcus aureus*

Author Block:

A. D. Berti[1], H. M. Turner[1], I. R. Monk[2], S. L. Baines[2], B. P. Howden[2], R. A. Proctor[1], W. E. Rose[1]; [1]Univ. of Wisconsin - Madison, Madison, WI, [2]Univ. of Melbourne, Melbourne, Australia

Abstract Body:

Background: *Staphylococcus aureus* exposed to daptomycin (DAP) frequently demonstrates increased carotenoid pigment production. The major carotenoid produced by staphylococci, staphyloxanthin, is biosynthesized by the *crtMNOPQ* pathway. Here we characterize the carotenoid content of *S. aureus* containing individual targeted mutations outside of the *crt* locus based on a panel of sequence variations identified following serial *in vitro* DAP exposure. **Methods:** Whole genome sequencing was performed on 25 experimental replicates of *S. aureus* strain J01 exposed to DAP with-or-without adjunctive antibiotics *in vitro* over a period of 28 days. Genetic loci containing mutation in at least 10% of the 25 replicates were selected for further study. Mutations in loci thus identified were introduced in isolation back into the original strain J01 via pIMAY-based allelic replacement. Carotenoid extracts were quantified spectrophotometrically and confirmed via chromatography and mass spectrometry. Membrane fluidity was determined using polarized spectrofluorometry with 1-6 diphenylhexatriene as a lipid-partitioning probe. Susceptibility to reactive oxygen species was assessed via peroxide tolerance assay. Minimum inhibitory concentration (MIC) to DAP was assessed by broth microdilution. **Results:** Nine loci were identified that contained mutation in at least 10% of replicates. Targeted mutation in *gerCC* (nt925 del A) or *rsh* (L68F) significantly increased carotenoid production. These genes encode a medium-chain length polyprenyl synthase and a GTP pyrophosphokinase, respectively. No other targeted mutations significantly altered carotenoid production. Mutation in either *gerCC* or *rsh* resulted in more rigid membranes. Strains with mutation in *rsh, but not gerCC,* were less susceptible to hydrogen peroxide-mediated killing. No mutation resulted in increases to the DAP MIC. **Conclusions:** Mutation in *gerCC* or *rsh* results in phenotypes frequently observed in both DAP nonsusceptible isolates and clinically persistent strains. While no mutation identified in this work significantly alters the DAP MIC, they likely play a role in survival under stress conditions including those induced by antibiotic exposure.

Author Disclosure Block:

A.D. Berti: None. **H.M. Turner:** None. **I.R. Monk:** None. **S.L. Baines:** None. **B.P. Howden:** J. Scientific Advisor (Review Panel or Advisory Committee); Self; Pfizer. **R.A. Proctor:** None.

W.E. Rose: C. Consultant; Self; The Medicines Company, Visante. E. Grant Investigator; Self; Merck (Cubist). L. Speaker's Bureau; Self; Merck (Cubist).

Poster Board Number:

SATURDAY-773

Publishing Title:

Loss of Antibiotic Resistance in Benzoate-Evolved *E. coli*

Author Block:

E. S. Ditmars, P. Basting, K. Creamer, S. Acero, I. Hamdallah, E. Eder, J. L. Slonczewski; Kenyon Coll., Gambier, OH

Abstract Body:

We conducted experimental evolution over 2000 generations on *Escherichia coli* K-12 to isolate clones with increased fitness under benzoate stress. 24 populations of *E.coli* were grown in semi-aerobic microplate wells and sub-cultured with increasing levels of benzoate in LBK at pH 6.5. 16 benzoate-evolved clones were selected based on growth rate in benzoate and on chloramphenicol sensitivity. Clones isolated from this experiment showed a number of mutations that suggested that benzoic acid stress could result in a decrease of antibiotic resistance, such as a 6,000-bp deletion that includes the entire Mar (multiple antibiotic resistance) operon. Benzoate-evolved clones were phenotyped in 8 ug/ml chloramphenicol 5 mM benzoate 100 mM MOPS pH 7 LBK. Phenotyping revealed a number of clones sensitive to chloramphenicol. Certain clones showed greater chloramphenicol sensitivity than a MarA knockout strain. One highly sensitive clone, G5-2, was selected for further phenotyping; its resequenced genome showed only one mutation known to be linked to antibiotic resistance in the gene for mar regulator, *rob*. By replacing this mutated gene with its ancestral allele, we determined that the G5-2 clone's decrease in antibiotic resistance was not *rob* mediated, implying that there is a secondary, uncategorized aromatic acid-induced antibiotic resistance system. Salicylate, the main inducer of the Mar regulon and the main circulating product of aspirin, conferred an even greater fitness advantage than benzoate in our evolved clones. By contrast, acetate and sorbate had no effect on division time. Our results may model evolutionary trends seen in the gut of individuals taking aspirin. This model suggests that there could be a connection between the health of the microbiome and the selective pressure of aspirin derivatives such as salicylate in individuals who take aspirin on a regular basis.

Author Disclosure Block:

E.S. Ditmars: None. **P. Basting:** None. **K. Creamer:** None. **S. Acero:** None. **I. Hamdallah:** None. **E. Eder:** None. **J.L. Slonczewski:** None.

Poster Board Number:

SATURDAY-774

Publishing Title:

Participation of *Saccharomyces cerevisiae* Monothiol Glutaredoxin (Grx6) in the Response against Cadmium Toxicity in *Schizosaccharomyces pombe*

Author Block:

Y. J. Choi[1], **K. Kim**[1], S. K. An[2], C. J. Lim[1]; [1]Kangwon Natl. Univ., Chuncheon, Korea, Republic of, [2]Jaema Trading Co., Wonju, Korea, Republic of

Abstract Body:

Glutaredoxins (Grxs), also known as thioltransferases (TTases), are thiol oxidoreductases that regulate cellular redox state in a variety of organisms. In the budding yeast *Saccharomyces cerevisiae*, Grx1 and 2 are cytosolic dithiol Grxs, while Grx3, 4 and 5 are monothiol Grxs. A gene encoding a new monothiol Grx, Grx6, was previously cloned from the genomic DNA of *S. cerevisiae* by PCR. The *S. pombe* cells harboring plasmid pFGRX6 containing the Grx6 gene could grow much better, after the shifts to the fresh medium with cadmium chloride (25, 50 and 100 μM), than the vector control yeast cells. They were identified to contain the lower reactive oxygen species (ROS) and nitrite content, an index of nitric oxide (NO), in the presence of cadmium, than the vector control cells. In the absence or presence of cadmium, changes in glutathione content, superoxide dismutase and glutathione peroxidase activities were also compared. In brief, the *S. cerevisiae* Grx6 participate the defense against cadmium toxicity in *S. pombe*.**:**

Author Disclosure Block:

Y.J. Choi: None. **K. Kim:** None. **S.K. An:** None. **C.J. Lim:** None.

Poster Board Number:

SATURDAY-775

Publishing Title:

Defensive Roles of a Second Protein Disulfide Isomerase (PDI2) against Heavy Metal Toxicity in *Schizosaccharomyces pombe*

Author Block:

J. Choi[1], **K. Ahn**[2], K. H. Park[1], K. Kim[1], C. J. Lim[1]; [1]Kangwon Natl. Univ., Chuncheon, Korea, Republic of, [2]Baekseok Culture Univ., Cheonan, Korea, Republic of

Abstract Body:

In the previous work, a second gene encoding protein disulfide isomerase (PDI2) was cloned from the *Schizosaccharomyces pombe* genome using the *E. coli*-yeast shuttle vector pRS316 to generate the recombinant plasmid pYPDI2. This work aimed to assess the protective roles of PDI2 against heavy metals, such as mercury and cadmium, using the PDI2-overexpressing recombinant plasmid pYPDI2. In the presence of mercury and cadmium (25 μM, 50 μM and 100 μM), the *S. pombe* cells harboring the plasmid pYPDI2 were able to grow significantly better than the vector control yeast cells. They were identified to contain the lower reactive oxygen species (ROS) and nitrite content, an index of nitric oxide (NO), in the presence of heavy meals, than the vector control cells. In the absence or presence of heavy metals, changes in glutathione content, superoxide dismutase and glutathione peroxidase activities were also compared. In brief, the *S. pombe* PDI2 possesses a defensive role against heavy metal stress.

Author Disclosure Block:

J. Choi: None. **K. Ahn:** None. **K.H. Park:** None. **K. Kim:** None. **C.J. Lim:** None.

Poster Board Number:

SATURDAY-776

Publishing Title:

***Staphylococcus aureus* Persisters Form Due to a Drop in ATP**

Author Block:

B. P. Conlon, **E. A. Zalis**; Northeastern Univ., Boston, MA

Abstract Body:

Background: We sought to examine the mechanism of persister formation in *Staphylococcus aureus*. *S. aureus* is responsible for a variety of difficult to treat biofilm associated infections. Persister cells are antibiotic tolerant sub-populations of cells believed to be responsible, at least in part, for the antibiotic tolerance of the biofilm. We hypothesized that persisters in an exponential phase population are cells entering the stationary phase before the bulk of the population**Methods:** We compared antibiotic tolerance of stationary phase and exponential phase populations. We used fluorescence activated cell sorting (FACS) of cells with stationary phase specific promoters (P*capA* and P*arcA*) downstream of GFP. We also measured ATP and examined its role in antibiotic tolerance.**Results:** We found that indeed stationary phase specific promoters fused to GFP facilitated sorting of persister cells from the population and generated a 1000 fold enrichment of persisters. We also found that persister formation and expression of these persister markers was dependent on ATP.**Conclusions:** ATP is the major energy currency of the cell. ATP drives the activity of major cell processes targeted by antibiotics. Our results demonstrate that ATP levels in a population are heterogeneous and a drop in ATP results in persister formation, presumably due to inactivity of antibiotic targets. This finding has major implications for the development of new treatments to kill persister cells and eradicate, not just for *S. aureus* but also other important, difficult to treat bacterial infections.

Author Disclosure Block:

B.P. Conlon: None. **E.A. Zalis:** None.

Poster Board Number:

SATURDAY-777

Publishing Title:

Metal Resistance Patterns in *Enterococcus spp.* Isolated from Irrigation Water

Author Block:

A. A. Ramos, L. A. Navedo, M. Díaz-Camacho, N. M. Rodriguez-Bonano, L. B. Mendez; Univ. del Este, Carolina, PR

Abstract Body:

Background: *Enterococcus spp.* are gram-positive commensal bacteria found in the GI tract of humans and animals. Resistance to antibiotics and heavy metals has been reported in environmental strains of *Enterococcus spp.* Their presence in the environment is of public health concern since enterococci are a common cause of community-acquired and foodborne diseases, causing approximately 800,000 infections per year in USA. The purpose of this study was to determine metal resistance patterns in environmental strains of *Enterococcus spp.* isolated from irrigation water. **Methods:** *E. faecalis* and *E. faecium* were isolated from water samples obtained in a farm in Puerto Rico, which uses well water for irrigation. The minimum inhibitory concentration for 8 metals (i.e. arsenic, cadmium, copper, iron, lead, nickel, and vanadium) was determined using the micro-broth dilution technique. Briefly, enterococci were incubated with increasing concentrations (0.03-1.6 mg/mL) of the metals dissolved in brain heart infusion broth supplemented with 0.5% nalixidic acid. The absorbance (595 nm) was measured after 24 hours of incubation.**Results:** Results showed differences in metal resistance patterns among *E. faecalis* and *E. faecium* isolates. Most of the strains showed resistance to As (III) while only two were resistant to Pb. Metal resistance correlated with previously observed antibiotic resistance patterns determined for the same strains. Specifically, lead-resistant isolates showed higher resistance to the previously tested antibiotics.**Conclusions:** These results are important to public health since in many instances foodborne diseases are associated with contaminated water used for irrigation.

Author Disclosure Block:

A.A. Ramos: None. **L.A. Navedo:** None. **M. Díaz-Camacho:** None. **N.M. Rodriguez-Bonano:** None. **L.B. Mendez:** None.

Poster Board Number:

SATURDAY-778

Publishing Title:

Molecular Mechanism of Bacterial Killing by Plasmid-Encoded Two-Component Bacteriolysin Bac41 in *Enterococcus faecalis*

Author Block:

J. Kurushima, H. Tomita; Gunma Univ., Gunma, Japan

Abstract Body:

Background: For decades, plasmid-mediated acquisition of drug resistances has increased severity of bacterial infection. Proteinaceous bacteriocin (bacteriolysin) Bac41 is encoded on plasmid of clinical strains of *Enterococcus faecalis* that is a causative agent of several opportunistic infections. Since Bac41 system selectively kills bacterial cells not carrying Bac41-coding plasmid, it is supposed to contribute propagation of Bac41-coding plasmid among *E. faecalis* population. Bac41 lytic effectors, $BacL_1$ and BacA, are secreted proteins responsible for specific bacteriolysis on *E. faecalis*. Here, we reported frequency of Bac41 in *E. faecalis* clinical strains and molecular functions of the Bac41 effectors, $BacL_1$ and BacA. **Materials and Methods:** Epidemiologic study for Bac41 was performed by PCR detection of Bac41-related genes and bacteriocinogenic activity against *E. faecalis*. Bactericidal assay was performed by soft-agar method using bacterial supernatant or recombinant protein prepared from *E. coli* expression system. For fluorescent imaging, *E. faecalis* cells were incubated together with the fluorescence-labeled recombinant proteins in THB broth and chemically fixed, following by analysis under the fluorescent microscopy. **Results:** Among 327 *E. faecalis* clinical isolates, 195 strains (59.6%) were Bac41 positives, showing that Bac41 is extensively propagated through *E. faecalis* in clinical environment. Mixture of $BacL_1$ and BacA recombinants induced bacteriolysis on *E. faecalis*. In contrast, single treatment of $BacL_1$ or BacA did not show any lytic activity, indicating that these effectors need each other to exert the bacteriolytic activity. Both $BacL_1$ and BacA have peptidoglycan hydrolase-like domains. Actually, $BacL_1$, but not BacA, showed an endopeptidase activity to degrade *E. faecalis* cell wall. Fluorescent imaging revealed that $BacL_1$ specifically localized in cell division loci of the target *E. faecalis* cell surface independently of BacA. BacA also localized the same region only when catalytic mutant of $BacL_1$ coexisted. However, the BacA targeting was not detected when coexistence of wild-type $BacL_1$ or BacA alone. **Conclusion:** These observations suggested working model that $BacL_1$ primarily attacked to target cell wall independently of BacA, and then BacA follows $BacL_1$ to rapidly trigger the bacteriolysis.

Author Disclosure Block:

J. Kurushima: None. **H. Tomita:** None.

Poster Board Number:

SATURDAY-779

Publishing Title:

The Essential Role of Stress Induced Sigma Factor E in Gram Negative Bacteria

Author Block:

A. M. Zappas; The Pennsylvania State Univ., University Park, PA

Abstract Body:

Background: Due to increasing antibiotic resistance, new antibiotic development has become essential research. Sigma factor E is a potential new target for antibiotic development. The alternative sigma factor E is activated when stress is induced on membrane or periplasmic proteins within most gram-negative bacteria. Though sigma E was found to be essential in the cell, mutations known as suppressors may allow cells to grow in absence of sigma E[1]. These suppressor mutations give insight to the role of sigma E in the cell and help to understand how sigma E could be targeted with an antibiotic. The project involves connecting a mutation known as prlF1 to the sigma E pathway, and analyzing if the mutation may replace or bypass the function of sigma E. This mutation was found to relieve hybrid jamming in outer membrane protein secretion in *Escherichia coli*[2]. **Methods:** To test the suppression effect of prlF1, the gene *rpoE* coding for sigma E is removed by gene knockout.In order to remove any other mutations from creating a suppression effect prlF1 was marked by a chloramphenicol marker. The marked strain was used to create a P1 lysate and then used for a phage transduction to transduce prlF1 into two wild type strains. The final strain was used to test the suppression effect of prlF1, the gene *rpoE* coding for sigma E is removed by gene knockout. The overexpression of the gene *prlF* has also shown a similar suppression effect as prlF1. The overexpression is done by an expression plasmid containing an IPTG promoter.**Results:** Sigma E is more easily removed in prlF1 strains than wild type, showing its suppression. The overexpression of *prlF* allows more growth of sigma E gene knockout products than in wild type strains**Conclusions:** Suppression is shown by both prlF1 and the overexpression of prlF. Identifying the connection of prlF1 and prlF to sigma E will help to understand how the envelope is being maintained by sigmaE, leading to better understanding of sigmaE as an antibiotic target.

Author Disclosure Block:

A.M. Zappas: None.

Poster Board Number:

SATURDAY-780

Publishing Title:

Fnr Regulates Important Virulence Factors in Apec (Avian Pathogenic *Escherichia coli*)

Author Block:

N. L. Barbieri, A. R. Baker, G. Li, C. M. Logue, L. K. Nolan; Iowa State Univ., Ames, IA

Abstract Body:

Avian pathogenic *Escherichia coli* (APEC) is the etiologic agent of colibacillosis, an important cause of morbidity and mortality in poultry. Though many virulence factors associated with APEC pathogenicity are known, their regulation remains unclear. FNR (fumarate and nitrate reduction) is a well-known global regulator that works as an oxygen sensor and has previously been described as a virulence regulator in bacterial pathogens. The goal of this study was to examine the role of FNR in the regulation of such APEC virulence factors and processes as adherence and invasion, type I fimbriae, type VI secretion, survival during oxidative stress, and growth in iron-restricted environments. To accomplish this goal, APEC O1, a well characterized, highly virulent strain of APEC with multiple virulence mechanisms, was compared to its FNR mutant for expression of various virulence traits. Deletion of FNR was found to affect APEC O1's adherence and invasion and expression of *ompT*, a plasmid-encoded outer membrane protein; type I fimbriae; and *aatA*, an autotransporter system. Indeed, the *fnr*$^-$ mutant showed an 8-fold reduction in expression of type I fimbriae and a highly significant reduction in expression of *fimA*, *ompT* (plasmid-borne) and *aatA* ($P<0.0001$). FNR also was found to regulate expression of the type VI secretion system, affecting the expression of *vgrG* and *clpV*. Further, FNR was found to be important to APEC O1's growth in iron-deficit media and survival during oxidative stress with the mutant showing a 4-fold decrease in tolerance to oxidative stress, as compared to the wildtype. Thus, our results suggest that FNR not only mediates APEC adaptation to anaerobic metabolism but also functions as an important regulator of APEC virulence.

Author Disclosure Block:

N.L. Barbieri: None. **A.R. Baker:** None. **G. Li:** None. **C.M. Logue:** None. **L.K. Nolan:** None.

Poster Board Number:

SATURDAY-781

Publishing Title:

Novel *Acinetobacter baumannii* Strategy to Regulate the Response to Dna Damage

Author Block:

C. Ching, K. Gozzi, B. Heinemann, V. G. Godoy; Northeastern Univ., Boston, MA

Abstract Body:

Acinetobacter baumannii (Ab) is an emerging multidrug-resistant, opportunistic pathogen. *Ab* survives desiccation, remaining on the surfaces of hospital equipment, reaching immunocompromised individuals. *Ab* antibiotic resistance acquisition has been linked to its DNA damage response (DDR) and DDR genes are involved in DNA damage and desiccation-induced mutagenesis. In *Escherichia coli*, RecA, the cells' main recombinase, binds single stranded DNA, the signal of DNA damage, to form the nucleoprotein (RecA*). Co-protease activity of RecA* promotes autocleavage and inactivation of LexA, the global DDR repressor, resulting in expression of DDR genes. In *Ab*, this well-known circuitry does not exist, in part because *Ab* lacks LexA. We have shown that conserved DDR genes in *Ab* form two phenotypic subpopulations in response to DNA damage: one with low and another with high expression. Bimodal DDR gene expression may provide a strategy for survival and plasticity in an ever-changing environment.We aim to elucidate the regulation of *recA* in *A. baumannii* as we hypothesize it underlies the bimodal DDR. We have found that the *recA* gene contains a *cis*-acting element in its 5' untranslated region (UTR) unlike any other known DDR gene in *A. baumannii* or other bacteria. *In-vitro* transcription demonstrated that this 5'UTR is structured. A plasmid-borne fluorescent reporter was constructed in which mKate is regulated by the UTR. Mutants of the UTR that delete repeated sequences or changes predicted stem and loop secondary structures were constructed using site-directed mutagenesis. Fluorescence microscopy experiments using the parental and mutant strains with and without DNA damage treatment have shown that the *cis*-acting regulatory element and its structure are important for *recA* regulation. Remarkably, the UTR senses DNA damage not only in *Ab* but also in other bacterial species. These findings provide insight into the evolution of a fundamental DDR regulation and how *Ab* is able to quickly acquire antibiotic resistances.

Author Disclosure Block:

C. Ching: None. **K. Gozzi:** None. **B. Heinemann:** None. **V.G. Godoy:** None.

Poster Board Number:

SATURDAY-782

Publishing Title:

Identification of Sigma / Anti-Sigma Factor Regulatory Pairs in the Cyanobacterium Nostoc Punctiforme

Author Block:

A. Amir Ali; California State Univ. Northridge, Glendale, CA

Abstract Body:

The filamentous cyanobacterium Nostoc punctiforme grows using plant-like oxygenic photosynthesis and can differentiate into spore-like akinetes, nitrogen-fixing heterocysts, or motile hormogonia in response to stress. Under optimal growth conditions, the genes involved in environmental stress are inactive but under stressful conditions, transcription of stress-responsive genes is induced. A subset of these stress responses are hypothesized to be regulated by the 12 alternative sigma factor subunits of RNA polymerase, previously identified in the genome, which belong to groups 1-4. Anti-sigma factors are hypothesized to sequester these alternative sigma factors until stress induces their release, but little is known about these specific interactions. Seven putative anti-sigma factors were identified in Nostoc punctiforme by sequence homology. We screened for the interaction of all sigma factors with these putative anti-sigma factors using a bacterial 2-hybrid assay and found 23 interactions. These interactions were then quantified by using β-galactosidase assays and 12 of the interactions were shown to have significant β-galactosidase activity. 2 of the 12 interactions with the highest β-galactosidase activity have been confirmed using GST-pulldown assays and Western Blotting, and 5 have yet to be confirmed. These data support the conclusion that multiple anti-sigma factors function in Nostoc punctiforme to regulate gene transcription. A putative anti-sigma factor antagonist belonging to the STAS (sulphate transporter and anti-sigma factor antagonist) superfamily has also since been identified using sequence homology. We hypothesize that the anti-sigma factor antagonist will interact with the putative anti-sigma factors. We plan to screen these interactions using bacterial 2-hybrid assays, quantify these interactions using β-galactosidase assays, and confirm them using GST-pulldown assays and Western Blotting. Larger implications of these findings will help our understanding of complex regulatory events involved in cellular differentiation and adaptation to stress in cyanobacteria and eventually increase our understanding of sigma/anti-sigma interactions and anti-sigma/anti-sigma factor antagonist interactions in all bacteria.

Author Disclosure Block:

A. Amir Ali: None.

Poster Board Number:

SATURDAY-783

Publishing Title:

Fitness Cost Associated With The Loss Of Major Porins In *klebsiella Pneumoniae*

Author Block:

A. Fajardo-Lubian, A. Agyekum, J. Iredell; The Westmead Inst. for Med. Res., the Univ. of Sydney and Westmead Hosp., Westmead, Australia

Abstract Body:

It has been suggested that the loss of certain porins could significantly affect the bacterial fitness and the susceptibility to antibiotics. Porins form water-filled channels that allow the passive transport of small molecules across the bacterial membrane, a vital process to cell survival. *Klebsiella pneumoniae* encodes two major porins, OmpK35 and OmpK36. In order to fully understand the role of porins in bacterial adaptation and antibiotic resistance, mutants in five porins (OmpK35, OmpK36, OmpK37, OmpK26 and PhoE) of *K. pneumoniae* wild-type strain ATCC 13883 were obtained by complete deletion or interruption of the gene. Antibiotic resistance tests and competition experiments were performed to evaluate the consequences of the loss of porins for antimicrobial susceptibility and bacterial fitness. The levels of expression of porins were measured in *K. pneumoniae* mutants by real time RT-PCR and compared with those in the wild type strain. Our data reveal that the loss of OmpK36 affects the metabolic fitness. The adaptation of the bacteria to external conditions in the absence of its major porins is a complex process that includes the up-regulation and down-regulation of "secondary" porins (*ompK37, ompK26, phoE* and l*amB*) depending on the environmental circumstances and bacterial growth phase.

Author Disclosure Block:

A. Fajardo-Lubian: None. **A. Agyekum:** None. **J. Iredell:** None.

Poster Board Number:

SATURDAY-784

Publishing Title:

Integrating Microbial Genome Annotation Research into the Undergraduate Biology Curriculum

Author Block:

K. Moitra; Trinity Washington Univ., Washington DC, DC

Abstract Body:

Background: The process of linking biological information to gene sequences is called genome annotation. It involves annotating predicted genes and connecting these genes to protein functions. The integration of microbial genomics research into the undergraduate biology curriculum provides students with the opportunity to become familiar with state-of-the-art bioinformatics tools and answer original research questions. Our central hypothesis is that by upscaling and integrating undergraduate research within the classroom experience we would enable larger numbers of students to have access to authentic research, which would, in turn have a high impact on student engagement, learning outcomes and student interest in pursuing future scientific research careers. **Methods:** Students in Introductory Genetics classes and Cell Biology classes over a period of 3 years annotated over 60 predicted ABC (ATP Binding Cassette) genes of 2 microbes: *Methanothermobacter thermautotrophicus* and *Methanobacterium sp. SWAN-1* utilizing the Genomics National Initiative- Annotation Collaboration Toolkit (GENI-ACT) developed by the Joint Genome Institute (JGI) to annotate microbial genomes. A number of pre-course and post-course tests and surveys were conducted to assess if the project engaged students, impacted student learning outcomes, and generated interest in scientific research. **Results:** Our results suggest that the project had a high impact in all three targeted areas. The post-course student surveys indicated that 93% of students acknowledged that genome annotation was a very positive and engaging experience. We also discovered from directed testing and grading of the gene annotations that the students exhibited a tremendous gain in knowledge of genome annotation and research skills over the semester. Finally, 81% of students agreed that they had more interest in pursuing an advanced degree/following a scientific research career, based on their experience with the genome annotation project. **Conclusions:** This suggests that the project has fulfilled its desired outcomes and can be instrumental in engaging students in undergraduate research and in encouraging them to pursue future scientific research careers.

Author Disclosure Block:

K. Moitra: None.

Poster Board Number:

SATURDAY-785

Publishing Title:

Using the Sea-Phages Program to Integrate Undergraduate Research Experience in Freshman Science Lab Courses

Author Block:

H-M. Chung; Univ. of West Florida, Pensacola, FL

Abstract Body:

Background: The SEA-PHAGES (Science Education Alliance-Phage Hunters Advancing Genomics and Evolutionary Science) program has been implemented nationwide to promote course-based undergraduate research experience. It is jointly administered by Graham Hatfull's group at the University of Pittsburgh and the Howard Hughes Medical Institute's Science Education division. The prototype of the program is a two-semester research course that begins with students digging in the soil to isolate, purify new phages and follows with sequencing the genome of newly purified phage genome and genome annotation. In the past nine years, the program has successfully increased undergraduate interest and retention in the biological sciences through the courses mostly offered by the Biology department or Honors programs in participating schools. UWF joined the SEA-PHAGES Program in 2015; we aim to engage students' interest in biological science with an inter-discipline approach that enables students to truly appreciate chemistry in biological science. **Methods:** With this in mind, we offered the SEA-PHAGES research project by integrating General Chemistry I - and II - and Biology I - and II laboratory courses in fall 2015 and spring 2016. Our goals are to identify novel phage(s) and annotate their genomes in the Bio I and II lab sessions in fall 2015 and spring 2016 semesters, and to explore experiments (and projects) of general chemistry that can be directly linked to microbiology, genetics, genomics and ecology through General Chemistry I and II lab activities in fall 2015 and spring 2016, and "Exploring Santa Rosa Island" projects occurring in Bio II lab session in spring 2016. **Results:** We will present the newly identified phages: Slimphazie and DarthP, novel elements of our course design, hurdles encountered when integrating biology and chemistry lab activities, as well as feedback from students in regard to the good and bad of the program. **Conclusions:** The findings of our endeavor will support the course-based undergraduate research model as the more effective platform for success in introductory biology.

Author Disclosure Block:

H. Chung: None.

Poster Board Number:

SATURDAY-786

Publishing Title:

Introducing a Mini-Course-Based Undergraduate Research Experience into a Microbiology Laboratory in Order to Identify Plants with Antimicrobial Properties and Help Students Identify as Scientists

Author Block:

P. J. Baynham; St. Edward's Univ., Austin, TX

Abstract Body:

The US faces a growing need for diverse Science Technology Engineering and Math (STEM) professionals with a shrinking pipeline for such individuals. A current threat in healthcare in the US and abroad is the growing resistance of bacteria to current antibiotics, with associated infections sickening 2 million people, and killing more than 23,000 people in the US annually. To address both of these situations, an abbreviated course-based undergraduate research experience (mini-CURE) was introduced into a junior level undergraduate microbiology lab required for biology and biochemistry majors at St. Edward's University (SEU) in spring 2015 (n=32). Over half the students in the sciences at SEU are from groups under-represented in STEM disciplines and 35% are Pell eligible. After gaining basic skills, students analyzed plant extracts from various countries obtained from the National Cancer Institute for antimicrobial properties using a Kirby-Bauer disk diffusion assay for three weeks. Students examined the literature to determine their solvent of choice and which bacterium they would test for inhibition of growth and/or quorum sensing. As they tested their samples they maintained an electronic laboratory notebook on Blackboard. The impact of this project on students was analyzed using David Loppato's pre and post CURE surveys (2008). Of 352 extracts tested 21 showed growth inhibition. The promising extracts will be further analyzed by future students. According to the surveys my students had large gains in "understanding how scientists work," "skill in interpretation of results" and "tolerance for obstacles faced." In the overall assessment of the course students indicated that the course was a good way to learn about the subject, about the process of scientific research and that it had a positive effect on their interest in science. Anecdotally, students seemed more engaged in the activities associated with the mini-CURE and raved about these in course evaluations compared with other lab assignments. These data suggest that this mini-CURE was successful in helping students learn, fostering their confidence while helping them to identity as scientists and in finding promising samples for further work. In spring 2016, this project is being expanded to include the entire semester.

Author Disclosure Block:

P.J. Baynham: None.

Poster Board Number:

SATURDAY-787

Publishing Title:

Genomic and Phenotypic Characterization of *Epilithonimonas diehli* Sp. Nov., Isolated from a Freshwater Creek

Author Block:

K. T. Jacobs; Lycoming Coll., Williamsport, PA

Abstract Body:

A Gram-Staining-Negative, yellow-orange pigmented bacterial strain, designated FH1 was isolated from Fox Hollow in Williamsport, PA during an undergraduate microbiology course. The 16S rRNA sequences of strain FH1 was most similar to that of *Epilithonimonas lactis* (98.1%) and *Epilithonimonas ginsengisoli* (97.1%) in the family *Flavobacteriaceae*. The genomes of FH1 and *E. lactis* were sequenced, assembled, annotated and compared to each other and the type species of the genus, *E. tenax*. The estimated DNA-DNA hybridization value between FH1 and *E. lactis* was 33.1 as calculated by the DSMZ Genome-Genome Distance Calculator. The Average Nucleotide Identity (ANI) was 85.6. Both values are well below the threshold for separate species. Comparison of annotated gene sets identified. The 4.0 Mbp genome of FH1 contained 632 genes not found in either of the other *Epilithonimonas* sequenced genomes. Phenotypic comparisons identified a several distinguishing characteristics that support the hypothesis that isolate FH1 represents a novel species in the genus *Epilithonimonas* for which the name *Epilithonimonas diehli* sp. nov. is proposed.

Author Disclosure Block:

K.T. Jacobs: None.

Poster Board Number:

SATURDAY-788

Publishing Title:

Cloning Of Unique β-Lactamases from *Elizabethkingia miricola* and *Elizabethkingia anopheles, E*xperiments in a Freshman Course

Author Block:

J. A. Matts, P. J. Canaan, **N. J. Torres**, J. E. Gustafson; Oklahoma State Univ., Stillwater, OK

Abstract Body:

The genus *Elizabethkingia* contains emerging human pathogens that express multiple antimicrobial resistance and are linked to water source outbreaks in hospitals. Modern genomics in combination with traditional approaches has demonstrated that there are at least five unique species of *Elizabethkingia*. The analysis of *Elizabethkingia* genomes also indicates these organisms harbor greater than 10 chromosomally-located β-lactamases genes, which makes treating *Elizabethkingia* infections with -lactams impossible. To date, only 2 unique metallo-β-lactamases and an extended-spectrum β-lactamase have been cloned from *Elizabethkingia meningoseptica* and characterized. We now report the cloning of additional *Elizabethkingia miricola* and *Elizabethkingia anopheles* β-lactamases which was carried out to provide a course-embedded research experience to freshman biochemistry students. Initially, amplicons of the putative β-lactamase genes were generated from genomic DNA with primers with *BamHI* (5') and *SacI* (3') restriction sites. These amplicons were then cloned into pSKB3 and transformed into *Escherichia coli* DH5α. Following sequence confirmation, the plasmids were then transformed into *E. coli* BL21 to characterize β-lactamase activity following induction with IPTG. For a positive control, the previously characterized β-lactamase BlaB3 was cloned from *E. meningoseptica*. As expected, BlaB3 overexpression in *E. coli* led to the hydrolysis of nitrocefin, a chromogenic β-lactamase substrate. Out of 15 putative serine- and metallo-β-lactamases examined, 7 hydrolyzed nitrocefin similar to BlaB3 (in 30 min) and 4 demonstrated slight nitrocefin hydrolysis (in 45 min). We have therefore identified 6 functional β-lactamases in *E. miricola* and 5 in *E. anopheles*. In addition, we have identified 3 β-lactamase genes identified by gene annotation in *E. anopheles* and 1 in *E. miricola* that do not express β-lactamase activity in a heterologous system. Furthermore, we provide evidence that this course embedded research was met with great enthusiasm by the students in our class.

Author Disclosure Block:

J.A. Matts: None. **P.J. Canaan:** None. **N.J. Torres:** None. **J.E. Gustafson:** None.

Poster Board Number:

SATURDAY-789

Publishing Title:

A Student-Led Investigation Exploring Bacterial Presence in Water Distribution Systems to Teach High School Microbiology Students About Scientific Writing and the Peer-Review Publication Process

Author Block:

K. Gonzalez; Lowell High Sch., Lowell, MA

Abstract Body:

Inquiry based learning allows students to develop knowledge of scientific concepts and ideas by allowing them to ask questions, seek answers, collect and analyze data, and communicate and defend their findings using evidence. An effective way to demonstrate learning gained from inquiry based science is to have the students conduct independent (although guided) research, and develop a manuscript of their work for publication.Microbiology students explored whether or not bacteria were present in different types of water distribution systems at our high school. Water samples (9-12) were collected from the three different water distribution systems on different days. One milliliter of each sample was pipetted onto nutrient agar and incubated at 35^{O}C for 24 hours. Colonies were counted after 24 hours to calculate Colony Forming Units (CFUs)/mL. A statistical difference among the mean CFUs/mL of the three water distribution systems was observed (p<0.021; Kruskal-Wallis ANOVA). These findings indicate that bacteria remain in drinking water after purification, however, more elaborate purification mechanisms appear to improve upon the removal of bacteria from the drinking water supply. Students divided the portions of the paper they would write based on the journal specifications. The lead author assembled and finalized the document for submission. The manuscript was submitted to the Journal of Emerging Investigators, a high school peer-reviewed science journal, in December 2015. This process has led to a self-reported increase in confidence performing scientific research, as well as a developing identity as a scientist. This experience has also led to two additional investigations being pursued. The students are currently determining whether or not yogurt affects the numbers of *Bacteroides thetaiotaomicron* in culture, and whether or not a nutritional supplement containing honey and ginger is an effective antimicrobial agent against *Escherichia coli* and *Staphylococcus epidermidis.* This research opportunity will continue to be offered to the microbiology students and may be incorporated into the core biology and microbiology curriculum at our school.

Author Disclosure Block:

K. Gonzalez: None.

Poster Board Number:

SATURDAY-790

Publishing Title:

Personal Microbiome Portraits: Using Next-Generation Sequencing for Inquiry-Based Learning

Author Block:

M. Hartman, H. DeBaets, K. Harrington, M. Fierman, D. Slonim, D. R. Walt; Tufts Univ., Medford, MA

Abstract Body:

Microbiomes are an excellent entry point to engage K-12 students in the life sciences and introduce a variety of subjects including microbiology, human health, and ecological communities. Recently, the study of microbiomes has been revolutionized by developments in next-generation sequencing (NGS) technology. Despite the relative simplicity and low cost offered by NGS, experience with microbiomes remains out of reach for most high school students due to the limited availability of specialized equipment and knowledge. To bridge this access gap, we have developed an inquiry-based module that uses NGS to bring microbial ecology to high-school classrooms as part of our larger BioSeq project (http://ase.tufts.edu/chemistry/walt/sepa/index.html). Our classroom-based project, the "Microbiome Portrait Experiment", emphasizes the personal aspects of the human microbiome. In this module, students sample microbial communities on their hard palate and retroauricular crease (behind the ear) and ask the basic research question: "will the microbiome from one site on my body be more similar to the microbiome on the other part of my body, or will it be more similar to the microbiome on the same body part of another person?" The specific result of this experiment is not pre-determined and will vary between students and classrooms, representing a more realistic scientific research experience. We have run the Microbiome Portrait experiment in seven local high school classrooms and reached over 100 students. Students were tested pre- and post-intervention, and results indicated consistent knowledge gains in the areas of genetics, bioinformatics, and laboratory protocols. Based on our assessment outcomes, we conclude that the Microbiome Portrait Experiment has been very successful in engaging high school students and demonstrating the personal relevance of science. As our program expands to more schools, we plan to continue refining our methods to address a broader range of research questions.

Author Disclosure Block:

M. Hartman: None. **H. DeBaets:** None. **K. Harrington:** None. **M. Fierman:** None. **D. Slonim:** None. **D.R. Walt:** A. Board Member; Self; Illumina. K. Shareholder (excluding diversitied mutual funds); Self; Illumina.

Poster Board Number:

SATURDAY-791

Publishing Title:

Public Health's Pit Crew: A Look Inside the Indiana State Public Health Laboratory

Author Block:

S. Matheson, J. Madlem, M. Glazier; Indiana State Dept. of Hlth.Lab., Indianapolis, IN

Abstract Body:

Laboratory science is a fundamental component of the public health infrastructure and is critical in responding to novel strains of disease, chemical spills, and other health emergencies. However, it is facing a serious workforce shortage. One major cause for this manpower crisis is laboratory science is a “faceless” profession and is invisible to the general public. In an attempt to showcase the Indiana public health laboratory and expose middle school students and their parents to professions available in public health laboratory science, the Indiana State Department of Health (ISDH) Laboratory kicked off the 2015 Association of Public Health Laboratories (APHL) annual meeting with an open house student event targeting grades 5-8 on May 17, 2015.The ISDH Laboratory student event in Indianapolis, entitled “Public Health’s Pit Crew”, was planned by the ISDH Laboratory and APHL. The event was marketed through local middle schools, ISDH, and through the Indiana Laboratory System database. Student activities ranged from solving an outbreak with staff laboratorians to performing Pulsed-Field Gel Electrophoresis. Students met members of the local 53rd Civil Support Team and toured their mobile laboratory to learn how they respond to biological and chemical incidents. Parents were invited to the event and were given a tour of ISDH Laboratories. They watched their children participate in various laboratory activities and viewed several public health documentaries. They were also introduced to Jerome Adams, MD, the ISDH Health Commissioner.Approximately, 40 middle school students attended “Public Health’s Pit Crew”, along with 30 parents. Feedback from attendees was positive, students displayed genuine interest in laboratory science, and requests for future events were received.Public health laboratory science protects our lives and must become visible and apparent to our youth. The extent to which public health laboratories can continue to make improvements in public health greatly depends on assuring a sufficient and competent workforce. “Public Health’s Pit Crew” was extremely successful, and the ISDH Laboratory will continue its outreach efforts to address the rapidly approaching workforce shortage in public health laboratory science. Future student events and open houses will be planned in Indiana.

Author Disclosure Block:

S. Matheson: None. **J. Madlem:** None. **M. Glazier:** None.

Poster Board Number:

SATURDAY-792

Publishing Title:

A Microbiological Platform for Investigation

Author Block:

J. Redfern, J. Verran; Manchester Metropolitan Univ., Manchester, United Kingdom

Abstract Body:

The Museum of Science and Industry (http://msimanchester.org.uk/) located in Manchester, UK hosts a contemporary science programme called Pi: Platform for Investigation. The platform provides a space for scientists to demonstrate their work and research to members of the public. The event typically attracts over 400 visitors, mainly families with children between 7 and 14 years of age attend on a single Saturday once a month.Microbiology researchers from Manchester Metropolitan University have delivered three Pi events in recent years; The Very Small World of Viruses (2012), Fuelling the future (2015) and Antimicrobial resistance (AMR, 2016). Each activity was designed to discuss and convey specific microbiological messages that were relevant to people's lives, and incorporated a range of hands-on activities designed to engage families. The Very Small World of Viruses aimed to convey three messages: the nature and size of viruses; treatment and antibiotics stewardship; and prevention of viral infection. Activities included drawing, microscopy, making models of viruses with clay to scale in comparison with a large cell floor covering, and the creation of a virus Christmas decoration (the event was held on World AIDS Day). Fuelling the Future focused on the importance and prevalence of fermented foods, touching on the gut microbiome and probiotics. Activities included an introductory guesstimate exercise on the percentage of 'good' bacteria; a ''Myths or Medicine' board, videos, a display of world fermented food (including foods to sample), and a map, encouraging additional contributions. The 2016 AMR event conveyed messages about bacterial infection, treatment (antibiotics), the origin of antibiotics, and antibiotic stewardship. This event was based on the Yale University Small Worlds Initiative, in collaboration with the UK Microbiology Society. Participants plated out soil they bought from home. A follow-up viewing and interpretation session was carried out at the University microbiology lab. Discussion with scientists was embedded in every activity. Each event was evaluated using a range of methods (counting outputs, ad-hoc conversations, written feedback). Each event was well attended and very successful, and evaluation strategy enabled better analysis and interpretation of success, as well as indicating strategies for future events.

Author Disclosure Block:

J. Redfern: None. **J. Verran:** None.

Poster Board Number:

SATURDAY-793

Publishing Title:

Teaching Microbiology Applications and Poster Presentations in Environmental Week Event

Author Block:

A. M. N. KORRES, F. B. Aponilário, J. R. Bringhenti; Inst. Federal de Educação, Ciência e Tecnologia do Espírito Santo, Vitoria, Brazil

Abstract Body:

This work reports an activity performed with 17 Environmental and Sanitary Engineering undergraduate students during Applied Microbiology discipline classes. Students were challenged to develop a theme in Microbiology associated with environmental and, or sanitary application to expose during the 2015 Environment Week of Campus Vitória, held between days 2 and 4 July. The event focused on "Four thousand dreams. One School. Consume with care", in reference to the United Nations campaign. Themes developed were biodigestor and biogas production, aseptic techniques, and virus in biological control. Themes were discussed for four weeks during and out of the classes. Students prepared posters, flies and other visual and printed materials to present during the technique-scientific exposition of Environment Week event in the Institution. Projects were followed by the teachers since early preparation to the presentation. One group prepared a plastic 20 L bioreactor to produce biogas using organic wastes 10 days before the presentation and a miniature of a bacteria to explain the exposition visitors the importance of this group of microorganism in the process. Other group prepared informative material about microorganism control in food and aseptic techniques to distribute among the show attendees and also provided a public utility service. The other group prepared a poster to explain an example of the use of virus in biological control of caterpillars *Spodoptera frugiperda* with Baculovirus, what can avoid or reduce the use of chemical control. The exposition occurred from 9 am to 7 pm during the poster show of the Environment Week of Ifes. Considering the importance of the issues studied, the involvement and dedication of students in the activity and the contribution of this participation to the professional life, the teachers concluded that the proposal of challenges that lead students to actively take part in the learning process should be valued and encouraged in the Engineering teaching. In addition, teachers considered the methodology used easy to replicate and enable the students a practical experience.

Author Disclosure Block:

A.M.N. Korres: None. **F.B. Aponilário:** None. **J.R. Bringhenti:** None.

Poster Board Number:

SATURDAY-794

Publishing Title:

Integrating a Unique Microbiology Educational Platform Highlighting Interactive Puppets: "The Culture Plate Gang"

Author Block:

J. G. Thomas; Allegheny Hlth.Network, Pittsburgh, PA

Abstract Body:

Background: The challenges continue in educating various audiences, magnified in clinical microbiology. In the past 51 years, we have integrated a variety of novel teaching techniques, initially focusing on post graduate education in medicine/dentistry. Here, we describe our most recent platform highlighting the use of 7 topic-selected 36-44 inch puppets addressing microbial phenotype, antibiotic resistance, probiotics, infection prevention, and history. **Methods:**Seven microbial/ infectious disease topics with matching puppet names were selected: first, by academic coursework at WVU Schools of Medicine/Dentistry; second, by invitation to ancillary medical/dental CE programs; third, public health interest including civil service organizations; and fourth, political and religious societies. Each topic was linked to 4-6 lectures depending on depth and breadth, emphasizing appropriate additional platform tools including: 3-D PowerPoints/ 3-D glasses, motion, sound, and 5 minute visual introduction based on author international travels, highlighted by representative clothing.**Results:** The seven topics with matching puppets included: 1) Biofilm Bradford, 2) Planktonic Phylis, 3) Antibiotic Resistant Rachel and CAT scan, 4) Probiotic Buster, 5) Infection Control Claire, 6) Candy Candida, and 7) Historical Louis. They were referred to as the "Culture Plate Gang"; only Candy Candida (Number 6) was species specific highlighting our VAP research and most recently Historic Louis (Number 7), a French speaking microbial personality . One hour lectures were best suited to 1/ 2 puppets, while 2/3 hour CE courses could accommodate 4-6. Most gratifying was growth in infection prevention and use in 'Ronald McDonald House-like' residences, or ICU's with adolescent patients, explaining cleanliness; Most surprising was growth in civil service, adult education and political meetings . To date, greater than 200 lectures have been evaluated. Success has been marked by a variety of significant awards received from universities, national and international organizations. **Conclusions:**Today's society is driven by visual and entertaining electronics; we harnessed this approach by incorporating standing puppets, matching infectious disease/microbial topics and lectures, unmasking a broad interest in microbiology, drawing parallels between human behavior and microbes.

Author Disclosure Block:

J.G. Thomas: None.

Poster Board Number:

SATURDAY-795

Publishing Title:

Testing an Application Based Service Learning Pedagogy

Author Block:

M. G. ANDERSON[1], J. M. Engle[1], R. Lamendella[2], J. M. Senko[3], G. E. Rowe[4], S. Grubb[5], S. K. Woodley[6], N. Trun[6]; [1]Mount Aloysius Coll., Cresson, PA, [2]Juniata Coll., Huntingdon, PA, [3]Univ. of Akron, Akron, OH, [4]La Roche Coll., Pittsburgh, PA, [5]Univ. of Pittsburgh, Pittsburgh, PA, [6]Duquesne Univ., Pittsburgh, PA

Abstract Body:

Background: Eight institutions have collaborated over three years to implement Application Based Service Learning (ABSL), a pedagogical approach engaging students in responding to community concerns. Disciplines include microbiology, geochemistry, physiology, chemistry, architecture, and communication.**Methods:** ABSL emphasizes five practices documented as having a high impact on student learning: learning communities, writing-intensive courses, collaborative projects, undergraduate research, and service learning. These practices are incorporated into courses in which students address a community-based problem, such as the possibility that feral cats carry pathogenic microbes, or that areas impacted by mining or manufacturing practices might have altered water quality or issues associated with their redevelopment for other uses. As part of their course work, students collect and analyze samples, and their findings are shared not only within the class but with other ABSL classes and the community at large.**Results and Conclusions:**Three years of assessment data using the Classroom Undergraduate Research Experience Survey (CURE), Student Assessment of Learning Gains (SALG), and the Critical Thinking Assessment Test (CAT) will be reported, indicating gains in scientific understanding and communication, gains in student learning and attitudes, improved technical skills, and increased motivation to work. Additionally, there was an increase in the quantity and quality of undergraduate research and honors theses completed.

Author Disclosure Block:

M.G. Anderson: None. **J.M. Engle:** None. **R. Lamendella:** None. **J.M. Senko:** None. **G.E. Rowe:** None. **S. Grubb:** None. **S.K. Woodley:** None. **N. Trun:** None.

Poster Board Number:

SATURDAY-796

Publishing Title:

Linking a Capstone Project in Microbial Physiology with Service Learning at Secondary School

Author Block:

C. Rios-Velazquez[1], B. Cabrera[2]; [1]Univ. of Puerto Rico at Mayaguez, Mayaguez, PR, [2]Residential Ctr. of Educative Opportunities of Mayagüez (CROEM), Mayaguez, PR

Abstract Body:

There are diverse emerging challenges for the students' transition processes from school to an academic community, especially the importance of team work and service learning. Also, there are opportunities for faculty and teachers to team and collaborate to guide these processes. A special capstone project (CP) was assigned to students in the Microbial Physiology class, to combine and link as a team, the knowledge acquired in class with service learning by developing microbial models as pedagogical instruments for secondary school. After choosing the school, the university professor and the science school teacher chose Microbial Physiology Structure and Morphology as the CP topic. A total of five teams of 5-6 students were organized and a month given for the task completion. The models prepared included: a gram-negative (GN), gram-positive (GP), Mycobacteria (MY), an Archaea (AR), and a fungus (FU). A rubric was designed to evaluate the projects in general terms, creativity, clarity, pedagogical functionality, and overall evaluation. The teams prepared an index card summarizing the model explanation and described it orally to the evaluators, which included the class professor, school teacher, class cohort, and visitors. A teams-self and project evaluation was also done. The Residential Center of Educative Opportunities of Mayagüez (CROEM) was the chosen secondary school. Considering all the individuals evaluations, the project scores ranged from 84% (GP) - 100% (AR and FU). Once averaged, the models ranked as follow: AR (98%), FU (98%), GN (95%), MY (92%) and GP (91%). The percentages in the ranking coincided with teams-self-evaluations. By categories: the GN and AR were the more creative (96%); the FU showed more clarity (96%), while pedagogically, the GN was the best (98%). Finally, the GN was chosen as the best in general terms (98%). Some of the written comments focused on ways to improve the models (better ID, size, materials used), and oral presentations. The teacher, evidenced by an educational plan, the use of the models in the classroom. This activity represent a creative way to support collaboration among different educational centers and could be implemented in any class, once a link between the faculty-teacher is done and an agreement of the topics is established.

Author Disclosure Block:

C. Rios-Velazquez: None. **B. Cabrera:** None.

Poster Board Number:

SATURDAY-797

Publishing Title:

Virtual Virus, a Semester-Long Interdisciplinary Project on the Crossroads of Creativity and Knowledge Integration

Author Block:

B. MARINTCHEVA; Bridgewater State Univ., Bridgewater, MA

Abstract Body:

Virtual virus is a semester-long interdisciplinary project offered as a part of upper level elective course in Virology. Students are challenged to apply key concepts from multiple biological subdisciplines to "synthesize" a plausible virtual virus. The project is executed as a scaffolded series of hands-on sessions and mini-projects during which students built physical models of their viruses and developed scenarios for viral life cycles and virus-host interactions on the cellular and organismal level. The outcomes of individual projects were integrated into continuous story via mock conference presentation and comprehensive report modeling article publication. Instructor and peer feedback were used as tools to prompt reflection and guide revisions of the final report. Student learning gains and attitudes toward the approach were studied by evaluating project work product and end of the semester survey. Outcome analysis demonstrated that students exit the course with elaborated conceptual understanding of viruses and ownership of their work. The project can be viewed as an approach to model the process of scientific discovery in fast-forward mode by combining active learning, creativity and problem solving to assemble and communicate a virtual virus story.

Author Disclosure Block:

B. Marintcheva: None.

Poster Board Number:

SATURDAY-798

Publishing Title:

Improving Civic and Scientific Literacy through Student Reading with Scaffolded Thematic Classroom Research Experiences

Author Block:

D. S. SMYTH[1], J. But[2]; [1]Mercy Coll., Dobbs Ferry, NY, [2]New York City Coll. of Technology, Brooklyn, NY

Abstract Body:

Our assessments to date have shown that for many of students the major hurdle to learning basic scientific content is their lack of one basic skill - reading. This has led to the establishment of Reading Effectively Across the Disciplines (READ), aimed at improving reading skills through faculty training, peer led team learning, student training in strategic reading and dissemination of the materials via a website.At Mercy College, READ has been expanded to include an additional focus, the inclusion of research experiences to encourage civic and scientific literacy. Whereas most authentic research experiences target the laboratory, we have targeted the lecture component of two sequential courses, Introductory Biology, and Microbiology. To encourage reading, students were given textbook surveys, strategic reading assignments and instructed in concept mapping. The classroom experience included think-pair-share moments. Students in both classes were assigned teams and engaged in research of civic import in both classes. In biology, students were tasked with developing a poster, in an area of civic and scientific importance. The posters were printed at the end of the semester and displayed publically in the library. The microbiology students utilized social media to generate an ePortfolio of a microorganism to understand bacterial classification. The second microbiology project involved a presentation and pitch for grant funding from the NIH for an area of importance in microbiology chosen by the students.Through these literature-based projects, students were able to take their first steps towards understanding and immersing themselves in the scientific method using authentic student-driven discovery. Student feedback was positive as measured using student feedback forms. Students enjoyed participating in the activities. Many students found concept mapping to be the most useful reading technique. Our students were not familiar with the generation of posters or bibliographies prior to the biology experience. The ePortfolio and engaging atmosphere of the debate was found to be the most exciting aspect of the microbiology experience. This pilot has shown the feasibility of intervening in the lecture to enhance civic engagement and scientific literacy and exposing all students to research and the scientific method.

Author Disclosure Block:

D.S. Smyth: None. **J. But:** None.

Poster Board Number:

SATURDAY-799

Publishing Title:

Active Learning for Undergraduate Medical Students: A First Encounter with Evidence-Based Medicine in the Microbiology Laboratory

Author Block:

N. A. Castillo, O. Candolfi, A. Davila, L. Arzamendi; Univ. Autónoma de Baja California, Tijuana, Mexico

Abstract Body:

Active learning is essential in the training of medical students. In the microbiology laboratory at the Autonomous University of Baja California we use project-based learning so that students acquire skills in the diagnosis and treatment of infectious diseases applying clinical practice guidelines, laboratory diagnosis protocols and evidence-based medicine. This proposal aims to strengthen the understanding of microbiology and diagnostic techniques, develop critical thinking skills, cultivate students' interest in scientific research and encourage collaborative work by conducting a project for the resolution of a case of infectious disease and case report. Medical students (n=75) were organized in teams to resolve a case of an infectious disease. Prior to the biosafety training students obtained a specimen with the quality criteria. As a hypothesis, students established a differential diagnosis based on clinical practice guidelines and evidence-based medicine. They identified the causative organism and antimicrobial susceptibility test was performed referring laboratory diagnostic procedures. The results were discussed and a diagnosis with a proposed therapy was established. Information was integrated into a clinical case report. The case report and the student's perception were assessed with a questionnaire. Students apply evidence-based medicine and clinical practice guidelines to establish a diagnosis with the set of clinical variables. Questionnaire results show that most students know the application of evidence-based medicine, clinical practice guidelines and laboratory diagnosis procedures. The majority of students are motivated to pursue research and agree on the importance of knowing all the steps in the diagnosis of infectious disease. Through project-based learning, students build their knowledge by a specific task to generate products for learning, applying the scientific method and quantitative reasoning, they learn to communicate collaboratively and understand the importance of science in medical practice.

Author Disclosure Block:

N.A. Castillo: None. **O. Candolfi:** None. **A. Davila:** None. **L. Arzamendi:** None.

Poster Board Number:

SATURDAY-800

Publishing Title:

Modeling of Cancer System Pathogenesis with *H. pylori*: A Novel Approach

Author Block:

S. Perez; Coll. of Med.- Univ. of Arizona at Phoenix, Phoenix, AZ

Abstract Body:

Background: Modeling behaviors of gastric cancer system with a H.Pylori co-morbidity allows scientists to describe pathogenesis through the lens of a system approach. Microscopic natural systems in the human body such as cellular structures exhibit behaviors that show it has elements of a systems approach such as inputs, processes, output feedback that occur.**Methods:** Insightmaker.com is a simulation and modeling program used to create the stock and flow models. This system based approach allows a user to create primitives such as stock and variables and add connections using link and flow elements. A simulation run provides a visualization of the defined stock against a timeline on a dynamic graph.**Results:** A generalized pathogenesis model was first generated in insightmaker.com. An organ specific model was also created to model how cancer cells interact with the different organs and cells in the human body. The organ specific model will help the scientist determine if new chemical pathways and relationships can be discovered to help explain organ specific cancer pathogenesis.**Conclusion:** The successful implementation of a system approach is the packaging of the instructional activity and its supporting content in bite size and incremental approach and explicit description of the activity's deliverables. The drag and drop interface and intuitive icon driven menu of Insightmaker is ideal for lowering the learning curve for a systems thinking instructional activity. The initial instructional activity was designed with an easy to follow demo of an actual working systems model in systems biology or population ecology. This allows for the content to be familiar to the student. A team of 4 students constructed a systems model of a portion of the cancer pathogenesis cycle such as tumor formation, tumor metastasis or tumor aptosis. An evaluation rubric was used to guide the development of the group created student model with evaluation categories such as narrative of the system model, attributes of the system, data flow and values used and a personal reflection of their experience.

Author Disclosure Block:

S. Perez: None.

Poster Board Number:

SATURDAY-801

Publishing Title:

Virtual Biotech: The Meat in the Sandwich?

Author Block:

D. Cameron, F. Holmwood, A. McElroy, **C. Craig**; The Res. Network Ltd, Sandwich, United Kingdom

Abstract Body:

There is an urgent clinical need to discover and develop novel approaches to treat infectious diseases (IDs). Innovative target validation and early drug discovery is increasingly available from academic institutions. Pharma companies can complete development, manufacture and distribution of therapies, but are primarily interested in external projects only after demonstration of clinical safety and efficacy. Optimized management of early stage discovery through to successful uptake by Pharma is key to efficient prosecution of new treatments. Networks of Pharma experienced scientists, united through flexible virtual biotech entities (VBs), can enable projects to bridge this gap with collaborations to gain access to grant and investment resources and through VB based project management of the lead generation and optimization, translational activities and early clinical trials.University and research institute business development departments publish projects seeking external 'bench to bedside' support. To quantify, qualify and identify those ID projects that would benefit most from VB support, a systematic regional survey of 1272 projects was carried out. Of these projects 154 targeted IDs (non-exclusive: 61 antibacterial; 73 antiviral; 20 anti-parasite, 5 antifungal). Additional major therapeutic areas represented included oncology (328), cardiovascular (210) and CNS (170) with 410 others. Antibacterial and antiviral approaches included respectively small molecule (31 and 28) vaccine (8 and 11) and other platform or diagnostic approaches (30 and 34). Antiviral approaches included HIV (n=24), with 2 specifically looking at HIV cure, influenza virus (n=8) HCV (n=6) and other viruses (n=14). Pan-antiviral approaches including immunomodulation were also common (n=21). Triage was performed and ID projects meeting criteria of high clinical need with available expertise and resource have become substrate for VBs and the establishment of consortia to facilitate further project development.VB companies providing Pharma based expertise can enable progression of discovery and development projects to attain Pharma support to enter the clinic. This form of bridging can effectively and efficiently provide the required expertise for managing and progressing scientific projects suitable for clinical development and treatment of patients.

Author Disclosure Block:

D. Cameron: None. **F. Holmwood:** None. **A. McElroy:** None. **C. Craig:** None.

Poster Board Number:

SATURDAY-802

Publishing Title:

Cost Analysis of Implementing Maldi-Tof with Real-Time Antimicrobial Stewardship Intervention for Bloodstream Infections

Author Block:

R. Kaakeh, T. Patel, **J. Nagel**, D. Newton, J. Stevenson; Univ. of Michigan, Ann Arbor, MI

Abstract Body:

Background: Studies evaluating rapid diagnostic testing with stewardship intervention have consistently demonstrated improved clinical outcomes for patients with bloodstream infections. However, the cost of implementing new rapid diagnostic testing can be significant, and usually does not generate additional revenue. There is minimal data evaluating the financial impact on total hospital costs following implementation of MALDI-TOF for rapid organism identification and allocating antimicrobial stewardship pharmacy resources. **Methods:** A cost analysis was performed utilizing patient data generated from the hospital cost accounting system, and factoring in additional costs of MALDI-TOF equipment, supplies and personnel, and the cost associated with allocating pharmacist time for blood culture review and antimicrobial therapy intervention. The cost analysis was performed from a hospital perspective for 3-month blocks before and after implementation of MALDI-TOF plus stewardship intervention. **Results:** A total of 480 patients with blood stream infections were included in the analysis: 247 in the pre-intervention group and 233 in the intervention group. Mortality was significantly improved in the intervention group (21% vs. 12%, $p<0.01$), and mean length of stay was numerically shorter (13 ± 16.5 vs. 12 ± 10.4 days). The total hospital cost per bloodstream infection was lower in the intervention group ($45,018 vs. $42,580). Intensive care unit costs accounted for the largest share of the total costs in each group and were also lower in the intervention group ($13,727 vs. $10,833). **Conclusion:** Implementing MALDI-TOF with real-time stewardship review and intervention decreased mortality for patients with bloodstream infections. Despite the additional costs of implementing MALDI-TOF and dedicating additional personnel time, the total hospital costs decreased by $2,438 per bloodstream infection, for an approximate annual cost savings of $2.34 million.

Author Disclosure Block:

R. Kaakeh: None. **T. Patel:** None. **J. Nagel:** None. **D. Newton:** None. **J. Stevenson:** None.

Poster Board Number:

SUNDAY-001

Publishing Title:

Microbial Community Response and Crude Oil Biodegradation in Different Deep Oceans

Author Block:

J. Liu[1], J. Fortney[1], S. Techtmann[2], D. Dominique[1], T. Hazen[1]; [1]Univ. of Tennessee, Knoxville, Knoxville, TN, [2]Michigan Technological Univ., Houghton, MI

Abstract Body:

Many studies have shown that microbial communities can play an important role in oil spill clean up. However, very limited information is available on the oil degradation potential and microbial community response to crude oil contamination in deep oceans. Therefore, we investigated the response of microbial communities to crude oil in various deep-sea basins from around the world where oil exploration is anticipated (Eastern and Central Mediterranean Sea, Great Australian Bight and Caspian Sea). In this study, microcosms were set up aerobically with three different treatments: seawater, seawater + oil and seawater + oil + oil dispersant. Samples were taken at three time points for the analysis of oil degradation by fluorescence and GC-MS, and microbial community changes by16S rRNA sequencing. CO_2 evolution followed a similar pattern among off the basins. The treatment of seawater + oil + dispersant had the highest CO_2 production. The amendment of oil lead to a more CO_2 accumulation than seawater treatment. However, it's much higher in the Gulf of Mexico (GOM) than other oceans. What's more, the dissolved organic mater analysis revealed that application of oil dispersant lead to better oil degradation, which was consistent with the GC-MS results. Oil biodegradation appears to occur rapidly in all of the sites. In addition, there was a clear succession of microbial communities during degradation of oil. The microbial diversity decreased in all of the microcosms over time. Oil amendment affected how quickly the diversity decreased. The relative abundance of *Proteobacteria* increased drastically while the relative abundance of archaea decreased. In particular, the percentage of *Betaproteobacteria* increased in samples from the Central Mediterranean Sea. However, *Gammaproteobacteria* increased in abundance in the microcosms from the Eastern Mediterranean Sea and Great Australian Bight, which was very similar to GOM.

Author Disclosure Block:

J. Liu: None. **J. Fortney:** None. **S. Techtmann:** None. **D. Dominique:** None. **T. Hazen:** None.

Poster Board Number:

SUNDAY-002

Publishing Title:

Dimethylsulphoniopropionate Catabolism by Bacteria Isolated from the East China Sea

Author Block:

J. Liu[1], S-H. Zhang[1], J. Liu[1], H-H. Zhang[1], J. D. Todd[2], G-P. Yang[1], **X-H. Zhang**[1]; [1]Ocean Univ. of China, Qingdao, China, [2]Univ. of East Anglia, Norwich, United Kingdom

Abstract Body:

Marine bacteria play a central role in the production of the environmentally important gas dimethylsulfide (DMS) from dimethylsulfoniopropionate (DMSP), an abundant compatible solute made by many marine phytoplankton. Here bacterial communities from four seawater samples in the East China Sea (ECS) were characterized by both culture-dependent and molecular techniques. Of 91 marine bacterial isolates screened, 37 and 2, respectively, had the ability to catabolise DMSP generating DMS or methanthiol (MeSH), with only a few isolates able to use DMSP or acrylate as sole carbon source for growth. Many of DMS-producing bacteria (32.4%) were Roseobacters, some of which have not been reported to catabolise DMSP. Genes homologous to *dddD*, *dddL* and *dddP*, previously implicated in DMSP degradation, were also characterized from some of these strains. The existence of *ddd* gene(s) of one *Labrenzia* strain and one *Ahrensia* strain were confirmed by gene cloning and expressing. For the first time, some Gram-positive *Actinobacteria*, closely related to species in *Agrococcus*, *Brevibacterium*, *Kytococcus*, *Microbacterium*, *Micrococcus* and *Phycicoccus* genera were found to make DMS. These results highlight the phylogenetic diversity of various DMS-producing bacteria. 16S rRNA gene clone libraries indicated that uncultured bacteria affiliated to Roseobacter were predominant in the sampling sites with higher DMSP concentration, such as the coastal site and surface seawaters. Both of the Roseobacter bacterial abundance and the proportion of DMS-producing bacteria in all cultivated bacteria showed positive correlations with DMSP and DMS concentration, indicating that the concentration of DMSP in seawater is likely to play a role in structuring bacterial communities and also confirming that bacterial cleavage of DMSP is probable a major source of DMS in seawater.

Author Disclosure Block:

J. Liu: None. **S. Zhang:** None. **J. Liu:** None. **H. Zhang:** None. **J.D. Todd:** None. **G. Yang:** None. **X. Zhang:** None.

Poster Board Number:

SUNDAY-003

Publishing Title:

Seasonal Dynamics Of A Phototrophic Microbial Mat In An Intertidal Marine Environment

Author Block:

U. Lopez, G. Flores; California State Univ. Northridge, Northridge, CA

Abstract Body:

Photosynthetic microbial mats are complex, stratified microbial ecosystems that can be found in a variety of environments including intertidal regions of salt marshes. Generally, phototrophic organisms dominate the top layers, while diverse heterotrophic organisms can be found throughout a well-developed mat. This study focuses on how seasonally fluctuating environmental factors (salinity, temperature, and pH) influence the diversity and composition of an intertidal microbial mat located in Southern California, USA. Using high-throughput sequencing of the 16S rRNA gene and the ITS1 region of fungi, we characterized the microbial communities of the top (0 - 0.5cm) and bottom layers (0.5 - 1.0cm), and the underlying sediments of a mat system. To date, field measurements and microbial samples have been collected and analyzed over six seasons (summer 2014 & 2015, fall 2014 & 2015, winter 2015, and spring 2015). Over the observation period, salinity (3.7% - 5.9%), temperature (18.0°C - 30.4°C) and pH (7.8 - 8.4) were variable, revealing a dynamic environment influenced by seasonal changes in tide height, solar radiation, and precipitation. Microbial communities were compared for changes in phylogenetic diversity and in community composition. The top layer of the mat was composed primarily of oxygenic-photosynthetic Cyanobacteria, purple sulfur bacteria, and aerobic heterotrophs, but the relative abundance of each varied seasonally. In the bottom layers of the mat, the depletion of oxygen and filtering of light selected for anaerobic heterotrophs, primarily sulfate reducing bacteria. Fairly consistent conditions within underlying sediments maintained a relatively constant microbial community. Similarly, fungal communities throughout the layers of the mat varied seasonally in the abundance of each lineage. Ongoing seasonal characterization of this mat system will provide foundational data that will allow us to identify factors structuring this mat system and will ultimately lead to a better understanding of how photosynthetic microbial mats contribute to carbon cycling within salt marsh environments.

Author Disclosure Block:

U. Lopez: None. **G. Flores:** None.

Poster Board Number:

SUNDAY-004

Publishing Title:

Diversity of Methane Associated Microorganisms in the Columbia River Estuary

Author Block:

K. Jenkins[1], M. Kim[1], D. Fachko[1], T. Peterson[2], G. Nyerges[1]; [1]Pacific Univ., Forest Grove, OR, [2]Oregon Hlth.& Sci. Univ., Portland, OR

Abstract Body:

Methanogens and methanotrophs play crucial roles within the global methane cycle. Methanotrophic activity is the most effective biological methane sink in the environment, and methanogenic activity contributes significantly to atmospheric methane levels. The diversity of these microbes is well studied in many environments, however, estuarine methanogens and methanotrophs are not well characterized, even though estuaries contribute significantly to the global methane budget. This study investigates methanogen and methanotroph diversity of water and sediment from the Columbia River estuary (CRE). 14 water filter and 13 sediment samples collected from three locations throughout the CRE were subjected to PCR using gene-specific primers targeting the mcrA gene in methanogens and the pmoA gene in methanotrophs. PCR products were cloned and sequenced. Sequences were analyzed using NCBI BLAST, then trimmed, translated, and aligned using CLC Main Workbench. Neighbor-joining amino acid phylogenetic trees were created using Geneious. The mcrA tree, with reference sequences from a peat bog and M. kandleri as the outgroup shows clustering of half of our samples with the reference sequences. The tree reveals a wide diversity of the mcrA genes, with matches to sequences mainly from river sediment, rice paddy soil, and wetland sediment. The pmoA tree, with reference sequences from the Newport Bay estuary, CA and N. oceani as the outgroup shows one large clade with a bootstrap value of 97 containing 24 of our samples and sparse clustering with reference sequences. The tree indicates limited diversity of pmoA sequences with all but six matching to sequences from river and lake sediment, and remaining sequences matching to sludge, oil field soil, and a coal mine. The trees indicate little similarity between methanogens and methanotrophs from the CRE and the referenced locations. With low percent identity matches revealed by BLAST, one *pmoA* sequence and six of our *mcrA* sequences are likely novel. The majority of sequences showed similarity to sequences from freshwater environments, indicating that the methanogenic and methanotrophic communities in the CRE are dominated by freshwater representatives.

Author Disclosure Block:

K. Jenkins: None. **M. Kim:** None. **D. Fachko:** None. **T. Peterson:** None. **G. Nyerges:** None.

Poster Board Number:

SUNDAY-005

Publishing Title:

Investigating Microbial Carbon Cycling in Sediments Using Natural Isotope Respirometry in a Novel, Carbon-Free Bioreactor

Author Block:

N. Mahmoudi[1], S. Beaupre[2], A. Pearson[1]; [1]Harvard Univ., Cambridge, MA, [2]Stony Brook Univ., Stony Brook, NY

Abstract Body:

Aquatic sediments are carbon-rich environments that harbor complex and diverse microbial communities which mediate the rate and extent of organic matter degradation. In order to gain insight into microbial carbon utilization and carbon cycling, we constructed a bioreactor system to measure carbon isotopes during microbial degradation of complex organic matter. Using sediments collected from Salt Pond (Falmouth, MA), we observed a successive pattern of microbial respiration such that several peaks appear over a 7-day time-series experiment. $\Delta^{14}C$ signatures of CO_2 fractions collected during incubation indicated that the microbial community is primarily respiring labile organic matter from fast cycling pools. The observation of different rates of respiration - corresponding to multiple peaks of CO_2, each with different $\Delta^{14}C$ signatures - suggest that the community may be using different suites of carbon compounds in succession. High-throughput DNA sequencing was carried out on daily samples to determine if organic matter was degraded by a succession of taxa or by a single dominant group. Bacilli were found to be the dominant group observed throughout the experiment. Within Bacilli, Planococcaceae accounted for over 90% of assigned sequences during days 1 to 4, when the majority of total carbon was respired. This suggests that the observed CO_2 peaks on these days are a result of changes in enzymatic activities rather than changes in community structure. During days 5 to 7, there was a steady increase in sequences assigned to Panenibaillaceae, coinciding with prominent CO_2 peak observed at this time. Concurrent enzymatic analyses are being carried out to determine the metabolic pathways that correspond to each peak.

Author Disclosure Block:

N. Mahmoudi: None. **S. Beaupre:** None. **A. Pearson:** None.

Poster Board Number:

SUNDAY-006

Publishing Title:

Microbial Interactions with Natural Organic Matter Extracted from the Oak Ridge FRC

Author Block:

X. Wu[1], S. Jagadamma[2], T. C. Hazen[2], N. Justice[1], A. P. Arkin[1], S. Jenkins[1], T. R. Northen[1], M. W. Fields[3], P. Fox[1], P. Nico[1], R. Chakraborty[1]; [1]Lawrence Berkeley Natl. Lab., Berkeley, CA, [2]Oak Ridge Natl. Lab., Oak Ridge, TN, [3]Montana State Univ., Bozeman, MT

Abstract Body:

Natural organic matter (NOM) is central to microbial food webs; however, little is known about the interplay between the physical and chemical characteristics of NOM and its turnover by microbial communities. Microbial activity changes structure and properties that influence further bioavailability of NOM. To date, our understanding of these interactions is insufficient, and it is critical to identify the important role NOM characteristics play to the structure and composition of microbial communities and to the metabolic potential of that community. In this study, we aimed to study the interactions between microbial communities and native NOM in background well FW305 at Oak Ridge Field Research Center, Oak Ridge, TN. The NOM was obtained by extracting FW305 sediment samples using MilliQ-water via shaking and sonication. The total organic carbon and inorganic carbon in these sediment samples were 0.071% and 0.011%, respectively. The extraction efficiencies were 3.2% for organic carbon and 1.6% for inorganic carbon. Results from UV and FTIR tests showed that the extracted NOM mainly contained polysaccharides and there were very few aromatic and unsaturated compounds. The NOM was then provided as the carbon source for microbes from FW305 groundwater. Analysis of the enriched microbial community, and transformed NOM metabolites was carried out. 16S rRNA sequencing and metatranscriptomics were performed to identify the changes of microbial communities. Several fine-scale chemical techniques including FTIR, LC-TOF-MS, and Orbitrap were also used to characterize the metabolites.

Author Disclosure Block:

X. Wu: None. **S. Jagadamma:** None. **T.C. Hazen:** None. **N. Justice:** None. **A.P. Arkin:** None. **S. Jenkins:** None. **T.R. Northen:** None. **M.W. Fields:** None. **P. Fox:** None. **P. Nico:** None. **R. Chakraborty:** None.

Poster Board Number:

SUNDAY-007

Publishing Title:

Genomic Sequence of *Enterococcus* Phage KKAS003-1

Author Block:

A. Gomez[1], C. Lyons[2], M. Shiaris[2]; [1]Cornell Univ., Ithaca, NY, [2]Univ. of Massachusetts, Boston, Boston, MA

Abstract Body:

Bacteriophages infecting bacteria in the genus *Enterococcus* are widespread and have been isolated from a variety of sources. There is growing interest in their use for phage therapy since strains in this genus are major nosocomial pathogens. Despite their abundance, these phages are not well characterized and only eight complete genome sequences are available. A plaque assay was used to isolate and *Enterococcus faecalis* phage from active sludge from an urban sewage treatment plant. Using Illumina paired-end next generation DNA sequencing, we characterized the novel *Enterococcus* phage. Sequencing yielded 128 scaffolds with a total length of 109,365 bp. Quality of the sequence data was low to assemble a complete genome. The assembly contains 148 putative open reading frames (ORFs). Of these, 120 match sequences in the NCBI database, with putative functions identified for 40. Most of the protein-encoding ORFs are structural or involved in capsid assembly or DNA processing. The phage lysed a variety of species in the genus *Enterococcus.* Phylogenetic analysis showed that ΦKKAS003-1 is a member of the *Spounavirinae* subfamily of the *Myoviridae* family. Therefore, the phage likely has a double-stranded DNA genome with an isometric capsid, long contractile tail and is an obligate lytic phage. It is most closely related to *Enterococcus* phages EFDG1, ΦEF24C, and ECP3. Higher quality sequence data is needed to validate and expand upon these results.

Author Disclosure Block:

A. Gomez: None. **C. Lyons:** None. **M. Shiaris:** None.

Poster Board Number:

SUNDAY-008

Publishing Title:

Prophages Related to Lytic Nontailed Viruses Are Widespread in Marine *Vibrio*

Author Block:

F. A. Hussain, K. M. Kauffman, M. F. Polz; Massachusetts Inst. of Technology, Cambridge, MA

Abstract Body:

The surface ocean is dominated by nontailed viral morphotypes (1), yet tailed viruses are most abundant in viral culture collections. Previous bioinformatic analyses identified putative Corticovirus-like prophages as widespread in the Proteobacteria (2) and suggested that they may contribute to the nontailed viruses. Here, we show these elements to also be prevalent in diverse *Vibrio* genomes, and further, that they are able to naturally excise from their host genome and occur protected in the extracellular milieu. By concentrating, nuclease treating, and then sequencing the supernatants of putative lysogenic cultures we find that active prophages excise during both mid-exponential and late-stationary phases, indicating continuous release during growth. Using a comparative genomics approach, we find that in their major capsid protein and packaging ATPase, these prophages show homology to those of their lytic counterpart, the Corticovirus PM2. Out of 849 fully sequenced *Vibrio* genomes, 401 (49%) harbor a prophage, and of these, 36% contain more than one prophage. The elements are themselves diverse, and cluster into at least 6 distinct groups based on sequence similarity of the major capsid protein. Similar elements are not necessarily found in closely related hosts, suggesting that the elements have a broad host range. Sequencing data suggest the prophages use a site-specific recombinase to integrate into the host genome directly upstream to a tRNA dihydrouridine synthase, and the surrounding genes, upstream and downstream of the integration site, may determine host specificity. Additionally, for several genomes with the element, we identify a partner strain that has identical upstream and downstream regions, but that is missing the element, leading us to believe these may be potential hosts for the corresponding element. The widespread distribution and observed natural continuous induction of these elements suggests that they play a significant role in the ecology of marine *Vibrio* and may contribute to the nontailed viral majority.

Author Disclosure Block:

F.A. Hussain: None. **K.M. Kauffman:** None. **M.F. Polz:** None.

Poster Board Number:

SUNDAY-009

Publishing Title:

Vector-Borne Viruses of Human Interest in Southern Ukraine

Author Block:

O. O. Yurchenko[1], D. O. Dubina[1], N. O. Vynograd[2]; [1]SB «Mechnikov Ukranian Anti-Plague Res. Inst. of the Ministry of Hlth.of Ukraine», Odesa, Ukraine, [2]Danylo Halytsky Lviv Natl. Med. Univ., Lviv, Ukraine

Abstract Body:

Background: Sporadic cases of vector-borne viral diseases - tick-borne encephalitis (TBE), Crimean-Congo hemorrhagic (CCHF) and West Nile (WNF) fevers have been registered in Ukraine. However, very little is known about natural vectors and hosts of the arboviruses or their prevalence among humans in the country. The goal of this study was to determine a spectrum and abundance of arboviruses circulating in natural foci and among humans in southern Ukraine.**Methods:** We examined 21,674 ixodid ticks, 45,652 mosquitoes, 515 avian brain tissue samples, 6 small mammals, and 813 human sera collected in southern Ukraine in 2000-2007. Arthropods and brain tissue suspensions were tested for arboviruses by intracerebral inoculation of newborn mice, in enzyme-linked immunosorbent assay (ELISA) or reverse-transcription polymerase chain reaction. Human sera were tested for antibodies by hemagglutination inhibition test and ELISA. The Envelope (E) protein gene of 7 tick-borne encephalitis virus (TBEV) strains, isolated from ticks in southern Ukraine in 1988-90, were sequenced by the Sanger method.**Results:** TBEV, Uukuniemi (UUKV), CCHF viruses were detected in ticks - the minimum infection rates (MIR) were 0.48%, 0.27% and 0.03%, respectively. Mosquitoes were infected with West Nile (WNV) and Sindbis (SINV) viruses - the MIRs were 0.16% and 0.09%, respectively. The main vectors of TBEV and UUKV were *Ixodes ricinus* ticks, WNV and SINV - *Culex pipiens* mosquitoes. TBEV, UUKV, WNV, SINV, Tahyna (TAHV) and Inkoo viruses were found in birds, while snowshoe hare virus (SSHV) - in hares. In humans, seroprevalence rate for WNV was up to 28.75%, and for TBEV - up to 11.17%. Antibodies to SINV, TAHV and SSHV were detected in single cases. WNF cases were serologically confirmed for 33 humans. All studied TBEV strains belonged to European genotype (TBEV-Eu) and had 4 marker amino acid replacements in E protein.**Conclusions:** In summary, WNV and TBEV are the most important vector-borne viruses regarding possible infection of humans in southern Ukraine. The circulation of TBEV-Eu may determine milder course and better outcome of the infection.

Author Disclosure Block:

O.O. Yurchenko: None. **D.O. Dubina:** None. **N.O. Vynograd:** None.

Poster Board Number:

SUNDAY-010

Publishing Title:

Molecular Phylogeography of Foot and Mouth Disease Virus in Bangladesh

Author Block:

M. Sultana, H. Ullah, M. A. Siddique, A. Rahman, S. T. Towhid, **M. A. Hossain**; Univ. of Dhaka, Dhaka, Bangladesh

Abstract Body:

Foot-and-Mouth Disease Virus (FMDV), etiological agent of FMD, causes an endemic animal disease in Bangladesh. Here we report circulatory FMDV serotypes and its genome-wide analysis to develop strategies of trans-border disease control and containment.RT-PCR was employed to amplify VP1 gene of viral RNA from blister tissue samples of infected animals collected from 24 districts of Bangladesh since 2011 to 2015. Bioinformatics analyses of cultured FMDV isolates' whole genomes and retrieved VP1 sequences was done along with Bayesian MCMC method to predict phylogeography of circulatory FMDV.FMD was predominant in female animals (55.87%) than males (44.13%) with increased susceptibility in aged cattle (51.17%) than the young animals (38.97%) and calves (9.86%). FMDV serotypes O, A and Asia 1were circulatory in Bangladesh with predominant serotype O (~80%) within the lineage ME-SA/Ind-2001. Within the Ind-2001 clade, the type O complete genome sequences were clustered with those of type O of Uttarkhand, India 2010; Assam, India 2012 and Karnataka, India 2013. FMDV type O from pig formed a sub-clade homologous to 2013 sequences of cattle. Sequences of serotype A retrieved from samples of Chittagong and Gazipur districts, belonged to genotype VII under topotype Asia clade. Serotype Asia 1 re-emerged in 2012 after last out breaks in 1996 and was confined only in certain areas of Jessore and Gazipur districts with sequences clustered within genetic lineage C. The Phylographic analysis confirmed the cross-border movement of FMDV from India to Bangladesh. VP1 sequences of Bangladesh-FMDV detected a notable 8 nucleotide substitutions in the immune-dominant epitope located in GH loop conferring antigenic heterogeneity; most likely the reason behind vaccination failure using imported vaccine.Unrestricted unidirectional animal movement from India is the main source of intrusion of FMDVs in Bangladesh. Presence of significant positive selection pressure within the VP1 antigenic sites implicates evolution of VP1 under high immune surveillance. Regular monitoring is necessary for checking genotype turnover and emergence of new antigenic types that might lead to new outbreaks.

Author Disclosure Block:

M. Sultana: None. **H. Ullah:** None. **M.A. Siddique:** None. **A. Rahman:** None. **S.T. Towhid:** None. **M.A. Hossain:** None.

Poster Board Number:

SUNDAY-011

Publishing Title:

Classification Of Bacteriophage Isolated From The Great Salt Lake Using Electron Microscopy

Author Block:

B. D. Nelson[1], M. J. Domek[1], D. M. Belnap[2]; [1]Weber State Univ., Ogden, UT, [2]Univ. of Utah, Salt Lake City, UT

Abstract Body:

Bacteriophage lyse bacteria and play crucial role in the recycling of nutrients in a halophilic environment such as the Great Salt Lake (GSL). A previous study showed that the bacteriophage CW02, isolated from the GSL, was a dsDNA bacteriophage with icosahedral head and a short non-contractile tail and belonged to the bacteriophage family podoviridae. CW02 was also shown to share a conservative protein fold in a capsid protein originally identified in bacteriophage HK97. Very few bacteriophage isolated from the GSL have been assigned within the bacteriophage classification scheme. In this study we attempt to classify recently isolated bacteriophage from the GSL based on morphology using transmission electron microscopy (TEM) and molecular techniques. Bacteriophage were isolated from water and soil in or near the GSL. Bacterial lysate containing bacteriophage were centrifuged and filtered to remove bacterial debris. The sample was concentrated using 100,000 molecular weight cut-off filters. Samples were further purified by CsCl density gradient ultracentrifugation. Six bacteriophages have been imaged using TEM. TEM has shown all bacteriophage infecting Salinivibrio costocola bacterium SA-39 to be icosahedral with no detectable tail while bacteriophage infecting Salinivibrio costicola bacterium SA-40 having a circular head with a long tail. This suggests that the structure and shape of the bacteriophage capsid play an important role in the specificity of the bacteriophage to host. Based on shapes found using TEM, the bacteriophage infecting SA-39 likely belongs to the Podoviridae group while bacteriophage infecting SA-40 possibly belong to either long-tailed bacteriophage families Myoviridae or Siphoviridae. Further characterization using cryogenic electron microscopy should enable us to determine the 3D characterization of the capsid.

Author Disclosure Block:

B.D. Nelson: None. **M.J. Domek:** None. **D.M. Belnap:** None.

Poster Board Number:

SUNDAY-012

Publishing Title:

What And Where Are The Native American Begomoviruses?

Author Block:

N. Jacko, S. Duffy; Rutgers Univ., New Brunswick, NJ

Abstract Body:

Begomoviruses are economically and socially important ssDNA plant viruses that are transmitted by the whitefly *Bemisia tabaci* and cause severe commercial crop losses which contribute to famine in developing countries. This project seeks to determine what specific begomoviruses are present along the eastern seaboard of the United States and where these viruses are located. Such information is imperative from an epidemiological standpoint in order to better control the spread of these dangerous plant pathogens. It has been previously determined that *B. tabaci* populations can over-winter outdoors as far north as South Carolina. However, we propose that increasing temperatures evident in the northeastern region of the United States have enabled *B. tabaci* populations to migrate further north. A comprehensive literature review and samples collected during the summer of 2014 revealed that *B. tabaci* populations have not reached New Jersey. Thus, samples of whiteflies were collected from states south of New Jersey and north of Florida as the begomoviruses in Florida have been studied extensively. To test for the presence of begomoviruses, methods similar to those used for vector enabled metagenomics were utilized and whitefly samples were analyzed via PCR with degenerate begomovirus detection primers. Protocols were tested using a model ssDNA virus, bacteriophage φX174, or total DNA from begomovirus-infected plants before implementing on begomovirus samples. Whitefly samples have so far been virus-free. Continued screening could prove very useful in the prevention of the spread of begomoviruses and the subsequent destruction of commercial crops along the eastern seaboard of the United States.

Author Disclosure Block:

N. Jacko: None. **S. Duffy:** None.

Poster Board Number:

SUNDAY-013

Publishing Title:

Isolation of Polyvalent Bacteriophages Using Sequential Multiple Host Approaches

Author Block:

P. Yu, J. Mathieu, M. Li, Z. Dai, P. J. Alvarez; Rice Univ., Houston, TX

Abstract Body:

Many studies on phage biology are based on isolation methods that may inadvertently select for narrow host-range phages. Consequently, broad host-range phages, whose ecological significance is largely unexplored, are consistently overlooked. To enhance research on such polyvalent phages, we developed two sequential multi-host isolation methods and tested both culture-dependent and culture-independent phage libraries for broad infectivity. Lytic phages isolated from activated sludge were capable of interspecies or even interorder infectivity without a significant reduction in the efficiency of plating (0.45 to 1.15). Two polyvalent phages (PX1 of the *Podoviridae* family and PEf1 of the *Siphoviridae* family) were characterized in terms of adsorption rate (3.54×10^{-10} to 8.53×10^{-10} ml/min), latent time (40 to 55 min), and burst size (45 to 99 PFU/cell), using different hosts. These phages were enriched with a nonpathogenic host (*Pseudomonas putida* F1 or *Escherichia coli* K-12) and subsequently used to infect model problematic bacteria. By using a multiplicity of infection of 10 in bacterial challenge tests, >60% lethality was observed for *Pseudomonas aeruginosa* relative to uninfected controls. The corresponding lethality for *Pseudomonas syringae* was 50%. Overall, this work suggests that polyvalent phages may be readily isolated from the environment by using different sequential hosts, and this approach should facilitate the study of their ecological significance as well as enable novel applications.

Author Disclosure Block:

P. Yu: None. **J. Mathieu:** None. **M. Li:** None. **Z. Dai:** None. **P.J. Alvarez:** None.

Poster Board Number:

SUNDAY-014

Publishing Title:

Biological Characterization of a Library of Mycoviruses Detected in Marine Fungi Isolated from *Posidonia oceanica* (L.)

Author Block:

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Abstract Body:

Previously we characterized molecularly 12 new viral species[1] in 6 different marine fungal isolates from *Posidonia oceanica*[2,3] present in the *Mycotheca Universitatis Taurinensis* (MUT) collection. These new viral species are distributed among different dsRNA and ssRNA taxa. Scope of our work is the dissection of biological impact of these viruses on their fungal host comparing cured or partially cured isolates to the original virus-infected ones. Curing was obtained with a mix of techniques comprising single spore colonies, hyphal tipping, and use of anti-viral drugs (ribavirin and cycloheximide). The first cured isolate was a *Penicillium janczewskii* (MUT4358) which originally contained a Chrysovirus. From a distinct *P. janczweskii* isolate (MUT4359) that contained a second Chrysovirus and an Alternaria longipes-like virus we obtained a semi-cured strain. We also obtained 3 semi-cured strains starting from *P. aurantiogriseum* var. *viridicatum* (MUT4330), a fungus in which we identified the presence of 6 different viral species: we cured a bipartite virus (similar to Curvularia thermal tolerance virus), then from this, we cured a fusari-like virus, and finally we removed a partiti-like virus. The isolate we obtained still contained three distinct virus species. This collection of *P. aurantiogriseum* isolates allowed us to determine variation in viral titers according to the complexity of the virome present in the isolate revealing virus-virus regulation. Finally we obtained a cured isolate from the basidiomycetes *Wallemia sebi* (MUT4935) infected by a virus related to *Ustilago maydis virus H1*. Each set of virus-infected, cured or partially cured strains were grown in different conditions (up to 63) by combining different media, salinity concentrations and temperatures. A vast array of growth and developmental differences, including effects on secondary metabolites, could be associated with presence/absence of viruses. We are currently evaluating possible aspects of fungal-fungal (intra and interspecific), and fungal-bacterial interactions influenced by presence/absence of mycoviruses.

Author Disclosure Block:

L. Nerva: None. **G.C. Varese:** None. **G. Gnavi:** None. **M. Ciuffo:** None. **M. Turina:** None.

Poster Board Number:

SUNDAY-015

Publishing Title:

High-throughput Sequencing Uncovers Low Homogeneity in the Biogeography of Ssdna Viruses

Author Block:

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Abstract Body:

Regular emergence and re-emergence of viral pathogens emphasizes the importance of understanding viral biogeography and migration. Using new sequencing technologies to investigate the inter- and intra-population dynamics of non-human viral pathogens provides insight on how to effectively contain and mitigate future human outbreaks. Investigations utilizing high-throughput sequencing have determined that local viral diversity is extremely high, but does not scale to produce an exponentially higher global diversity. It follows that similar genotypes can be found great distances apart, although they may not be permanent constituents of any single population. Transient genotypes have been observed in temporal surveys of closed systems, where genotypes migrate between individual populations. This study focused on the population dynamics of single-stranded DNA (ssDNA) viruses in open systems at a single time-point; sampling wastewater treatment plants (WWTPs) from three neighboring cities in Northwest Florida which receive constant inflow and potentially receive the same viruses from the local environment. Given the increased potential for migration, expectations were that populations would be mostly homogenous with relatively few viruses that are unique to individual WWTP. Isolated ssDNA viruses were rolling circle amplified and sequenced on an Illumina HiSeq. Viral genotypes with genetic similarity to *Circoviridae*, *Geminiviridae*, and *Microviridae* were recovered from all three WWTPs, however <5% match genotypes previously recovered from the study area. It has been determined that <10% of the genotypes were present in all three plants and the majority of genotypes were specific to one WWTP. This result highlights the high level of diversity within each population, while the high observed heterogeneity indicates limited migration opportunities between the WWTPs.

Author Disclosure Block:

V. Pearson: None. **D. Rokyta:** None.

Poster Board Number:

SUNDAY-016

Publishing Title:

Host Range Mutation Narrows Further Host Range Mutation Spectrum of Bacteriophage Phi6

Author Block:

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Abstract Body:

Host range mutations are the key step in viral emergence, and are thus frequently studied in microbial evolution. Fortunately, host range mutants have easily distinguishable phenotypes with which to determine mutational spectra, reflecting underlying viral evolvability. However, the epistatic effect of preexisting host range mutations may suppress or spur further viral evolvability. We tested whether having an expanded host range mutation affected further host range expansion using the fast-evolving *Pseudomonas* dsRNA bacteriophage phi6, a model RNA virus frequently used in experimental evolution studies. The average per base mutation rate of phi6 is approximately 10^{-6} per generation and it has several known novel hosts available for these experiments. In this study, the mutations permitting growth on *Pseudomonas syringae* pv. *atrofaciens* (PA) were determined for two phi6 genotypes: wildtype and one carrying a host range mutation allowing infection of *P. syringae* pv. *tomato* and *P. pseudoalcaligenes* ERA (mutant E8G in the P3 attachment protein). Fifty single plaques of each genotype were isolated and raised on their typical laboratory hosts to obtain high titer lysates, which were then plated on PA. One host range mutant plaque per lysate was isolated at random and the P3 attachment protein was Sanger sequenced to identify mutation(s). Wild type phi6 has a considerably more diverse selection and dispersed distribution of mutations in its PA host range mutation spectrum than its isogenic E8G mutant. Eight single mutations appeared in the 50 wild type phi6 isolates, however only two of those showed up in the 50 E8G isolates. The same mutation (c398t) was the most frequent in both populations: 90% of E8G isolates, 26% of the wild type. In conclusion, novel host range mutation spectrum narrowed when a host range mutation is already present in the P3 attachment protein in phi6. Preexisting host range mutations may greatly influence development of further host range mutation spectra in RNA viruses.

Author Disclosure Block:

L. Zhao: None. **D. Stemate:** None. **S. Duffy:** None.

Poster Board Number:

SUNDAY-017

Publishing Title:

Unique High Molecular Weight Phage Tail-Like Bacteriocins of Listeria Monocytogenes

Author Block:

G. Lee, U. Chakraborty, G. Govoni, D. Gebhart, D. Scholl; AvidBiotics Corp, S. San Francisco, CA

Abstract Body:

Introduction: *Listeria monocytogenes* is a foodborne human pathogen that can cause severe disease in certain high-risk individuals. These bacteria produce high molecular weight, phage tail-like bacteriocins (PTLBs), "monocins", upon induction of the SOS system. **Methods:** We purified, and characterized monocins and using MS we found peptides which allowed us identify the genetic locus encoding them. The monocin gene cluster which is 11.6 Kb in length and encodes 18 ORFs was cloned and expressed in *Bacillus subtilis*, producing high yields of functional bactericidal particles. Monocins were screened against a panel of *L. monocytogenes* isolates to ascertain the killing spectrum. The receptor binding protein, which determines target cell specificity, was identified and engineered to change the bactericidal spectrum. **Results:** Unlike the F-type pyocins of *Pseudomonas* which are related to lambda-like phage tails, we find that monocins are more closely related to TP901-1-like phage tails; structures not previously known to function as bacteriocins. The structures consist of an 80nm tube of stacked discs, at one end of which is a complex baseplate structure. One wild type monocin, M35152, was found to kill mainly serotype 4b strains. We were able to retarget the specificity to serotype 1/2a by fusing a phage receptor binding protein to the monocin counterpart. **Conclusions:** This discovery represents a new example of an envelope-penetrating nano-machine that has diverged to function as either DNA delivery (phage tail) or as a membrane-disrupting bacteriocin. While it may be assumed that these structures were co-opted from phages, we cannot rule out the opposite - that ancient phages co-opted complex bacteriocins from the cell which then underwent adaptions to become efficient at translocating DNA. Either way it appears that that multiple classes of phage tails and their related bacteriocins have co-evolved separately in parallel. A combination of a wild-type monocin and an engineered monocin together have a bactericidal spectrum that covers all of the major pathogenic serotypes of *L. monocytogenes*. We plan to develop this combination as a weapon to combat food born listeriosis.

Author Disclosure Block:

G. Lee: D. Employee; Self; AvidBiotics. **U. Chakraborty:** D. Employee; Self; Avidbiotics. **G. Govoni:** D. Employee; Self; Avidbiotics. **D. Gebhart:** D. Employee; Self; Avidbiotics. **D. Scholl:** D. Employee; Self; Avidbiotics.

Poster Board Number:

SUNDAY-018

Publishing Title:

Comparative Analyses of Forty-Four *Gordonia* Phage Genomes

Author Block:

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Abstract Body:

Presented here is the characterization of 44 Gordonia phages. This includes plaque and virion particle morphologies along with comparative genomic analyses. Phages were isolated from soil using modified protocols developed for the isolation of mycobacteriophages. Forty-two of the 44 phages described here were isolated using *Gordonia terrae*, the remaining two phages were isolated using *Gordonia neofelifaecis*. In ongoing host range studies that include six different Gordonia species, these phages showed varying infection patterns across and amongst these hosts.Like the mycobacteriophages, the sequenced *Gordonia* phages were grouped together in clusters based on nucleotide similarity and protein families. The sequenced *Gordonia* phages comprise 13 new clusters, as well as 16 singletons. Interestingly, three *Gordonia* phages are included as members of Cluster A, a cluster with 376 mycobacteriophages. This is the first example of phages isolated on different Actinobacteria genera that share greater than 50% nucleotide similarity. The sequenced *Gordonia* phages exhibit a wide range of genome sizes, ranging from 17118 - 98136 bp. *Gordonia* phages also possess a gamut of GC%, ranging from 47.0% to 68.6%. From the comparative analyses of 44 *Gordonia* phages it is apparent that the population of *Gordonia* phages is as immense, diverse, and dynamic as that of mycobacteriophages, and provide a glimpse of the broader evolutionary relationship of the Actinobacteriophages.

Author Disclosure Block:

D. Jacobs-Sera: None. **M. Montgomery:** None. **C.A. Guerrero:** None. **D.A. Russell:** None. **J. Lapin:** None. **P. Rimple:** None. **A. Stanton:** None. **W.H. Pope:** None. **G.F. Hatfull:** None.

Poster Board Number:

SUNDAY-019

Publishing Title:

A Longitudinal Survey for Phage-Encoded Toxin Genes in the Sewage-Impacted Environment Along the San Diego Coastline

Author Block:

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Abstract Body:

It is not uncommon for public beaches along San Diego's coastline to be closed after rain due to contamination. Sources of contamination include untreated sewage flowing from the Tijuana River and urban runoff from throughout San Diego. *Escherichia coli*, known to carry the phage-encoded shiga toxin gene (*stx*), is commonly found in human and animal feces and is a known human pathogen. We were interested in investigating and comparing the presence of *stx* in sewage-impacted areas along the San Diego coast during the dry and rainy seasons. From 2007-2015, sediment and water samples from the Tijuana River National Estuarine Research Reserve (TRNERR) and neighboring Imperial Beach were collected and screened via molecular assays for *stx*. Sediment and water samples from both locations frequently tested positive for *stx*. Overall, *stx* was detected in 78% of all samples. The *stx* gene was detected most frequently in samples collected from the area most highly impacted by sewage input—the estuary. The *stx* gene was detected more frequently in the water samples from the estuary (90%), versus the sediment samples, and vice versa for the Imperial Beach samples (82% in sediment vs. water). Comparing samples collected in the rainy season (October through March) versus the dry season (April through August), *stx* was detected most in the rainy season samples, with the greatest number detected in the samples from the estuary. No detectable variation in *stx* gene frequency was observed between samples collected from 2007 to 2015. Overall, the data indicates an increased presence of phage-encoded *stx* in the highly sewage-impacted area of the Tijuana River Estuary during the rainy season when local and international wastewater treatment is insufficient at treating the influx of raw sewage after heavy rains. Phage are highly mobile genetic elements and when they carry virulence genes such as *stx*, the transfer of these genes to new bacteria could lead to the evolution of novel human pathogens. It is therefore important to continue to monitor these impacted environments for phage-encoded genes, especially during the rainy season.

Author Disclosure Block:

T. Condeff: None. **V. Casas:** None. **S. Maloy:** None.

Poster Board Number:

SUNDAY-020

Publishing Title:

Assessment of Bacteriophage Diversity and Their Potential Controls on Heterotrophic Decomposition in Peatlands

Author Block:

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Abstract Body:

Little is known about the diversity and role of bacteriophages in carbon (C) rich ecosystems such as peatlands in tropical and temperate regions. To better understand how bacteriophages influence organic C cycling to final products like CO_2 and CH_4, phage communities and phage like particles were isolated or manipulated from freshwater sources from Amazon or Northern peatlands. Here we present initial findings on bacteriophages and their effects on heterotrophic bacteria, which in turn affects the decomposition and the C cycling towards CH_4 in peatlands. To assess diversity, phages were enriched from soil samples from temperate sites and water samples from Amazon peatlands by filtering to remove organic matter and bacteria, and iron flocculation to bind the phages and allow for capture on a filter. Phage community enrichments were screened using the cross-streaking method against 100 heterotrophic bacterial isolates obtained from the same sites to identify phage hosts. Once a host was found, the phage was isolated and a basic characterization was performed determining plaque morphology, and phage morphology via electron microscopy. Selected phages will be sequenced for accurate identification. To assess phage effects on decomposition, *in vitro* soil slurry incubations were done with the addition of tea extract and iron sulfate (TeaF), a virucidal agent, and rates of CO_2 and CH_4 formation were measured using temperate peat soils. In comparison to controls, slurries with TeaF had a major increase in CO_2 formation. The average rate of CO_2 production with the TeaF agent was 1.637 µg/hour while the control was 0.0958 µg/hour. The average rate of CH_4 with the TeaF was 0.207 µg/hour while the control was 0.111 µg/hour. This indicates that the putative TeaF inhibition of phages allowed for an increase in overall CO_2 production by heterotrophs from a reduced predation pressure exerted by phages. Nevertheless, the effects increased CH_4 formation rates were also apparent but after a lagging time of several days to a week while effects on CO_2 rates were noticeable within hours. Similar inhibition tests are underway for Amazon peatland soils. Our results provide the first assessment of bacteriophage diversity and abundance in Amazon peatlands and also evaluates their quantitative contribution or influence of organic carbon decomposition in peatland soils.

Author Disclosure Block:

J. Spring: None. **H. Cadillo-Quiroz:** None. **A. Sarno:** None.

Poster Board Number:

SUNDAY-021

Publishing Title:

Detection of Halophilic Bacteriophage in Soils Near the Great Salt Lake

Author Block:

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Abstract Body:

Bacteriophages that target halophilic bacteria from the Great Salt Lake (GSL) have been isolated and studied previously; however, little is known about the distribution of bacteriophages in the soil surrounding the GSL. The objective of this study was to determine the presence of halophilic bacteriophage in the soils surrounding the GSL, facilitating a greater understanding of the role they play in the terrestrial ecosystem. A technique was developed to amplify bacteriophage from soil using host-bacteriophage pairs isolated from the GSL. The technique tested bacteriophage survival and attachment in soil and was also used to isolate new bacteriophage. Soil samples were collected from Antelope Island (in GSL) at various distances from the water. Previously isolated *Salinivibrio costicola* strains (SA36, SA39, SA40 and SA50) and their respective bacteriophage (DB01, NS01, JM01 and CW02) were incubated with GSL soil and halophilic media. After an overnight incubation the soil extract was diluted and a spot test assay was performed to detect the titer of bacteriophages. The technique tested known bacteriophage-host pairs and demonstrated that strains SA39 and SA40 amplified markedly more than the other bacteriophages suggesting that certain bacteriophages bind to soil particles or are inactivated by soil components. Bacteriophages in the GSL may be important in controlling the growth and ecology of the microbial populations in this highly saline environment. Additionally, two potentially new bacteriophages were isolated and amplified, then observed with an electron microscope.

Author Disclosure Block:

M.J. Domek: None. **G. Ward:** None. **M.D. Culumber:** None.

Poster Board Number:

SUNDAY-022

Publishing Title:

Microbial Community Dynamics During Lake Ice Formation

Author Block:

T. Butler, A. Baldwin, A. Dwyer, **S. M. Techtmann**; Michigan Technological Univ., Houghton, MI

Abstract Body:

Microbial communities experience dramatic environmental changes on a seasonal basis. Many aquatic environments can transition from 20°C in summer months to persistent ice cover during winter months. The dynamics of microbial communities is poorly understood in aquatic settings and in particular little is known about changes in microbial community structure and abundance during times of persistent ice cover. The goal of this study is characterize the temporal dynamics of microbial communities during seasonal transition. Here we sought to perform a high-resolution time-series of the Keweenaw Waterway. Triplicate samples were collected five days per week from surface water in the Keweenaw Waterway between November and April. *In situ* environmental variables (Temp, pH, DO, Salinity, ORP) were measured at the time of sampling. Samples were collected for analysis of dissolved organic carbon, inorganic nutrients, microbial cell counts, and microbial community analysis. Abundance of bacteria (bacterial 16S rRNA), Archaea (Archaeal 16S rRNA), fungi (fungal ITS) and eukaryotes (eukaryotic 18S) were determined using qPCR. Microbial community structure was determined using massively parallel sequencing of the 16S rRNA gene using the Illumina MiSeq. The microbial community at initial time points was dominated by members of the Actinobacteria in the orders Actinomycetales (24% of recovered reads) and Acidomicrobiales (12%). Members of the Verrucomicrobia were also abundant during the initial time points (8%). Members of the Burkholderia (9%) and the Planctomycetes (7%) were also present at high abundance. We expect that there will be little variation in the microbial community from day-to-day, but will show dramatic shift in microbial abundance and diversity during the transition from fall into winter and from winter into spring. The results of this on-going study will help to elucidate how microbial abundance and diversity change over drastic seasonal transition and how ice cover affects microbial abundance and diversity. In the long term we hope to link temporal changes in environmental parameters with functional differences to better understand how seasonal variations affects biogeochemical cycling.

Author Disclosure Block:

T. Butler: None. **A. Baldwin:** None. **A. Dwyer:** None. **S.M. Techtmann:** None.

Poster Board Number:

SUNDAY-023

Publishing Title:

Flood and Infectious Diseases in an Endemic Setting - A Report from Chennai

Author Block:

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Abstract Body:

Background: Following a flood, there exists the potential for transmission of water-borne diseases and for increased levels of vector-borne diseases. These infectious diseases outbreaks may have significant societal impacts, however, there is very little data from the developing world. This study aimed to quantify the infectious diseases reported from a tertiary care hospital before and after major floods.**Methods**: A cross sectional study was conducted in a tertiary care hospital, in Chennai (heavily flooded district in Southern India, during December 2015 floods). Information was collected from hospital notifiable disease records. The numbers of cases of acute gastroenteritis (AGE), typhoid fever, leptospirosis, dengue and malaria reported in the month of November 2015 and December 2015 were analyzed.**Results**: A total of 103 infectious cases were reported after floods (December), when compared to 145 cases reported before floods (November). However, there is major differences in the disease profile reported as shown in the table -1 below**Table-1: Infectious Disease Reported Before and After Floods**

	AGE	Typhoid	Leptospirosis	Dengue	Malaria	Total
November 2015	17	0	0	126	3	146
December 2015	43	3	0	56	1	103

Conclusion: There is nearly 30% (146 to 103) decrease in infectious diseases reported following floods in South India. However, there is 150 % (17 to 43) increase in the number of AGE cases reported and 55 % (126 to 56) drop in dengue cases reported after floods. These findings should be confirmed across other health care institutions and in community settings for better understanding and infectious disease preparedness for future disasters.

Author Disclosure Block:

D. Sureshkumar: None. **R. Gopalakrishnan:** None. **V. Ramasubramanian:** None. **A. Rajalakshmi:** None. **S. Saujanya:** None.

Poster Board Number:

SUNDAY-024

Publishing Title:

Short-Term Temporal Variation of Grassland Soil Microbial Communities Enlarged under Experimental Warming

Author Block:

M. M. Yuan, Z. Shi, J. Li, L. Wu, Z. He, J. Van Nostrand, J. Zhou; Univ. of Oklahoma, Norman, OK

Abstract Body:

Background: How microbial communities change over time is a fundamental, yet unsolved question in ecology, especially for highly diverse communities with complex structures. Previous studies have suggested that the long-term (over years) turnover rates for soil microbial communities are actually similar to macroscopic plant and animal communities. However, the temporal decay rates of microbial communities under strong seasonal fluctuations have not yet been evaluated.**Methods:** To assess such short-term diversity changes, and the influence of climate warming, soils were collected in a field experiment in a central Oklahoma tall grassland, where the humid subtropical climate features clear seasonal patterns. In 2012, surface (0-15cm) soil was sampled monthly from four replicated experimentally warmed (+3 °C continuously for 4 years) and control plots, and analyzed by MiSeq sequencing of 16S rRNA gene amplicons.**Results:** A total of 1.66 million high quality 16S rRNA amplicon sequences were obtained and clustered into 70,022 OTUs (97% identity cutoff). A comparison including all the samples revealed that both warming and sampling month significantly ($P<0.005$ by Adonis with Bray-Curtis distance) affected the soil microbial community structure. Separating the samples by treatment generated different turnover patterns in control and warmed soils. The changes in community structures (distance matrices) were correlated (by Mantel test $r = 0.085$, $P = 0.058$) with days between observations in warmed plots, but not in control plots. The species time relationship exponent (STR-w) was slightly higher in warmed than in control plots ($w = 0.609$ for control, $w=0.622$ for warming, $P = 0.076$ by t-test), suggesting a faster species accumulation over time in response to warming. Also, warmed communities had a higher β-diversity (Sørensen index, $P=0.02$) than control, confirming the higher temporal divergence of warmed communities.**Conclusions:** These results indicate that soil microbial communities change in structure along seasonal environmental fluctuations, and that experimental warming enlarged the short-term temporal variation.

Author Disclosure Block:

M.M. Yuan: None. **Z. Shi:** None. **J. Li:** None. **L. Wu:** None. **Z. He:** None. **J. Van Nostrand:** None. **J. Zhou:** None.

Poster Board Number:

SUNDAY-025

Publishing Title:

Effects of Warming on Soil Microbial Communities in a Grassland Ecosystem

Author Block:

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Abstract Body:

Elevated temperature causes ecosystem-level responses, including decreased soil moisture, increased primary productivity and litter decomposition. However, little is known about how soil microbiomes respond to warming. In this study, we surveyed the taxonomic and functional potential changes of soil microbial communities in a grassland field exposed to experimental warming (+3℃ at 1 cm belowground) for four years by MiSeq sequencing of 16S rRNA and internal transcribed spacer (ITS) gene amplicons, and by a functional gene array (GeoChip 5.0). Dissimilarity analysis of all samples showed that warming significantly ($P<0.05$) changed the overall functional composition, structure and potential of soil microbial communities, but not their corresponding taxonomic structures. The abundance of OTUs in the bacterial phyla, such as *Armatimonadetes*, *Firmicutes* and *Verrucomicrobia* was increased by warming, while that in *Acidobacteria, Proteobacteria, Phenylobacterium, Bacteroidetes* was decreased at 95% confidence interval. Besides, warming also altered the adundance of OTUs in the fungal phyla, e.g., *Ascomycota, Glomeromycota* and *Zygomycota*. The key functional genes involved in carbon degradation (e.g., *amyA, cdh, vdh, chitinase* gene) were enriched under warming, as well as the genes in nutrient-cycling process such as denitrification, ammonification and phosphorus utilization. Canonical correspondence analysis (CCA) indicated that microbial functional potential was highly correlated with annual temperature, carbon/nitrogen ratio, nitrate-nitrogen and aboveground plant and root biomass. These results provide new insights into our understanding of the response and feedback of soil microbial communities to warming.

Author Disclosure Block:

X. Zhou: None. **Z. He:** None. **L. Wu:** None. **J.D. Van Nostrand:** None. **M. Yuan:** None. **D. Tilman:** None. **X. Liu:** None. **J. Zhou:** None.

Poster Board Number:

SUNDAY-026

Publishing Title:

Characterization of Microbial Communities and Soil Organic Carbon Degradation Associated with the Depth and Thawing Effects on Tundra Soil in Alaska

Author Block:

H. Park, H. Park, D. Kim; Korea Polar Res. Inst., Incheon, Korea, Republic of

Abstract Body:

Background: In high-latitude regions, temperature has risen twice as fast as the global average (0.3^{o}C per decade) and this leads to the increase in microbial degradability against soil organic carbon (SOC). Furthermore, the decomposed SOC is converted into green-house gases (CO_2 and CH_4) and their release could further increase the rate of climate change. Thus, understanding the microbial diversity and their functions linked with SOC degradation in soil-thawing model is necessary.**Methods:** In this study, we divided SOC-rich tundra soil from Council, Alaska into two depth regions (30−40 cm and 50−60 cm of depth) and incubated that for 108 days at 0^{o}C. We adopted a pyrosequencing technique to investigate the microbial community changes in parallel withdifferent depths and incubation period. Subsequently, humic acids (HA), main component of SOC, were extracted from the identical soils and analyzed to examine the changing patterns of SOC.**Results:** A total of 111,804 reads were obtained through a pyrosequencing-based metagenomic study during the microcosm experiments, and 574−1,128 of bacterial operational taxonomic units (OTUs) and 30−57 of archaeal OTUs were observed. Taxonomic analysis showed that the distribution of bacterial taxa was significantly different between two samples, while archaea was similar. In detail, the relative abundance of phyla *Actinobacteria* and *Firmicutes* largely increased in 30−40 cm and 50−60 cm of soil depths, respectively. Genera *Oryzihumus* (30−40 cm) and *Desulfosporosinus* (50−60 cm) were predominant in *Actinobacteria and Firmicutes*, respectively. Weight measurement and gel permeation chromatography of the SOC extracts demonstrated that further polymerization of HA occurred in both depths during the microcosm experiments.**Conclusions:** Taken together our results indicate that these two bacterial phyla could play a key function in SOC degradation and utilization in cold tundra soil.

Author Disclosure Block:

H. Park: None. **H. Park:** None. **D. Kim:** None.

Poster Board Number:

SUNDAY-027

Publishing Title:

Diversity and Distribution of Soil Fungal Community in the Ny-Ålesund Region, Svalbard (High Arctic)

Author Block:

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Abstract Body:

Background: The Arctic tundra is particularly sensitive and vulnerable to climate change. Understanding the structure of soil fungal communities is essential for predicting the response of the Arctic soil environment to climate change.**Methods:** This study assessed the fungal community composition and its relationships with properties of surface soils in the Ny-Ålesund Region (Svalbard, High Arctic). A total of thirteen soil samples were collected and soil fungal community was analyzed by 454 pyrosequencing with fungi-specific primers targeting the rDNA internal transcribed spacer (ITS) region. The following eight soil properties were analyzed: pH, organic carbon (C), organic nitrogen (N), ammonium nitrogen (NH_4^+-N), silicate silicon (SiO_4^{2-}-Si), nitrite nitrogen (NO_2^--N), phosphate phosphorus (PO_4^{3-}-P) and nitrate nitrogen (NO_3^--N).**Results:** A total of 57,952 reads belonging to 541 operational taxonomic units (OTUs) were found. Of these OTUs, 343 belonged to Ascomycota, 100 to Basidiomycota, 31 to Chytridiomycota, 22 to Glomeromycota, 11 to Zygomycota, 10 to Rozellomycota, whereas 24 belonged to unknown fungi. The dominant orders were Helotiales, Verrucariales, Agaricales, Lecanorales, Chaetothyriales, Lecideales, and Capnodiales. The common genera (>8 soil samples) were *Tetracladium*, *Mortierella*, *Fusarium*, *Cortinarius*, and *Atla*. Distance-based redundancy analysis (db-rda) and analysis of similarities (ANOSIM) revealed that soil pH ($p=0.001$) was the most significant factor in determining the soil fungal community composition. Members of Verrucariales were found to predominate in soils of pH 8-9, whereas Sordariales predominated in soils of pH 7-8 and Coniochaetales predominated in soil samples of pH 6-7.**Conclusions:** The results suggest the presence and distribution of diverse soil fungal communities in the High Arctic, which can provide reliable data for studying the ecological responses of soil fungal communities to climate changes in the Arctic.

Author Disclosure Block:

T. Zhang: None. **X. You:** None. **L. Yu:** None.

Poster Board Number:

SUNDAY-028

Publishing Title:

Study of *Microcystis* Cyanophage in Singapore's Reservoir Over 2 Years Through Quantitative PCR

Author Block:

L. L. Loh[1], J. R. Thompson[2], S. H. Te[1], W. Li[1], K. Y. H. Gin[1]; [1]Natl. Univ. of Singapore, Singapore, Singapore, [2]Massachusetts Inst. of Technology, Boston, MA

Abstract Body:

Cyanophages (i.e. viruses that infect cyanobacteria) are crucial in regulating the abundance of their host cells, affecting the clonal diversity as well as the composition of the cyanobacterial community. This study focuses on a 2-year monthly monitoring of a tropical reservoir in Singapore. Surface monthly water samples were collected from three separate sites in the reservoir and the assemblage of cyanobacteria and cyanophage were characterized by QPCR based quantification of *Microcystis* cells and the g91 gene of the Ma-LMM01-type cyanophage, infectious to *Microcystis aeruginosa*. Our findings revealed higher host and Ma-LMM01-type phage abundance during a prolonged dry spell and lower concentrations after a rainy season. During the extended period of low rainfall in 2014, higher *Microcystis* concentration and lower total nitrogen levels were observed. *Microcystis* cyanobacterium do not have heterocysts and hence, TN were likely utilized by the *Microcystis* to increase its biomass. Interestingly, during the dry spell, elevated levels of total microcystin were detected, coinciding with similar or higher viral to host concentrations in the sampled sites. The higher cyanophage abundance were hypothesized to be the result of successful infection, host cell lysis and the subsequent release of microcystin into the water. Secondly, during the high rainfall period, lower host and phage abundance were observed, likely due to unfavorable conditions for the proliferation of cyanobacteria and subsequently, averting successful phage infections. Therefore, this 2-year monthly monitoring provided an insight into the long term occurrence and dynamics of host-viral interaction coupled with environmental water quality.

Author Disclosure Block:

L.L. Loh: None. **J.R. Thompson:** None. **S.H. Te:** None. **W. Li:** None. **K.Y.H. Gin:** None.

Poster Board Number:

SUNDAY-029

Publishing Title:

Functional and Compositional Shifts in Soil Microbial Communities in Response to Long-Term Warming in Alaska

Author Block:

C. Wang[1], L. Wu[1], J. Zhou[1], E. A. G. Schuur[2], R. Bracho[3], M. Yuan[1]; [1]The Univ. of Oklahoma, Norman, OK, [2]Northern Arizona Univ., Flagstaff, AZ, [3]Univ. of Florida, Gainesville, FL

Abstract Body:

Permafrost, which stores around half of the total soil organic carbon over the world, is generally believed to be sensitive and responsive to global warming. Potential positive feedback has been anticipated since increased temperature could induce the release of old carbon through changed microbial communities. However, this process is poorly understood. We studied how the soil microbial communities respond to 5 years of experimental warming in CiPEHR (Carbon in Permafrost Experimental Heating Research) project site in Alaska. We performed comprehensive functional gene analysis using GeoChip 5.0, an advanced functional gene array, and 16S and ITS rRNA sequencing. In the organic profile, the abundance of over 80% of functional genes involved in Carbon degradation was significantly increased after warming. Warming also increased the abundance of genes involved in nutrient cyclings such as N and P. The increase could contribute to the observed higher ecosystem respiration and gross primary productivity. Also, 16S rRNA analysis showed the composition of microbial communities changed significantly in response to warming. Altogether, our results showed both the functional and phylogenetic composition of the soil microbial communities responded actively to experimental warming and the response is depth-dependent.

Author Disclosure Block:

C. Wang: None. **L. Wu:** None. **J. Zhou:** None. **E.A.G. Schuur:** None. **R. Bracho:** None. **M. Yuan:** None.

Poster Board Number:

SUNDAY-030

Publishing Title:

Successional Dynamics of Grassland Microbial Communities in Response to Annual Clipping

Author Block:

X. Guo, X. Zhou, M. Yuan, L. Wu, Z. He, J. D. V. Nostrand, J. Zhou; The Univ. of Oklahoma, Norman, OK

Abstract Body:

Plant tissue removal via grazing, mowing and clipping is a central issue in land use practices for agriculture. However, predicting the effect of clipping on the structure and function of belowground microbial communities is hampered by the lack of long term data sets tracking annual clipping manipulation. Here, using metagenomics technologies, we examined the dynamic response of soil microbial communities to annual clipping for five years (2010-2014) at a grassland ecosystem in the Great Plains of North America. Our results indicated that root and microbial activity were significantly ($P < 0.05$) increased under annual clipping based on soil respiration and heterotrophic respiration. The abundance of six bacterial phyla, including *Actinobacteria* and *Bacteroidetes*, and fungal phyla *Ascomycota* and *Zygomycota* were significantly ($p < 0.05$) changed with clipping treatment, though the overall microbial community didn't significantly change. Based on GeoChip analysis, the annual background variation within the microbial community was greater than the significant changes introduced by clipping, but cumulative effects of annual clipping were still observed over time. Five years of annual clipping significantly ($P < 0.05$) increased the abundance of genes involved in the degradation of labile C and some types of recalcitrant C. More importantly, the abundance of genes involved in the degradation of recalcitrant C, but not labile C, were consistently significantly increased in the last two years, suggesting that recalcitrant C degradation was triggered under annual clipping. Other genes involved in nutrient-cycling processes including nitrogen (N) cycling and phosphorus utilization were also significantly increased by annual clipping. The shifts in the functional composition and structure of the microbial community were significantly correlated with soil C and N contents and plant productivity. This study provides new insights into successional dynamics of soil microbial communities and associated feedback responses to annual clipping.

Author Disclosure Block:

X. Guo: None. **X. Zhou:** None. **M. Yuan:** None. **L. Wu:** None. **Z. He:** None. **J.D.V. Nostrand:** None. **J. Zhou:** None.

Poster Board Number:

SUNDAY-031

Publishing Title:

Rdp: Tools and Data for Gene-Targeted Metagenomics

Author Block:

B. Chai, S. Gunturu, L. Tift, T. Soares, Z. Xing, M. O'Keefe, Q. Wang, J. Tiedje, **J. R. Cole**; Michigan State Univ., East Lansing, MI

Abstract Body:

RDP offers aligned and annotated sequence data and analysis tools for rRNA genes and environmentally important protein-coding genes to the research community through its websites (http://rdp.cme.msu.edu and http://fungne.cme.msu.edu). As of January 2016, RDP's data collections include 3,224,600 16S rRNA and 108,901 fungal 28S rRNA sequences. In addition, most RDP tools are available as open source command-line versions through RDP's GitHub repository (https://github.com/rdpstaff/). This repository offers additional options for customizing high-throughput analysis.RDP's FunGene Pipeline & Repository provide databases for 264 protein coding genes useful as phylogenetic markers and for following important ecological functions. In addition to the aligned and annotated gene and protein sequences, FunGene provides online analysis functions and tools for selecting subsets of sequences for download and further analysis.To facilitate the use of the FunGene data in targeted metagenomics, we have developed and are testing efficient high throughput primer design tools. Protein-coding genes are, in general, less conserved than structural RNA genes, meaning that often no single probe or primer pair is able to target a gene's full range of diversity. New highly-parallel qPCR platforms make it cost-effective to use multiple primer pairs. Our tool helps with the design of these multiple primers. It calculates Tm values for candidate primers based on thermodynamic properties, including Tm estimates for primer mismatches and it offers several diversity weighting schemes to maximize the diversity covered by a selected number of primers of targeted diversity. We have used this tool to develop better primers targeting antibiotic resistance genes and genes involved in nitrogen cycling.RDP's mission includes user support; email rdpstaff@msu.edu or call +1(517) 432-4998.

Author Disclosure Block:

B. Chai: None. **S. Gunturu:** None. **L. Tift:** None. **T. Soares:** None. **Z. Xing:** None. **M. O'Keefe:** None. **Q. Wang:** None. **J. Tiedje:** None. **J.R. Cole:** None.

Poster Board Number:

SUNDAY-032

Publishing Title:

Metagenomic and Metatranscriptomic Profiling of Human Gut Microbiota

Author Block:

P. Widmann, E. Haenssler, K. Kowalewski, N. Brinker-Krueger, **D. O'Neil**, M. Sprenger-Haussels; QIAGEN GmbH, Hilden, Germany

Abstract Body:

The human gastrointestinal tract is inhabited by a diverse community of microorganisms that affect human health and disease. Microbial communities can be comprehensively characterized based on their nucleic acid compositions via 16S rDNA-, metagenomic-, and metatranscriptomic approaches applying Next Generation Sequencing (NGS) technologies. The final information content derived from these analyses depends on high-quality starting material in the form of purified nucleic acids. The present study aimed to profile human gut microbiota based on genetic and transcriptomic levels. In order to reduce sample to sample heterogeneity which can naturally occur within stool samples and to be able to generate maximal data output from limited sample material nucleic acids were isolated from single aliquots.16S-rDNA, DNA shotgun, and RNAseq libraries were prepared and sequenced on an Illumina MiSeq system. Analysis of library and sequencing quality parameters showed that the simultaneous application of mechanical and chemical cell disruption methods in combination with an inhibitor removal component was best suited to isolate high quality RNA and DNA for NGS-downstream applications. Microbial community compositions, functional potentials and activities were determined by mapping quality controlled reads to bacterial reference genomes using the BWA-MEM alignment algorithm. Sample specific signatures confirmed that the chosen approach of nucleic acid isolation is a reliable method for extracting the collective microbial genetic material from human stool samples. The chosen approach enabled a comprehensive exploration of human gut microbiota.In conclusion, in-depth analysis of human gut microbiota compositions, functional potentials and activities were conducted based on RNA and DNA from single stool aliquots, separated into single eluate fractions. Comparative metagenomic and metatranscriptomic analyses particularly benefited from this procedure as nucleic acids for Whole Genome Sequencing and RNAseq were isolated from the same aliquot, thus eliminating aliquot-dependent heterogeneities in microbiome structure and allowing detailed analyses of limited input material.

Author Disclosure Block:

P. Widmann: D. Employee; Self; QIAGEN GmbH. **E. Haenssler:** D. Employee; Self; QIAGEN GmbH. **K. Kowalewski:** D. Employee; Self; QIAGEN GmbH. **N. Brinker-Krueger:**

D. Employee; Self; QIAGEN GmbH. **D. O'Neil:** D. Employee; Self; QIAGEN GmbH. **M. Sprenger-Haussels:** D. Employee; Self; QIAGEN GmbH.

Poster Board Number:

SUNDAY-033

Publishing Title:

Genomic and Functional Stability of the Human Gut Microbiome

Author Block:

R. S. Mehta[1], G. Abu-Ali[2], A. Subramanian[2], J. Lloyd-Price[2], D. A. Drew[1], P. Lochhead[1], A. D. Joshi[1], K. L. Ivey[2], H. Khalili[1], E. B. Rimm[2], J. Izard[3], C. Huttenhower[2], A. T. Chan[1];
[1]Massachusetts Gen. Hosp., Boston, MA, [2]Harvard T.H. Chan Sch. of Publ. Hlth., Boston, MA, [3]Forsyth Inst., Cambridge, MA

Abstract Body:

Background: Characterizing the temporal stability of the human gut microbiome is integral to identifying diagnostic biomarkers and modifiable microbiome components for disease prevention and treatment strategies. However, we have a limited insight into the temporal dynamics of the gut microbiome and the determinants of stability that are capable of influencing transitions between health and disease states. **Methods:** We conducted metagenomic and metatranscriptomic sequencing of the human gut microbiome among 308 male participants enrolled in a sub-study of the Health Professionals Follow-up Study. Using a previously validated self-sampling collection method, participants provided up to four stool samples - one from each of two consecutive bowel motions collected 24-72 hours apart, followed by a second set collected approximately 6 months later. DNA was extracted from 929 samples and RNA was reverse-transcribed to cDNA from a subset of 378 samples. Both were sequenced with the Illumina HiSeq platform. Metagenomic and metatranscriptomic read data were profiled using the HUMAnN2 and MetaPhlAn2 platforms, respectively.**Results:** Within-person variation in taxonomic and metagenomic composition over time was consistently lower than between-person variation at any given time point, in contrast to metatranscriptomic profiles. In exploring the determinants of stability, feature prevalence and relative abundance appeared to be highly correlated with feature stability. Metagenomic stability accounted for approximately 70% of metatranscriptomic stability. Moreover, 80% of differentially regulated pathways were consistently over- or underexpressed.**Conclusions:** In one of the largest studies to date, we describe the major underpinnings of within- and between-person variation in the gut microbiome. Gut organismal composition and metagenomic profiles remain highly personalized over time while metatranscriptomic profiles are more variable. Nonetheless, these results suggest that prospective characterization of the gut microbiome at a single time-point can provide a suitable measure for long-term follow-up of disease incidence.

Author Disclosure Block:

R.S. Mehta: None. **G. Abu-Ali:** None. **A. Subramanian:** None. **J. Lloyd-Price:** None. **D.A. Drew:** None. **P. Lochhead:** None. **A.D. Joshi:** None. **K.L. Ivey:** None. **H. Khalili:** None. **E.B. Rimm:** None. **J. Izard:** None. **C. Huttenhower:** None. **A.T. Chan:** None.

Poster Board Number:

SUNDAY-034

Publishing Title:

Clustering Proteins Into Functional Categories to Elucidate the Temporal Functional Stability of the Human Gut Metaproteome

Author Block:

J. A. Blakeley-Ruiz[1], W. Xiong[2], Y. Song[3], C. M. Fraser-Liggett[3], R. Hettich[2]; [1]Univ. of Tennessee, Knoxville, TN, [2]Oak Ridge Natl. Lab., Oak Ridge, TN, [3]Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract Body:

Microbes living in the human gut form a complex and dynamic ecosystem that plays an important role in human physiology; however, the details of population and functional balances of the microbiome remain poorly understood. High-throughput microbiome sequencing and high performance mass spectrometry enable measurement of the composition and metabolic activities of this ecosystem using a combination of genetic and protein information. One fundamental question is how stable is this microbiome ecosystem over time? Comparing samples over time, however, is challenging due to the necessity of acquiring and integrating separate metagenomes and metaproteomes for each sample. To address this dilemma, we undertook two informatics procedures: 1) clustering all the proteins identified in a study by sequence identity in order to remove redundancy, and 2) clustering proteins into functional groups based on annotation databases such as pfam, eggNOG, gene ontology, and KEGG to garner metabolic information. Our testbed for this work are fecal samples from two Crohn's disease patients, collected on days 0, 30, and 180 after surgical resection. The gut proteome for each sample was measured in duplicate via 2D-LC-MS/MS using an LTQ-Orbitrap-Elite-MS. Proteins for each sample were identified using a concatenated human/microbial database derived from the metagenome of each sample. Metaproteomes across samples were clustered by sequence identity at varying resolutions using the *Uclust* algorithm. Metaproteomes across samples were also clustered by functionality using HMMER on the pfam and eggNOG databases. Clustering by protein sequence identity to remove redundancy was found to be too limited for meaningful information on microbiome temporal stability. Of the approximately 3,580 (median) non-redundant microbial proteins identified in each sample, very few were found across all six samples. Depending on the resolution of the functional categories, clustering by functional groups leads to a 30-70% overlap of all the functional groups identified across all six samples, thereby yielding much more detailed information about microbiome variation over time post-surgery.

Author Disclosure Block:

J.A. Blakeley-Ruiz: None. **W. Xiong:** None. **Y. Song:** None. **C.M. Fraser-Liggett:** None. **R. Hettich:** None.

Poster Board Number:

SUNDAY-035

Publishing Title:

Metagenomic Analysis Reveals a Highly Dynamic Human Microbiome during Pregnancy

Author Block:

D. Aliaga Goltsman[1], C. L. Sun[1], D. B. DiGiulio[2], B. C. Thomas[3], J. F. Banfield[3], D. A. Relman[1], March of Dimes Prematurity Research Center at Stanford University School of Medicine; [1]Stanford Univ., Palo Alto, CA, [2]Stanford Univ., Stanford, CA, [3]Univ. of California Berkeley, Berkeley, CA

Abstract Body:

The human microbiome plays key roles in human health and disease, yet the impact of the microbiome on pregnancy, and of pregnancy on the microbiome, is largely unknown. Previous characterizations of the human microbiome during pregnancy have largely focused on taxonomic composition of vaginal microbial communities. Although analyses of the community genomic composition at different body sites may generate important insight about the possible role of the microbiome in gestational health, detailed functional analyses have been limited. Here we describe a longitudinal study of the vaginal, gut and oral microbiome from ten subjects during pregnancy. In total, 293 samples collected approximately every 3 weeks of gestation were subject to Illumina paired-end metagenomic sequencing. 1.53 Gb of non-human sequences were assembled into contigs and functionally annotated for pathway analysis. The community composition from vaginal, gut, and oral samples was estimated from assembled genes for 16S rRNA and single-copy ribosomal proteins. In addition, organismal bins were determined for vaginal samples. An average species richness of 9 ± 2, 101 ± 15, and 42 ± 11 was observed in vaginal, stool, and saliva samples, respectively; Shannon's index of diversity indicates a highly dynamic microbiome composition during pregnancy. Metabolic pathway analyses suggest stability of core pathways over time, but high inter-individual variability for pathways that are likely contributed by lower abundance community members. For example, non-metric multidimensional scaling separates pathways contributed by high diversity from low diversity communities in the vaginal microbiome. Our results highlight the importance of assembly-driven metagenomic and functional analyses for understanding the dynamics of the human microbiome during pregnancy.

Author Disclosure Block:

D. Aliaga Goltsman: None. **C.L. Sun:** None. **D.B. DiGiulio:** None. **B.C. Thomas:** None. **J.F. Banfield:** None. **D.A. Relman:** None.

Poster Board Number:

SUNDAY-036

Publishing Title:

Dietary Drivers of the Gut Microbiome in Children

Author Block:

N. S. Rhoades, G. Flores, D. Herman; California State Univ. Northridge, Northridge, CA

Abstract Body:

The human gut is home to trillions of microbial cells whose functions are essential for normal human physiology. However, quantitative linkages between diet and gut microbiome composition are lacking, particularly in post-weaned, pre-pubescent children (ages 2-8 years). To address this knowledge gap, we conducted a community-based research project at a children's center located in the San Fernando Valley, approximately 30 miles north of downtown Los Angeles, CA. Study participants (n=90) provided three fecal samples and three dietary recalls of the foods consumed 24-hours prior to fecal sampling. Microbial communities were characterized using Illumina amplicon sequencing of the variable region 4 (V4) of the 16S rRNA gene, and functional diversity was assessed using shotgun metagenomic sequencing. Dietary data was disaggregated and converted to macro- and micronutrients using the ASA24 a program developed by the USDA, which uses individual-level nutrient estimates based on the Food and Nutrient Database for Dietary Studies (FNDDS). For each individual, dietary and microbiome data were averaged across the three sampling days to account for day-to-day variability in microbiome and diet. We found that older children tended to have more diverse microbiomes but diversity did not level off by the age of three, as others have reported. When we look at the population as a whole, significant relationships between higher level dietary features such as Whole grains, Total dairy, and gut microbiome composition were found. When we bin individuals by diet related age groups (2-3 and 4-8 years), many age specific trends were revealed. For example, in children between the ages of 2-3 years, micronutrients such as selenium, niacin, and calcium were strongly related to the abundance of specific bacterial families. In the 4-8 age group, several bacterial families were differentially influenced by the amount of fatty acids consumed. While analysis of these data is ongoing, results show that diet can be used to predict features of the microbiome in an age specific way. Only by understanding these relationships in diverse populations of healthy individuals during different life-stages will we be able to link subtle changes in the gut microbiome with human health outcomes later in life.

Author Disclosure Block:

N.S. Rhoades: None. **G. Flores:** None. **D. Herman:** None.

Poster Board Number:

SUNDAY-037

Publishing Title:

The Oral Microbiome During Cancer Chemotherapy and Mucositis Development

Author Block:

B-y. Hong[1], A. K. Dupuy[2], L. Choquette[1], A. L. Salner[3], P. Schauer[3], J. A. Burleson[1], L. Strausbaugh[2], A. Dongari-Bagtzoglou[1], D. E. Peterson[1], P. I. Diaz[1]; [1]UConn Hlth., Farmington, CT, [2]Univ. of Connecticut Storrs, Storrs, CT, [3]Hartford Hosp., Hartford, CT

Abstract Body:

Background: Cancer chemotherapies can cause a painful complication known as oral mucositis, which can subsequently compromise treatment outcomes. It is still not clear if chemotherapy directly alters the balance of normal flora in the oral cavity, and if these alterations are related to the development of oral mucositis. The goal of this study was to evaluate changes in the oral bacterial microbiome during chemotherapy and to investigate the relationship between these changes and oral mucositis. **Methods:** Forty-nine subjects diagnosed with cancer and scheduled to receive a cycle of 5-fluorouracil or doxorubicin-based chemotherapy, and 30 non-cancer controls were enrolled. Oral mucositis incidence and severity, and the salivary bacterial microbiome were evaluated pre-chemotherapy at baseline (V1) and during one chemotherapy cycle (V2-V4). Healthy controls were followed at V1 and V4 across the same period. Bacterial DNA isolated from saliva was used to construct PCR amplicon libraries targeting hypervariable regions 1-2 of the 16S rRNA gene, which were sequenced on the 454 platform.**Results:** Overall incidence of oral mucositis among cancer subjects was 80% with severity peaking at V3. The salivary microbiome showed decreased alpha-diversity during chemotherapy (V1 vs V3-V4), independently of antibiotic treatment, while no changes occurred in non-cancer controls. Decreased diversity correlated with mucositis incidence and severity at V4. Interestingly, subjects with sustained mucositis at V4 showed, on average, significant changes in the abundance of 16% of operational taxonomic units (OTUs) in their microbiomes, while subjects who recovered from mucositis by V4 had changes in only 3% of OTUs.**Conclusions:** This study demonstrates an association between salivary bacterial diversity and oral mucositis duration and severity. Our findings suggest that the degree of perturbation of the normal oral flora during cancer chemotherapy can constitute a risk factor for oral mucositis severity and/or persistence.

Author Disclosure Block:

B. Hong: None. **A.K. Dupuy:** None. **L. Choquette:** None. **A.L. Salner:** None. **P. Schauer:** None. **J.A. Burleson:** None. **L. Strausbaugh:** None. **A. Dongari-Bagtzoglou:** None. **D.E. Peterson:** None. **P.I. Diaz:** None.

Poster Board Number:

SUNDAY-038

Publishing Title:

Assessment of Rumen Microbiome in Indian Surti Buffalo (*Bubalus bubalis*) Adapted to a High Roughage Diet Using Shotgun Sequencing

Author Block:

A. B. Patel[1], A. K. Patel[2], N. V. Patel[2], B. Reddy[2], R. K. Shah[1], A. T. Hinsu[2], D. Madamwar[1], C. G. Joshi[2]; [1]Sardar Patel univ., Bakrol, Gujarat, India, [2]Anand Agricultural Univ., Anand, Gujarat, India

Abstract Body:

Indian buffaloes contribute significantly to the national economy by providing dairy products and meat. Ruminants mainly depend on rumen microbiota for feed digestion and energy production. Uncovering microbial diversity and functional capacity of buffalo rumen can play a vital role in the animal health and productivity. Eight healthy Indian Surti buffaloes were gradually adapted to an increasing roughage diet (4 animals each with green and dry roughage) containing 50:50 (S1), 75:25 (S2) and 100:0 (S3) roughage to concentrate mix proportion for 6 weeks. In the present study, metagenomic approach was applied to divulge rumen microbiome using shotgun sequencing by Ion Torrent PGM platform and MG- RAST annotation pipeline. Our results showed that bacteria were the major community whereas archaea, eukaryota and viruses made up the minor communities in the overall microbial diversity. A total of 34 abundant to rare bacterial phyla were detected, among them Bacteroidetes, Firmicutes, Proteobacteria, Fibrobacteres and Actinobacteria were the most predominant in decreasing order, respectively. Genera such as *Prevotella, Bacteroides,* and *Clostridium* were dominant in buffalo rumen. Functional analysis indicated abundance of protein (~26.60%) and carbohydrate (~22.64%) metabolism in buffalo rumen. Rumen fluid metabolite study revealed that nitrogen content reduced and total volatile fatty acids raised in high roughage diet fed animals. Mining of metagenome sequence by CAZyme Analysis Toolkit identified a total of 70033 contigs encoding complex plant polysaccharides degrading enzymes representing potential source of enzymes for biofuel production and other industrial applications. Analysis reveals that composition of diet and fractions of rumen content have significant impact on rumen microbiome at taxonomic as well as functional level. Our study provided a base for designing strategies to develop feed diet for successful management of rumen micoflora and improvement in animal growth performance.

Author Disclosure Block:

A.B. Patel: None. **A.K. Patel:** None. **N.V. Patel:** None. **B. Reddy:** None. **R.K. Shah:** None. **A.T. Hinsu:** None. **D. Madamwar:** None. **C.G. Joshi:** None.

Poster Board Number:

SUNDAY-039

Publishing Title:

Functional Type I-F of CRISPR-CAS System is Present in the Gut Metagenome of Malaria Vector Mosquitoes

Author Block:

J. Xu, D. Pei, W. Yu; New Mexico State Univ., Las Cruces, NM

Abstract Body:

Background: Mosquitoes can transmit diseases like malaria and dengue fever. Mosquito control is critical for preventing mosquito-borne diseases. Mosquito harbors a complex microbiome in the gut ecosystem. The microbial community contributes significantly to various mosquito life traits, such as fecundity and immunity. In a metagenomic ecosystem, horizontal transfer of genetic elements and host defense system like CRISPR-CAS machinery are known to be one of the crucial parameters that influence the diversity, stability and resilience of a microbial community. Understanding how a microbial homeostasis is maintained would facilitate to develop novel strategy to alter microbiome as desired to manipuate mosquito traits.**Methods:** We have generated a metagenomic assembly by NGS shotgun sequencing mosquito gut microbial DNA. We also sequenced genomes of 12 bacterial strains that were isolated from the mosquito gut microbial community. CRISPR sequences were identified by using CRISPR finder, and phage sequences were identified by PHAST. Functionality of the I-F type of CRISPR-CAS was tested by efficiency of transformation of plasmids with engineered protospacers.**Results:** We have identified type I-F and I-E subtypes of CRISPR-CAS immune system as well as prophages in the metagenomic assembly from the mosquito gut. The sequences were confirmed in the single bacterial genomes of *Serratia* and *Acinetobacter*, both were isolated from the mosquito gut. The I-F type of CRISPR-CAS in *Serratia* strain was functional, which was demonstrated by the ability to degrade plasmids with engineered protospacers that match with the spacers in the CRISPR arrays. Prophage sequences were present in the Serratia genome, which was inducible upon the treatment with mitomycin C *in vitro*. The interactions between CRISPR-CAS and phages are under investigation.**Conclusions:** Phage delivered CRISPR machinery as antimicrobials has been used for eliminating target bacteria in a metagenome. This approach can be used for manipulating mosquito gut microbiome by targeting specific bacterial members, which may lead to a synthetic microbiome for mosquito control.

Author Disclosure Block:

J. Xu: None. **D. Pei:** None. **W. Yu:** None.

Poster Board Number:

SUNDAY-040

Publishing Title:

Microbial Diversity Recovery in Reclaimed Surface Coal Mine Soils in the Appalachian Mountains, West Virginia

Author Block:

D. H. Huber, A. Hass, S. A. Malkaram, K. Barry, N. Montenegro-Garcia, U. K. Reddy; West Virginia State Univ., Institute, WV

Abstract Body:

Surface coal mining in the forested Appalachian Mountain region is followed by reclamation methods that are expected to restore the landscape and vegetation to a state similar to the original. Soil restoration is an important objective as well because soil structure is also destroyed. However, the extent to which current reclamation methods lead to the recovery of soil microbial diversity following severe disturbance is still not well known. We tested the hypothesis that soil structure (organic matter) will recover and microbial diversity will increase with time following standard reclamation protocols. We sampled surface coal mine sites near Charleston, West Virginia that differed in the amount of time since reclamation was begun. Four different mine sites showed a positive correlation ($R^2 = 0.81$) between time since reclamation and accumulation of soil organic matter. Metagenomic sampling was done from two sites separated by nineteen years post-reclamation: site 4M is recent, site W94 is older. Illumina sequencing produced 8 Gb of sequence from each site, representing triplicate sampling. Sequences were analyzed using the metAMOS suite of programs. Taxonomic profiling was done using Metaphyler which uses a set of 31 phylogenetic marker genes rather than simply 16S rRNA. The dominant bacterial phylum in both sites was Proteobacteria, ranging from 54 to 61% abundance. Actinobacteria were more abundant in the recently disturbed 4M site (23%) compared to W94 (12%). In contrast, Acidobacteria increased 10-fold in the older site W94 (2.3%) compared to 4M (0.23%) which is consistent with their known widespread occurrence in natural soils. Based on 16S rRNA genes, the Acidobacteria were also found to be more diverse in the older reclaimed soil. Verrucomicrobia, which are known to be a component of natural soils, also increased in abundance over time: none detected in 4M samples and 0.2% detected in W94. In conclusion, time since reclamation was associated with an increase of soil organic matter and an increase in the diversity and abundance of Acidobacteria and Verrucomicrobia.

Author Disclosure Block:

D.H. Huber: None. **A. Hass:** None. **S.A. Malkaram:** None. **K. Barry:** None. **N. Montenegro-Garcia:** None. **U.K. Reddy:** None.

Poster Board Number:

SUNDAY-041

Publishing Title:

Species-specific and Temporal Shifts in Carbon Utilization Strategies Among Filamentous Ascomycete Fungi

Author Block:

C. A. Zeiner[1], S. O. Purvine[2], E. Zink[2], S. Wu[3], L. Pasa-Tolić[2], D. L. Chaput[4], C. M. Santelli[5], C. M. Hansel[6]; [1]Boston Univ., Boston, MA, [2]Pacific Northwest Natl. Lab., Richland, WA, [3]Univ. of Oklahoma, Norman, OK, [4]Smithsonian Inst., Washington, DC, [5]Univ. of Minnesota, Minneapolis, MN, [6]Woods Hole Oceanographic Inst., Woods Hole, MA

Abstract Body:

Fungi generate a wide range of extracellular oxidative enzymes and reactive metabolites, collectively known as the secretome, that synergistically drive carbon (C) degradation in the environment. While secretome studies of model organisms have greatly expanded our knowledge of these oxidative enzymes, few have extended secretome characterization to environmental isolates or directly compared temporal patterns of enzyme utilization among phylogenetically diverse species. Thus, the mechanisms of C degradation by many ubiquitous soil fungi remain poorly understood. Here we use a combination of quantitative iTRAQ proteomics and custom bioinformatic analyses to compare the protein composition of the secretomes of four Mn(II)-oxidizing Ascomycete fungi over a three-week time course. We demonstrate that although the fungi produce a similar suite of extracellular enzymes, they exhibit striking differences in the regulation of these enzymes among species and over time, revealing species-specific and temporal shifts in C utilization strategies as they degrade the same substrate. Specifically, our findings suggest that *Paraconiothyrium sporulosum* AP3s5-JAC2a and *Alternaria alternata* SRC1lrK2f employ sequential enzyme secretion patterns concomitant with decreasing resource availability, *Stagonospora* sp. SRC1lsM3a preferentially degrades proteinaceous substrate before switching to carbohydrates, and *Pyrenochaeta* sp. DS3sAY3a utilizes primarily peptidases to aggressively attack C sources in a concentrated burst. This work highlights the diversity of operative metabolic strategies among cellulose-degrading Ascomycetes and enhances our understanding of their role in C turnover in the environment.

Author Disclosure Block:

C.A. Zeiner: None. **S.O. Purvine:** None. **E. Zink:** None. **S. Wu:** None. **L. Pasa-Tolić:** None. **D.L. Chaput:** None. **C.M. Santelli:** None. **C.M. Hansel:** None.

Poster Board Number:

SUNDAY-042

Publishing Title:

Strain-Specific Metatranscriptomic Evidence of Pervasive and Diverse Chemolithoautotrophy Relevant to C, S, N, and Fe Cycling in a Shallow Alluvial Aquifer

Author Block:

H. R. Beller, T. N. M. Jewell, U. Karaoz, E. L. Brodie, K. H. Williams; Lawrence Berkeley Natl. Lab., Berkeley, CA

Abstract Body:

Groundwater ecosystems are conventionally thought to be fueled by surface-derived allochthonous organic matter and dominated by heterotrophic microbes living under often-oligotrophic conditions. However, in a two-month study of nitrate amendment to a perennially suboxic aquifer in Rifle (CO), strain-resolved metatranscriptomic analysis revealed pervasive and diverse chemolithoautotrophic bacterial activity relevant to C, S, N, and Fe cycling. Prior to nitrate injection, anaerobic ammonia-oxidizing (anammox) bacteria accounted for 16% of overall microbial community gene expression, whereas during the nitrate injection, two other groups of chemolithoautotrophic bacteria collectively accounted for 80% of the metatranscriptome: (1) members of the Fe(II)-oxidizing Gallionellaceae family and (2) strains of the S-oxidizing species, *Sulfurimonas denitrificans*. Notably, the proportion of the metatranscriptome accounted for by these three groups was considerably greater than the proportion of the metagenome coverage that they represented. Transcriptional analysis revealed some unexpected metabolic couplings, in particular, putative nitrate-dependent Fe(II) and S oxidation among nominally microaerophilic Gallionellaceae strains, including expression of periplasmic (NapAB) and membrane-bound (NarGHI) nitrate reductases. The three most active groups of chemolithoautotrophic bacteria in this study had overlapping metabolisms that allowed them to occupy different yet related metabolic niches throughout the study. Overall, these results highlight the important role that chemolithoautotrophy can play in aquifer biogeochemical cycling, a finding that has broad implications for understanding terrestrial carbon cycling and is supported by recent studies of geochemically diverse aquifers.

Author Disclosure Block:

H.R. Beller: None. **T.N.M. Jewell:** None. **U. Karaoz:** None. **E.L. Brodie:** None. **K.H. Williams:** None.

Poster Board Number:

SUNDAY-043

Publishing Title:

Bacterial Growth In Coastal Marine Waters Estimated By Metagenomics And Metatranscriptomics

Author Block:

B. J. CAMPBELL[1], D. Kirchman[2]; [1]Clemson Univ., Clemson, SC, [2]Univ. of Delaware, Lewes, DE

Abstract Body:

Background: Microbial abundance is typically used as an indicator for microbial contributions to ecosystem processes. However, abundance does not account for differences in growth rates or activity of microbes. Based on prior work with 16S rRNA:rRNA gene ratios, we hypothesized that the most abundant groups may not be the most active. **Methods:** We used estimates of the expression and abundance of genes found in different cellular processes to explore seasonal activity and growth rates of bacterial taxa in coastal Delaware waters. We calculated the relative abundance of different classes of genes found in the metagenomes and metatranscriptomes from three different seasons based on both unassembled and assembled datasets. The gene classes included all assigned functional genes, different growth-related genes, rRNA, as well as those for common carbon and other metabolic pathways.**Results:** Most taxa had equal representation in the metagenome and metatranscriptome in December. However, growth-related gene clusters of many taxa were significantly more or less represented in the metatranscriptomes of samples collected in March or May. Our results suggest that abundant SAR11 and *Flavobacterales* clades grew slower than their abundance would indicate, while many *Rhodobacterales* and *Gammaproteobacteria* grew faster than expected, especially in March and May.**Conclusions:** These results highlight the power of comparative metagenomics/metatranscriptomics to explore growth rates of individual taxa and their contributions to ecosystem processes.

Author Disclosure Block:

B.J. Campbell: None. **D. Kirchman:** None.

Poster Board Number:

SUNDAY-044

Publishing Title:

Impact of Seven Years of Glyphosate-Resistant Corn and Glyphosate Applications Under Conventional and Reduced Tillage on Bulk and Rhizosphere Soil Nitrification Rates and Distribution of Archaeal and Bacterial Nitrifying Communities

Author Block:

M. B. Jenkins[1], M. A. Locke[1], K. N. Reddy[2], D. S. McChesney[1], R. W. Steinriede[1]; [1]USDA-Agriculture Res. Service, Oxford, MS, [2]USDA-Agriculture Res. Service, Stoneville, MS

Abstract Body:

Background: Conservation tillage practices across the country have been implementing genetically engineered glyphosate resistant corn (GM) crops along with applications of the herbicide glyphosate (gly). We tested the null hypothesis that six and seven years of glyphosate applications to both glyphosate resistant and non-resistant corn (nonGM) under conventional tillage (CT) and reduced tillage (RT) would not impact the soil process of nitrification and the distribution of archaeal and proteobacterial nitrifying communities.**Methods:** The experimental design was a randomized complete block with glyphosate resistant (GR) and non-GR corn, with and without glyphosate applications under CT and RT. Nitrification rates of bulk and rhizosphere soils were determined and the data generated were regressed against four exoenzymes (exoenzymes , β-glucosidase (BG), β-N-acetylglucosaminidase (NAG), leucine aminopeptidase (LAP), acid phosphatase (AP)) involved in the initial steps in organic matter mineralization, and their nutrient acquisition ratios. With qPCR total archaeal and proteobacterial components of the soil microbiomes, and archaeal and proteobacterial ammonia monooxygenase gene (*amo*A) were determined.**Results:** In 2013 the nonGM-nogly treatment associated with both bulk and rhizosphere soils under RT displayed greater ($P < 0.05$) nitrification rates than other treatments. In 2014 nitrification rates of GM-nogly treatments associated with rhizosphere soil and RT were greater ($P < 0.05$) than other treatments. In 2013 correlations were observed between nitrification rates and BG:AP acquisition ratios in bulk soil no-gly treatments. In 2014 analogous correlations were observed in bulk soil +gly BG:AP and (LAP+NAG):AP ratios. Preliminary data on qPCR ratios between archaeal and proteobacterial *amo*A gene indicated differences between +gly and nogly treatments.**Conclusions:** Glyphosate applications appeared to affect nitrification rates, and the distribution of the arachaeal and proteobacterial nitrifying communities, but not the relation between nitrification and nutrient acquisition ratios related to C:P and N:P.

Author Disclosure Block:

M.B. Jenkins: None. **M.A. Locke:** None. **K.N. Reddy:** None. **D.S. McChesney:** None. **R.W. Steinriede:** None.

Poster Board Number:

SUNDAY-045

Publishing Title:

Primer Development to Target N_2O-Producing Fungal Community

Author Block:

H. Chen, W. Shi; North Carolina State Univ., Raleigh, NC

Abstract Body:

Background: Fungal denitrification has been increasingly investigated as an important biological source of N_2O, an important greenhouse gas and ozone-depleting substance. But the community ecology of N_2O-producing fungi is still poorly understood due to the lack of culture-independent tools, such as PCR primers.**Methods:** Degenerate primers that target fungal nitrite reductase gene (*nirK*) and nitric oxide reductase gene (P450nor), the two important genes for regulating fungal N_2O-production were designed based on the conservative protein and DNA regions in public databases and evaluated for primer specificity and efficiency using a number of N_2O-producing fungal cultures and agricultural soil samples.**Results:** Four pairs of fungal *nirK*-targeting primers (FnirK_F1/R1, F1/R2, F2/R2, and F3/R2) were developed, among which FnirK_F3/R2 appeared to be more efficient as this pair amplified ~ 80% of tested fungal cultures, including *Aspergillus*, *Fusarium*, *Penicillium*, and *Trichoderma*, etc. The soil clone sequences amplified by FnirK_F3/R2 were distributed not only in the clusters of fungal cultures isolated from the soil sample (e.g., *Fusarium* and *Aspergillus*), but also in the clusters of *Chaetomium*, *Metarhizium*, and *Myceliophthora*, which were failed to be isolated from the soil sample in our previous work. However, some bacterial *nirK* could also be amplified due to high similarities between fungal *nirK* and bacterial type II *nirK*. A pair of degenerate primers that target P450nor genes was also proved to be able to amplify diverse groups of N_2O-producing fungi from fungal cultures and soil samples. The amplicons were shown to phylogenetically relate to known denitrifying fungi.**Conclusions:** Our primers that target fungal *nirK* and P450nor genes were proved to have the wide-range capability of amplifying diverse denitrifying fungi from the environment. However, the P450nor-targetting primers may be better than *nirK*-targeting primers to explore N_2O-producing fungal community in the environment, given that high similarities between fungal *nirK* and bacterial type II *nirK* could limit the specificity of fungal *nirK* primers.

Author Disclosure Block:

H. Chen: None. **W. Shi:** None.

Poster Board Number:

SUNDAY-046

Publishing Title:

Circular Resource Flow of Wood Ash into Forest Can Have Detrimental Effects on Soil Bacterial Community

Author Block:

J. T. Nielsen[1], T. Bang-Andreasen[2], **C. S. Jacobsen**[2]; [1]Copenhagen Minicipality, Copenhagen, Denmark, [2]Aarhus Univ., Copenhagen, Denmark

Abstract Body:

Background: The high alkalinity of the waste product and fertilizer wood ash might have detrimental effects on forest soil microbial communities, hence the many essential microbial driven processes which maintain soil quality. Here we investigated responses in bacterial community in a Spruce forest soil to wood ash addition using both culture-dependent and -independent methods to obtain high resolution insight into bacterial responses.**Methods:** Microcosms were set up using soil from the O horizon of a Danish spruce plantation mixed with ash, acquired from a Danish heating plant, to the final doses of 0, 3, 12 and 90 t ash · ha^{-1} and incubated for 42 days at 10 °C. Different soil parameters were monitored during incubation: pH; quantification of viable bacteria, spore-forming bacteria and *Pseudomonas* species using agar plates with general and selective media; changes in bacterial community by Illumina MiSeq paired-end sequencing of 16S rRNA gene amplicons.**Results:** Results showed increase in pH with increasing doses of wood ash. Stimulation of the number of culturable bacteria were observed up to the dose of wood ash 12 t ha^{-1}, while a drastic decrease were observed at 90 t ha^{-1}. Substantial change in the dominant taxonomic groups was observed following the addition of wood ash and the different ash doses resulted in clearly distant bacterial community structures. Decrease in bacterial richness was observed for soil with added ash doses of 12 and 90 t ha^{-1}.**Conclusions:** Wood ash in high levels added to forest soil has detrimental effects on soil bacteria

Author Disclosure Block:

J.T. Nielsen: None. **T. Bang-Andreasen:** None. **C.S. Jacobsen:** None.

Poster Board Number:

SUNDAY-047

Publishing Title:

Bacterial Communities in Cultivated and Wild Cranberry Bogs

Author Block:

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Abstract Body:

Background: The effects of cultivation on soil microbial populations are largely unknown. Transitions in microbial population structure between fallow and cultivation, or between virgin and cultivated soils may be an important early determinant of the fertility and capacity for disease-suppression of the cultivated soil, as well as a predictor of recovery following a period of intensive agriculture. Cranberry bogs are a good model system for comparing microbes associated with native and cultivated soils because of the genetic similarity between wild and domesticated cranberry plants, and their location in the same geographic region (*e.g.* southeastern Massachusetts).**Methods:** Soil samples were collected from wild and cultivated cranberry bogs over the course of the growing season for several years to determine the diversity and composition of the respective phytobiomes in native and cultivated cranberry bogs. Population structures were determined by pyro-sequencing of the amplified V4 region of 16S rRNA genes. Paired-end reads were assembled using PANDAseq, and subsequently analyzed using the QIIME software package to call operational taxonomic units and to calculate richness and diversity indices.**Results:** Bacterial communities in both cultivated and wild bogs are composed of five dominant phyla, *Proteobacteria*, *Acidobacteria*, *Bacteriodetes*, *Verrucomicrobia* and *Actinobacteria*, but with different relative abundances, indicating possible descriptive population structures for each bog type.**Conclusions:** Principal Components Analysis based on soil bacterial populations among sampled sites suggest that sampling location or soil type are more important than cultivation effects in determining bacterial community structure.

Author Disclosure Block:

G. Ebadzad: None. **S.R. Gadagkar:** None. **S. Soby:** None.

Poster Board Number:

SUNDAY-048

Publishing Title:

Abundance and Diversity of Nitrite-Dependent Anaerobic Methane-Oxidizing Bacteria in a Flooded Rice Paddy Supplied with Different Amounts of Urea

Author Block:

S. Jeong, H. Lee, C. Jeon; Chung-Ang Univ., Seoul, Korea, Democratic People's Republic of

Abstract Body:

The nitrite-dependent anaerobic methane oxidation (n-damo) process that oxidizes methane using nitrite under anaerobic conditions is known to be accomplished by "*Candidatus* Methylomirabilis oxyfera" belonging to the candidate phylum NC10. *M. oxyfera*-like bacteria have been detected in rice paddies, but the ecology of *M. oxyfera*-like bacteria in rice paddies was rarely explored. The aim of this study was to investigate the abundance and diversity of n-damo bacteria in a flooded rice paddies supplied with different amounts of urea. Different amounts of urea (0, 15, 30, and 60 $kgha^{-1}$) were applied three times to a flooded rice paddy at - 1, 15, and 45 days based on the transplantation of a rice cultivar (*Oryza sativa*). Soil cores were sampled from each treatments at a flowering stage (63 days after rice transplantation, 18 days after third urea application). The soil cores were divided into top (0-5 cm), middle (10-15 cm), and bottom (20-25 cm) layers and the diversity and abundance of *M. oxyfera*-like bacteria were investigated using t-RFLP (terminal-Restriction Fragment Length Polymorphism) and quantitative PCR approaches, respectively. A 16S rRNA gene clone library of *M. oxyfera*-like bacteria in the rice paddy was constructed and a phylogenetic tree was built, showing that *M. oxyfera*-like bacteria were divided into five clades (group a, b, c, d and e). Using an online program (Restriction Enzyme Picker Online), *NciI* was selected as a suitable restriction enzyme to discriminate the *M. oxyfera*-like bacterial groups by t-RFLP. The clade d was not detected and the relative abundance of clades a and e were detected as minor clades, but abundance of *M. oxyfera*-like bacteria in a flooded rice paddy was not significantly different depending on the amount of urea application. However, the T-RFLP analysis showed that the clades b and c were dominant clades in all depths; in particular, the relative abundance of clade c were higher along a depth gradient in all urea applications, suggesting that *M. oxyfera*-like bacterial community differed depending on the depth of rice paddy.

Author Disclosure Block:

S. Jeong: None. **H. Lee:** None. **C. Jeon:** None.

Poster Board Number:

SUNDAY-049

Publishing Title:

Incidence and Ecology of *Salmonella enterica* in Public Access Watersheds in the Leafy Green Growing Region of Central California

Author Block:

L. Gorski, A. S. Liang, S. Walker, K. F. Romanolo; USDA, ARS, WRRC, Albany, CA

Abstract Body:

Consumption of contaminated produce accounts for nearly half of the foodborne illness outbreaks in the United States, and leafy greens account for a majority of produce outbreaks and recalls. Produce can become contaminated pre-harvest via contaminated water or through wildlife, and public access waterways provide a central reservoir for pathogen contamination. We are in year 4 of a 5-year survey of several public access watersheds in a major leafy green production region of Central Coastal California to determine the prevalence of foodborne pathogens, including *Salmonella enterica*. The watershed is never used for field irrigation directly, but is available to wildlife, and serves as an indicator of the levels of these foodborne pathogens in the environment.At least twice monthly Moore swab samples were collected from lakes, streams, rivers, and ponds for up to 24 hours to allow sampling of flowing water and sediment over an extended period of time. Sampling was done at 30 sites within a 500 square mile region of the Salinas Valley. Following enrichment and selection on XLD and MSRV media, *Salmonella*-presumptive colonies were confirmed by PCR. Subtyping analysis consists of rep-PCR, Pulsed-field gel electrophoresis (PFGE), and serotype analysisAfter 4 years and analysis of over 2500 samples, overall *Salmonella* prevalence was 56% among 5 interconnected watersheds, and statistical analysis indicated no significant differences between watersheds or the season. To date 1567 individual isolates of *Salmonella* are being analyzed for serotype. Over 50 different serotypes have been identified, and the top 3 are monophasic 6,8:d:-, followed by Give, and Typhimurium. Other serotypes of note that have been identified with regularity were Oranienburg, Infantis, Enteritidis, and Heidelberg. Some serotypes were localized to certain watersheds, and others were distributed among the whole region. Comparisons of PFGE profiles of *Salmonella* isolates isolated over 7 years of surveys indicates that some strains persist in the region, possibly cycling between water and wildlife. Comparison of our results with those from studies of *Salmonella* serotype distribution in other regions of the United States indicates that there may be regional biases in serotype.

Author Disclosure Block:

L. Gorski: None. **A.S. Liang:** None. **S. Walker:** None. **K.F. Romanolo:** None.

Poster Board Number:

SUNDAY-050

Publishing Title:

Agricultural Soil Management Effect on Plant Growth Promoting Rhizobacteria Diversity

Author Block:

B. Lee, A. Caldwell, C. Ouverney; San Jose State Univ., San Jose, CA

Abstract Body:

Substituting agrochemicals with beneficial microbes, such as plant growth promoting rhizobacteria (PGPR), is a promising strategy to fertilize soils and mitigate the detrimental effects of agricultural intensification. PGPR have intrinsic properties, such as promoting phosphate (P) solubilization that can be utilized in PGPR poor systems to improve plant growth. Culture-based studies of PGPR provide a limited understanding of the complex interactions within the rhizosphere, regions near plant roots. We implemented culture-independent techniques to survey the bacterial diversity and to identify key PGPR groups in the rhizosphere of coffee plants grown under different agricultural practices. We were interested in bacterial groups associated with P cycling as a source of novel bio-inoculant candidates. Genomic DNA was extracted from 90 soil samples collected from three coffee farms, in Brazil, with distinct farming practices: Intensive, transitional, and organic. Amplicons of the V4 hypervariable region of the 16S rRNA marker gene were generated by Illumina sequencing. After performing quality control on the sequences with Trimmomatic, millions of pair-ended reads were further analyzed with the mothur pipeline and visualized with R. The bacterial consortia statistically differed in each farm based on the Analysis of Molecular Variance of the Operational Taxonomic Units (OTUs) clustered at 97% similarity ($p<0.001$). Of the OTUs discovered in the coffee rhizosphere, 3% of them were unique to each coffee farm. These discriminatory sequences could serve as signature identifiers of agricultural practices. *Arthrobacter*, *Bacillus*, *Bradyrhizobium*, and *Pseudomonas* were the dominating P-solubilizing genera identified in the coffee farms. *Bradyrhizobium* positively correlated with organic and transitional practices, while *Bacillus* and *Pseudomonas* positively correlated with intensive practices. Most of the bacteria in the three farms are not yet characterized. Our findings indicate that agricultural practices have a significant influence on the bacterial diversity in the rhizosphere. Furthermore, novel bacteria identified from the farms could serve as potential PGPR inoculants to enhance P utilization efficiency, improving the sustainability of high agricultural demands.

Author Disclosure Block:

B. Lee: None. **A. Caldwell:** None. **C. Ouverney:** None.

Poster Board Number:

SUNDAY-052

Publishing Title:

N_2O Consumption by G*emmatimonas aurantiaca* under Aerobic and Anaerobic Culture Conditions

Author Block:

R. A. Sanford[1], J. Chee-Sanford[2]; [1]Univ. of Illinois Urbana-Champaign, Champaign, IL, [2]USDA-ARS, Urbana, IL

Abstract Body:

Bacteria affiliated with the phylum Gemmatimonadetes are abundant in many soil microbial communities. In addition, soil metagenomes have revealed that the nitrous oxide reductase (*nosZ*) gene associated with this group is also common in soils, which is of interest since N_2O is a greenhouse gas. NosZ catalyzes the terminal step in denitrification. The only cultured representative of this group, *Gemmatimonas aurantiaca*, has never been reported to grow anaerobically with N_2O as the electron acceptor or under any denitrifying growth conditions even though it possesses a *nosZ* gene. Our experimental objective was to evaluate this organism's ability to use N_2O as an electron acceptor for growth. The ability to denitrify using nitrate or nitrite was also tested even though no nitrate reductase gene has been identified in the genome. Cultures started under anaerobic conditions on nitrate, nitrite or N_2O failed to grow or show depletion of these substrates. Nitrate and nitrite also failed to be used even when cells were grown aerobically with the O_2 allowed to deplete first. N_2O reduction only commenced in the presence of O_2 and continued to be depleted when refed to the culture. *G. aurantiaca* cells grown aerobically with N_2O were diluted by 50% into fresh anaerobic, microaerobic (5% O_2) or fully aerobic medium containing 1% N_2O. After 48h, 36.8%, 30.5%, and 15.4 % of the N_2O added was reduced in anaerobic, microaerobic and aerobic cultures, respectively. After 7 days, the microaerobic N_2O reduction reached 81.6%, slightly greater than that observed anaerobically (79.3%) even though O_2 was still detected in the headspace. Controls showed no loss of N_2O. CO_2 generation occurred under all conditions. Results show that *G. aurantiaca* does use N_2O as an e- acceptor, however it does not grow as a typical denitrifier. Evidence also suggests that this aerobic organism may reduce N_2O even in the presence of O_2 and that only under high O_2 conditions is the N_2O reduction rate reduced. Our results demonstrate that N_2O consumption may be mediated in soil by members of the Gemmatimonadetes, incapable of typical denitrification, but representing a potential sink for this greenhouse gas.

Author Disclosure Block:

R.A. Sanford: None. **J. Chee-Sanford:** None.

Poster Board Number:

SUNDAY-053

Publishing Title:

Farming Systems Modify the Impact of Inoculum on Soil Microbial Diversity

Author Block:

S. L. Ishaq[1], S. P. Johnson[1], Z. J. Miller[2], E. A. Lehnhoff[3], C. J. Yeoman[1], F. D. Menellad[1]; [1]Montana State Univ., Bozeman, MT, [2]Montana State Univ., Conrad, MT, [3]New Mexico State Univ., Las Cruces, NM

Abstract Body:

Background: This study hypothesized that bacterial diversity in soil growing redroot pigweed (*Amaranthus retroflexus* L.) or wild oat (*Avena fatua* L.) would differ using soil inoculum from organic or conventional farms. **Methods:** Eight paired USDA-certified organic (tillage-intensive diverse crop rotation) and conventional (no-tillage wheat-fallow rotation) farms in north-central Montana, growing wheat or *Triticum turanicum* Jakubz. (var. Kamut), a close relative, had soil sampled and used as living or sterile (autoclaved for two 1 hr sessions 24 hr apart) inoculant into potting soil to examine biotic influences. *A. retroflexus* or *A. fatua* was planted and grown for 16 wk, after which the 16S rRNA gene V3-V4 region was sequenced by Illumina MiSeq, and analyzed using PANDAseq, MOTHUR, R, and PRIMER-E. **Results:** Treatments clustered (AMOVA, ANOSIM, UniFrac, $P < 0.05$), with living/sterile inoculum being the largest delineating factor (ANOSIM $R = 0.492$). Living was more diverse than sterile (Shannon, Inverse Simpson, $P < 0.05$), shared the most taxa within the treatment, and had more discriminant taxa ($P < 0.05$). Organic had more diversity (Shannon, $P < 0.05$) and discriminant taxa ($P < 0.05$) than conventional. The most abundant genus, of 854 found, was *Arthrobacter* (up to 13% abundance). Proteobacteria was most abundant (35 - 50% abundance), followed by Actinobacteria (11 - 24% abund.). Farm pair 1 (organic and conventional) with sterile inoculum and *A. fatua* had more Firmicutes (19.7% and 22.6% abund., respectively) than other samples (2.3 - 6.9% abund.). Living soil had more ($P < 0.05$) Chloroflexi, unclassified bacteria, and especially Acidobacteria (mean 7.2% versus sterile mean 3.8% abund.), and sterile had more Bacteroidetes, Firmicutes, Gemmatimonadetes, and especially Verrucomicrobia (mean 3.9% versus living mean 1.5% abund.). Cyanobacteria were higher in soil from three organic farms growing *A. retroflexus* for living (9.8 - 11.6% abund.) and sterile soil (8.9 - 10.5% abund.), than other samples (1 - 7.7% abundance). **Conclusions:** Living organic soil inoculum increased diversity and selected for certain bacteria, consistent with previous studies. A complementary study indicated that diverse soil microbiota is associated with increased crop-weed competitive ability.

Author Disclosure Block:

S.L. Ishaq: None. **S.P. Johnson:** None. **Z.J. Miller:** None. **E.A. Lehnhoff:** None. **C.J. Yeoman:** None. **F.D. Menellad:** None.

Poster Board Number:

SUNDAY-054

Publishing Title:

Microbial Activity of Cemetery and Agricultural Soils on Maryland's Eastern Shore

Author Block:

E. A. B. Emmert, C. H. Briand, A. Hawkins, G. Seho-Ahiable, A. Evans, K. Baker, A. Mrozinski, S. Geleta; Salisbury Univ., Salisbury, MD

Abstract Body:

Anthropogenic activities on the Eastern Shore of Maryland have caused soil erosion and degradation due to deforestation, intensive agriculture, and poor soil conservation practices. Geleta et al (2014) compared the physical and chemical properties of agricultural soil to that of soil in small, undisturbed family cemeteries on Maryland's Eastern Shore. Farm fields surrounding the cemeteries were eroded with the cemeteries as elevated regions within the fields. Agricultural fields contained lower organic matter, carbon, and total nitrogen compared to the cemetery soils. Since microbes are essential for maintaining soil fertility, we examined the microbial activity in these soils to determine the effect of human activity and agriculture on soil health. We sampled seven sites in Wicomico County, Maryland containing a family cemetery and a surrounding agricultural field (5 sites) or suburban lawn (2 sites). We collected soil monthly for a year from both the cemeteries and adjacent fields or lawns and measured microbial activity in all soils via fluorescein diacetate hydrolysis (FDA) and dehydrogenase activity. FDA results were more consistent among sites compared to dehydrogenase activity. Of the 83 soil sample pairs across all 12 sampling dates and 7 sites, 57% exhibited significantly greater ($P \leq 0.05$) FDA hydrolysis in the cemetery soil compared to the agricultural or lawn soil. However dehydrogenase activity was only significantly higher in cemetery soils for 27% of the soil sample pairs. Much less frequently did the agricultural or lawn soil have significantly higher microbial activity compared to the cemetery soil (10% of sample pairs for FDA and 7% for dehydrogenase). Both measurements for soil microbial activity were highest in the spring and lowest in late summer at all seven sites. Based on the more consistent measurement of FDA hydrolysis, microbial activity is greater in cemetery soils, supporting the earlier work of Geleta et al, and providing additional evidence to suggest human activity has greatly altered the physical, chemical, and biological properties of these soils.

Author Disclosure Block:

E.A.B. Emmert: None. **C.H. Briand:** None. **A. Hawkins:** None. **G. Seho-Ahiable:** None. **A. Evans:** None. **K. Baker:** None. **A. Mrozinski:** None. **S. Geleta:** None.

Poster Board Number:

SUNDAY-055

Publishing Title:

Impact of Winter Cover Crops on Microbial Soil Activities in Tillage and no Tillage Soybean Fields

Author Block:

H. L. TYLER; USDA-ARS, Stoneville, MS

Abstract Body:

Conventional agriculture has negative effects on soil quality and several conservation practices have been implemented to alleviate these effects. The practices of no tillage and winter cover crops can improve soil structure and lead to the accumulation of plant residues in soil. Agricultural management practices impact soil microbial communities, which play an important role in nutrient mineralization and organic matter turnover. The effect of different tillage and cover crop treatments on soil microbial activity in soybean (*Glycine max* L.) fields was examined in a study conducted in Stoneville, Mississippi. The experiment was set up as a randomized block with four blocks of tillage or no tillage plots, each planted with winter cover crops of elbon rye (*Secale cereal* L.), crimson clover (*Trifolium incarnatum* L.), or no cover crop. Soil samples were collected prior to cover crop planting in the fall, at spring soybean planting, and once a month over the course of the summer growing season. All samples were assayed for the activities of enzymes linked to organic matter processing (cellobiohydrolase and β-glucosidase), nutrient mineralization (phosphatase and N-acetylglucosaminidase [NAGase]), and general microbial activity (fluorescein diacetate [FDA] hydrolysis). Differences between treatments varied over the course of the growing season, but with the exception of phosphatase and FDA, cover crops had a greater impact on enzyme activities than tillage. All enzyme activities assayed were significantly higher in rye than non-cover crop plots for most of the growing season ($p<0.05$), up to 84 and 87% in the cases of cellobiohydrolase and NAGase. With crimson clover, NAGase, phosphatase, and FDA hydrolysis were higher than non-cover crop plots ($p<0.05$), but tended to be lower than rye plots, increasing activity by no more than 50%. These results indicate the organic matter deposited by cover crops, particularly rye, fuels microbial organic matter turnover and nutrient cycling over the entire soybean growing season. This enhancement of microbial activity can promote soil fertility and could potentially minimize the need for fertilizer application. Future work will examine microbial activity and community composition, as well as soil nutrient levels, over successive growing seasons to gain a better understanding of how cover crops and no tillage enhance soil biological quality.

Author Disclosure Block:

H.L. Tyler: None.

Poster Board Number:

SUNDAY-056

Publishing Title:

Differential Responses in Nitrogen Cycling Processes By Soil Microorganisms Exposed to Diurnal- versus Constant Temperature Conditions

Author Block:

J. C. Chee Sanford[1], L. Connor[1], E. Claussen[2], J. Marqui[2], R. Sanford[2]; [1]USDA-ARS, Urbana, IL, [2]Univ. of Illinois, Urbana, IL

Abstract Body:

The recurring spatiotemporal patterns observed in agricultural soil microbial communities largely occur due to legacy effects from long-term responses to annual climate cycles and cropping systems. Temperature (T) effects on soil microbes are well known but often observed using a fixed mean T, yet large diurnal swings occur seasonally, especially in surface (0-5cm) soils. Here, we looked closely at how soil diurnal T influences microbial N- and C- cycling and related gene expression. Triplicate microcosms with soils taken at 0-5 cm and 20-30 cm from an agricultural field (Havana, IL) were amended with urea-ammonium-nitrate and incubated under a 24h T regimen typical of June diurnal cycles; 18.9°C-37°C (large T flux, mean 25°C, 0-5 cm depth) and 23°-25°C (near constant T flux, mean 24°C, 20-30 cm depth). Unamended controls (No N added) were set up in parallel. N-compounds and CO_2 were measured over 21d along with community T-RFLP profiling and qPCR of 16S rRNA-, *nosZ, amoA, nrfA* genes (DNA) and gene transcripts (cDNA). The net cumulative N_2O concentrations were highest in the 0-5 cm soil under large diurnal T flux throughout the 21d with the highest net N_2O production rate occurring after 24h (76 nmol/d), greater than any other soil treatment. Net cumulative N_2O in the 0-5 cm soil under near constant T were next highest and were always greater than any 20-30 cm soil microcosm at any time. Net CO_2 production was highest in the 0-5 cm soils, however a greater flux was observed with the 18°C diurnal T change, 1250- vs 1131 nmol CO_2 after 21d, respectively. Measured processes were similar in the 20-30 cm soils regardless of T. Microbial communities showed distinctions at soil depths, but shifted accordingly with activity measurements upon exposure to treatments. Collectively, the data suggests surface soils harbored populations that were differentially active in response to T and distinct from those at the 20-30 cm soil depth, the latter exhibiting similar characteristics regardless of temperature. Overall, we showed significant differences in how microbial communities can respond to natural diurnal temperature conditions in contrast to fixed temperatures, with impact particularly attributed to surface soils on gaseous N-losses and greenhouse gas emissions.

Author Disclosure Block:

J.C. Chee Sanford: None. **L. Connor:** None. **E. Claussen:** None. **J. Marqui:** None. **R. Sanford:** None.

Poster Board Number:

SUNDAY-058

Publishing Title:

Ancient Gut Resistomes are Reservoirs of Antimicrobial Resistance Genes

Author Block:

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Abstract Body:

Background: Up to 50% of antimicrobials in the USA are inappropriately prescribed, leading to antimicrobial-resistant (AMR) bacteria, a public health hazard. Also, antimicrobials in animal feed as growth promoters exacerbate this problem. Recent data provide insights into ancient human gut microbiomes as reservoirs of AMR genes and represent an opportunity to study the evolution of AMR genes prior to the antimicrobial era.**Materials and Methods:** Metagenomic analyses were conducted to identify sequences homologous to antibiotic-resistance genes in seven mummified human gut remains. DNA was sequenced using an Illumina MiSeq and the pair-ends joined using QIIME 1.9. Contigs were assembled using the CLC software and BLASTx carried out against the CARD database. The results were parsed based on the type of antibiotic.**Results:** AMR classes identified included β-lactamases, mutated penicillin-binding proteins (PBPs), multidrug transporters and resistance to quinolones, tetracyclines and vancomycin. Several taxa, including genera in the Enterobacteriaceae and Clostridiaceae carried the *mutS* and *mutL*, which mediate mutator phenotypes. Categories associated with β-lactamases, resistance to tetracycline and vancomycin were the most abundant. Four classes of β-lactamases were identified including Class A, C and D, and metallo-β-lactamases, with the Proteobacteria as the major reservoir. 30 different tetracycline-resistance determinants, including efflux pump proteins, ribosomal protection and inactivating enzymes were present. Firmicutes, Proteobacteria, Actinobacteria and Bacteroidetes were the primary reservoirs. Vancomycin-resistance genes associated with the VanA, VanB, VanC and VanD phenotypes were identified, as well as genes of the vancomycin-resistance operon, including *vanB, vanR, vanH* and *vanS*. *Enterococcus faecalis* and *E. faecium* were the primary reservoirs of resistance genes in the mummified gut remains.**Conclusions:** This study suggests that antibiotic-resistance may not necessarily be solely associated with antibiotic misuse and predates antibiotic therapy, and that the gut microbiome can serve as reservoirs for AMR genes in humans.

Author Disclosure Block:

T. Santiago-Rodriguez: None. **J. Pace:** D. Employee; Self; ATCC-CTM @ ILSE. **G. Fornaciari:** None. **S. Luciani:** None. **G.A. Toranzos:** None. **R.J. Cano:** D. Employee; Self; ATCC-CTM @ ILSE.

Poster Board Number:

SUNDAY-059

Publishing Title:

Profiling of Gut Microbiota and Antibiotic Resistant Bacteria in Zoo Animals

Author Block:

Y. Li, L. Zhang, H. Wang; The Ohio State Univ., Columbus, OH

Abstract Body:

Background: Besides antibiotic selective pressure, the host gastrointestinal tract system also has a recognized contribution to antibiotic resistance (AR) ecology.**Methods:** To assess the potential impact of non-food animals and diet on AR ecology, we investigated the prevalence of AR gene reservoir, profile of antibiotic resistant bacteria in zoo animals using metagenomic approach and traditional culture dependent/independent methods. Twenty-seven zoo animal fecal samples were collected from a local zoo from 2013 to 2015. Fecal DNAs were extracted and subjected to 16S metagenomics analysis on an Illumina Miseq. Metagnomic data were processed and analyzed with Mothur (v1.36.1) and further statistical analysis was performed using R (v3.2.3). Meanwhile, representative AR gene reservoirs were examined by Taqman qPCR, and antibiotic resistant bacteria were recovered on Columbia blood agar (CBA), supplemented with antibiotics including tetracycline, erythromycin, or cefotaxime.**Results:** Our data showed that herbivores possess significantly more diversified gastrointestinal microbiota compared to carnivores, with 106 ± 25 to 63 ± 18 OTUs (p=0.015), respectively. PCA analysis and cluster analysis with Ward's Method showed that gut microbiota of herbivores and carnivores clustered to distinct groups. In addition, antibiotic resistant bacteria were found prevalent in zoo animal fecal samples, with up to 17% of recovered bacteria resistant to at least one of Tet, Erm or Ctx. Up to 9.0, 8.7, 8.2 $\log_{10}$ copies/g of *tetM*, *ermB*, and bla_{TEM} genes, respectively, were found in the zoo animal fecal samples, though the abundance of the AR gene pools varied significantly among subjects.**Conclusions:** Data from this study illustrated that zoo animal feces also disseminate antibiotic resistant bacteria and AR genes, even though these animals were not exposed to growth promoting antibiotics. Furthermore, there is a clear difference in the profiles of gut microbiota between herbivores and carnivores, which suggests that changing diet may modulate gut health.

Author Disclosure Block:

Y. Li: None. **L. Zhang:** None. **H. Wang:** None.

Poster Board Number:

SUNDAY-061

Publishing Title:

Presence of Class 1 Integron and Multidrug Resistance in *Escherichia coli* Carried by Flies

Author Block:

T. S. Alves[1], M. M. G. Ferraz[1], J. Gurgel[1], G. H. B. Lara[2], M. G. Ribeiro[2], **D. S. Leite**[1];
[1]UNICAMP, Campinas, Brazil, [2]UNESP, Botucatu, Brazil

Abstract Body:

The flies are known for their role as vectors of various pathogens. Furthermore, recently flies have been identified as vehicles of multidrug resistant bacteria. This study aimed to characterize *Escherichia coli* isolates from flies regarding the presence of integrons, occurrence of antimicrobial resistance and virulence factors. The strains were obtained from the external surface of flies collected in dairy farm in Brazil and analyzed through antimicrobial susceptibility testing. The antibiotic resistance genes, *intI* gene and variable region 5'CS-3'CS, associated with class 1 integron, and virulence factors related to bovine diseases were investigated by PCR. Integron-positive *E. coli* were subjected to restriction fragment-length polymorphism analysis of the variable region and classified into phylogenetic groups. Statistical analysis was performed using Fisher's exact test. We isolated 135 *E. coli* from 57 flies. From these, 89/135 (65.9%) had at least one resistance gene. The highest relative frequencies were obtained for the genes *gyrA* (49.6%), bla_{TEM} (36.3%), *parC* (28.1%), and *tetA* (14.8%). Multidrug resistance (MDR, resistant to ≥ 3 antimicrobial classes) was observed in 52/89 (58.4%) isolates, and 8/89 (9%) showed class 1 integron. This eight integron-positive strains generated the same pattern in RFLP assay and were allocated in the phylogenetic group B1 (6/8), A (1/8) and D (1/8). In this study, virulence factors *hlyA* (12.4%), STa (5.6%) and *stx1* (7.9%) were associated with the absence of multidrug resistance, whereas, *eae*, K99 (F5) and LTII no showed any association. Our results indicate that flies can carry multiresistant non-pathogenic strains which contain mobile genetic elements and thus it can constitute an essential element in the spread of antimicrobial resistance in the environment and to animals of farms.

Author Disclosure Block:

T.S. Alves: None. **M.M.G. Ferraz:** None. **J. Gurgel:** None. **G.H.B. Lara:** None. **M.G. Ribeiro:** None. **D.S. Leite:** None.

Poster Board Number:

SUNDAY-062

Publishing Title:

Metagenomic Survey for Important Reservoirs of Antibiotic Resistance Genes

Author Block:

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Abstract Body:

Background: Use and abuse of antibiotics have increased the abundance of resistance genes in bacteria associated with humans and animals over the last 70 years. It is also possible that use of biocides and metals has contributed to the promotion of antibiotic resistance via co-selection. However, knowledge about the environments outside of the human body that may be important reservoirs of resistance genes (i.e. resistome) is currently scarce, and reliable large-scale quantitative data on the abundance and diversity of resistance genes and taxa is lacking. **Methods**: We screened metagenomes covering external environments (n=358) and animal-associated environments (n=145) from MG-RAST, and human body sites (n=350) from the Human Microbiome Project. We also conducted shotgun metagenomics for 11 samples collected from antibiotic-polluted sites in India. In total, 864 metagenomic Illumina datasets with >10 million reads (9.2 Tb of data) were investigated. Resistance genes were examined in all metagenomes using our recently developed databases of resistance genes to antibiotic (Resqu) and biocide and metals (BacMet) and taxonomic affiliations of 16s rRNA from all metagenomes were assigned by Metaxa2. **Results & conclusions:** We identified potential hotspots for resistance development, such as antibiotic-polluted environments and wastewater treatment plants, where abundance and diversity of resistance genes and mobile genetic elements (MGEs) were high. Human microbiota carried a comparatively much lower number of MGEs than do all other investigated animal and external environments, indicating a higher potential of external environments for horizontal gene transfer via MGEs. Resistance profiles of different environments clustered by habitat, as did taxonomic profiles. Most external environments hosted a larger diversity of taxa than the human microbiota, suggesting that the resistome not yet encountered in pathogens is large. Surprisingly, high numbers of resistance genes and MGEs were detected in Beijing smog, suggesting that air pollution may be important for resistance transmission. **Significance:** These results expand our knowledge of human, animal and external environmental resistomes and can aid in identifying risk environments, thereby providing guidance on risk-reducing actions.

Author Disclosure Block:

C. Pal: None. **J. Bengtsson-Palme:** None. **E. Kristiansson:** None. **D. Larsson:** None.

Poster Board Number:

SUNDAY-063

Publishing Title:

Antibiotic Resistance Profiles Derived from Metagenome Sequencing Data are Highly Dependent on the Database Used for Analysis

Author Block:

E. Gómez-Sanz[1], S. Jaenicke[2], R. Wittwer[3], A. Goesmann[2], M. van der Heijden[3], B. Duffy[1], T. H. Smits[1]; [1]Zurich Univ. for Applied Sci., Waedenswil, Switzerland, [2]Justus-Liebig-Univ., Giessen, Germany, [3]Agroscope, Zürich, Switzerland

Abstract Body:

Here we compared the output of commonly used databases from the analysis of antimicrobial resistance (AMR) in (meta)genome sequencing data from 6 different agricultural soil systems. Four AMR databases were used: MvirDB (Zhou *et al.,* 2006), ARDB (Liu *et al.,* 2009), CARD (McArthur *et al.,* 2013) and ARG-ANNOT (Kumar Gupta *et al.,* 2014). Two soil systems were located at the Swiss alpine Glaspass: one free of and one where manure was applied over 6 centuries. The other 4 systems belong to a Swiss experimental farming trial: organic (manured) and conventional (NPK fertilization) systems ploughed and no tilled. Soil DNA was extracted and sequenced using a single run of Illumina MiSeq per per soil management system. The four databases give a relative low but consistent number of read counts for all six systems, with percentages between 0.011-0.016% (ARDB), 0.005-0.006% (ARG-ANNOT), 0.125-0.151% (CARD), and 1.115-1.694% (MVirDB). However, from the predominant hits per database and per soil, we concluded that i) number and nature of hits differed dependent on the database used, ii) hits to efflux pumps were highly abundant, and iii) resistance to fluoroquinolones, tetracyclines, aminoglycosides, macrolides-lincosamides-streptogramins, and β-lactams were most common. Comparing hits for the different soil systems from each database showed that these were very similar. MVirDB and CARD retrieved hits to a broader dataset, including more general transporters and housekeeping genes, while ARG-ANNOT gives the most specific output based on gene names. On the other hand, the reliability of the hits can be questioned for the used cutoff value in the database search. Although appearing quite widespread, AMR genes are present only at low copy numbers in Swiss soils. TaqMan qPCR assays on shared genes from the database searches are now under development to consolidate the specificity and abundance in soil samples.

Author Disclosure Block:

E. Gómez-Sanz: None. **S. Jaenicke:** None. **R. Wittwer:** None. **A. Goesmann:** None. **M. van der Heijden:** None. **B. Duffy:** None. **T.H. Smits:** None.

Poster Board Number:

SUNDAY-064

Publishing Title:

A Sewage Microbiome is Dominated by *Arcobacter cryaerophilus* that Expresses Multiple Drug Resistance and Virulence Genes

Author Block:

J. A. Millar, R. Raghavan; Portland State Univ., Portland, OR

Abstract Body:

Background: Wastewater treatment plants (WWTP) are good indicators of a community's microbial burden. WWTP are also important hubs for horizontal gene transfer (HGT) of antibiotic resistance genes (ARGs) and virulence factors (VFs). *Arcobacter*, a bacterium commonly found in sewage communities, has become a growing public health concern in the past decade due to its pathogenicity and growing antibiotic resistance. Our study explored the bacterial diversity of a WWTP, and characterized the putative ARGs and VFs present in an *Arcobacter* species that dominates this microbiome.**Methods:**DNA and RNA were extracted from untreated sewage samples from Tucson, AZ over 2 days. Contigs and draft genome were assembled using IDBA and Albertsen *et al.* 2013. Gene expression was measured in CLC. ARGs and VFs were annotated using PHMMER and the ARDB and PATRIC databases. Putative HGT events were detected using HGTector and phylogenetic analyses.**Results:**15 phyla were present and stable across the three samples. We identified 162 ARGs belonging to 60 antibiotic types and 177 VFs belonging to 46 virulence categories. The emerging human pathogen *Arcobacter cryaerophilus* dominated all samples. Its draft genome contained 48 putative ARGs against 27 antibiotic types, dominant expression belonging to Tetracycline. We also found 74 putative VFs belonging to 22 virulence categories, highest expression relating to Adhesion and Invasion.The *A. cryaerophilus* genome also contained genes related to mobile genetic elements (phages, 227; transposases, 58); in sum, about 4% of its genome appears to be horizontally acquired from outside the order of Campylobacterales. Of interest, about 5% of VFs show signs of HGT, mostly relating to Intracellular Survival and Replication.**Discussion:**Our results show that even with the continuous influx of sewage, the microbial diversity in a WWTP remained constant across 25 hours, suggesting that the urban population hosts a fairly stable microbial community. Additionally, high levels of expression of ARGs among the sewage microbes indicate the presence of large number of antibiotics in the urban sewage, highlighting the urgent need to better manage antibiotic use. *A. cryaerophilus*, which contains multiple ARGs, thrive in this environment. As treatment of sewage is not always very effective, this emerging human pathogen could pose a threat to public health.

Author Disclosure Block:

J.A. Millar: None. **R. Raghavan:** None.

Poster Board Number:

SUNDAY-065

Publishing Title:

Barriers to Dissemination of Antibiotic Resistance Genes from Wastewater

Author Block:

J. Z. Kubicek-Sutherland[1], C. Weibel[1], M. O. A. Sommer[2], D. I. Andersson[1]; [1]Uppsala Univ., Uppsala, Sweden, [2]Technical Univ. of Denmark, Hørsholm, Denmark

Abstract Body:

Background: Horizontal gene transfer contributes to the spread of antibiotic resistance among bacterial pathogens. It is thought that wastewater treatment plants (WWTPs) provide an ideal location for the dissemination of resistance genes from environmental reservoirs due to the accidental exposure to pathogenic bacteria in effluent. However, recent metagenomic studies have shown that despite isolating a large number of functional resistance genes in WWTPs, these genes display little to no homology with genes found in pathogens indicating that dissemination of these genes occurs rarely. Here, we investigate some of the possible barriers of dissemination by characterizing the expression of these genes in *Escherichia coli*, a common human pathogen. **Methods:** Resistance genes that were previously isolated from several WWTPs in Denmark were cloned into a single copy vector under the control of an inducible promoter. Assessments of resistance and bacterial growth rate were made as a function of the level of induced gene expression.**Results:** A large fraction, 44% (28/64), of the WWTP genes identified by functional screens displayed no resistance compared to wild type when expressed in our system. 19% (12/64) showed intermediated resistance levels (2-5 fold over wild type). 37% (24/64) were highly resistant to antibiotics (over 5-fold) with 16 genes displaying over 50-fold increased resistance. Fitness assays indicate that over 70% of these genes exhibit a growth defect of at least 20% compared to wild type. However, 5 of 16 genes displaying high-level resistance exhibit little to no fitness defect in *E. coli*.**Conclusions:** These results indicate that the majority of resistance genes found in the WWTPs display low levels of resistance and/or a high fitness cost both of which likely circumvent their utility in pathogenic isolates. However, we did identify several genes conferring high-level resistance in *E. coli* with minimal fitness effects, which should be characterized further as these may be of future clinical importance. Understanding the dynamics of horizontal gene transfer between pathogens and their environment will aid in the development strategies to control the emergence of antibiotic resistant pathogens.

Author Disclosure Block:

J.Z. Kubicek-Sutherland: None. **C. Weibel:** None. **M.O.A. Sommer:** None. **D.I. Andersson:** None.

Poster Board Number:

SUNDAY-066

Publishing Title:

High Diversity Of Environmental A*cinetobacter S*pecies Recovered from Mexican Rivers Uncovered with A Novel MLSA Scheme Reveals Contrasting Patterns Of Antimicrobial Resistance And Mobile Genetic Elements

Author Block:

L. Ochoa Sánchez, J. Rivera, P. Vinuesa Fleischmann; Univ. Natl. Autónoma de Mèxico-CCG, Cuernavaca, Morelos, Mexico

Abstract Body:

Background: *Acinetobacter* is an important genus because some species cause extremely serious problems in hospitals due to antimicrobial and antiseptic resistance. Little is currently known about the genetic diversity and distribution of the genus in natural environments. There isn't an MLSA scheme available for this genus. An MLST scheme is available only for *A. baumanni*. Here we report an MLSA study of environmental *Acinetobacter* strains isolated from rivers and sediments with contrasting levels of contamination.**Methods:** *Acinetobacter* strains were isolated on different media supplemented or not with antibiotics (ceftazidime, cefotaxime, imipenem, ciprofloxacin), and their resistance profiles against 11 antibiotics in five families were determined. Sampling of the water column and sediments of two heavily contaminated and two conserved rivers was performed in the state of Morelos (Central Mexico). The core genome of the genus was computed with GET_HOMOLOGUES using all available genome sequences. Six loci were selected to design PCR primers using Primers4clades. ML phylogenies were inferred using PhyML3. Statistical analyses were performed in R. Profile of plasmid were visualized using Kaisser's method.**Results:**All Acinetobacter strains (n=35), reported here in were MDR and were classified in 8 known and 3 possibly new species. The mean number of resistances was not significantly higher (chi-squared $p < 0.17$, df=1) in strains recovered from contaminated rivers. However, the number of plasmids was significantly higher in those rivers.**Conclusions:** A high diversity of MDR environmental Acinetobacter species (11 out of 35 isolates) diversity was recovered and robustly classified with the new MLSA scheme. Isolates from contaminated sites displayed a significantly higher number of plasmids.

Author Disclosure Block:

L. Ochoa Sánchez: None. **J. Rivera:** None. **P. Vinuesa Fleischmann:** None.

Poster Board Number:

SUNDAY-067

Publishing Title:

Multiple Genes Encoding Resistance to Human Clinical Antibiotics Identified on Captured Stream Plasmids

Author Block:

K. G. Libuit[1], C. Kapsak[1], E. Gehr[1], S. Turner[2], J. Herrick[1]; [1]James Madison Univesity, Harrisonburg, VA, [2]Univ. of Virginia, Charlottesville, VA

Abstract Body:

Background: Plasmids in agriculturally-impacted bodies of water may play a significant role in the dissemination of antibiotic resistance (AR). High bacterial loads in stream sediment and selective pressures introduced by agricultural practices may facilitate the exchange and recombination of genetic material, creating reservoirs of AR genes that can potentially be accessed by fecal and other animal and human pathogens. **Methods:** Transmissible plasmids were captured "exogenously" from stream sediment samples by conjugating sediment cells with a rifampicin-resistant strain of *Escherichia coli*. Transconjugants were selected on tetracycline- and rifampicin-amended medium. Plasmids were isolated from transconjugants using an alkaline-based plasmid prep developed in our lab, electroporated into an electrocompetent *E. coli* strain, and tested for decreased antibiotic susceptibility, relative to the un-electroporated strain, using a modified Stokes disk diffusion method. Plasmid pEx1-20 was sequenced on the Oxford Nanopore MinION and Ion Torrent PGM DNA sequencers. MinION reads were assembled using PBcR, polished with PGM reads using Pilon, typed using PlasmidFinder and annotated using both automated (Prokka) and manual approaches.**Results:** Twenty-three of thirty captured plasmids conferred decreased susceptibility to multiple antibiotics in addition to tetracycline. One plasmid, pEx1-20, conferred resistance to tet, kan, tic, pip, and cip. MinION and PGM sequencing, assembly, and polishing of pEx1-20 resulted in a single, 90kb contig. The web tool PlasmidFinder was used to classify pEx1-20 into the incompatibility (inc) group IncP1-β, a group of plasmids capable of transfer and replication in nearly all Gram-negative bacteria. Automated and manual annotation revealed multiple AR genes (*tetA, tetR, tetG, aadA9, sul1, floR*, and *pse-4*) clustered on transposable genetic elements (transposons and integrons) throughout the plasmid genome.**Conclusions:** Captured stream plasmids harbor a surprising suite of linked genes encoding resistance to multiple late-generation clinical antibiotics. The presence of these AR genes suggests that there may be a significant reservoir of AR genes in streams capable of transmission to human and animal bacterial pathogens and commensals.

Author Disclosure Block:

K.G. Libuit: None. **C. Kapsak:** None. **E. Gehr:** None. **S. Turner:** None. **J. Herrick:** None.

Poster Board Number:

SUNDAY-068

Publishing Title:

Antibiotic Resistance Scenario in Bangladesh; Mechanism and Ecological Impact

Author Block:

M. Sultana[1], S. Bashar[1], Y. Ahmed[1], T. A. Siddiquee[2], M. Khan[2], M. A. Hossain[1]; [1]Univ. of Dhaka, Dhaka, Bangladesh, [2]BCSIR, Dhaka, Bangladesh

Abstract Body:

Clinical overuse or misuse of antibiotics, inappropriate clinical liquid waste (CLW) management and it's discharge to ecological water (EW), and mismanagement of clinical patients create antibiotic resistance situation alarming in developing countries; and this antibiotic resistance markers may spread beyond the boarder. Here we report antibiotics and resistome pollution, mechanism of resistance harborage and its spreading scenario in Bangladesh.Active antibiotics in CLW, admixing point of EW (Buriganga River) and sediment were analyzed using Liquid Chromatography Mass Spectrometry (LCMS/MS) after solid phase extraction. Resistome marker integron class 1 and resistant genes were analyzed using PCR in total DNA isolated from CLW, EW and from phenotypically screened MDR bacteria.CLW and admixing point of Buriganga river (10 m down) contained Ciprofloxacin, Cloxacillin, Amoxicillin and Tetracycline at sub-MIC level. Accumulation of antibiotics at sediment soil (per gm) around admixing point amounted high Ciprofloxacin (4.255 ng), Cloxacillin (4.365 ng) and Tetracycline (5.55 ng). CLW is also loaded with MDR bacteria ($1x10^5$~$1.2x10^7$cfu/ml) and resistant gene pool *qnr*S, bla_{CTX-M} and integron class 1. About 47.13% isolates showed MDR properties among which 31.2% were ESBL-positive harboring specific genes (bla_{CTX-M}, bla_{TEM} and bla_{SHV}). On the other hand, 96.7% ESBL isolates showed resistance towards Ciprofloxacin with harborage of *qnr*S gene in plasmid and/or the presence of *acrA*, *acr*B and tolC genes encoding AcrAB-TolC efflux pump. Imipenem resistance was screened among 434 clinical isolates of which 16.8% showed Imipenem resistance with MIC_{IMP} value as high as 256 µg/ml. The carbapenem resistant bla_{VIM-2} gene was detected to be encoded in chromosomally harbored Integron class 1 associated gene cassette in resistant *Pseudomonas stutzeri*.Antibiotics and resistome pollution is increasing alarmingly in Bangladesh and may spread beyond its origin to environments. Selective pressure of antibiotics in EWB and mixing of autochonous species with allochthonous may influence horizontal gene transfer (HGT) resulting origin of new species of clinical importance.

Author Disclosure Block:

M. Sultana: None. **S. Bashar:** None. **Y. Ahmed:** None. **T.A. Siddiquee:** None. **M. Khan:** None. **M.A. Hossain:** None.

Poster Board Number:

SUNDAY-069

Publishing Title:

Antibiotics and Antibiotic Resistance Genes in Surface Waters Associated with Livestock Operations

Author Block:

C. D. Ecker[1], C. E. Givens[2], J. W. Duris[2], D. W. Kolpin[3], M. J. Focazio[4], M. T. Meyer[5], S. K. Haack[2]; [1]United States Geological Survey, Columbus, OH, [2]United States Geological Survey, Lansing, MI, [3]United States Geological Survey, Iowa City, IA, [4]United States Geological Survey, Reston, VA, [5]United States Geological Survey, Lawrence, KS

Abstract Body:

Antibiotic resistant bacteria have rapidly become the focus of a well-documented global medical challenge. To assess the potential contribution of livestock production to antibiotic resistance genes in the environment, 78 samples (stream water, bed sediment, and manure) were collected from 19 strategically selected sites across 12 states. Fifteen sites were selected at locations with small (<32 km^2) drainage areas with one primary livestock type (poultry, beef, dairy or swine). In addition, four sites were selected as control sites that had similar agricultural land use (e.g. crop production), but had no livestock operations or manure application practices occurring within the drainage area (i.e. rural background sites). At each site, one bed sediment, representative manure and two stream water samples (i.e. before manure application and after manure application/runoff) were collected and analyzed for the following: fecal indicator bacteria (FIB), 33 antibiotics by HPLC/MS-MS, and 9 antibiotic resistance genes. Antibiotic resistance genes analyzed included 6 subfamilies in the β-lactamase *ampC* family and quinolone resistance genes *qnrABS*. There were few detections (5 of 36) of antibiotics in stream water samples. Almost all of stream water samples having an antibiotic detected (4 of 5) were samples collected during runoff conditions. Dairy and poultry sites showed a statistically higher number of detections ($p = 0.03$) for the $ampC_{CIT}$ gene family in runoff water samples as compared to runoff water samples from rural background sites. Other statistically significant ($p < 0.05$) findings included an increase in *qnrABS* genes in poultry runoff, and *ampC* genes in dairy and swine runoff as compared to rural background runoff samples. Overall, livestock operations appear to be contributing more antibiotic resistance genes to stream waters than agricultural practices that do not involve manure application. Identifying hotspots of antibiotic resistance genes and their potential sources is critical in ultimately understanding the spread of antibiotic resistance genes to pathogenic bacteria in the environment.

Author Disclosure Block:

C.D. Ecker: None. **C.E. Givens:** None. **J.W. Duris:** None. **D.W. Kolpin:** None. **M.J. Focazio:** None. **M.T. Meyer:** None. **S.K. Haack:** None.

Poster Board Number:

SUNDAY-070

Publishing Title:

Persistence of DNA Encoding Antibiotic Resistance Genes in Poultry Litter

Author Block:

S. Young[1], B. Nayak[2], J. Weidhaas[3], V. Harwood[1]; [1]Univ. of South Florida, Tampa, FL, [2]Pinellas County, Largo, FL, [3]West Virginia Univ., Morgantown, WV

Abstract Body:

Antibiotic use in animal agriculture such as poultry farming can contribute to the spread of antibiotic resistance through fecal pollution released into the environment. Understanding survival and persistence of antibiotic resistant bacteria and associated resistance genes can help assess the role of the environment in the spread of antibiotic resistance. Fecal indicators *Escherichia coli* and enterococci are commonly used as water quality determinants, but their association with antibiotic resistance genes in poultry waste is unclear. The persistence of antibiotic resistance genes was assessed in fresh and saltwater mesocosms inoculated with fecal-contaminated poultry litter (bedding) over seven days. Mesocosms were inoculated with litter from broiler chickens raised in conventional poultry houses at West Virginia University. Antibiotic resistance genes were chosen for analysis based on their mechanisms of resistance and their use in poultry production. PCR was performed on water and sediment samples to detect genes that carry resistance to β-lactams ($bla_{\text{CMY-2}}$), tetracyclines (*tetB*, *tetM*), quinolones (*qnrS*), erythromycin (*ermB*) and aminoglycosides (*aadA*). Fecal indicators were quantified by culture and qPCR. Antibiotic resistance genes relevant to human health and animal agriculture persisted for up to seven days in water and sediment from both marine and freshwater. Presence or absence of antibiotic resistance genes *tetB*, *tetM* and *ermB* were not significantly correlated to the level of enterococci by culture (p=0.329), *E. coli* by culture (p=0.137) or the poultry marker LA35 by qPCR (p=0.295). Other genes will be tested for correlation to FIB and MST markers to determine whether these can predict the presence of antibiotic resistance genes in the environment. Preliminary results reinforce the need for integrative approaches in assessing antibiotic resistance in environmental matrices. FIB and MST markers are important for regulatory purposes and assessing contamination sources, but may not correlate well with resistance genes. These methods can be used as part of a larger toolkit that includes identification of potential pathogens carrying antibiotic resistance genes.

Author Disclosure Block:

S. Young: None. **B. Nayak:** None. **J. Weidhaas:** None. **V. Harwood:** None.

Poster Board Number:

SUNDAY-072

Publishing Title:

Triclosan Affects Bacterial Community Composition and the Quantities of Antibiotic Resistance Genes in Model Wastewater Treatment Bioreactors

Author Block:

J. W. Beni[1], B. B. McGivern[2], J. J. Donato[2], T. M. LaPara[1]; [1]Univ. of Minnesota, Minneapolis, MN, [2]Univ. of St. Thomas, St. Paul, MN

Abstract Body:

Triclosan is a widely used antimicrobial found in numerous household items from cosmetics and soap to plastics and fabrics. Triclosan is also commonly detected in untreated wastewater where it could act as a selective agent for antibiotic resistance in bioreactors used for treatment. The goal of our research is to understand the effect of triclosan on wastewater microbial communities. Triplicate enrichment cultures were grown in the presence of different concentrations of triclosan (0 to 15 μg/L) for eight weeks. Bacterial community composition was determined by deep sequencing (Illumina MiSeq) of PCR-amplified 16S rRNA gene fragments. Triclosan concentrations were determined using liquid chromatography tandem mass spectrometry (LC-MS/MS). Antibiotic resistance levels were determined by both shotgun metagenomics (Illumina HiSeq) and by microfluidic qPCR targeting more than 25 genes encoding resistance to a myriad of antibiotics. Chemical analysis confirmed a range of triclosan concentrations in the biomass samples. Bacterial community composition exhibited statistically significant differences in response to triclosan concentrations according to an Adonis test of Bray-Curtis dissimilarity matrix. Antibiotic resistance levels exhibited similarly different levels as a function of triclosan concentration. From these results we conclude that triclosan demonstrably and significantly alters microbial community composition and the selection of antibiotic resistance genes.

Author Disclosure Block:

J.W. Beni: None. **B.B. McGivern:** None. **J.J. Donato:** None. **T.M. LaPara:** None.

Poster Board Number:

SUNDAY-073

Publishing Title:

An *In Vitro* Study to Assess the Effects of Tetracycline on the Human Intestinal Microbiota

Author Block:

J. Y. Jung[1], Y. Ahn[1], S. A. Piñeiro[2], C. E. Cerniglia[1]; [1]Natl. Ctr. for Toxicological Res., U.S. FDA, Jefferson, AR, [2]Ctr. for Vet. Med., U.S. FDA, Rockville, MD

Abstract Body:

Background: The human intestinal microbiota community is a stable ecosystem that could be altered by the ingestion of antibiotic residues in or on foods derived from food-producing animals. These potential perturbations are evaluated by national regulatory authorities as part of the safety evaluation of veterinary antimicrobial agents used in food-producing animals. **Methods:** We investigated using an *in vitro* culture test system the effects of 0.15, 1.5, 15, and 150 µg/ml tetracycline on the intestinal microbiota composition, the prevalence of tetracycline resistance genes (TRGs), and fecal binding after 1 (short-term) and 40 (long-term) days of exposure in 3% human fecal suspensions collected from two individuals. **Results:** Binding of tetracycline to fecal suspensions showed that 41.4 ± 14.8% of tetracycline was bound. Bacterial community analysis using rRNA-based pyrosequencing revealed that *Firmicutes* and *Bacteroidetes* were the most dominant phyla in fecal samples; however, interpersonal variation occurred between the two subjects in terms of the ratio of phylotypes. While the impact of tetracycline on intestinal microbiota varied between the two subjects, evaluation of changes at the genus level from control to tetracycline-treated fecal samples showed that a fraction of the members of *Bacteroides* that belong to *Bacteroidetes* increased at low concentrations as well as higher concentrations of tetracycline in both short- and long-term exposure for both subjects. Principal component analysis confirmed the pyrosequencing findings of interpersonal variability of intestinal microbiota and despite that interpersonal variability, one genus of *Bacteroides* was the most affected group of the microbial community. Among the 23 TRGs screened, four *tet* genes (*O*, *Q*, *W*, and *X*) were found as major TRGs in fecal samples. Quantitative real-time PCR showed that *tetX*, which is responsible for inactivating tetracycline, had a high positive relationship with an increase in tetracycline concentration. In addition, *Bacteroides* had positive correlations with the increase of *tetX*. **Conclusions:** Overall, this pilot study indicated interpersonal variability regarding the effects of tetracycline on microbial composition and prevalence of TRGs at various dose levels of tetracycline and times of exposure.

Author Disclosure Block:

J.Y. Jung: None. **Y. Ahn:** None. **S.A. Piñeiro:** None. **C.E. Cerniglia:** None.

Poster Board Number:

SUNDAY-074

Publishing Title:

Widely Used Benzalkonium Chloride Disinfectants Co-select for Bacterial Antibiotic Resistance

Author Block:

M. Kim, S. Oh, M. R. Weigand, J. K. Hatt, R. Krishnan, K. T. Konstantinidis; Georgia Inst. of Technology, Atlanta, GA

Abstract Body:

Background & Materials: Whether disinfectant exposure promotes antibiotic resistance (Ab^R) has been a long-standing debate with major practical consequences. To obtain insights into this issue, we exposed a microbial community originating from a contaminated river sediment (Calcasieu River, LA, USA) to benzalkonium chlorides (BAC; a family of quaternary ammonium compounds widely-used as disinfectants) for 3 years in fed-batch bioreactors. From bioreactors, one with dextrin peptone as the sole carbon source and no BAC present (DP) and one with dextrin peptone plus BAC (DPB), two *Pseudomonas aeruginosa* strains from the same ancestor were isolated and used to study adaptive evolution that occurs during BAC exposure.**Results:** Metagenomics of the bioreactors, coupled with genetic manipulations of derived isolates revealed that BAC exposure induced the spread of Ab^R in several species by selecting for the horizontal transfer of mobile DNA elements that encode a BAC efflux pump together with Ab^R genes. Although several BAC-exposed isolates exhibit higher resistance to certain antibiotics, other isolates did not likely due to their intrinsic resistance mechanisms. To further investigate the underlying molecular mechanisms, the two *P. aeruginosa* isolates were exposed to increasing concentrations of BAC, up to 1640 mg/L, when no growth was observed due presumably to BAC toxicity. Transcriptomics revealed overexpression of several Ab^R genes such as the MexCD-OprJ multidrug efflux genes, encoded in chromosomal DNA of the evolved strain relative to the ancestor in response to BAC presence and also higher MIC values for tetracycline and ciprofloxacin with supplementation of BAC were observed in the BAC-evolved DPB populations.Fixed mutations in the histidine kinase A domain of the *pmrB*, which regulates resistance to polymyxin B, were identified in BAC-evolved DP populations by whole genome sequencing. Consistent with this finding, MIC tests showed higher MIC values for polymyxinB resistance in the BAC-evolved DP populations.**Conclusion:** All together, our results showed that BAC exposure could co-select for increased resistance to antibiotics, and the underlying molecular mechanisms varied based on the genetic make-up of the species considered and the concentration of BACs used.

Author Disclosure Block:

M. Kim: None. **S. Oh:** None. **M.R. Weigand:** None. **J.K. Hatt:** None. **R. Krishnan:** None. **K.T. Konstantinidis:** None.

Poster Board Number:

SUNDAY-075

Publishing Title:

WGS Characterization of *Campylobacter* and *Enterococcus* Mutants from Exposure to Low Concentrations of Erythromycin

Author Block:

Q. Yang, C. Li, K. Domesle, T-T. Tran, **B. Ge**; U.S. Food and Drug Admin., Laurel, MD

Abstract Body:

Macrolides, such as erythromycin and azithromycin, remain the frontline agents for treating human bacterial infections. The mechanisms underlying the development of macrolide resistance in *Campylobacter* and *Enterococcus* are not yet fully understood. In this study, a collection of macrolide-resistant *C. jejuni /C. coli* (*n* = 67) and *E. faecium /E. faecalis* (*n* = 85) mutants selected by exposure to low concentrations of erythromycin was analyzed using WGS and an in-house bioinformatics pipeline for mutations in target genes (23S rRNA, ribosomal proteins, *cmeR*) and the acquisition of new resistance genes (*erm*(B) and others). For *Campylobacter*, no mutations in L4, L22, *cmeR*, or acquisition of new resistance genes were observed in mutants when comparing to corresponding parent strains. Point mutations in the 23S rRNA gene, consisting of A2074C (4/67, 6.0%), A2074G (9/67, 13.4%), and C2627G (37/67, 55.2%), occurred in a total of 50 (74.6%) mutants. Interestingly, no mutants possessed more than one of these mutations and the A2074C (or G) mutations were only observed in *C. coli* mutants. In the majority (11 out of 13) of *C. coli* mutants, mutations at the A2074 position were found in two out of three copies of the 23S rRNA gene except in two cases where the mutation (A2074G) was homozygous. The majority of mutations occurred in mutants that were isolated in later transfers, which also coincided with having an erythromycin MIC greater than 512 µg per ml. To our knowledge, the high prevalence of C2627G mutation occurred in the 23S rRNA gene has not been reported before. For *Enterococcus* mutants, no mutation was found in L16 or acquisition of new resistance genes when comparing to corresponding parent strains. Point mutations in the 23S rRNA gene including A2058, A2059, G2581 and G2535 were not detected either. In summary, WGS is a powerful tool to efficiently identify gene mutations and new resistance genes in a large panel of bacterial strains. Further analysis on mutants lacking point mutations in the 23S rRNA gene is warranted.

Author Disclosure Block:

Q. Yang: None. **C. Li:** None. **K. Domesle:** None. **T. Tran:** None. **B. Ge:** None.

Poster Board Number:

SUNDAY-076

Publishing Title:

Survival of Daptomycin-Resistant MRSA Under Different Conditions

Author Block:

I. Kanesaka[1], S. Fujisaki[1], A. Kanayama[2], A. Kaneko[3], H. Takahashi[2], I. Kobayashi[2]; [1]Toho Univ., Chiba, Japan, [2]Toho Univ., Tokyo, Japan, [3]Tokai Univ., Kanagawa, Japan

Abstract Body:

Background: We have previously reported on the isolation of daptomycin (DAP)-resistant MRSA isolates from patients on daptomycin therapy. As reports of outbreaks due to DAP-resistant isolates are extremely rare, we studied the viability of DAP resistant MRSA in different conditions in order to determine whether DAP resistant organisms are less adapted to survive in the environment compared to DAP sensitive MRSA.**Materials and Methods:** A total of 10 isolates were studied: 4 DAP-resistant MRSA isolates recovered from patients undergoing DAP therapy; 6 DAP-sensitive MRSA isolates, *Staphylococcus aureus* ATCC 25923 and *S. aureus* ATCC 43300. A total of 20 Polypropylene (PP) and Silicone (SI) plates (1 cm^2) were used for adherence of isolates. Each of the 10 PP and 10 SI plates were seeded with 10^5 CFU of the test isolate. After allowing plates to dry, viability at 20 °C was assessed at days 1, 2, 3, 7, 15 and 30 by vigorous mixing to suspend the organisms following by plating for colony counts. Further, Each organism were suspended with distilled water and physiological saline to achieve the desired final inoculum (10^5 CFU/mL) and incubated at 20 °C up to 30 days to assess viability.**Results:** ATCC and DAP-susceptible MRSA isolates were viable for over 15 days. There was no difference in viability of DAP-resistant and DAP-susceptible MRSA applied to PP using a saline suspension; however, DAP-resistant MRSA attached to SI was not detected viability after 2 to 3 days. Cells viability were not observed at 6 days for some isolates in DW. DAP-resistant MRSA isolates with an amino acid substitution at *mprF* and increased cell-wall thickness showed decreased viability over time.**Conclusions:** Our results indicate that DAP-resistant MRSA possessing a *mprF* mutation and increased cell-wall thickness have greater difficult of surviving in the environment which may account for decreased likelihood of outbreaks caused by DAP-resistant MRSA.

Author Disclosure Block:

I. Kanesaka: None. **S. Fujisaki:** None. **A. Kanayama:** None. **A. Kaneko:** None. **H. Takahashi:** None. **I. Kobayashi:** None.

Poster Board Number:

SUNDAY-077

Publishing Title:

Intrinsic Resistance of *Burkholderia cepacia* Complex to Benzalkonium Chloride

Author Block:

Y. AHN[1], J. Kim[1], O. Kweon[1], S-J. Kim[1], R. C. Jones[2], K. Woodling[1], G. G. d. Costa[1], J. J. LiPuma[3], D. Hussong[4], B. S. Marasa[4], C. E. Cerniglia[1]; [1]NCTR / US FDA, Jefferson, AR, [2]MS Bioworks LLC, Ann Arbor, MI, [3]Univ. of Michigan, Ann Arbor, MI, [4]CDER / US FDA, Silver Spring, MD

Abstract Body:

Background: Pharmaceutical products that are contaminated with *B. cepacia* complex (BCC) may pose serious consequences to vulnerable patients. Benzyldimethylalkylammonium chloride (BZK) cationic surfactants are extensively used in medical applications and have been implicated in the co-selection of antimicrobial resistance.**Methods:** The capability of BCC to degrade BZK, tetradecyldimethylbenzylammonium chloride (C_{14}BDMA-Cl), dodecyldimethylbenzylammonium chloride (C_{12}BDMA-Cl), decyldimethylbenzylammonium chloride (C_{10}BDMA-Cl), hexyldimethylbenzylammonium chloride (C_6BDMA-Cl), benzyltrimethylammonium chloride (BTMA-Cl) was determined by incubation in 1/10 diluted Tryptic Soy Broth (TSB) to determine if BCC has the ability to survive and inactivate these disinfectants . For BZK, C_{14}BDMA-Cl, and C_{12}BDMA-Cl, growth inhibition was observed for the 20 BCC strains in disinfectant solutions that ranged from 64 to 256 µg/ml.**Results:** The efflux pump inhibitor carbonyl cyanide-m- chlorophenylhydrazone (CCCP) increased the sensitivity of bacteria to 64 µg/ml of BZK. The 20 BCC isolates grew well in 1/10 diluted TSB medium with BZK, C_{12}BDMA-Cl, and C_{10}BDMA-Cl and degraded these compounds. Formation of benzyldimethylamine (BDMA), and benzylmethylamine (BMA) as initial metabolites suggested that the cleavage of C alkyl-N bond occurred as the first step of BZK degradation by BCC. Proteomic data further confirmed that efflux pump activity and metabolic inactivation are involved in the resistance of BCC to BZK.**Conclusions:** This study provides a systematic methodological approach to determine the intrinsic resistance mechanisms of BCC to BZK.

Author Disclosure Block:

Y. Ahn: None. **J. Kim:** None. **O. Kweon:** None. **S. Kim:** None. **R.C. Jones:** None. **K. Woodling:** None. **G.G.D. Costa:** None. **J.J. LiPuma:** None. **D. Hussong:** None. **B.S. Marasa:** None. **C.E. Cerniglia:** None.

Poster Board Number:

SUNDAY-078

Publishing Title:

Characterization of Plasmids Harboring *bla*$_{VEB}$ in *Vibrio parahaemolyticus* Strains

Author Block:

R. Li[1], L. Ye[2], D. Lin[1], Y. Zhou[1], E. W. Chan[1], S. Chen[1]; [1]The Hong Kong Polytechnic Univ., Hung Hom, Hong Kong, [2]Hong Kong PolyU Shen Zhen Res. Inst., Shenzhen, China

Abstract Body:

Background: *Vibrio parahaemolyticus* is one of the important foodborne pathogens worldwide and causes severe infections, which may need antimicrobial therapy. Extended-spectrum beta-lactamases (ESBLs) producing producing *V. parahaemolyticus* has been reported, while the data is still very limited. In this study, for the first we detected *bla*$_{VEB}$ in *V. parahaemolyticus* isolates and depicted the genetic structure of *bla*$_{VEB}$. **Methods:** *V. parahaemolyticus* were isolated from different food products in Shenzhen, China. Twenty-one ESBL-producing *V. parahaemolyticus* isolates were subjected to MIC determination, screening of resistance genes, conjugation and S1-PFGE characterization. The plasmids encoding *bla*$_{VEB}$ were sequenced by Illumina and Pacbio platforms. Annotation was performed with RAST tool.**Results:** Two *V. parahaemolyticus*(VP72, VP92) were found positive for *bla*$_{VEB}$. S1-PFGE indicated the *bla*$_{VEB}$ genes are located on plasmids (ca. 60K, 320K) and the second plasmid was transferable by conjugation. After sequencing, *bla*$_{VEB-2}$ were positive for VP92. A new *bla*$_{VEB}$ -like variant was found in VP72 and harboring three substitutions (V19A, T104M and N294D) compared with *bla*$_{VEB-1}$. One complete plasmid (Genbank no. KU356480) with 338, 538bp in length was obtained and the core *bla*$_{VEB}$-like gene harboring MDR region was got for another plasmid. Annotations shown that the *bla*$_{VEB-2}$ was located on a class 1 integron (*bla*$_{VEB-2}$- *aadB- arr2- cmlA-bla*$_{OXA-10}$- *aadA1*) and *bla*$_{VEB}$ -like gene was in a genetic environment of *mobA-aac(6')-II-bla*$_{VEB}$-like-*aadB-tnpA*. After BLAST, the *bla*$_{VEB-2}$ genetic environment was identical to that of *bla*$_{VEB-1}$ in *Acinetobacter baumannii*(CU459141). The plasmid backbone of *bla*$_{VEB-2}$ was only similar to a *V. cholera* plasmid pNDM-116-14(99% identity in 78% coverage). *bla*$_{VEB}$-like genetic arrangement was novel and similar *bla*$_{VEB}$-*aadB* sequences were found in NCBI database.**Conclusions:** The genetic structure of *bla*$_{VEB-2}$ indicates this structure may derived from *A. baumannii*, incorporated into a *Vibrios* plasmid and evolved into a *bla*$_{VEB-2}$. *bla*$_{VEB}$-like gene was supposed to originate from integron structure and recombine into new plasmid by *bla*$_{VEB}$-*aadB* circular cassette. The emergence of *bla*$_{VEB}$ in *Vibrios* constitutes a potential public health threat.

Author Disclosure Block:

R. Li: None. **L. Ye:** None. **D. Lin:** None. **Y. Zhou:** None. **E.W. Chan:** None. **S. Chen:** None.

Poster Board Number:

SUNDAY-079

Publishing Title:

Molecular Characterization of the Mechanisms of Carbapenem Resistance in *E. coli* Isolated from Pig in China

Author Block:

D. Lin[1], R. Li[1], M. Xie[2], E. W. Chan[1], S. Chen[1]; [1]PolyU, Hong Kong, China, [2]PolyU Shen Zhen Res. Inst., 南京, China

Abstract Body:

Background: The prevalence of carbapenem-resistance in organisms recovered from animals was reported to be extremely low. This study aims to investigate the prevalence of carbapenem-resistant strains of Enterobacteriaceae in animals and the underlying resistance mechanisms concerned. **Methods:** 220 faecal samples from pigs were tested. 11 carbapenem-resistant E. coli strains were isolated from 5 faecal samples in 1 farm located in Henan province. PCR assay showed that all these strains harboured blaNDM-1. A total of 5 PFGE patterns and 4 ST types were detectable, with identical PFGE and ST types detectable in different samples and organisms of different PFGE and ST types recoverable from the same sample. Conjugation experiments indicated that the carbapenem resistance phenotypes could be transferred to the E. coli J53 strain. All the plasmids belonged to the IncFII type, but two different sizes of plasmids were identified by S1-PFGE. Complete plasmid sequence was obtained for one each of these two different sizes of plasmid, pHNEC55 with size of 81.498kb and pHNEC46 of 74.046kb, using the illumina and SMAT sequencing platforms. These two plasmids were shown to share the similar backbone as a plasmid harbouring multiple resistance genes, namely pHN7A8, which was previously recovered from E. coli isolated from animal in China several years ago. The major structural difference between them was that the blaCTX mobile element fragment in pHN7A8 was replaced by the blaNDM-1 mobile element fragment of pHNEC55. The difference between pHNEC55 and pHNEC46 was that the smaller plasmid, pHNEC46, lacked the fosA3 mobile element fragment. **Conclusions:** The study characterized two new plasmids carrying $bla_{\text{NDM-1}}$ in *E. coli* isolated from pig. The backbone of these two plasmids was different from the common blaNDM-1-borne plasmid recoverable from clinical Enterobacteriaceae strains, but was highly similar to blaCTX-M encoding plasmid isolated from animals, suggesting that these two new plasmids may be formed by inserting the blaNDM-1 mobile element into the common IncFII plasmid in animal *E coli* strains. Successful detection of these plasmids in different *E. coli* strains constitutes evidence of effective transmission of these plasmids among animal bacterial isolates.

Author Disclosure Block:

D. Lin: None. **R. Li:** None. **M. Xie:** None. **E.W. Chan:** None. **S. Chen:** None.

Poster Board Number:

SUNDAY-080

Publishing Title:

***Escherichia coli* Producing CTX-M-Type Extended-Spectrum β-Lactamase (ESBL) Isolated from an Urban River, Drinking Water and Raw Vegetables in Quito, Ecuador**

Author Block:

D. Ortega-Paredes[1], P. Barba[2], N. Espinel[1], S. Mena[1], V. Crespo-Pérez[1], **J. Zurita**[1]; [1]Pontificia Univ. Catolica del Ecuador, Quito, Ecuador, [2]BioMed. Res. Unit. Zurita & Zurita Lab., Quito, Ecuador

Abstract Body:

Background: *Enterobacteriaceae* producing Extended-Spectrum β-lactamases (ESBL) is a global concern. In Latin America, the variant CTX-M-15 is the most frequently reported in clinical settings. In recent years, increasing evidence about food and environmental location of *E. coli* producing ESBL indicate a growing problem of dissemination. This issue compromises food safety and environmental integrity. The aim of this study was to identify and characterizing *E. coli* producing CTX-M-type ESBL in the principal urban river, drinking water and fresh vegetables in Quito-Ecuador.**Methods:** Quito city is located in the Andean Region of Ecuador at an altitude of 2,850 m. Most of the wastewater of the city is discharged in Machángara river. The drinking water collected in the highlands is treated and distributed to city. The fresh vegetables mostly come from the valleys and highlands around Quito. In 2015, nine points were sampled in the river, twenty one samples of drinking water were collected in different points of the city and nine fresh vegetables mostly raw consumed were sampled. *E coli* was isolated using filtering method in ColiBlue24® medium supplemented with cefotaxime 5 µg/ml. Species identification were achieved by MALDI-TOF. ESBLs production was confirmed with Double Disk Test and MIC was made by Vitek2. ESBL genes and integron 1 variable region were amplified and sequenced.**Results:** *E. coli* producing ESBLs were isolated in the points at the river with anthropogenic disturb. Only lettuce and alfalfa were positive for *E. coli* ESBL. All drinking water samples were negative for all *Enterobacteriaceae* studied. CTX-M-15 was the most prevalent ESBL identified in the urban river, followed to CTX-M-18, CTX-M-3, CTX-M-65 and CTX-M-20. Isolates from fresh vegetables presented only CTX-M-15. Variable region of integrons class 1 presented several variants of *dfrA* (trimethoprim resistance) and *aadA* (aminoglycoside resistance) gene cassettes. These findings were related with multi-resistance profile of isolates.**Conclusions:** Our results show that the urban river and fresh vegetables contribute to the presence of multi-drug-resistant *E. coli* in urban environment.

Author Disclosure Block:

D. Ortega-Paredes: None. **P. Barba:** None. **N. Espinel:** None. **S. Mena:** None. **V. Crespo-Pérez:** None. **J. Zurita:** None.

Poster Board Number:

SUNDAY-081

Publishing Title:

Detection of *Klebsiella pneumoniae* Carbapenemase and New Delhi Metallo-Beta-Lactamase Genes in Wastewater from Pretoria, South Africa

Author Block:

R. Dos Santos[1], M. Ehlers[2], M. de Jesus[1], **M. Kock**[2]; [1]Univ. of Pretoria, Pretoria, South Africa, [2]Univ. of Pretoria/Natl. Hlth.Lab. Service, Pretoria, South Africa

Abstract Body:

Bacterial antibiotic resistance is a major threat to human health and is a phenomenon that is on the rise. The aquatic environment, including wastewater, is often regarded as a reservoir for antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs) and is a suitable environment for the transfer of antibiotic resistance determinants. The lack of removal of antibiotic resistance determinants from wastewater treatment plants (WWTPs) may allow its spread into the environment and may negatively impact on treatment of community-associated infections. The aim of this study was to identify beta-lactamase genes, including *Klebsiella pneumoniae* carbapenemase (KPC) and New Delhi metallo-beta-lactamase (NDM) genes from wastewater collected from three WWTPs (in and around the Pretoria/Tshwane area, South Africa) in winter and late spring/early summer in 2013 from the influent and effluent zones. Total genomic DNA was extracted directly from the wastewater using a phenol/chloroform extraction method. Selected beta-lactamase genes, which included Cefotaximase-Munich (CTX-M), Sulfydryl variable (SHV), Oxacillinase-48-(OXA-48)-like, Temionera (TEM), OXA-1-like, KPC and NDM were tested for using molecular methods. The wastewater treatment process of two of the three WWTPs showed promising results in the removal of the targeted β-lactamase genes but the wastewater treatment process of the third WWTP was less efficient as several genes were detected in the effluent. Identifying the KPC and NDM genes within wastewater was a significant find. The identification of the β-lactamase genes over both collection periods highlights that wastewater is a reservoir for β-lactamase genes. If the wastewater treatment process does not remove ARGs, as was the case in one of the WWTPs, the effluent can become a reservoir for the ARGs. This study identified a dissemination route of β-lactamase genes into the environment, which can lead to more resistant bacteria in the community and in turn exacerbate community-associated infections. The possibility of implementing additional treatment of raw sewage before its entrance into WWTPs should also be considered. To our knowledge, this is the first identification of NDM-1 in wastewater in South Africa.

Author Disclosure Block:

R. Dos Santos: None. **M. Ehlers:** None. **M. de Jesus:** None. **M. Kock:** None.

Poster Board Number:

SUNDAY-082

Publishing Title:

Presence Of β-Lactamases In *Acinetobacter baumannii* Isolated from the Antarctic Continent

Author Block:

A. Opazo-Capurro[1], C. Cigarroa[1], A. Fuentealba[1], C. Lima[1], M. Dominguez-Yevenes[1], H. Bello-Toledo[1], L. Vergara[2], **G. Gonzalez-Rocha**[1]; [1]Univ. of Concepcion, Concepcion, Chile, [2]San Sebastian Univ., Concepcion, Chile

Abstract Body:

Background: *A. radioresistens* (Ara) possesses a remarkable ability for surviving in extreme environmental conditions being rarely associated with clinical infections. Despite its low incidence in human infections compared to other members of the *Acinetobacter* genus, it is important to remark its role as a reservoir of antibiotic resistance genes. The carbapenemase-encoding gene *bla*$_{\text{OXA-23-like}}$ has been identified in the chromosome of this bacterium, being a potential source of carbapenems-resistance genes. Due to the above, the aim of this work was to investigate the presence of β-lactams resistance genes in Ara isolates collected in the Antarctic continent. **Methods:** Two Ara isolates, A145 and A154, were collected from soil samples at Ardley Island in the Fildes Peninsula, Antarctica, in 2013. The species identification was achieved by the 16S rRNA gene sequencing. The antibiotic susceptibility patterns to different classes of antibiotics were determined by disk diffusion. The genetic relatedness analysis was carried out by rep-PCR and the presence of β-lactamases genes, such as those encoding ESBLs and carbapenemases, and the presence of the IS*Aba1* element was investigated by standard PCR.**Results:** Both isolates showed identical rep-PCR patterns, being classified as clones. Both isolates resulted resistant to diverse β-lactams, such as ampicillin, cefuroxime, cefotaxime, ceftazidime and cefepime, and susceptible to carbapenems. The *bla*$_{\text{TEM-1}}$ and *bla*$_{\text{PER-2}}$ genes were found in both isolates, which could explain the resistance to aminopenicillins, second- and third-generation cephalosporins. Both isolates were found to carry *bla*$_{\text{OXA-23-like}}$ although none was resistant to imipenem or meropenem. PCR to detect IS*Aba1* upstream to *bla*$_{\text{OXA-23-like}}$ proved negative .**Conclusions:** This work represents the first description of β-lactam resistance genes in Ara from Antarctica, which is a place without the selective antibiotic pressure that occurs in hospitals and which nonetheless represents a potential source of antibiotic-resistance genes. In conclusion, the results reassure the potential role of Ara as a reservoir of antibiotic-resistance genes, which could have an important impact in the nosocomial environment.

Author Disclosure Block:

A. Opazo-Capurro: None. **C. Cigarroa:** None. **A. Fuentealba:** None. **C. Lima:** None. **M. Dominguez-Yevenes:** None. **H. Bello-Toledo:** None. **L. Vergara:** None. **G. Gonzalez-Rocha:** None.

Poster Board Number:

SUNDAY-083

Publishing Title:

Molecular Epidemiology of Carbapenem-Resistant *Burkholderia cepacia* Complex from Pharmaceutical Wastewaters from South-Western Nigeria

Author Block:

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Abstract Body:

The occurrence of antibiotics and other pharmaceuticals in the environment has become an increasing public concern. The emergence of antimicrobial-resistant bacteria presents a major threat to public health because it reduces the effectiveness of antimicrobial treatment, leading to increased morbidity, mortality and healthcare expenditure; therefore we assayed for carbapenemase production in *Burkholderia cepacia* complex isolated from pharmaceutical wastewaters in Nigeria.Untreated Wastewaters from ten pharmaceutical industries located in Nigeria were sampled during April 2011 to June 2012. Gram Negative bacteria were isolated, Gram stain, morphology on blood agar, test on ashdown agar, oxidase test, growth at 42^{o}C and lactose fermentation test were carried out. Identification of isolates and antibiotic susceptibility test on eighteen antibiotics were done with the VITEK 2 machine. The Hodge test was carried out to determine the production of the carbapenemase enzyme. Molecular analysis with *recA* gene and sequencing was carried out to identify the strains. Detection of the presence of carbapenamase genes *VIM, KPC, OXA-48, NDM, IMP* and *GIM* was also determined by PCR.Ninety-seven Gram-negative bacteria were isolated. Two isolates of *Burkholderia cepacia* complex were resistant to imipenem amongst other bacterial isolates; they were also resistant to the cephalosporins and the fluoroquinolones. However the hodge test and the PCR results on the production of the carbapenamase genes were negative for both isolates. Further results with *recA* PCR and sequencing clearly identified the strains as *Burkholderia cepacia* and *Burkholderia cenecepacia*.The results showed that the resistance mechanism of *Burkholderia cepacia and Burkholderia cenocepacia* to imipenem was not the production of carbapenamase enzyme, but likely because of their intrinsic characteristics, mutation, porin loss or efflux pump resulting to their ability to be resistant to imipenem.

Author Disclosure Block:

A.I. Obasi: None. **E.O. Ugoji:** None. **S.C. Nwachukwu:** None. **C. Kohler:** None. **K. Breitbach:** None. **A. Gholer:** None. **V. Balau:** None. **Y. Pfeifer:** None. **I. Steinmetz:** None.

Poster Board Number:

SUNDAY-084

Publishing Title:

Detection of *meca* Gene in *Staphylococcus aur*eus Isolated from Animal and Sea Foods

Author Block:

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Abstract Body:

Background: In recent times, methicillin resistant *Staphylococcus aureus* (MRSA) has become a serious problem and challenge to health care facilities. Rapid and accurate diagnosis is the key factor for the successful therapeutic intervention and to reduce morbidity and mortality due to this pathogen. Molecular techniques are increasingly popular to detect the presence of specific drug resistant genotype in clinical isolates but due to economic and/or diagnostic sensitivity issues, time consuming culture methods are still in practice. With the increased and indiscriminate use of antibiotics in food industry, we hypothesized that there is a high propensity for drug resistant strains among the *S. aureus* animal and sea foods isolates. Simultaneously, we have also planned to screen these isolates for *mecA* gene (confers methicillin resistance) and its use in deducing the drug resistance profile of these isolates in comparison to conventional techniques.**Methods:** A total of 57 samples drawn from meat and sea foods were screened for *S. aureus* using standard microbiological techniques. *S. aureus* food isolates were tested for their antibiotic sensitivity/resistance profile using standard Kirby-Bauer disc diffusion method. These bacterial isolates were also investigated for the presence of *mecA* gene with standard PCR technique. This was also tested for PBP2 protein on SDS-PAGE to confirm the expression profile of the *mecA* gene harbored in these isolates. Attempt was made to understand the diagnostic value of conventional disc diffusion technique, *mecA* PCR and the combination.**Results:** Among the food samples (n=57), 47 (84%) were found positive for *S. aureus*. Of these *S. aureus* food isolates, 32 (68%) were methicillin resistant (MRSA) by disc diffusion technique. Among these MRSA isolates, 96.8% (31/32) were *mecA* positive by PCR. SDS-PAGE confirmed the *mecA* expression profile based on PBP2 detection.**Conclusions:** Presence of MRSA in animal food product is indeed a cause of concern and warrants stringent monitoring. *mecA* PCR is an extremely useful MRSA diagnostic technique with just 6 hour turnaround time. Establishing appropriate laboratory diagnostic guidelines on the use of *mecA* PCR especially in developing and underdeveloped countries may help reduce the MRSA disease burden and transmission.

Author Disclosure Block:

R. Anandan: None. **D. Lakshmipriya:** None. **V. Nithyakalyani:** None. **C. Banurekha:** None. **S. Rajkumar:** None.

Poster Board Number:

SUNDAY-085

Publishing Title:

Specificity of Bacteriophage for Antibiotic-Resistant *Salmonella typhimurium*

Author Block:

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Abstract Body:

Background: The rapid spread of new antibiotic-resistant bacteria has become a global concern because of frequent failure in treatment and delay in antibiotic development. Recently, bacteriophage therapy has received much research attention as a viable alternative to conventional antibiotics. Therefore, this study describes a new effort toward understanding the interaction mechanisms between antibiotic-resistant *Salmonella* Typhimurium and phages.**Methods:** The antibiotic susceptibility, β-lactamase activity, bacterial motility, gene expression, and lytic activity were evaluated in ciprofloxacin-induced antibiotic-sensitive *Salmonella* Typhimurium ($ASST^{CIP}$) and ciprofloxacin-induced antibiotic-resistant *S.* Typhimurium ($ARST^{CIP}$), which were compared to the parent strains ($ASST^{WT}$) and $ARST^{WT}$).**Results:** The multiple resistance to different classes of antibiotics was developed in the $ARST^{CIP}$, showing the increased MIC values of ampicillin (AMP) from 512 to >512 μg/ml, norfloxacin (NOR) from 0.125 to 16 μg/ml, chloramphenicol (CHL) from 4 to 16 μg/ml, and tetracycline (TET) from 128 to 256 μg/ml, respectively. The lowest and highest lactamase activities were observed in $ASST^{WT}$ (6-20 μmol/min/ml) and $ASST^{CIP}$ (46-49 μmol/min/ml), respectively. The inducible β-lactamase activity was more considerably increased in the $ASST^{CIP}$ (49 μmol/min/ml) than $ARST^{CIP}$ (7 μmol/min/ml). The *acrA, lpfE,* and *hilA* genes were significantly upregulated by more than 10-fold in in both $ASST^{CIP}$ and $ARST^{CIP}$. The induction of multiple antibiotic resistance resulted from the increased efflux pump activity (AcrAB-TolC). The highest phage adsorption rates were more than 95% for $ASST^{WT}$, $ASST^{CIP}$, and $ARST^{WT}$, while the lowest adsorption rate was 52% for $ARST^{CIP}$ at 15 min of infection. The least lytic activity of phage was 20% against the $ARST^{CIP}$, followed by $ASST^{CIP}$ (30%). The adsorption rate of phage against $ARST^{CIP}$ was 52% at 15 min of infection, which resulted in the decrease in lytic activity (12%).**Conclusions:** Understanding the interaction of phage and bacteria is essential for the practical application of phage to control and detect antibiotic-resistant bacteria. The results provide useful information for understanding the binding specificity of phages for multiple antibiotic-resistant pathogens.

Author Disclosure Block:

J. Kim: None. **A. Jo:** None. **L. Jung:** None. **J. Ahn:** None.

Poster Board Number:

SUNDAY-086

Publishing Title:

Efficacy of Colistin Combined with Meropenem, Gentamicin or Rifampicin Against Carbapenem-resistant *Pseudomonas aeruginosa* Biofilms

Author Block:

A. Geladari, M. Simitsopoulou, I. Stamouli, C. Antachopoulos, **E. Roilides**; Aristotle Univ. of Thessaloniki, Thessaloniki, Greece

Abstract Body:

Background: While biofilm (BF)-associated infections due to carbapenem-resistant *Pseudomonas aeruginosa* (CR-Pa) are a therapeutic challenge, new antibiotics are lacking. Since colistin monotherapy can lead to emergence of resistance, combination therapies are employed. We investigated the bactericidal activities of colistin (COL), meropenem (MER), gentamicin (GEN) and rifampicin (RIF) in the double combinations of COL-MER, COL-GEN and COL-RIF against CR-Pa mature BF. **Methods:** Four CR-Pa BF-producers, isolated from the sputum of CF patients, were incubated at 5x10^6 CFU/mL in microtiter plates at 37°C for 48h. BF were then incubated with COL, MER, GEN and RIF at 0.007-256mg/L for 24h. Drug combinations of COL (0.06-32mg/L) with MER, GEN or RIF (0.007-64mg/L) at 37°C for 24h were examined using a checkerboard microdilution method. BF damage measured as % reduction of metabolic activity was assessed by XTT assay. The MIC50 for BF was determined as the minimum concentration that caused ≥50% bacterial damage compared to that for the untreated controls. The combination effect was defined according to Bliss model as synergistic, antagonistic or indifferent when the observed BF damage was significantly higher than, lower than or equal to the expected damage, respectively. All the isolates were tested in triplicate.**Results:** All the 4 CR-Pa isolates produced weak biofilm. BF MIC50 of COL, MER, GEN and RIF for the 4 CR-Pa isolates was 64mg/L, 16mg/L, 64mg/L and 16mg/L, respectively. Synergistic activity was found for one isolate at 0.25-2mg/L of COL combined with 0.5-2mg/L of RIF (mean ΔE value of significant interactions, 23% [range, 8% to 38%]; mean SE, 2% [range, 0.09% to 3.76%]). For this isolate antagonistic interactions were observed at 1-2mg/L of COL combined with 4-16mg/L of GEN (mean ΔE value of significant interactions, -15% [range, -2% to -22%]; mean SE, 1.3% [range, 0.4% to 2.5%]). For the remaining three isolates, drug combinations showed indifferent results.**Conclusion:** Although CR-Pa strains were weak BF producers, high BFMIC50 were observed. Interactions of COL with MER, GEN or RIF are mostly indifferent; occasionally COL-RIF combination may be synergistic and COL-GEN antagonistic.

Author Disclosure Block:

A. Geladari: None. **M. Simitsopoulou:** None. **I. Stamouli:** None. **C. Antachopoulos:** None. **E. Roilides:** None.

Poster Board Number:

SUNDAY-088

Publishing Title:

Adherence and Proliferation of *Propionibacterium acnes* on Materials of Orthopaedic Relevance

Author Block:

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Abstract Body:

Background: Wound infections after shoulder surgery substantially affect patient outcomes; deep infections typically result in loss of shoulder strength and range of motion[1]. *Propionibacterium acnes* is a slow-growing, anaerobic-aerotolerant, Gram-Positive bacteria commonly found as part of the natural flora of the large intestine, conjunctiva, oral cavity and in the pilosebacous follicles of the skin[2]. An opportunistic pathogen most notably recognized for its role in acne vulgaris, it is the most common organism to colonize the skin surface around the shoulder. Due to requirement of extended culture of at least 13 days, *P. acnes* is often under diagnosed as a cause of pain, loosening and chronic inflammation. In this study we aimed to develop an understanding of the adherence times required by *P. acnes* on five common implant materials.**Methods:** Five common orthopaedic spinal implant materials: Polyether Ether Ketone (PEEK), Cobalt Chromium, Stainless Steel, Titanium and Titanium Alloy were inoculated with $1x10^7$ CFU/ml *Propionibacterium acnes*. Hours allowed for adherence and proliferation of *P. acnes* were varied over a 24 hour period. Implants were fixed, dehydrated, submitted to critical point dry, sputter coating, and visualized using Scanning Electron Microscopy (SEM). SEM images and data were processed using Microsoft Excel and ImageJ.**Results:** Adherence to PEEK increases significantly over the first 24 hours with respect to the other materials. Preliminary biofilm formation occurs exclusively on PEEK in the first 24 hours of growth with significant formation occurring when adherence allowed is 20 hours. Stainless steel was shown to be the most resistant to adherence of *P. acnes.***Conclusion:** The results observed in this study are consistent with previous studies conducted with other pathogens in our laboratory suggesting rougher surfaces are more permissive for bacterial adherence. The data presented in this study may help surgeons and manufacturers make more informed decisions when designing implants at risk of *P. acnes* colonization.

Author Disclosure Block:

C.K. Mayfield: None. **A. Zega:** None. **D.G. Deckey:** None. **D. Garcia:** C. Consultant; Self; Materials Science Associates, LLC. **A. Green:** A. Board Member; Self; Deputy Editor Journal of Bone and Joint Surgery. C. Consultant; Self; Tornier, Inc. N. Other; Self; Tornier, Inc- Royalties. **J. Jarrell:** D. Employee; Self; BioIntraface, Inc., Materials Science Associates, LLC.. K.

Shareholder (excluding diversitied mutual funds); Self; BioIntraface, Inc.. **R. Hayda:** None. **C. Born:** C. Consultant; Self; BioIntraface, Inc.. K. Shareholder (excluding diversitied mutual funds); Self; BioIntraface, Inc..

Poster Board Number:

SUNDAY-089

Publishing Title:

Characterization of Antimicrobial Peptides for Treatment of *Pseudomonas aeruginosa*

Author Block:

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Abstract Body:

Background: Dental infections including gingivitis and periodontitis are major issues in the military. In deployed military personnel, 12% of dental emergencies involve gingivitis or periodontitis. *Pseudomonas aeruginosa* is one bacteria associated with these infections. Because traditional periodontal treatment is time consuming and repetitive, innovative antimicrobial and antibiofilm compounds need to be discovered for the effective prevention and eradication of bacterial biofilms in dental plaques. Antimicrobial peptides (AMPs) have novel modes of antibacterial activity allowing for effective treatment of biofilm and drug resistant infections.**Objectives:** Screen the antimicrobial activity of AMPs against *P. aeruginosa*, investigate variances in reported minimal inhibitory concentrations (MICs), determine the cytotoxicity of AMPs against human gingival epithelial cells (HGEPs), and measure AMP antibiofilm activity determining minimal biofilm inhibitory concentrations (MBICs).**Materials/Methods:** Two laboratory strains (PAO1 and PA14) and two drug-resistant clinical *P. aeruginosa* strains which were isolated locally, were tested using five AMPs including 1018, KSL-W, K4K20S4, K4S(1-15), and K6L9. MIC values in multiple growth media and the LIVE/DEAD bacterial viability assay were used to assess antimicrobial activity. Measurement of AMP cytotoxicity using lactate dehydrogenase and water soluble tetrazolium cell proliferation assays were performed with HGEPs. MBICs were determined using standardized biofilm assays.**Results:** AMPs possessed a broad range of activity against all *P. aeruginosa* strains, with less effective results in enriched media compared with minimal media. The most effective antimicrobial AMPs were K4K20S4, K4S(1-15) and K6L9, with some activity in 1018 and KSL-W. However, the AMPs rich in lysine residues proved to be cytotoxic to HGEPs, with more tolerance present in KSL-W and 1018. Antibiofilm results for 1018 showed a range of antibiofilm activity from 10-20 µg/ml.**Conclusions:** KSL-W and 1018 appear to be suitable candidates for use against gingival and periodontal pathogens. The peptide 1018 also appeared to have activity against *P. aeruginosa* biofilms. Further testing is underway to identify additional AMPs against periodontal pathogens.

Author Disclosure Block:

M. Kay: None. **J. Titus:** None. **Y. Hwang:** None.

Poster Board Number:

SUNDAY-090

Publishing Title:

Multidrug Resistance and Biofilm Formation of *Staphylococcus aureus* and Coagulase-Negative *Staphylococci* Species Isolated from Clinical Specimens

Author Block:

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Abstract Body:

Staphylococci are ubiquitous skin microbiota, and important nosocomial and community-acquired pathogens, and becoming increasingly resistant to many commonly used antibiotics. Staphylococcal biofilm is one of the virulence factors and resistant to host innate immune response and systemic antimicrobial therapeutic treatment. A total of 28 *Staphylococcus* species isolates from nasal and perirectal swabs of patients were obtained from a tertiary health care facility in Karachi, Pakistan. These isolates were identified as 15 *S. aureus*, 8 *S. sciuri*, 3 *S. hemolyticus*, 1 *S. lentus* and 1 *S. epidermidis*. They were resistant to multiple antibiotics. The highest antibiotic resistances were to lincomycin (85.7%), followed by oxacillin (82.1%), and ciprofloxacin and erythromycin (78.6%), tetracycline and cefazolin (71.4%), gentamicin (67.9%), and kanamycin (53.6%). All isolates were resistant to ampicillin and penicillin while susceptible to vancomycin. The antimicrobial resistance rates varied between *S. aureus* and coagulase-negative *Staphylococci* isolates. The resistance percentage of *S. aureus* isolates was higher than coagulase-negative *Staphylococci* for all the antibiotics tested except for oxacillin and lincomycin. Alpha and beta hemolysins were present in 5 (17.9%) and 10 (35.7%) of isolates, respectively. Both DNase and gelatinase were produced more in *S. aureus* isolates than coagulase-negative *Staphylococci*. By microtiter plate method, we found that most of the isolates were able to form biofilm and two *S. sciuri* (3S and 56S) and one *S. aureus* (41S) were strong biofilm producers. The adhesin genes varied among different strains. The *fnbB* and *fib* genes were only detected in one and three strains, respectively. The detection rates of other adhesin genes were as follows: *eno* (92.9%), *icaA* (82.1%), *cna* and *bap* (75.0%), *bbp* (60.1%), *ebpS* and *clfA* (57.1%), *clfB* (53.6%), and *fnbA* (46.4%). There was no direct correlation in distribution of adhesin genes and biofilm formation. This study indicates multidrug-resistant coagulase-positive and -negative clinical *Staphylococcus* species have the great ability to produce biofilm and virulence potential, and health risks posed by such isolates should not be underestimated.

Author Disclosure Block:

K. Sung: None. **J. Chon:** None. **R. Bensen:** None. **S. Iram:** None. **S. Khan:** None.

Poster Board Number:

SUNDAY-091

Publishing Title:

Identification of Membrane Proteins Related to Quorum Sensing Mechanism of Uropathogenic *E.coli* Se15, a Common Causative Pathogens Indwelling Catheters

Author Block:

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Abstract Body:

The organisms of interest in catheter associated urinary tract infections (CA UTIs) are *E. coli*, *P. aeruginosa* and *S. aureus.* Especially, uropathogenic *E.coli* (UPEC) is a major pathogen which account for more than 80% in UTIs. These bacteria are common causative pathogens which colonize the catheter by quorum sensing (QS) mechanism. In case of pathogenic bacteria, OMPs interact with other bacteria or host and act as adhesions or receptors to facilitate colonization. Consequently, the study of inner and outer membrane proteins (IMPs/OMPs) is essential to understand microbe-microbe interactions and to identify key targets for development of therapeutic agents. Therefore, this study is focused on expressional changes of membrane proteins of uropathogenic *E.*coli. In addition, this study also compares and identify membrane proteins that are differentially expressed under *E.coli* single culture and mixed culture of *E.coli*, *P. aeruginosa* and *S. aureus*. Two-dimensional gel electrophoresis and matrix associated laser desorption ionization-time of flight mass spectrometry analysis was adopted to analyze the membrane proteins involved in quorum sensing mechanisms. It was observed that, in mixed cultures, IMPs/OMPs of uropathogenic *E.coil* showed differential expression of virulence related proteins (ColE, CvaA, FocC, StfD, TomB) and QS related proteins (LsrA, OppD, FbpC, DorA and FabG). Interestingly, the proteins (LsrA, and OppD) which import the QS signal molecules to cytoplasm were increased in the mixed culture conditions than those in the single culture of *E.coli*. And also, the virulence factors related to biofilm formation (TomB) and fimbrial chaperone proteins (StfD and FocC,), were highly expressed in the mixed culture condition in compare to single culture of *E.coli*. Based on this study, it was observed that receptor proteins related to QS and virulence proteins were more affected by mixed cultures than those of the single culture of *E.coli*. Overall, these findings indicate that uropathogenic *E.coli* dominates the interspecies competition between common pathogens indwelling catheters.

Author Disclosure Block:

S. Kang: None. **H. Choe:** None. **S. Lee:** None.

Poster Board Number:

SUNDAY-092

Publishing Title:

***streptococcus Agalactiae* Biofilm Production Is Influenced By Environmental Ph, Glucose, And Zinc Availability**

Author Block:

R. S. Doster[1], V. Kothary[1], L. M. Rogers[1], C. A. Wakemen[1], E. P. Skaar[1], S. D. Manning[2], D. M. Aronoff[1], J. A. Gaddy[3]; [1]Vanderbilt Univ. Sch. of Med., Nashville, TN, [2]Michigan State Univ., East Lansing, MI, [3]Tennessee Vally Hlth.care System, Nashville, TN

Abstract Body:

Background: Group B *Streptococcus* (GBS) infections are important causes of preterm birth and neonatal sepsis. Approximately 30% of pregnant women are colonized with GBS, but recto-vaginal colonization can be transient or persistent. We hypothesize that biofilm production is important to maintain vaginal colonization by GBS. We sought to define environmental characteristics that influence GBS biofilm production *in vitro*.**Methods:** Clinical GBS isolates were grown in Todd Hewitt Broth that varied in pH, glucose content, and zinc availability. *In vitro* biofilm production at 24 hours was determined by measuring the OD_{600} (bacterial biomass) and OD_{540} after crystal violet staining. Biofilms were also grown in a lateral drip flow reactor and on explanted fetal membranes. These samples were evaluated by scanning electron microscopy and confocal microscopy to visualize biofilm production.**Results:** 6 clinical GBS isolates were evaluated for biofilm production at pH 5, 6, and 7.8 with or without 1% glucose supplementation. 5/6 of these isolates grew the most biofilm at pH 5.0 with 1% glucose. We then completed a more detailed biofilm comparison using 2 of these strains, GB37, an invasive capsular type V, and GB590, a colonizing capsular type III strain. These were compared in a lateral drip flow reactor, where marked differences in biofilm production were seen with GB37 producing more biofilm with a higher carbohydrate content. We assessed these strains' ability to form biofilms in media with limited zinc availability. In the presence of TPEN, a zinc chelator, both strains produced greater than 3 times amount of biofilm/biomass compared to media alone. Biofilm production returned to baseline with the addition of exogenous zinc. Biofilm production on explanted fetal membranes was also reduced at 24 hours with the addition of 10 μM zinc.**Conclusions:** GBS clinical isolates vary in their ability to produce biofilm. *In vitro* biofilm production was improved with low pH (5.0), glucose supplementation, and limited zinc availability. These environmental conditions may be important for vaginal colonization of GBS during pregnancy and subsequent ascending infections.

Author Disclosure Block:

R.S. Doster: None. **V. Kothary:** None. **L.M. Rogers:** None. **C.A. Wakemen:** None. **E.P. Skaar:** None. **S.D. Manning:** None. **D.M. Aronoff:** None. **J.A. Gaddy:** None.

Poster Board Number:

SUNDAY-093

Publishing Title:

Antimicrobial Activity and Phytochemical Properties of C*roton zambesicus* on S*taphylococcus aureus* and S*treptococcus* Species

Author Block:

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Abstract Body:

Background: Antimicrobial activity of *Croton zambesicus* on *Staphylococcus aureus* and *Streptococcus* species was determined during the study. *Croton zambesicus* belongs to the family, Euphorbiaceae and order, Malpighiales. It has its common name as 'Ajekobale' (Yoruba language, Nigeria). **Methods:** About 200g of the powdered leaves of the *Croton zambesicus* plant were soaked in 400ml of 95% Ethanol, Distilled water and Petroleum ether in a 500ml reagent bottle and stoppered. This was allowed to stand for 14 days to permit full extraction of the active ingredients. It was filtered and the extracts were rotary dried to obtain the concentrate kept in fridge prior to use. A 2.0µg/ml solution of each extract was prepared with DMSO (dimethyl sulfoxide) and fractionated into 0.6µg/ml, 0.4µg/ml and 0.2µg/ml concentrations needed for the bioassay. The antimicrobial properties of the extracts were determined using the agar diffusion method on Mueller Hinton agar culture.**Results:** Ethanolic extract of *Croton zambesicus* has the highest antimicrobial effect on *Staphylococcus aureus* with the 0.6g/ml concentration giving the highest zone of inhibition. However, aqueous extract of *Croton zambesicus* on *Staphylococcus aureus* and *Streptococcus* species respectively was low. Phytochemical screening of *Croton zambesicus* shows the presence of constituents such as Cyanogenic Glucoside, Steroid, Anthraquinone, Phenol, Saponins and Flavonoids, except alkanoid and tanins. Quantitative analyses of mineral elements in *Croton zambesicus* shows the presence of Sodium (Na), Potassium (K), Calcium (Ca), Magnesium (Mg), Zinc (Zn), Iron (Fe), Lead (Pb), Cupper (Cu), Manganese (Mn) and Potassium (P). Magnesium has the highest composition and lead was not detected. Its proximate nutrients have highest composition of carbohydrate.**Conclusions:** Observations made in this study will help to identify some active agents responsible for antimicrobial potentials of *Croton zambesicus* especially in subduing aetiologic agents tested under this context among others. Thus, the study ascertains the value of this medicinal plant in the development and production of new drugs or antibiotics.

Author Disclosure Block:

O.A. Ajayi: None.

Poster Board Number:

SUNDAY-094

Publishing Title:

Quorum Sensing-Mediated Social Cooperation is Required for *Streptococcus mutans* to Compete with Related Species in Biofilms

Author Block:

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Abstract Body:

Background: Natural life of bacteria in biofilms often involves complex interactions that can be either competitive or cooperative. Competition between species is a well-known ecological force to drive bacterial metabolism, diversity and evolution. However, relatively little is known of bacterial cooperative activities in inter-species interactions. **Methods:** In this study, we first examined quorum sensing (QS)-mediated cooperation for bacteriocin production in *Streptococcus mutans* UA159 (Sm) and its isogenic mutant SmΔCDE (QS negative). We then examined interspecies interactions of Sm with Ss (*S. sanguinis* SK108) and An (*Actinomyces naeslundii* WVU627) in dual- or three-species biofilms.**Results:** The results showed that SmΔCDE was defective in QS-mediated bacteriocin activity, but had the higher growth yield than the parent, suggesting that QS-mediated cooperation was metabolically costly. We also found that QS-activated population (Sm) was susceptible to exploitation by QS mutant (SmΔCDE) in a mixed culture system. Interestingly, the populations began to shift with dominance by Sm (wt) over SmΔCDE when the culture was introduced with a competing species, either Ss that was sensitive to bacteriocins produced by Sm or An that was more effective to form a biofilm but not sensitive to bacteriocins. Even more interestingly, all four populations of Sm, SmΔCDE, Ss and An (three species) co-existed with frequencies of 40% of Sm, 10% of SmΔCDE 30% of Ss and 20% of An in the biofilm. The results suggest that QS-mediated social cooperation in *S. mutans* may be limited by metabolic cost or by the invasion of a QS mutant or 'cheater'. However, the presence of one or more competing species can limit or reduce the spread of QS mutants in the community.**Conclusions:** We conclude that QS-mediated social cooperation is required for *S. mutans* to compete with related species in biofilms.

Author Disclosure Block:

Y. Li: None. **X. Tian:** None.

Poster Board Number:

SUNDAY-095

Publishing Title:

Characterizationof a Biofilm Formation-associated Histidine Kinase in *Listeria monocytogenes*Egd-e

Author Block:

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Abstract Body:

Background: *Listeria monocytogenes* biofilm formation is a concern in food processing plants and as a result their presence has been has been widely studied. However, identification of genes associated with biofilm formation in *L. monocytogenes* is limited. Histidine kinases (HKs) can act as sensors for cell to cell communication responsible for environmental stresses in *L. monocytogenes*. Through our preliminary study, we found that *L. monocytogenes* serovar 1/2a food-related isolates (FRIs) produce more biofilm than serovar 4b FRIs, and 4b strains do not have an ortholog corresponding to *lmo1061*, a putative HK of *L. monocytogenes* EGD-e (serovar 1/2a). We then hypothesized that *lmo1061* may play a significant role in biofilm formation. **Methods:** Using a reverse genetic approach, an in-frame deletion mutant of *lmo1061* (Δ*lmo1061*) was constructed to characterize the role of *lmo1061* in biofilm formation. The abilities of *L. monocytogenes* EGD-e wild-type (WT) strain, the Δ*lmo1061* strain, and the complemented strain on cell growth, biofilm formation using the crystal violet binding assay, and antimicrobial resistance were further determined. **Results:** The results demonstrate that Δ*lmo1061* produced less biofilm than WT and complemented strains for the whole experiment period of 5 weeks at 4 °C. However, a significant difference in antimicrobial resistance between WT and *Δlmo1061*strains was not found. The growth of Δ*lmo1061* tended to decrease with increased time when compared to WT strain. After three weeks, the OD_{600} value of Δ*lmo1061* strain was significantly less than that of WT strain with downregulation in the mRNA expression level of genes, encoding sugar uptake transporters. **Conclusions:** Data from our biofilm-associated phenotypes suggests that a HK *lmo1061*may be involved in biofilm formation. However, a notable difference in the abilities of the WT and mutant strains on cell growth and stress adaptation was not found. A further study may be required to examine functional redundancy among *lmo1061* paralogs with the molecular mechanisms involved in biofilm formation by the gene.

Author Disclosure Block:

A.A. Khan: None. **D. Bae:** None. **H. Abdelhamed:** None. **A. Karsi:** None. **T. Zhang:** None. **C. Wang:** None.

Poster Board Number:

SUNDAY-096

Publishing Title:

Comparison of *Salmonella enterica* typhimurium and *Escherichia coli* o157:H7 Survival and Adaptation to Desiccation, Low Water Activity, and Thermal Tolerance

Author Block:

A. Maserati[1], F. Diez-Gonzalez[2], A. Lourenco[2], **R. C. Fink**[3]; [1]Univ. of Minnesota, St. paul, MN, [2]Univ. of Minnesota, St. Paul, MN, [3]St. Cloud State Univ., St. Cloud, MN

Abstract Body:

Background: *Salmonella* and *Escherichia coli* O157 are important foodborne pathogens, responsible for multiple outbreaks every year in the US. *Salmonella* species are different to *E. coli* because they are more resistant to desiccation and develop thermal tolerance when exposed to low water activity. The objective of this study was to compare the morphological and phenotypical characteristics of both pathogens. **Methods:** The ability to survive desiccation and low water activity (a_w 0.11) was tested by inoculating *Salmonella* ser. Typhimurium and *E. coli* O157:H7 cultures on micro glass-beads and polystyrene microplates. Cells were dried at 38.5°C for 4 days and then equilibrated to a_w 1.0 and 0.11 for 7 d. Cell counts were determined for each strain and treatment. Scanning electron microscopy (SEM) was used to analyze phenotypic and morphological changes of cells under each condition. For thermal treatment, cells equilibrated on micro glass beads were exposed to 75°C.**Results:** On both beads and microplates, *E. coli* O157 counts were reduced at least 2 log CFU after drying compared to *Salmonella* ($p \leq 0.05$). *E. coli* was never recovered at a_w 0.11 in both matrices after 7 d, but *Salmonella* counts remained at more than 10% of the initial count. SEM images of cells dried on glass beads showed differences in biofilm formation during drying between *Salmonella* and *E. coli* O157. *Salmonella* cells were covered by a thick layer of exopolisaccharides, whereas *E. coli* produced very little glycocalyx. No differences between the two were detected at a_w 1.0. At a_w 0.11, *E. coli* biofilm appeared flattened and damaged compared to *Salmonella*'s, where a thick layer of matrix was still present. After thermal treatment, the *E. coli* biofilm presented damaged and detached spots.**Conclusions:** Our results show that, differently from *E. coli* O157, *Salmonella* is able to readily produce a structured biofilm during the early stages of desiccation. *E. coli* eventually forms a biofilm morphologically similar to *Salmonella*. However, the different thermal tolerance of these structures suggests that there may be important chemical factors that play a role.

Author Disclosure Block:

A. Maserati: None. **F. Diez-Gonzalez:** None. **A. Lourenco:** None. **R.C. Fink:** None.

Poster Board Number:

SUNDAY-097

Publishing Title:

Attachment and Viability Properties of *Acinetobacter baumanii* with Different Antimicrobial Susceptibility Profiles

Author Block:

I. Kanesaka[1], A. Kanayama[1], A. Kaneko[2], H. Takahashi[1], I. Kobayashi[1]; [1]Toho Univ., Tokyo, Japan, [2]Tokai Univ., Kanagawa, Japan

Abstract Body:

Background: Attachment to environment surfaces and long-term survival of bacteria in the hospital environment poses a serious risk of hospital-acquired infections as transmission of resistant organisms from the inanimate hospital environment can occur. In this study, we studied the viability of *A. baumannii* after artificial contamination of surfaces of material commonly used in the hospital environment. **Materials and Methods:** A total of 8 *A. baumannii* isolates consisting of seven clinical isolates of *A. baumannii* including 4 multi-drug resistant *Acinetobacter* (MDRA) and ATCC 15308 were applied to polypropylene (PP) and silicone (SI) plates (1 cm^2). A 10^5 inoculum of each organism was added to PP and SI plates for a total of 16 plates. After allowing plates to dry, viability at 20 °C was assessed at days 1, 2, 3, 7, 15 and 30. One ml of BHI broth was added and plates were shaken to remove attached organisms followed by quantitative culture. **Results:** Including the ATTC strain and 3 clinical isolates of non-MDRA, about 10^2 CFU were recovered on PP at 7 days and 15 days on SI. All MDRA isolates were not detected on PP and SI after 1 day (p<0.01). The viability of A. baumannii differed based on the material to which attachment occurred. Furthermore, non-MDRA isolates had longer survival compared to MDRA. **Conclusions:** The data shows that viability of *A. baumannii* attached to environmental surfaces differs based on whether it is non-MDRA and MDRA.

Author Disclosure Block:

I. Kanesaka: None. **A. Kanayama:** None. **A. Kaneko:** None. **H. Takahashi:** None. **I. Kobayashi:** None.

Poster Board Number:

SUNDAY-098

Publishing Title:

Biofilm Formation Is Clonal Specific among *Staphylococcus aureus* Isolated from Selected Vermont Dairy Farms

Author Block:

R. Mugabi, J. Barlow; Univ. of Vermont, Burlington, VT

Abstract Body:

Staphylococcus aureus remains an animal and public health burden; in cattle, it causes mastitis one of the leading causes of production losses in the dairy sector. The ability of *S. aureus* to form biofilm is believed to contribute to chronicity and persistence of infections. The objective of this study was to evaluate whether clonal lineage and surface type significantly affect biofilm formation. Isolates used in this study were from two previous epidemiologic studies in Vermont.One hundred isolates were from bulk tank milk of 43 organic dairy farms and fifty-nine from various sources (cow milk, teat end, human hand and nose, dog and bulk tank) of four farmstead cheese conventional dairy farms. All the isolates (159) were strain typed by multilocus sequence typing (MLST) and assigned to their respective clonal complexes using MLST eBURST software. The isolates were PCR screened for presence of icaA, icaD and Bap genes. Biofilm formation was evaluated using the standard microtiter plate *in Vitro* assay using two 96 well plate type surfaces (treated and untreated). All isolates were positive for icaA and icaD but negative for Bap gene. Among the organic isolates, CC97 isolates significantly formed biofilm ($p<0.05$) on untreated plates in comparison to other clonal complexes (CC8, CC151) whereas CC8 isolates formed strong biofilms on treated plates. In a linear regression model, plate surface and clonal lineage were significant predictors of biofilm formation. There was no significant difference in biofilm formation among *S. aureus* isolates from the four conventional farms on the two plate surfaces regardless of the source. However CC8 isolates significantly ($p<0.05$) formed biofilm compared to CC5, CC30 and CC97 on the two plate surface types.Overall, this work shows that *S. aureus* biofilm formation is lineage specific and may contribute to the dominance of a given lineage. The difference seen in the isolates between the two studies (conventional vs organic) may be attributed to differences in diversity. Organic isolates had twenty strain types from 43 farms compared to 8 strain types from 4 conventional dairy farms. This work lays a platform to investigate *S. aureus* surface markers that drive the differences in biofilm formation between surface types and within *S. aureus* lineages.

Author Disclosure Block:

R. Mugabi: None. **J. Barlow:** None.

Poster Board Number:

SUNDAY-099

Publishing Title:

Listeria Adhesion Protein Augments Antilisterial and Antibiofilm Activity of Peptide Functionalized Gold Nanoparticles

Author Block:

A. K. SINGH, M. A. R. Amalaradjou, A. K. Bhunia; Purdue Univ., West Lafayette, IN

Abstract Body:

Background: Tunable surface chemistry of citrate-stabilized gold nanoparticles[1] (GNP) provides suitable and flexible platform for stable conjugation and targeted delivery of various biomolecules in theranostic applications. Gold nanoconjugates can be applied as an antibacterial and antibiofilm agents. Bacterial infections such as listeriosis caused by foodborne pathogen, *Listeria monocytogenes* poses a serious threat to human health worldwide. In this study, we synthesized and assessed the antilisterial and antibiofilm properties of a novel gold nanocomposite, functionalized with antilisterial peptide Pediocin AcH[2] and *Listeria* adhesion protein[3] (LAP) for targeted destruction of *L. monocytogenes*. **Methods:** Biophysical (TEM, Zeta potential, FTIR, Spectroscopy, Fluorimetry) methods were adopted to characterize the nanocomposite. Bioassay, plate counts, and mammalian cell culture-based cytotoxicity assays were used to assess antilisterial and antibiofilm activity of GNP composites. Biocompatibility of the composite was also assessed using the LDH-based cytotoxicity assay on Caco-2 cells after treatment with GNP alone or gold nanocomposites.**Results:** TEM results revealed that the GNP and the nanoconjugate sizes were about 20 nm and 40 nm, respectively. Biophysical experiments underscored electrostatic interactions between citrate-stabilized GNP and biomolecules (Pediocin, LAP). Compared to the GNP-Pediocin conjugate, treatment of *L. monocytogenes* with the GNP-Pediocin-LAP conjugate resulted in 11.1% higher zone of inhibition in bioassay, and higher reduction (1.5 $\log_{10}$ CFU/mL) in bacterial counts. In the antibiofilm test, similar treatments resulted in 24% and 31% more reduction in *Listeria* counts after 24 h and 48 h of incubation, respectively. Also, in cytotoxicity assay, *L. monocytogenes* induced cytotoxicity was lowered by 16% for pre-treatment of Caco-2 cells with the GNP-Pediocin-LAP conjugate compared to the GNP-Pediocin alone. Neither GNP nor nanocomposite exposure resulted in any significant cytotoxicity (p <0.05) on the Caco-2 cells.**Conclusions:** Gold nanocomposites were biocompatible and when functionalized with both the Pediocin and the LAP, revealed higher antilisterial and antibiofilm activities compared to just Pediocin functionalized GNP or Pediocin alone.

Author Disclosure Block:

A.K. Singh: None. **M.A.R. Amalaradjou:** None. **A.K. Bhunia:** None.

Poster Board Number:

SUNDAY-100

Publishing Title:

The Effect of Nicotine and Sugar on the Growth and Biofilm Formation of *Pseudomonas aeruginosa*

Author Block:

G. A. De Leon, N. Chalongwongse, J. K. Chan; Cal Poly Pomona, Pomona, CA

Abstract Body:

Nicotine is the addictive chemical found in tobacco and is consumed by millions of people daily. It has been proven that smokers experience more bacterial infections than non- smokers. In addition, sugars are also known to be a health risk and can lead to obesity and tooth decay. Pseudomonas aeruginosa (PA) is a gram-negative opportunistic bacterium that is found in the human body, including the oral cavity. The ability of PA to form biofilms allows the bacteria to survive in adverse conditions and to be more tolerant to antibiotics than stationary-phase planktonic bacteria. The objective of this study is to examine how nicotine and various sugars affect the growth and biofilm formation of PA. To determine the effect of nicotine on the growth, different concentrations of nicotine ranging from $1x10^{-1}$ M to $1x10^{-11}$ M were added to a flat bottom 96-well microtiter plate containing a 1:10 dilution of the 24 hour culture of PA in M63 liquid media. After 48 hours of incubation, the growth of the PA was evaluated using a spectrophotometer. The results were analyzed using a sample t-test to observe for significant differences ($p<0.05$). To study the effect of nicotine and sugars on biofilm formation, the crystal violet biofilm assay was used. After 48 hours of incubation of the fresh culture PA, the non-adhered PA bacteria were washed and decanted and the adhered PA bacteria were stained with crystal violet. The amount of stain in the bacteria was released with acetic acid and was then measured and analyzed using a sample t-test for significance ($p<0.05$). All data represented quadruplicate runs and the results indicated that nicotine at the concentrations used had no significant effect on the growth of PA, but significantly decreased the biofilm formation, with higher concentrations of nicotine suppressing biofilms formed ($p<0.05$). Results also showed that of the sugars tested, sucrose, maltose and galactose all significantly suppressed biofilm formation of PA, while glucose had no significant effect. When combined with nicotine at a concentration of $1x10^{-3}$ M, the results showed that the suppressing effect of nicotine with sugar is not additive.

Author Disclosure Block:

G.A. De Leon: None. **N. Chalongwongse:** None. **J.K. Chan:** None.

Poster Board Number:

SUNDAY-101

Publishing Title:

Coexistence of *Streptococcus gordonii* and *Streptococcus mutans* in a Cariogenic Mixed-Species Biofilm

Author Block:

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Abstract Body:

Background: *Streptococcus mutans* is associated to the pathogenesis of dental caries while *Streptococcus gordonii* is linked to healthy biofilms, as an antagonist of *S. mutans*. Hence, we studied whether *S. gordonii* (Sg) and *S. mutans* (Sm) co-exist in a cariogenic mixed-species biofilm, and whether specific *Sg* genes affect the formation of mixed-species biofilm.**Methods:** The strains *S. gordonii* DL-1 (parental and knockout mutant strains for genes *ccpA, gtfG,* and *pox), Actinomyces naeslundii* ATCC 12104 and *S. mutans* UA159 were used to grow mixed-species biofilms on saliva-coated hydroxyapatite discs in the presence of 1% (w/v) sucrose and 25% whole saliva. Biofilms were evaluated at 3 distinct ages (29, 67 and 115h) for viable population, and for architecture and biomass composition using confocal microscopy.**Results:** In the mixed-species biofilm formed with parental strains, both *Sm* and *Sg* populations increased over time. However, the proportions of the three mutant strains did not increase while *Sm* thrived in these biofilms. *A. naeslundii* population declined in the biofilm formed with *Sg* parental strain, while it increased in biofilms containing *Sg* mutant strains. Moreover, the mixed-species biofilms with the *Sg* parental strain lacked well-defined microcolonies (vs. biofilm with *mutant* strains). The amount of bacteria and exopolysaccharides matrix biomass were distinct for mixed-species biofilms formed with *Sg* parental strain (*vs*. the three mutant strains; $p<0.05$).**Conclusions:** Therefore, *S. gordonii* can co-exist well in a cariogenic mixed-species biofilm with *S. mutans*, and the disruption of *S. gordonii* genes *ccpA*, *gtfG* and *pox* affects the fitness of this species in biofilms.

Author Disclosure Block:

M.I. Klein: None. **C. Getaz:** None. **J. Kreth:** None.

Poster Board Number:

SUNDAY-102

Publishing Title:

Differential Production of Pyocins by Environmental and Cf-Associated Pseudomonas Aeruginosa

Author Block:

J. J. Bara, A. W. Diddle, S. K. Remold; Univ. of Louisville, Louisville, KY

Abstract Body:

Bacteriocins are narrow spectrum, anticompetitor toxins, produced by bacteria in response to environmental stress and are important mediators of competitive interactions. The opportunistic pathogen *Pseudomonas aeruginosa,* which is the most dominant bacterium causing chronic infections in individuals with cystic fibrosis (CF), is distinct among studied bacteria in the extent to which strains produce bacteriocins, known in *P. aeruginosa* as pyocins. However, despite their ubiquitous production by *P. aeruginosa*, we lack a comprehensive understanding regarding the role of pyocins during chronic infection of the CF upper respiratory tract. Furthermore, recent studies suggest that pyocin production may be pleiotropically linked to performance in response to certain abiotic stressors that induce their expression. In this study, we used a set of 63 environmental and CF-associated *P. aeurginosa* strains collected from sites in and around 15 different homes in the Louisville, KY metropolitan area to investigate 1) the number and diversity of pyocins found in environmental and CF strains 2) the ability of environmental and CF strains to inhibit the growth of a competitor via pyocins, and 3) the correlation between pyocin production and sensitivity to ciprofloxacin, or oxidative stress. Using a standard PCR protocol, we screened each *P. aeruginosa* strain for the presence of 5 S-type pyocin genes (S1, S2, S4, S5, and AP41), as well as the R/F-type pyocin gene cluster. Using a subset of 29 *P. aeruginosa* strains we conducted a bidirectional inhibition assay to assess the ability of the strains to inhibit or be inhibited of via pyocins. For each strain, we determined the ciprofloxacin and hydrogen peroxide minimum inhibitory concentration using a broth microdilution method in 96-well plates. We found no significant difference in the number or composition of pyocin genes in environmental and CF strains, but found that CF strains were more susceptible and inhibited growth at a significantly lower rate relative to environmental strains. Additionally, we found no significant correlation between pyocin inhibition rate and sensitivity to either ciprofloxacin or hydrogen peroxide. Our results suggest that while environmental and CF strains have a similar array of pyocins, they may be differentially expressed or regulated, as inhibition and susceptibility rates were significantly different.

Author Disclosure Block:

J.J. Bara: None. **A.W. Diddle:** None. **S.K. Remold:** None.

Poster Board Number:

SUNDAY-103

Publishing Title:

The Role of *Rpoe* Gene in Biofilm Formation and Virulence of S*almonella typhimurium*

Author Block:

D. Peng, J. Huang, S. Chen; Coll. of Vet. Med., Yangzhou Univ., Yangzhou, China

Abstract Body:

Salmonella Typhimurium is one of the most important food-borne pathogens for humans, and its formation of a biofilm may improve its resistance to environmental stress. However, the roles of the *rpoE* gene in biofilm formation and virulence of *S. Typhimurium* remain unclear. In this study the *rpoS* gene-dependent or -independent strain for biofilm formation was determined by crystal violet staining and catalase test. The Real-time PCR method was established to compare the expression of six different Sigma factors during the period of biofilm formation. Deletion mutants were constructed using the Red recombination system, and their biofilm-forming ability and LD_{50}s were determined. The results showed that the expression of *rpoE* gene was the highest in an *rpoS*-independent strain S016 after an 24-hour incubation, while the expression of *rpoS* gene was the highest in an *rpoS*-dependent strain S025. Both S025ΔrpoS and S025ΔrpoE mutants could not produce biofilm. However, S016ΔrpoS kept the biofilm-forming ability, while S016ΔrpoE could not produce biofilm. The LD_{50}s in chickens of all ΔrpoS mutants were similar as that of the wild-type strains, while LD_{50}s of all ΔrpoE mutants were lower than that of the wild-type strains. Our data indicate that **t**he *rpoE* gene acts as a regulator in biofilm formation and virulence of *S. typhimurium*.

Author Disclosure Block:

D. Peng: None. **J. Huang:** None. **S. Chen:** None.

Poster Board Number:

SUNDAY-104

Publishing Title:

D-Tagatose Inhibits *Streptococcus mutans* Biofilm Formation

Author Block:

K. Hasibul, T. Ogawa, H. Nakayama-Imaohji, T. Kuwahara, M. Miyake, M. Tokuda; Faculty of Med., Kagawa Univ., Miki, Kita, Japan

Abstract Body:

Background: Dental caries is one of the important global oral health burdens. Mutans streptococci have been established as a major cariogenic bacterial species. Among the hundreds of rare sugars, some (e.g. D-allose & D-pscicose) show the pharmacological effects that could be applied to medicine. We previously reported that a rare sugar, D-tagatose, suppresses the acid production & water insoluble glucan synthesis in *Streptococcus mutans*.**Methods:** In this study, we evaluated the inhibitory effect of D-tagatose on the *S. mutans* biofilm formation. Effect of different percentages of D-glucose, xylitol & D-tagatose on *S. mutans* GS5 biofilm production was compared by microplate assay with crystal violet. Growth monitoring of *S. mutans* until 24-h incubation showed that 1% D-tagatose only prolonged the lag-phase without interference in the final cell growth, indicating the *S. mutans* biofilm inhibition by this rare sugar is due to the mechanisms other than bacteriostatic effect.**Results:** D-tagatose (1.0 to 4.0%) significantly decreased *S. mutans* biofilm after 72-h incubation and its effect was dose-dependent. Consistently, 4% D-tagatose reduced insoluble glucan production from *S. mutans* in the presence of 1% sucrose to 17% of control. Scanning electron microscopy showed that D-tagatose was more effective for the *S. mutans* biofilm mass reduction than xylitol.**Conclusions:** From these findings, we conclude that the foods or preparations containing D-tagatose are useful tools for oral health care, which inhibit the initial stage of cariogenesis.

Author Disclosure Block:

K. Hasibul: None. **T. Ogawa:** None. **H. Nakayama-Imaohji:** None. **T. Kuwahara:** None. **M. Miyake:** None. **M. Tokuda:** None.

Poster Board Number:

SUNDAY-105

Publishing Title:

Bacterial Attachment and Biofilm Formation by Pathogenic Bacteria are Hindered by Small Nanoscale Pores

Author Block:

Y. Cheng[1], G. Feng[1], S. Wang[2], D. A. Borca-Tasciuc[2], R. W. Worobo[1], C. I. Moraru[1]; [1]Cornell Univ., Ithaca, NY, [2]RPI, Troy, NY

Abstract Body:

Background: Prevention of biofilm formation by bacteria is a prominent challenge in many areas affecting human health and life, including medicine, dentistry, food processing, and water treatment. This work showcases an effective solution for reducing attachment and biofilm formation by several pathogenic bacteria frequently implicated in foodborne illnesses and medical infections, by enhancing the repulsive force exerted on the bacterial cells.**Methods:** Using anodization, alumina surfaces featuring cylindrical nanopores with pore diameters of 15 to 100 nm were created and evaluated for attachment by *Escherichia coli* ATCC 43894, *Listeria monocytogenes* 10403S, *Staphylococcus aureus* 9144, and *Staphylococcus epidermidis* ATCC 35984. The attachment studies were conducted in tryptic soy broth, at 37 °C, in static mode. After 48 h, the attached cells were stained with a fluorescent dye and attachment evaluated by Confocal Laser Scanning Microscopy. Surface properties of the cells and anodic substrates (zeta potential, surface energy), size and topography were determined experimentally and used to calculate the cell-surface interaction forces based on the extended Derjaguin-Landau-Verwey-Overbeek theory. The maxima of the interaction forces were then correlated with the attachment data. The study was replicated a minimum of three times. **Results:** Anodic alumina surfaces with pore diameters of 15 and 25 nm were shown to effectively reduce bacterial attachment or biofilm formation by all strains, while larger pore sized increased attachment. The observed effects are likely due to the amplification of repulsive cell-surface interactions by the large surface area of the high density, small-diameter pores. Biological factors, including cellular appendages and the exopolysaccharide secreted by the cells, are also likely to affect attachment.**Conclusions:** This work enhances our understanding of the effect of nanoscale topography on bacterial attachment to abiotic surfaces. Moreover, this demonstrates how to optimize the elements of surface topography and further enhance the observed bacteria-repelling effects, which could have implications and applications in the health care field and food industry.

Author Disclosure Block:

Y. Cheng: None. **G. Feng:** None. **S. Wang:** None. **D.A. Borca-Tasciuc:** None. **R.W. Worobo:** None. **C.I. Moraru:** None.

Poster Board Number:

SUNDAY-106

Publishing Title:

Inactivation of *Reca* in *Enterococcus faecalis* Decreases Biofilm Formation on Various Surfaces

Author Block:

S. M. Lee, L. Chua; Monash Univ. Malaysia, Petaling Jaya, Malaysia

Abstract Body:

Enterococcus faecalis is a major nosocomial pathogen worldwide. Many *E. faecalis* isolates are able to produce biofilms which increase their recalcitrance to antibiotic therapy. Interestingly, subinhibitory concentrations of ciprofloxacin (an antibiotic which inhibits DNA replication) can actually stimulate biofilm production in other bacteria such as *Pseudomonas aeruginosa*. This phenomenon is linked to the SOS response, where the RecA protein acts as a sensor for DNA damage. Furthermore, repression of bacterial motility appears to play a role in the repression of SOS-inducible biofilm formation. The aim of this study was to determine if the inactivation of the *recA* gene would have an impact on the ability of *E. faecalis*, which are non-motile bacteria, to attach to various surfaces and to form biofilms. In this study, three strains were used: Wild type, *E. faecalis* JH2-2 (WT), insertionally inactivated *recA* mutant (M) and a complemented *recA* mutant strain (C). All three strains had growth rates that were not significantly different from one another. When all three strains were treated with subinhibitory concentrations of ciprofloxacin (MIC = 4 µg/ml) and levofloxacin (MIC = 2 µg/ml), the WT and C strains did not show increased biofilm production on 96-well microtiter plates. However, the M strain showed significantly lower biofilm production compared to the other two strains when grown with or without antibiotics. Additionally, all three strains were able to form biofilm on various surfaces such as glass, stainless steel and polypropylene. However, the *recA* mutant had significantly and consistently lower ($p<0.05$) biofilm formation for all surfaces compared to the WT and C strains. Similarly, the recA mutant has significantly lower ($p<0.05$) cell surface hydrophobicity (via contact angle measurement), initial attachment to polystyrene and aggregation ability. However there was no significant difference in terms of cell surface charge among the three strains when measured with a zetasizer. The results of this study suggest that RecA can affect the cell surface properties of *E. faecalis*, which in turn can affect the bacteria's ability to attach to surfaces and form biofilms.

Author Disclosure Block:

S.M. Lee: None. **L. Chua:** None.

Poster Board Number:

SUNDAY-107

Publishing Title:

The Influence of Culture Media on Biofilm Formation by Dermatophytes

Author Block:

C. B. Costa-Orlandi[1], R. H. Pires[2], J. D. Nosanchuk[3], A. M. Fusco-Almeida[1], M. J. S. Mendes-Giannini[1]; [1]UNESP - Univ.e Estadual Paulista, Araraquara, SP, Brazil, [2]UNIFRAN - Univ.e de Franca, Franca, SP, Brazil, [3]Albert Einstein Coll. of Med., Bronx, NY

Abstract Body:

Background: Over the last few years, filamentous fungi, particularly the dermatophytes, have been recognized as biofilm-forming microorganisms. Nutrients in the environment can influence the development and final chemical composition of biofilms, suggesting the correlation between metabolic substrates and biofilm architecture. This work aims to evaluate the influence of four different culture mediums on biofilm formation by *Trichophyton rubrum* and *T. mentagrophytes*.**Methods:** The biofilms of two ATCC strains and two clinical isolates of *T. rubrum* and *T. mentagrophytes* were observed following cultivation in RPMI 1640, Brain-heart infusion (BHI), Dulbecco's Modified Eagle's media (DMEM) and serum free medium for keratinocytes culture. After 72 hours of growth, the biomass and the extracelular matrix (ECM) were quantified using the crystal violet (CV) and safranin methods, respectively. The ultrastructures of the mature biofilmes were visualized by scanning electron microscopy (SEM) and confocal laser scanning microcopy (CLSM).**Results:** Overall, cultivation in RPMI, DMEM and BHI resulted in the most robust production of biomass and ECM. The biofilms of *T. rubrum* ATCC 28189, *T. rubrum* 143 and *T. mentagrophytes* 66 produced higher amounts of ECM compared to *T. mentagrophytes* ATCC 11481 ($p<0.05$). SEM images showed that the biofilms formed in RPMI, BHI and DMEM exhibited a highly organized ECM, covering and connecting the hyphae. CLSM images showed that RPMI and DMEM produced thicker biofilms, while the BHI produced denser biofilms.**Conclusions:** These results demonstrate that *T. rubrum* and *T. mentagrophytes* are able to form biofilms in diverse culture mediums with varying characteristics and these variations can contribute significantly to future studies of dermatophyte biofilms.

Author Disclosure Block:

C.B. Costa-Orlandi: None. **R.H. Pires:** None. **J.D. Nosanchuk:** None. **A.M. Fusco-Almeida:** None. **M.J.S. Mendes-Giannini:** None.

Poster Board Number:

SUNDAY-108

Publishing Title:

Biodegradation of Environmental Contaminants by Acidophilic Methanotrophs

Author Block:

Y. Shao; Texas A&M Univ., COLLEGE STATION, TX

Abstract Body:

Methanotrophs, ubiquitous in the environment, can oxidize methane by expressing soluble methane monooxygenase (sMMO) or particulate methane monooxygenase (pMMO). Compared to pMMO, sMMO is nonspecific and known for its ability to degrade a wide range of pollutants, including halogenated hydrocarbons. Among many acidophilic methanototrophs recently identified, *Methylocella* is one genus of methanotrophs which possess only sMMO. *Methylocella* species not only grow on one-carbon compounds like methane and methanol but also multicarbon compounds like acetate, pyruvate, succinate, malate, and ethanol. However, using multicarbon compounds, like acetate, may repress the expression of sMMO in some *Methylocella* species. The goal of this study is to investigate the ability of known Methyocella strains to degrade common groundwater contaminants in the absence and the presence of multicarbon substrates.Methylocella strains, *Methylocella tundra* and *Methylocella silvestris* BL2 and another facultative methanotrophs, *Methylocystis bryophila* are used as model strains, and trichloetheylene (TCE), 1,4 dioxane, and 1,2,3-trichloropropane (TCP) are used as model contaminants in this study. Our preliminary results show that *Methylocella tundra* growed on both methane and acetate. Bacteria grew faster when acetate as carbon source. The optical density at 600 nm (OD_{600}) reached 0.28 after 48 hours, while OD_{600} was only 0.13 growing on methane. *Methylocella silvestris* BL2 grew slowly than *Methylocella tundra*. It took *Methylocella silvestris* BL2 two weeks to reach OD_{600} 0.21. *Methylocystis bryophila* also grow on both carbon sources. OD_{600} reached 0.14 growing on methane and 0.18 growing on acetate. Three strains can survive at low pH..The results of naphthalene assay shows the *Methylocella tundra* can constitutively express sMMO with only acetate. *Methylocella silvestris* can express sMMO to degrade TCE. *Methylocella silvestris* also can grow with propane, with the express of sMMO, which may shows another metabolism involved in multicarbon compounds utility.The findings of this study are valuable to further study future research on the biodegradation of TCE in many environments where multicarbon compounds occur.

Author Disclosure Block:

Y. Shao: None.

Poster Board Number:

SUNDAY-109

Publishing Title:

Probing Active Acidophilic Methanotrophs Involved in Chlorinated Solvent Biodegradation

Author Block:

Y. Shao; Texas A&M Univ., College Station, TX

Abstract Body:

Chlorinated solvents, including trichloroethylene (TCE), are common groundwater contamination. Aerobic and anaerobic biodegradation of chlorinated solvent occurs under neutral pH. However, many of acidic aquatic systems (pH < 5.5) in the United State exist and degradation of chlorinated under acidic environment is thus potentially inhibited.Methanotrophs are capable of degrading TCE through cometabolic reactions using methane monooxygenase (MMO). Several groups of acidophilic methanotrophs, capable of growing well under acidic conditions, have been recently identified. However, little is known about the potential the acidophilic methanotrophs during TCE biodegradation in acidic aquifers. The goal of this study is to use molecular techniques (stable isotope probing and real-time-t-RFLP) to identify active methanotrophs capable of degrading TCE under acidic conditions.A series of microcosms were constructed with soil and groundwater from acidic aquifers, ^{13}C- methane or ^{12}C- methane as carbon source, and TCE. Samples were collected over time for TCE, methane, and microbial community analysis. Our preliminary data showed methane consumption of 2000 µg/L in 72 hours in microcosms incubated. Biodegradation of TCE was observed after 3 weeks of incubation, with 80% removal of TCE initially spiked on week 8^{th}. Our on-going research efforts to separate the heavier DNA (^{13}C-DNA) fractions separated from genomic DNA of collected samples, use the ^{13}C-DNA fractions as templates for determining microbial community structures in the microcosms, and for identification of active acidophilic methanotrophs via cloning and sequencing.The findings of this study will provide a better understanding of potential of using acidophilic methantorophs for TCE biodegradation in acidic aquifers.Keyword : Identification; Active Acidophilic Methanotrophs; SIP; Sequencing; TCE Biodegradation;

Author Disclosure Block:

Y. Shao: None.

Poster Board Number:

SUNDAY-110

Publishing Title:

***Dehalococcoides* and General Microbial Ecology of Differentially Trichloroethene Dechlorinating Flow-through Columns**

Author Block:

B. Mirza, D.L. Sorensen, R.R Dupont, J.E. McLean; Utah State Univ., Logan, UT

Abstract Body:

The microbial ecology of partial or complete organohalide respiration of the common groundwater contaminant trichloroethene (TCE) has been poorly understood. The ecology of general microbial communities and *Dehalococcoides mccartyi* (*Dhc*) populations that developed in the TCE-dehalogenating layer (0–10 cm) of flow-through columns continuously fed TCE for 7.5 years was studied. Duplicate columns biostimulated with one of three different carbon sources and duplicate non-stimulated controls developed into complete (whey amended), partial- (Newman Zone® nonionic surfactant oil formulation with lactate), limited- (Newman Zone® oil standard surfactant formulation with lactate) and non-TCE dehalogenating systems (controls). Bioaugmentation with Bachman Road culture four years prior to dismantling the columns did not influence the extent of TCE dehalogenation. Three distinct microbial communities were established; one for whey, oils and control treatments. The whey columns exhibited relatively low concentrations of *Proteobacteria* sequences and high *Bacteriodetes* sequences. A significant increase in bacterial genera *Dehalobacter*, *Desulfitobacterium*, *Sulfurospirillum*, *Desulfuromonas*, and *Geobacter* was observed in the columns with limited and partial TCE dehalogenation. These genera may include members capable of partial TCE dehalogenation and use of alternative terminal electron acceptor (Fe^{III}, NO_3, SO_4).*Dhc* formed two taxonomic clusters. Whey column sequences were dominated by Cornell group strains, while all other dehalogenating columns contained Pinellas group strains. Different biostimulation treatments influenced general microbial communities that altered *Dhc* populations either directly through their syntrophic interaction or indirectly by altering predominant column biogeochemical processes. Change in the *Dhc* composition rather than their overall abundance was critical for complete TCE dehalogenation.

Author Disclosure Block:

B. Mirza: None.

Poster Board Number:

SUNDAY-111

Publishing Title:

Complete Mineralization of Methyl Parathion and Lindane by *Pseudomonas putida* kt2440

Author Block:

T. Gong, Environmental Microbiology and Microbial Manufacture; Nankai Univ., Tianjin, China

Abstract Body:

Currently, we can use the approaches of synthetic biology to creat a strain that processes various catabolic pathways that do not exist in the same strain in nature. Here, we describe the metabolic engineering of a biosafety *Psudomonas putida* stain KT2440 for complete mineralization of methyl parathion (MP) and lindane. A chromosomal DNA scarless modification method (I.e., suicide plasmid in combination with a counter-selection marker *upp*) was adopted to construct a stable and unmarked engineering strain *P. putida* KT2440 can efficiently mineralize MP and lindane. The engineered strain can effiently mineralize 100mg/l MP and 25mg/l lindane within 24 and 60 h, respectively, using MP or lindane as the sole carbon source and inoculum cell density of OD_{600}=0.05. Such a strain not only would reduce the toxicity of MP and lindane but also would prevent the accumulation of potentially toxic intermediates in the environment. A significant metabolic bottleneck, i.e., growth inhibition by the intermediates *p*-nitrophenol and 2,5-dichlorohydroquinone was resolved by introducing two mineralization pathways into *P. putida* KT2440. Due to heterologous expression of *Vitreoscilla* hemoglobin, the engineered strain showed a higher growth rate than wild-type strain in oxygen-limited conditions. Due to heterologous expression of green fluorescent protein, the fate and activity of engineeried strain in soil could be easily monitored by fluorescence. The inoculation of engineered strain (10^6 cells/g) to soil treated with MP and lindane resulted in a higher degradation rate than in noni oculated soils. MP (50 mg/kg) and lindane (25 mg/kg) could be degraded completely in soil within 6 and 11 d, respectively. Soil remediation experiments to demonstrate the viability of the engineered strain in soil may highlight the power of synthetic biology to create novel strains for *in situ* bioremediation of organics contaminated soil.

Author Disclosure Block:

T. Gong: None.

Poster Board Number:

SUNDAY-112

Publishing Title:

Transcriptomic and Proteomic Studies of *Dehalobacter* UNSWDHB Reveal Major Changes During Its Organohalide Respiration

Author Block:

B-E. Jugder, H. Ertan, Y. K. Wong, N. Braidy, M. Manefield, C. P. Marquis, M. Lee; Univ. of New South Wales, Sydney, Australia

Abstract Body:

Background: *Dehalobacter* strain UNSWDHB is an organohalide respiring bacteria (ORB) capable of respiring with chloroform (CF, or trichloromethane) as an electron acceptor for the generation of cellular energy and as a consequence, dechlorinating CF to dichloromethane. **Methods:** Comparative genomic, transcriptomic and label-free quantitative proteomic analyses were undertaken to investigate the global response of UNSWDHB strain to CF respiration with a particular focus on its respiratory pathway and formate metabolism.**Results:** We detected the up-regulation of key proteins involved in dehalogenation and electron shuttling, including a CF reductive dehalogenase with its membrane docking protein and putative transcriptional regulator (TmrABC), different types of uptake hydrogenases (membrane-associated Hup-, Hyc- and Ech-type Ni-Fe hydrogenase complexes as well as soluble Hym- and Hyd-type Fe-Fe hydrogenases), formate dehydrogenases (a periplasmic Fdh-N/Fdh-O and a cytoplasmic Fdh-H), a 11-subunit complex I and a pyrophosphate-energized proton pump. Our analysis on the Wood Ljungdahl pathway and its proposed association with formate metabolism expands current knowledge of its role in ORB and it paves the way for future studies on its functional role.**Conclusion:** Taken together, this study provides a broader view on the energy conservation and general physiology of *Dehalobacter* UNSWDHB cells during its respiration utlising CF as an electron acceptor.

Author Disclosure Block:

B. Jugder: None. **H. Ertan:** None. **Y.K. Wong:** None. **N. Braidy:** None. **M. Manefield:** None. **C.P. Marquis:** None. **M. Lee:** None.

Poster Board Number:

SUNDAY-114

Publishing Title:

Biotransformation of Hexabromocyclododecane with the Haloalkane Dehalogenase $LinB_{UT}$ Isoform

Author Block:

T-H. Chang[1], **Y-H. Peng**[1], S. Wu[1], C-H. Hsu[1], C-W. Chou[1], Y. Nagata[2], Y-h. Shih[1]; [1]Natl. Taiwan Univ., Taipei, Taiwan, [2]Tohoku Univ., Sendai, Japan

Abstract Body:

Brominated flame retardants (BFRs) have been widely used in many industries over decades. Hexabromocyclododecane (HBCD) is one of the most commonly used BFRs, and it has just listed on the table of persistent organic pollutants. In order to develop cost-effective remediation methods for this emerging pollutant, we evaluate the transformation ability of $LinB_{UT}$, a haloalkane dehalogenase from *Sphingomonas paucimobilis* UT26, toward HBCD. The catalysis activity of purified $LinB_{UT}$ was analyzed according to the concentration of residual HBCD and releasing bromide ion after the reaction. The results showed that degradation and debromination of HBCD catalyzed by $LinB_{UT}$ were both dose-dependent. From the kinetics under different enzyme/substrate ratios, the Michaelis-Menten parameters, K_m (0.75 μM) was deduced and the k_{cat} (4.5×10^{-4} s^{-1}) was calculated. The affinity of $LinB_{UT}$ toward HBCD was high while the turn over number was low, leading to similar catalytic activity when compared to other subtracts. According to circular dichroism spectra, the protein structure of $LinB_{UT}$ was loosening at 50 °C, which explained the optimum temperature for enzyme activity. The key residuals for better transformation efficiency of $LinB_{UT}$ than $LinB_{B90A}$ isoform (derived from *Sphigobium indicum* B90A) was also discussed. In this study, the HBCD biotransformation activity of $LinB_{UT}$ was characterized, which provides an alternative remediation strategy for treating HBCD in the environment with one possible effective detoxifying agent.

Author Disclosure Block:

T. Chang: None. **Y. Peng:** None. **S. Wu:** None. **C. Hsu:** None. **C. Chou:** None. **Y. Nagata:** None. **Y. Shih:** None.

Poster Board Number:

SUNDAY-115

Publishing Title:

Anaerobic Dechlorination of Lightly Chlorinated Dibenzo-*p*-Dioxins in Contaminated Sediments

Author Block:

D. E. Fennell, C. Schneider, H. Zhen, J. Liu, R. Caba, S. Ogungbile, V. Krumins, L. Rodenburg; Rutgers Univ., New Brunswick, NJ

Abstract Body:

Background: During remediation of environmental media contaminated with organohalide pollutants, removal of all chlorines from the organic molecule through organohalide respiration is desired. This may detoxify the compound and ultimately allow further biodegradation to occur. We examined the dechlorination of polychlorinated dibenzo-p-dioxins (PCDDs) with four or fewer chlorines in aquatic sediments. **Methods:** Sediment samples from several sites including Gulf Island Pond, ME and Lake Roosevelt, WA, originally contaminated with environmental polychlorinated dibenzo-p-dioxins (PCDDs), were placed in serum bottles with anaerobic minimal medium. The sediments were amended with 1,2,3,4-tetrachlorodibenxo-p-dioxin to study methods for enhancing reductive dechlorination. Triplicate microcosms were amended with electron donor (organic acids), and other treatments were amended with alternate soluble organohalides intended to enhance organohalide-respiring bacteria that may also dechlorinate dioxin. GC-MS was used to monitor dioxins. **Results:** After prolonged incubation (>5 years) abundant 2-monochlorobibenzo-p-dioixn was detected in most treatments and lesser amounts of non-chlorinated dibenzo-p-dioxin (up to 9.8 mol% of the original parent compound) was detected in some treatments. Analysis of sediment microbial communities is being conducted to determine whether specific organohalide-respiring bacteria are present.**Conclusions:** Production of abundant mono-chloro and non-chlorinated dibenzo-p-dioxin from TetraCDD after long incubation times was observed in sediments from multiple locations. Detection of non-chlorinated dioxin raises the possibility that anaerobic dechlorination could result in complete removal of chlorines from polychlorinated dioxins. This would lessen toxicity and allow further degradation of the dioxin molecule.

Author Disclosure Block:

D.E. Fennell: None. **C. Schneider:** None. **H. Zhen:** None. **J. Liu:** None. **R. Caba:** None. **S. Ogungbile:** None. **V. Krumins:** None. **L. Rodenburg:** None.

Poster Board Number:

SUNDAY-116

Publishing Title:

Dioxin Substrate Range of Dibenzofuran-degrading Bacteria Isolated from Contaminated Sediments

Author Block:

H. AL mnehlawi, D. E. Fennell; Rutgers Univ., New Brunswick, NJ

Abstract Body:

Background/Objectives: The Passaic River/Newark Bay in New Jersey is heavily polluted by hazardous contaminants such as dioxins, polychlorinated biphenyls and heavy metals that are contained in the sediments. Highly chlorinated dioxins may be anaerobically dechlorinated in aquatic sediments and the daughter products of this process may be lightly chlorinated dioxin congeners and non-chlorinated dioxin. The goal of this project is to identify bacteria that aerobically biotransform non- and lightly-chlorinated dioxins.**Approach/Activities:** Sediment samples were collected from the mouth of the Passaic River as it enters Newark Bay. Sediment aliquots were incubated aerobically with dibenzofuran as the sole source of carbon and energy. After activity was noted, enrichments were transferred to fresh minimal medium several times with dibenzofuran as the sole carbon and energy source. Finally, enrichments were plated on minimal agar media and single colonies were transferred to fresh agar plates to obtain pure cultures. Single colonies were transferred back to minimal medium with dibenzofurans the sole carbon and energy source and growth was confirmed by OD increase. The resulting isolates were identified by sequencing partial 16S rRNA genes. The ability of the isolates to use non- and lightly-chlorinated dioxins as a sole energy and carbon sources is being investigated.**Results:** Several bacterial isolates characterized as dibenzofuran degraders, were enriched from the sediments. According to 16S rRNA gene sequencing analysis, they belong to the following genera: *Panibacillus, Janibacter* and *Achromobacter.* The isolates' ability to use non- and lightly-chlorinated dioxin congeners including 2,7-dichlorodibenzo-*p*-dioxin, 2-mon-chlorodibenzo-*p*-dioxin, and dibenzo-*p*-dioxin is being investigated.In conclusion, several dibenzofuran degrading bacterial isolates have been obtained. If the transformation of highly chlorinated dioxins occurs in the river sediment and produce lightly chlorinated dioxins, further degradation or complete aerobic degradation of the daughter compounds by these or other organisms would be an important mechanism to ensure complete removal of the contaminants.

Author Disclosure Block:

H. AL mnehlawi: None. **D.E. Fennell:** None.

Poster Board Number:

SUNDAY-117

Publishing Title:

***Frankia* sp. Strains Eui1c and Eun1f Have the Potential to Degrade Dioxins and Dioxin-like Compounds**

Author Block:

E. C. Swanson[1], M. Rehan[2], L. S. Tisa[1]; [1]Univ. of New Hampshire, Durham, NH, [2]Kafrelsheikh Univ., Kafr El Sheikh Governorate, Egypt

Abstract Body:

Frankia sp. are soiling dwelling, nitrogen-fixing, actinobacteria that form facultative symbiotic associations with 8 families of actinorhizal plants. *Frankia* sp. are resistant to numerous heavy metals and toxic organic pollutants which makes the *Frankia*-plant symbiosis a promising candidate for bioremediation applications. We tested the ability of *Frankia* to resist and degrade three dioxin-like organic compounds: biphenyl (BP), 4-chlorobiphenyl (4CBP), and dibenzofuran (DBF). A comparative genomics approach was used to detect regions in *Frankia* genomes homologous to a known dioxin-degrading operon (*bph*) in a closely related species, *Rhodococcus* RAH1. Only 2 of the 19 available *Frankia* genomes, strains EuI1c and EUN1f, contained a putative dioxin-degrading operon homologous to the *bph* operon in *Rhodococcus* RHA1. Growth assays demonstrated that *Frankia* strains EuI1c and EUN1f were able to resist concentrations of BP, 4CBP, or DBF up to 5mM. However, *Frankia* strain CN3, a representative strain lacking the *bph* operon, is only able to resist BP concentrations up to 2mM. Both strains EuI1c and EUN1f also exhibited linear growth with BP or DBF as a sole carbon and energy source at concentrations up to 1.0mM. However, neither strain was able to grow with 4CBP as a sole carbon and energy source. Expression of the putative *bph* operon in strain EuI1c in response to BP exposure was determined through RT-qPCR. This analysis revealed that three representative genes (*bphA, bphC*, and *bphE*) of the *bph* operon were upregulated in response to BP exposure. These findings suggest that *Frankia* sp. strains EuI1c and EUN1f degrade dioxin-like compounds and could be used to remediate land contaminated by dioxin-like compounds.

Author Disclosure Block:

E.C. Swanson: None. **M. Rehan:** None. **L.S. Tisa:** None.

Poster Board Number:

SUNDAY-118

Publishing Title:

Identification and Characterization of Bacterial Genes Utilized in Triclosan Degradation

Author Block:

T. K. O'Neal, S. M. NI CHADHAIN; Univ. of South Alabama, Mobile, AL

Abstract Body:

Triclosan is an antimicrobial incorporated into a wide variety of personal care products including soaps, toothpastes, cleaning agents and medical instruments. It is now known that triclosan can bio-accumulate in higher organisms. Studies have suggested that this may lead to negative effects such as disruption of T4 thyroid hormone. This research project is focused on both identifying and characterizing the genes bacteria utilize to degrade triclosan within contaminated environments. Enrichment cultures were previously established using soil and water collected on the University of South Alabama campus in Mobile, AL. Incubations were supplemented with triclosan as the sole source of carbon and energy and serially diluted onto agar plates in order to obtain pure cultures of triclosan degrading bacteria. Amplified ribosomal DNA restriction analysis (ARDRA) and ribosomal intergenic spacer analysis (RISA) of the culture collection was performed to separate the bacteria into different operational taxonomic units (OTU). Forty-eight triclosan degrading bacterial isolates were obtained and grouped into 18 different OTUs. The 16S rRNA gene from representatives of each OTU was sequenced to identify the bacteria to genus or species. The majority of the bacteria were classified as pseudomonads (60%) with other gammaproteobacteria, betaproteobacteria, and bacilli also identified. Triclosan degradation assays indicated that select isolates were capable of degrading triclosan when grown alone with triclosan as the sole carbon source. The isolates were screened by PCR for the recently described *tcsA* and *tcsB* triclosan degradation genes. Ten isolates tested positive for the *tcsA* amplicon. PCR primers were also designed to chlorophenol-4-monooxygenase (*tftD*) and 2,4-dichlorophenol 6-monooxygenase (*tfdB*), genes involved in degradation of the previously identified triclosan degradation intermediates 3-chlorophenol and 2,4-dichlorophenol. To date 10 isolates have yielded *tftD* amplicons which are currently being sequenced. Our results indicate that the ability to degrade triclosan is widespread among bacteria. Furthermore, while PCR results indicate that some of our isolates use previously described degradation genes, it is likely that others utilize novel degradation genes.

Author Disclosure Block:

T.K. O'Neal: None. **S.M. Ni chadhain:** None.

Poster Board Number:

SUNDAY-119

Publishing Title:

Exploring Microbial Genomes in Search of Novel Rieske Oxygenase Guided by Evolutionary Studies

Author Block:

J. Chakraborty[1], C. Suzuki-Minakuchi[1], K. Okada[1], T. K. Dutta[2], H. Nojiri[1]; [1]Univ. of Tokyo, Tokyo, Japan, [2]Bose Inst., Kolkata, India

Abstract Body:

Background: Degradation by bacterial aromatic ring-hydroxylating Rieske oxygenases (ROs) is a major pathway by which wide range of aromatic compounds are mineralized in the environment. Though their structure, mechanism and involved metabolic pathways have been widely studied, a complete understanding of the diversity and evolution of this enzyme system have never been explored. In this study, phylogenetic analyses with set of homologous sequences were used to propose a rational classification scheme and to generate a mathematical model describing the evolution of multi-component RO system. The dataset has further been extended to construct a database and prediction server.**Methods:** Representative sequences from each subgroup of RO family were used as probes to identify homologous sequences using an iterative psi-blast search against GenBank. ClustalX and MEGA were used for phylogenetic analyses. The relational database was built on Apache HTTP web server executing SQL queries. Sequence-dependent search has been implemented using blast algorithm while for cheminformatics, JME molecular editor, Open Babel and Chemminer have been used.**Results:** 411 RO homologues were identified from 260 bacterial strains. As per their phylogenetic relationships and combination of electron transport components, ROs were categorized into 11 different types, 4 of them being identified for the first time, falling within distinct 'similarity classes'. A 'trace-back' approach was followed to predict the components of each peripheral node, gradually proceeding towards preceding nodes using a heuristic algorithm, effectively depicting the entire pathway of evolution of ROs. A manually curated database, RHObase, was developed to provide comprehensive information on all biochemically characterized bacterial ROs, along with integral prediction tools. It is currently being used to characterize ROs from environmental isolates as well as to scan genome sequences of thermophiles with an aim to identify novel ROs.**Conclusion:** Apart from exploring the potential of novel ROs, this study is believed to aid in designing new bioremediation strategies along with application in industrial biosynthetic processes.

Author Disclosure Block:

J. Chakraborty: None. **C. Suzuki-Minakuchi:** None. **K. Okada:** None. **T.K. Dutta:** None. **H. Nojiri:** None.

Poster Board Number:

SUNDAY-120

Publishing Title:

Bacterial Community Analysis of a Wastewater Treatment Plant in Colombia and Screening for Lipid-Degrading Microorganisms

Author Block:

L. M. Silva-Bedoya[1], J. Morales-Rodríguez[1], M. S. Sánchez-Pinzón[2], G. E. Cadavid-Restrepo[1], **C. X. Moreno-Herrera**[1]; [1]Univ. Natl. de Colombia, Medellín, Colombia, [2]Compañía Natl. de Chocolates S. A. S., Rionegro, Colombia

Abstract Body:

Background: Removal of organic pollutants in the form of fats, oils and greases is a demanding procedure in wastewater treatment. Microorganisms present in wastewater play essential roles in the degradation and removal of these compounds and the analysis of their population structure and function has become the basis for problem solving and optimization of existing and new WWTPs. Microbial lipases have distinctive industrial attention because they can catalyze other synthetic reactions like transesterification making them an environmentally relevant class of enzymes, contributing to the improvement of wastewater treatment and biofuel synthesis. In Colombia, wastewater bacterial community studies have been focused on cultivable populations and there is little information regarding the general bacterial communities that inhabit these ecosystems. **Methods:** In this study, culture-dependent, rDNA based molecular techniques and enzymatic methods were used to estimate bacterial diversity, monitor temporal and spatial bacterial community changes and to screen for lipolytic microorganisms. **Results:** TTGE microbial community analysis revealed predominant bacterial families Acetobacteraceae, Bacillaceae, Prevotellaceae, Pseudomonadaceae and Veillonellaceae. Bacterial and fungal isolates were affiliated to the genera Cronobacter, Leclercia, Enterobacter, Klebsiella, Bacillus, Enterococcus, Escherichia, Kosakonia, Serratia, Chromobacterium, Mucor and Trichoderma. Several bacterial and fungal isolates tested positive for lipase enzyme production by qualitative and quantitative methods. **Conclusions:** Culture dependent and culture independent methods are complementary techniques that should be used hand in hand for a better understanding of bacterial communities and for studies regarding the potential functional properties of the isolates.

Author Disclosure Block:

L.M. Silva-Bedoya: None. **J. Morales-Rodríguez:** None. **M.S. Sánchez-Pinzón:** None. **G.E. Cadavid-Restrepo:** None. **C.X. Moreno-Herrera:** None.

Poster Board Number:

SUNDAY-121

Publishing Title:

Involvement of Laccase-Like Multicopper Oxidase in Humic Substances Degradation by Diverse Polar Soil Bacteria

Author Block:

H. Park, H. Do, J. Lee, H. Park, **D. Kim**; Korea Polar Res. Inst., Incheon, Korea, Republic of

Abstract Body:

Background: Soil humic substances (HS) are widely distributed in cold environments and comprise a significant fraction of soil organic carbon. Although bacteria dominate cold environment, there have been few studies on the mechnisms of HS biodegradation and alteration by them. Until recently, the HS degradatiopn has been focused on fungi associated with their nonspecific oxidizing enzymes such as laccase.**Methods:** Bacterial strains (n=281) were isolated at 15°C using a minimal medium containing humic acids (HA), a principal component of HS, from various polar soil samples: 217 strains from the Antarctic and 64 strains from Arctic soil. Among them, 73 potential HA-degrading bacteria were phylogenetically identifiedand then laccase-like multicopper oxidase (LMCO) gene fragments were obtained from them by PCR.**Results:** The 73 selected bacteria were affiliated with phyla *Proteobacteria* (73.9%), *Actinobacteria* (20.5%), and *Bacteroidetes* (5.5%). Most of them degraded HA at 10°C to 25°C, but not at temperatures over 30°C, indicating their cold-adapted degradative abilities. Thirty unique LMCOgene fragments were PCR-amplified from 71% of the isolated HA-degrading bacteria, all of which included the conserved copper binding regions (CBR) I and II, both essential for laccase activity. The bacterial LMCO sequences differed from known fungal laccases; for example, a cysteine residue between CBR I and CBR II of fungal laccases was not detected in the bacteria LMCOs. In addition, computer-aided molecular modeling showed these LMCOs contain a highly conserved copper-dependent active site formed by three histidine residues between CBR I and CBR II.**Conclusions:** Phylogenetic- and modeling-based methods confirmed the wide occurrence of LMCO genes in HA-degrading polar soil bacteria and linked their putative gene functions with initial oxidative reaction for HA degradation.

Author Disclosure Block:

H. Park: None. **H. Do:** None. **J. Lee:** None. **H. Park:** None. **D. Kim:** None.

Poster Board Number:

SUNDAY-123

Publishing Title:

Identification of Bacterial Consortia Members Able to Grow in a Polyether-Polyurethane Varnish

Author Block:

H. Loza-Tavera, A. Savín-Gámez, L. Domínguez-Malfavón, M. Vargas-Suárez, C. Peña-Montes, M. Quirasco; Facultad de Química, Univ. Natl. Autónoma de México, México, D.F., Mexico

Abstract Body:

Background: Polyurethanes (PUs) are synthetic polymers widely used in modern life due to their high durability and versatility. Because of their diverse chemical composition, recycling is complex and toxic, generating accumulation in landfills. There are two types of PUs: polyester (Ps) and polyether (Pe), being PePU the most recalcitrant. While there are many reports on bacterial and fungal species that degrade PsPUs, there are fewer reports on microorganisms able to degrade PePUs. Here we explored PePU biodegradation by microbial consortia.**Methods:** Eight microbial consortia (BP1-BP8) were obtained by culture enrichment in mineral medium with a PePU varnish (Polykack®) as the sole carbon source, inoculated with PU foams collected in a dumpsite. PePU biodegradation by these microbial consortia was evaluated by microbial growth, and analyzed by Fourier Transform Infrared Spectroscopy (FTIRS). Bacterial diversity analysis was performed by PCR-DGGE using consortia's V3 region of 16S rDNA gene. Selected DGGE bands from five consortia were cloned for bacterial identification.**Results:** Consortia BP5 and BP6 generated the largest biomass. FTIRS analysis showed that all the consortia were able to reduce or eliminate carbonyl (1726 cm^{-1}), and urethane (1532 and 1223 cm^{-1}) signals. Consortia BP3, BP6, BP7, and BP8 were also able to reduce ether signal (1108 cm^{-1}). Based on the reduction or elimination of FTIRS signals, consortia BP8, BP4, BP2 and BP6 were the most PePU degradative. Six uncultured bacteria and 16 different genera such as *Paracoccus*, *Advenela*, *Microbacterium*, *Propionibacterium*, *Lysinibacillus*, *Acinetobacter* and *Pseudomonas*, previously reported as xenobiotic degraders, were identified. The genera present in the most degradative consortia were *Mesorhizobium*, *Paracoccus* and *Bartonella* and an uncultured member of the *Jonesiaceae* family.**Conclusions:** These results show that microbial consortia present in PU foams collected from a dumpsite were able to attack Polylack's urethane, carbonyl and ether bonds. They represent microorganisms that could interact to obtain organic molecules from PePU for their living, and that can be useful for developing biotechnological processes for PU biodegradation.

Author Disclosure Block:

H. Loza-Tavera: None. **A. Savín-Gámez:** None. **L. Domínguez-Malfavón:** None. **M. Vargas-Suárez:** None. **C. Peña-Montes:** None. **M. Quirasco:** None.

Poster Board Number:

SUNDAY-124

Publishing Title:

***In Vivo* and *In Vitro* Degradation Studies for Poly(3-Hydroxybutyrate-*co*-3-Hydroxyhexanoate) Biopolymer**

Author Block:

A. Kehail, V. Boominathan, K. Fodor, T. Ferreira, V. Chalivendra, C. Brigham; Univ. of Massachusetts - Dartmouth, Dartmouth, MA

Abstract Body:

Polyhydroxyhexanoates (PHAs) are a family of naturally synthesized polymers that have shown promise as replacements for conventional, petrochemical polymers. These biopolymers are synthesized by bacteria like the Gram-negative betaproteobacterium *Ralstonia eutropha* under conditions of non-carbon nutrient stress. Typically, *R. eutropha* produces the homopolymer poly(3-hydroxybutyrate) [PHB]. However, recently, strains of *R. eutropha* have been developed that can biosynthesize the copolymer poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate) [P(HB-*co*-HHx)]. Studies have shown that this copolymer possesses favorable mechanical properties for use in medical devices and products (*e.g.*, sutures, scaffolds, bone plates). One of the major under-addressed issues associated with the use of biodegradable, bio-based PHA polymers in resorbable medical products is the correlation between the mechanical properties and the *in vivo* material degradation over time. In this study, P(HB-*co*-17%mol HHx) matrices were mechanically tested after either incubation in cultures of human embryonic kidney cells (HEK) for *in vitro* degradation studies for up to 4 weeks, or inserted into *Danio rerio* (zebrafish) tissues for *in vivo* degradation studies for up to 7 weeks. Young's modulus, tensile strength, elongation at break, toughness, and scanning electron microscopy (SEM) images of these samples were examined and compared to control samples that were not exposed to tissues by using a microindenter, tensile-tester setup, and SEM. Experiments have shown that Young's modulus of P(HB-*co*-17mol%HHx) during *in vitro* studies dropped from 3.26 MPa to 2.42 MPa within 4 weeks, and *in vivo* breakdown resulted in a significant decrease in Young's modulus with a drop from 3.26 MPa to 0.51 MPa within 7 weeks. Plasticization and recrystallization were observed and they would be reason for change in mechanical properties.

Author Disclosure Block:

A. Kehail: None. **V. Boominathan:** None. **K. Fodor:** None. **T. Ferreira:** None. **V. Chalivendra:** None. **C. Brigham:** None.

Poster Board Number:

SUNDAY-125

Publishing Title:

Accelerated Biodegradation of Polystyrene and Polyethylene by Mealworms (Larvae of *Tenebrio molitor)* from Various Sources in the USA and China

Author Block:

S. Yang[1], Y. Yang[2], A. Brandon[1], Z. Wang[1], N. Ren[3], J. Yang[2], C. S. Criddle[1], **W. Wu**[1]; [1]Stanford Univ., Stanford, CA, [2]Beihang Univ., Beijing, China, [3]Harbin Inst. of Technology, Harbin, China

Abstract Body:

Polystyrene (PS) and polyethylene (PE) have long been considered resistant to biodegradation. Mealworms (larvae of *Tenebrio molitor*) are common food storage pests and have been cultivated commercially for use as animal and bird food and as a source of insect protein. In 2015, a Beihang-Stanford research team demonstrated PS biodegradation in mealworms obtained in Beijing, China. From a mass balance, the PS ingested by mealworms was converted to CO_2 (up to 48%) and the balance excreted as granule-like fecula (ID < 0.2 mm). PS depolymerization within the guts was demonstrated by a decrease in the average molecular weight and by formation of likely daughter products. The generality of plastic eating behavior of mealworms has been further confirmed by cultivation of mealworms from seven different sources in China (Beijing; Qinghuangdao, Heibei; Shanghai) and the USA (Ham Lake, MN; Marion, IL; Newport News, VA; Trexlertown, PA). All of the mealworms tested to date consume PS, but at different relative rates. At ambient temperature (20-24 ^{o}C), the average daily consumption rate of PS ranged from 0.01 to 0.3 mg per 100 mealworms when fed PS alone. The mealworms also eat low density polyethylene (LDPE) foam as well as high density polyethylene (HDPE) in provided as powder particles as sole diet food. When mealworms (120 in each incubator) from Marion, IL, USA, were fed PS alone, the consumption rate and total amount consumed increased as a function of temperature from 20 to 30 ^{o}C by approximately 3.5±0.3%. Supplementing the diet with normal food (bran) enhanced the PS consumption rate and mass consumed. Higher ratios of bran to PS increased the percentage of added PS (1.80 g) that degraded over a 32 day period: 51.0±0.7% degraded at a ratio of 0:1; 59.1±1.5% at 1:6; 65.5% at 1:3; and 82.5±1.4% at 1:1 . Microbial community analysis indicated that the microbial structure changed significantly after the diet was switched from normal food bran to PS or PS plus bran. No biodegradation was detected when antibiotics were added to the feed, implicating gut microbial activity as the mechanism of PS biodegradation.

Author Disclosure Block:

S. Yang: None. **Y. Yang:** None. **A. Brandon:** None. **Z. Wang:** None. **N. Ren:** None. **J. Yang:** None. **C.S. Criddle:** None. **W. Wu:** None.

Poster Board Number:

SUNDAY-126

Publishing Title:

Environmental Microbial Community Tolerance and Adaptation to Biocides Use in Hydraulic Fracturing Operations

Author Block:

M. F. Campa[1], S. Techtmann[2], M. Patterson[1], A. Garcia de Matos Amaral[3], R. Lamendella[4], C. Grant[4], T. C. Hazen[1]; [1]Univ. of Tennessee, Knoxville, TN, [2]Michigan Technological Univ., Houghton, MI, [3]Federal Univ. of Bahia, Salvador, Brazil, [4]Juniata Coll., Huntingdon, PA

Abstract Body:

Hydraulic fracturing (HF) in the process of injecting a cocktail of chemicals, sand, and water into an underground rock formation to create fractures and release natural gas. Commonly, biocides are added to prevent biofouling of equipment and prevent microbial growth during gas extraction and gas souring. During extraction some of the injected water and chemicals returns to the surface; this flowback has been accidentally released into the environment, potentially exposing streams to HF chemicals such as biocides. To understand the effect biocides have in steams, microcosms were inoculated using stream water impacted by HF operations (three streams), and as control pristine stream water from the same area were used. Two groups of microcosms were set using the two more commonly used biocides, glutaraldehyde and DBNPA. The microcosms were incubated at ambient temperature over a period of 8 weeks. The microbial community adaptation to biocide was tracked by sampling every two weeks and performing 16s rRNA amplicon sequencing as compared to a biological control (no biocide added). Chemical degradation of the biocide was tracked every week by HPLC, as compared to an autoclaved control to track abiotic degradation. Finally, RNA was sampled at time zero and at the end of the experiment to understand what transcripts were upregulated by the presence of biocide, and hence allow the bacteria to adapt and tolerate the biocide. Our aim is that the results of this experiment will help understand the pathways of biocide resistance and the effect they have in the native microbial community.

Author Disclosure Block:

M.F. Campa: None. **S. Techtmann:** None. **M. Patterson:** None. **A. Garcia de Matos Amaral:** None. **R. Lamendella:** None. **C. Grant:** None. **T.C. Hazen:** None.

Poster Board Number:

SUNDAY-127

Publishing Title:

Effect of Cathode Depth on Sediment Microbial Fuel Cell Performance

Author Block:

J. M. PISCIOTTA, P. Minka; West Chester Univ., West Chester, PA

Abstract Body:

Sediment microbial fuel cells (sMFC) are a type of bioelectrochemical system (BES) that generate electric current coupled with catabolism of organic material in sediments. In sMFC a conductive anode is buried in anoxic sediment and electrically connected to a cathode placed in the overlying aerobic water column. SMFCs harness microbial breakdown of organic matter in sediment using autochthonous exoelectrogens to generate an electrical current. SMFCs have been demonstrated to power small-scale devices but most studies to date have been carried out under laboratory conditions. Understanding the microbial communities that develop in field sites and their role as exoelectrogens is important for optimizing sMFC performance. In this study, four sMFC were constructed and deployed for 80 days in a freshwater pond in Chester County, PA to study the power output and electrode microbial communities that develop under field conditions. Anodes were buried in sediment in water 1 m deep. We hypothesized that an illuminated cathode 5 cm below the water's surface would generate higher power output compared to the less well illuminated cathode 750 cm below the water's surface. A weatherproofed datalogger recorded voltage and temperature, and the resulting power output was analyzed. Triplicate electrode biofilm scrapings were collected from three locations on each anode totaling 36 samples analyzed, and triplicate samples were also taken from each harvested cathode. Interestingly, sMFCs with dark submerged lower cathodes resulted in higher power output (0.66 mW/m^2) in duplicate reactors and showed less operating variability over the 80 day experiment compared to duplicate sMFC with cathodes near the pond sun-lit surface. Fluctuations in voltage observed in sMFCs with upper cathodes may be due to greater environmental variability at the water's surface. PCR and denaturing gradient gel electrophoresis (DGGE) was used to compare the microbial communities present on the electrodes. These results indicate that pond sMFCs featuring an illuminated near-surface cathode have a quicker start up time, but that sMFC submerged cathodes produce a higher and more stable electrical current.

Author Disclosure Block:

J.M. Pisciotta: None. **P. Minka:** None.

Poster Board Number:

SUNDAY-128

Publishing Title:

Activity-Based Protein Profiling of Ammonia Monooxygenase in *Nitrosomonas europaea*

Author Block:

K. Bennett[1], N. C. Sadler[2], A. T. Wright[2], C. Yeager[3], M. R. Hyman[1]; [1]North Carolina State Univ., Raleigh, NC, [2]Pacific Northwest Natl. Lab., Richland, WA, [3]Los Alamos Natl. Lab., Los Alamos, NM

Abstract Body:

The nitrifying bacterium *Nitrosomonas europaea* oxidizes ammonia (NH_3) to nitrite (NO_2^-) through the sequential activities of ammonia monooxygenase (AMO) and hydroxylamine oxidoreductase (HAO). 1,7-octadiyne (17OD) was evaluated as a mechanism-based inactivator of AMO and used as an activity-based protein profiling probe for AMO. Inactivation of NH_4^+-dependent O_2 uptake by *N. europaea* was time and concentration dependent in the presence of 17OD, and there was no effect of 17OD on HAO activity. The effects of 17OD were irreversible and *de novo* protein synthesis was required for the recovery of ammonia-oxidizing activity following exposure to 17OD. Cells exposed to 17OD were reacted with Alexafluor 647-azide using a copper-catalyzed azide-alkyne cycloaddition reaction and analyzed by SDS-PAGE and IR scanning. A fluorescent 28 kDa polypeptide consistent with the mass of AmoA was observed in cells exposed to 17OD but not in cells that were pretreated with either allylthiourea or acetylene before 17OD. The 28 kDa polypeptide was membrane-associated and aggregated when SDS-solubilized samples were heated in the presence of β-mercaptoethanol. The same fluorescent polypeptide was also observed in cells pre-treated with several other diynes, but not in cells that were pre-treated with the structural homologs of these diynes, which contained only a single ethynyl functional group. Proteomic analyses including in gel digestion and MALDI-TOF/TOF analysis of the fluorescent polypeptide and on-bead digestion of biotin/streptavidin purified proteins from 17OD-treated cells confirmed the target of 17OD was AmoA, the active-site-containing polypeptide of AMO.

Author Disclosure Block:

K. Bennett: None. **N.C. Sadler:** None. **A.T. Wright:** None. **C. Yeager:** None. **M.R. Hyman:** None.

Poster Board Number:

SUNDAY-129

Publishing Title:

Characterization of Lipase from *Bacillus subtilis* Isolated from Oil-Contaminated Soil

Author Block:

T. O. Femi-Ola; Ekiti State Univ., Ado Ekiti, Nigeria

Abstract Body:

Lipases are glycerol ester hydrolases (EC: 3.1.1.3) which hydrolyses ester linkages of glycerides at water-oil interfaces. They occupy a prominent place among biocatalysts owing to their novel and divergent industrial applications. The interest in microbial lipase production has increased mainly because of its vast potential application in various industries ranging from pharmaceutical, bioremediation and waste water treatment. An extracellular lipase from *Bacillus subtilis* isolated from oil polluted soil was partially purified and characterized in this study. The enzyme was purified to 19.36 fold and the molecular weight was estimated to be 48.63 kDa by SDS-PAGE. The temperature optimum was 60 ^{o}C, while the enzyme exhibited appreciable thermostability retaining 70 % of activity at 70 ^{o}C for 1h. The lipase was most active in the pH range of 7-9 with an optimum activity at pH 8.0. The enzyme activity declined in the presence of Al^{3+} and Fe^{2+}, while Na^{+} stimulated the activity. Olive oil was found to be the preferred substrate. The maximum velocity V_{max} and K_m of the lipase during the hydrolysis of olive oil were 39.45 μmol/min/mil and 20.01 mM respectively.

Author Disclosure Block:

T.O. Femi-Ola: None.

Poster Board Number:

SUNDAY-130

Publishing Title:

Reduction of *Salmonella* Populations on Cucumber Fruit by Application of Lytic Bacteriophages

Author Block:

M. Sharma[1], G. Dashiell[2], E. T. Handy[1], C. East[1], R. Reynnells[3], P. D. Millner[1], C. White[4], F. Hashem[5], E. Nyarko[6], S. Micallef[7], A. Collins[8]; [1]USDA Agricultural Res. Service, Beltsville, MD, [2]Univ. of Maryland Eastern Shore, Princess Anne, MD, [3]Univ of Maryland Eastern Shore, Beltsville, MD, [4]Unviersity of Maryland Eastern Shore, Princess Anne, MD, [5]Univ. of Marlyand Eastern Shore, Princess Anne, MD, [6]Unviersity of Delaware, Newark, DE, [7]Univ. of Maryland, College Park, MD, [8]State Univ. of Pennsylvania, Manheim, PA

Abstract Body:

Background: Foodborne illness outbreaks of *Salmonella enterica* associated with consumption of cucumbers (*Cucumis sativus*) in the U.S. in 2014 and 2015 sickened over 1000 people and caused 5 deaths. Whole and fresh-cut cucumbers are susceptible to *Salmonella* contamination during growing and harvesting. We evaluated the effectiveness of applying lytic bacteriophages, a non-chemical intervention, to reduce *Salmonella* populations on cucumbers.**Methods:** Unwaxed cucumbers (greenhouse-grown, 'Lisboa' variety, or mini-cucumbers purchased at retail) were inoculated with 5 log CFU/cucumber *Salmonella* Newport, and then sprayed with 3.2 mL of 9 log PFU/ml SalmoFresh, a *Salmonella*-specific bacteriophage preparation (phage), or phosphate-buffered saline (control). Whole cucumbers were stored at 10 or 22^{o}C for 7 days. Mini-cucumbers were sliced with a sterile knife to investigate *Salmonella* transfer to mesocarp, and fresh-cut pieces were stored at 4^{o}C for 2 days. *Salmonella* populations from whole and fresh-cut cucumbers were recovered on selective media. Three replicates of each trial were conducted, and statistical analysis (Student *t*-Test) was performed.**Results:** Populations (log CFU/cucumber) of *Salmonella* on phage-treated whole cucumbers stored at 10^{o}C were 2.44, 1.72, and 1.56, which were significantly ($p < 0.05$) lower than those on control-treated cucumbers (4.27, 3.20, and 2.33) on days 0, 1, and 4, respectively. There were no significant differences in *Salmonella* populations between phage- and control-treatments of a) whole cucumbers at 22^{o}C, or b) fresh-cut cucumbers stored at 4^{o}C.**Conclusions:** Direct application of lytic bacteriophages (through spraying) to whole cucumbers was more effective in reducing *Salmonella* populations than indirect transfer of phages (through slicing) to the mesocarp of cucumbers. The greatest reduction in *Salmonella* populations occurred immediately after spraying phages onto cucumbers.

Author Disclosure Block:

M. Sharma: None. **G. Dashiell:** None. **E.T. Handy:** None. **C. East:** None. **R. Reynnells:** None. **P.D. Millner:** None. **C. White:** None. **F. Hashem:** None. **E. Nyarko:** None. **S. Micallef:** None. **A. Collins:** None.

Poster Board Number:

SUNDAY-131

Publishing Title:

***Salmonella*-Phage Diversity in Different Animals: Are Phages from Cattle, Pigs, and Wild Animals the Same?**

Author Block:

D. Rivera[1], F. Duenas[1], R. Tardone[1], C. Caceres[1], V. Toledo[1], C. Hamilton-West[2], **A. I. Moreno Switt**[1]; [1]Univ. Andres Bello, Santiago, Chile, [2]Univ. de Chile, Santiago, Chile

Abstract Body:

Salmonella is a widely distributed zoonotic pathogen that can be transmitted to humans as foodborne or by animal contact. *Salmonella* has 2,600 serovars that show distinct distribution in different animals and geographic regions; being a few serovars (e.g., Enteritidis) globally distributed. While phages infecting *Salmonella* are very abundant, our understanding on *Salmonella*-phage diversity is very limited. The aim of this study was to investigate the diversity of *Salmonella* -phage systems in different animals and geographic locations in Chile. *Salmonella* and phages were isolated from samples obtained on continental Chile of 49 llamas and small ruminants, 10 pig farms, 35 backyard poultry, 80 wild birds, and 28 reptiles, 8 dairy farms, along with backyard cows from Eastern Island. Isolates representing the most common serovars (Enteritidis, Typhimurium, Infantis, and Heidelberg) were used to isolate phages. On the isolated phages we tested the host range using i) a set of foreign isolates (26 *Salmonella* serovars obtained from the US), and ii) a set of native isolates (31 *Salmonella* obtained from the locations studied above). We have isolated 105 *Salmonella* isolates (9 from pigs, 31 from poultry, 31 from wild bird, 28 from reptiles, and one from a goat). Phages were more abundant than *Salmonella* with 201 phage isolates obtained. Most of the phages (40-60%) were isolated in *S.* Enteritidis, which was also the most susceptible serovar. Host range characterization with foreign isolates displayed that phages show a narrow host range (mean=4 isolates lysed), but with native isolates, phages show a wide host range (mean=13 isolates lysed). All *Salmonella* of serogroups D were the most susceptible to phages. Clustering also showed a group of widely distributed phages that are very specific to *Salmonella* of the serogroups D. Phages from remote location as Eastern Island showed distinct host specificity. *Salmonella* phages appears to be adapted to local isolates; while wide host range and narrow host range phages are co-existing on animals, resistant and susceptible *Salmonella* are also present indicating a dynamic that need to be further investigated.

Author Disclosure Block:

D. Rivera: None. **F. Duenas:** None. **R. Tardone:** None. **C. Caceres:** None. **V. Toledo:** None. **C. Hamilton-West:** None. **A.I. Moreno Switt:** None.

Poster Board Number:

SUNDAY-132

Publishing Title:

Evaluation of the Lytic Efficacy of Novel Bacteriophage Φ241 Against *Escherichia coli* O157:H7 in Model Food Systems

Author Block:

H. Deepnarain[1], Z. Lu[2]; [1]Kennesaw State Univ., Smyrna, GA, [2]Kennesaw State Univ., Kennesaw, GA

Abstract Body:

Background: Bacteriophages are emerging as alternative biocontrol agents against foodborne pathogens such as *Escherichia coli* O157:H7. A novel bacteriophage Φ241 specific to *E. coli* O157:H7 was previously isolated from a cucumber fermentation. Although the phage showed a great potential as a biocontrol agent of *E. coli* O157:H7, its lytic activity in real foods has not yet been studied. The objective of this study was to evaluate the lytic efficacy of Φ241 against *E. coli* O157:H7 in three model food systems (cucumber juice, beef broth, and apple juice). **Methods:** The lytic activity of Φ241 in each food was evaluated at three different multiplicities of infection (MOIs 1, 10, and 100). A filter-sterilized food system was inoculated with E. coli O157:H7 to achieve the initial cell concentration of 1.2 x 106 CFU/mL and with an appropriate amount of phage stock to reach the target MOI. The inoculated food was incubated at 37°C, and sampled in 1-h intervals for 10 h. The cell concentration in each sample was determined by the plate count method. The phage titer at the end of the experiment was determined through a plaque assay. **Results:** In cucumber juice, the phage infection at MOI 100 caused a rapid cell lysis within 1 h, resulting in 4.6-log-unit reduction in cell concentration compared with the control. Infection at MOIs 10 and 1 resulted in 4.8- and 2.5-log-unit reduction in cell concentration within 2 h, respectively. Infection at any of the three MOIs caused at least 5.3-log-unit reduction in cell concentration compared with the control. In beef broth, phage infection at MOIs 100, 10, 1 caused 2, 1, and 0.2 log reduction in cell concentration within 1 h, respectively, compared with the control. Infection for 3 h at MOIs 100, 10, and 1 caused 3, 4.3, and 3.7 log reduction, respectively. In contrast, the cell concentration in apple juice remained unchanged regardless of the presence or absence of the phage over the 10-h period.**Conclusions:** The data showed that the infection by Φ241 caused a rapid decrease in *E. coli* O157:H7 concentration in both cucumber juice and beef broth within 1 to 3 h depending on the MOI. This suggested that phage Φ241 could be used as a biocontrol agent for *E. coli* O157:H7 in certain vegetables and meats.

Author Disclosure Block:

H. Deepnarain: None. **Z. Lu:** None.

Poster Board Number:

SUNDAY-133

Publishing Title:

Effect Of Combined Stressors Added Sequentially Or Simultaneously On *listeria Monocytogenes* growth

Author Block:

L. Henderson, V. Guariglia-Oropeza, M. Wiedmann; Cornell Univ., Ithaca, NY

Abstract Body:

Listeria monocytogenes (*Lm*) is a bacterial pathogen that not only stands out due to its ability for extended survival and growth in a variety of environments, but also causes the third highest number of deaths among the major foodborne pathogens, making it essential to find ways to prevent transmission. On foods, *Lm* is exposed to many stresses, and is able to survive and grow under high salt and low temperature conditions. This research focuses on controlling *Lm* in the food supply, specifically reducing or eliminating its ability to multiply under conditions of high salt and low pH. We are interested in determining the efficiency of combined stresses on *Lm*, specifically whether the efficiency changes when stressors are added simultaneously or sequentially. We hypothesized that increased salt concentration and decreased pH work synergistically to inhibit *Lm* growth. However, prior exposure to salt would improve *Lm* growth in both WT and mutant strains. A "checkerboard" assay was used to determine the combined effects of salt and pH on WT (10403S) and sigma factor mutant (ΔBCHL) strains of *Lm*. Both strains were grown at 37C to mid-log phase (OD 0.4) in BHI and then inoculated in BHI containing 0, 2, 4, or 8% salt and pH 2, 3, 4, 5, 6, or 7. Growth was determined using a plate reader (OD 600). Data showed no difference in growth between strains that had prior exposure to salt and those that did not. The mutant strain is killed more quickly under combined stressors, and pH 3 medium showed the greatest amount of killing, regardless of strain. Surprisingly, strains grown in pH 4 medium showed a protective trend. Understanding stress conditions under which *Lm* can survive and grow can lead to different approaches to prevent contamination of foods and infections caused by foodborne pathogens.

Author Disclosure Block:

L. Henderson: None. **V. Guariglia-Oropeza:** None. **M. Wiedmann:** None.

Poster Board Number:

SUNDAY-134

Publishing Title:

Environmental Factors Impact the Expression of *Listeria monocytogenes* Attachment Factors

Author Block:

S. Levadney Smith, B. M. Prüß, T. M. Bergholz; North Dakota State Univ., Fargo, ND

Abstract Body:

Foodborne illness attributed to *Listeria monocytogenes* and contaminated fresh produce has increased significantly within the past two decades. *L. monocytogenes* is able to attach to produce and various surfaces in the processing environment. Once attached, *L. monocytogenes* can form biofilms that allow the bacteria to survive stressful conditions, such as low temperature, osmotic stress, and low nutrients. Attachment factors have been identified, such as Lcp, a cellulose binding protein, known to aid in the attachment of *L. monocytogenes* to the surface of produce. However, little is known about how environmental stresses impact the expression of attachment factors. Understanding environmental signals that affect attachment will allow targeted development of control measures aimed at reducing pathogens in the food supply. The expression of a known attachment factor, Lcp, in *L. monocytogenes* was evaluated upon exposure to stress.Single copy chromosomal reporter fusions were constructed in isolates from fresh produce outbreaks using pMJG2, and β-glucuronidase (GUS) as the reporter protein to assay the promoter activity of *lcp*. Reporter fusions were also constructed from the promoter of *uspA*, a SigB regulated gene, known to be induced under NaCl stress, as a positive control. The promoter activity was observed under conditions, including lettuce lysate, 5 ppm chlorine, 4.5% NaCl and 4°C. Cells were harvested at 6 time points, lysed, exposed to 4-MUG (4-Methylumbelliferyl-b-D-glucuronide), and promoter activity was reported as µM MUG/log CFU/Min. Log CFU was obtained by cell count.With initial exposure to lettuce lysate at 25°C, expression of GUS by the *lcp* promoter was elevated 1.43 fold compared to the control (minimal media, 25°C). There was no induction of GUS by the *lcp* promoter at 4°C. There was an initial increase followed by an overall reduction in GUS by the *uspA* promoter for all stresses at 4°C and NaCl at 25°C.Our findings indicate that expression of attachment factors are impacted by stress, such as the initial induction of GUS by the *lcp* promoter for lettuce lysate at 25°C and the induction of, followed by gradual decrease in expression of GUS by the *uspA* promoter, which indicates adaptation to stress over time. Finding conditions that can reduce the expression of attachment factors could be substantial in developing control measures that prevent initial attachment.

Author Disclosure Block:

S. Levadney Smith: None. **B.M. Prüß:** None. **T.M. Bergholz:** None.

Poster Board Number:

SUNDAY-135

Publishing Title:

Internalization and Survival of *Salmonella enterica* and *E. coli* O157:H7 in Growing Lettuce (*Lactuca sativa*)

Author Block:

D. Deng[1], Z. Shen[2], M. Reed[1], **G. ZHENG**[1]; [1]Lincoln Univ., Jefferson City, MO, [2]Univ. of Missouri-Columbia, Columbia, MO

Abstract Body:

Background: Foodborne disease outbreaks associated with fresh produce have been increasing in recent years in the U.S., due to increasing consumption of these products as a healthy diet. The current post-harvesting washing and sanitization processes do not always effectively reduce pathogen contamination of produce. Some of human pathogens, such as *Salmonella* spp. and *E. coli* O157:H7, can internalize in produce and therefore, are protected by plant tissues from disinfectant washes. It is believed that understanding the internalization and survival of the pathogens in produce can facilitate developing novel methods to reduce pathogen contamination of produce.**Methods:** Salad bowl green lettuce (Lactuca sativa) were seeded and grown in soil or hydroponic media and red florescent-protein-expressing *Salmonella enterica* ATCC 13036 or *E. coli* O157:H7 ATCC700728 was spiked into the growth media at different stages of growing lettuce. In a separate test, the pathogens were injected into the growing lettuce by syringes. The internalization and survival of the pathogens in the growing lettuce were then monitored by recovering the microbes from the edible portions of the vegetables.**Results:** Cells of *S. enterica* and *E. coli* O157:H7 appeared to be able to internalize into lettuce via root-to-shoot pathway, but the internalization efficacy of this route decreased with the maturing of the lettuce. The internalization rate (CFU/per lettuce) of the pathogens was higher in the hydroponic than in the soil system. Overall, internalized pathogens decayed rapidly in 20 days after the spike or injection.**Conclusions:** The results of this study suggest that cells of *S. enterica* and *E. coli* O157:H7 may be uptaken by growing lettuce via roots or wounds and that the survival of internalized pathogens may be longer than 20 days inside the vegetable, underlining the importance of preharvest controlling management.

Author Disclosure Block:

D. Deng: None. **Z. Shen:** None. **M. Reed:** None. **G. Zheng:** None.

Poster Board Number:

SUNDAY-136

Publishing Title:

Development of Predictive Model for the Growth of *Salmonella* spp. in Liquid Whole Egg, Egg Yolk, and Egg White

Author Block:

J-S. Moon; Animal and Plant, Quarantine Agency, Anyang-si, Gyeonggi-go, Korea, Republic of

Abstract Body:

Background: The food-borne illness by *Salmonella* in egg has recently become an issue in the United States. With the increasing demand of consumers on the safety of eggs in Korea, it was also important for us to systematically evaluate the risk of egg. Therefore, the objective of this study was to determine the risk of *Salmonella* by investigating the growth rate according to the time of various storage temperatures of the liquid whole egg, egg yolk, and egg white.**Methods:** We investigated and analyzed the growth rate of each samples. Fresh eggs (< 1 day after lay) collected were prepared by liquid whole egg, egg yolk, and egg white, and 10 ml aliquots of each sample were transferred to corresponding tubes. Samples were inoculated with a mixed culture (approximately 2.0-3.0 log CFU/ml) containing equal proportions of five species of *Salmonella* (S. bareilly, S. richmond, S. typhimurium monophasic, S. enteritidis and S. gallinarum). **Results:** After inoculating *Salmonella* onto the liquid whole egg, liquid yolk, and liquid egg white, the effects of storage temperatures (5, 10, 15, 20, 25, 30, 35, and 40℃) on the growth of *Salmonella* mixture were investigated. Lag time decreased and specific growth rate increased with a storage temperature (10℃<40℃) but not changed 5℃ in the liquid whole egg, liquid yolk, and liquid egg white. The growth of *Salmonella* was hardly made in the egg white. A Baranyi model was fitted for each temperature growth data and corresponding maximum growth rates were estimated as primary model. Pseudo-R2 values were greater than 0.98 for primary models. The maximum growth rates were obtained from each primary model in liquid whole egg, liquid egg yolk, and egg white and then plotted against temperature. The maximum growth rate was modeled using the modified Baranyi model for the secondary model, respectively. Combination of primary and secondary models resulted in a dynamic model whose predictions were also validated for two sinusoidal profiles. **Conclusions:** The model developed in this study can be used for predicting the growth of *Salmonella* spp. in liquid egg under a variety of temperature conditions in Korea.

Author Disclosure Block:

J. Moon: B. Collaborator; Self; Y. J. Kim, Y. H. Kim, B. R. Song, J. S. Lim, E. J. Heo, H. J. Park, S. H. Wee.

Poster Board Number:

SUNDAY-137

Publishing Title:

The Potential Binding of Tulane Virus to Human Intestinal Caco-2 Cells

Author Block:

K. Kniel, Q. Wang, J. Teichman; Univ. of Delaware, Newark, DE

Abstract Body:

The newly discovered Tulane virus (TV) is structurally similar to human norovirus (huNoV), and can recognize and bind to specific cellular receptors (e.g., HBGAs); however, specific binding has not been observed using human or animal cell lines rather only with chemical modulators. Here the binding between TV and cultured human Caco-2 cells is studied to better understand the early processes of infection during huNoV replication. This study aims to evaluate the binding capability of TV to differentiated and undifferentiated Caco-2 cells. The role of heparin sulfate during binding was also measured. After Caco-2 cells (ATCC #HTB-37) were seeded in 6-well plates. Undifferentiated Caco-2 (U-Caco-2) cells was obtained after reaching confluency, whereas differentiated Caco-2 cells (D-Caco-2) were obtained at 10 days post-confluency. Cells were washed with HBSS 3x and chilled to 4°C. Virus samples included ten-fold series dilutions of TV and TV pre-incubated in heparin sodium salt solutions (0-200 µg/ml) for 1 h at 37 °C. Virus samples were added into wells and incubated at 4°C for 1 h with gentle agitation. After three washes with cold PBS to remove the unbound TV, binding was determined by plaque assay. The data (3-replicates/3-samples each) were analyzed by ANOVA on JMP. TV can bind both U- and D- Caco-2 cells significantly, and the binding levels decreased with the initial inoculum concentration. With initial inoculum titers of 5.48 ± 0.18 log PFU, it was determined the binding levels were 2.23± 0.07 log PFU and 2.94 ± 0.15 log PFU for U- and D- Caco-2 cells, respectively. D-Caco-2 cells bound TV more significantly than U-Caco-2 cells ($p<0.05$). In addition, with increasing levels of heparin sulfate salts, the binding for both U-and D- cells was substantially inhibited from 2.85 ± 0.46 (at 0 µg/ml) to 2.19 ± 0.36 (at 200 µg/ml), and from 3.03 ± 0.66 (at 0 µg/ml) to 1.88 ± 0.24 (at 200 µg/ml), respectively. TV is more likely to bind D-Caco-2 cells indicating these cells may express molecules during differentiation that enhance binding affinity. Heparin sulfate may play a role in binding, or may act as a receptor.

Author Disclosure Block:

K. Kniel: None. **Q. Wang:** None. **J. Teichman:** None.

Poster Board Number:

SUNDAY-138

Publishing Title:

Comparison of the Activity of Monomeric, Dimeric and Polymeric Polyphenols from Blueberries Against Hepatitis A Virus

Author Block:

S. Joshi[1], A. Howell[2], **D. H. D'Souza**[1]; [1]The Univ. of Tennessee-Knoxville, Knoxville, TN, [2]Rutgers Univ., Chatsworth, NJ

Abstract Body:

Background: Blueberry polyphenols are reported to have antiviral properties against foodborne human norovirus surrogates. Blueberry polyphenols include anthocyanins, flavonoids and proanthocyanidins (PAC), also known as condensed tannins. PACs are dimers, oligomers, and polymers of catechins that contain linkages between C4 and C8 (or C6). PACs in blueberries vary in profile and quantity that contain B-type linkages compared to cranberry PACs that have A-type linkages. These linkages and structures affect activity. The objective of this research was to elucidate the mode of action of blueberry polyphenols against HAV by (a) comparing the anti-HAV activity of monomeric catechin, procyanidin B2, blueberry B-type PAC and A-type PAC from cranberries (C-PAC) at 37ºC using plaque assays; (b) determining the effect of B-PAC on viral adsorption and replication using pre- and post-infection treatment; and (c) determining the effects of B-PAC on HAV structure using Transmission Electron Microscopy (TEM).**Methods:** Equal amounts of HAV at 5 log PFU/ml were mixed with equal amounts of catechin hydrate, procyanidin B2, or B-PAC and treated for 3, 6 and 24 h at 37ºC. Viral infectivity of three replicate treatments carried out in duplicate was evaluated using standardized plaque assays and treated viruses were also visualized by TEM. Pre- and post-infection treatments of host cells with B-PAC were carried out and data were statistically analyzed.**Results:** No significant effect with 1 mg/ml catechin or 1 mg/ml procyanidin B2 was observed on HAV titers after 24 h. Both polymeric B-PAC and C-PAC reduced HAV titers to non-detectable levels after 3 h at 37ºC. Viral infection of host cells after treatment with 0.5 mg/ml B-PAC showed that HAV titers were reduced by 0.34 log PFU/ml indicating that B-PAC had modest effects on preventing viral adsorption. However, no significant reduction was obtained after post-infection treatment and thus was ineffective against viral replication. TEM observations revealed moderate effect on viral structure with either damaged viral capsid or binding by PAC, possibly preventing their attachment to host cells.**Conclusions:** These results provide insights on the mode of action and information for use in developing preventive antiviral therapies.

Author Disclosure Block:

S. Joshi: None. **A. Howell:** None. **D.H. D'Souza:** None.

Poster Board Number:

SUNDAY-139

Publishing Title:

Vacuum Steam Pasteurization for Inactivation of Pathogens on Flaxseed and Improvement of Shelf Life

Author Block:

M. K. Shah[1], B. Eklund[1], G. Asa[2], K. Graber[2], J. Sherwood[1], T. M. Bergholz[1]; [1]North Dakota State Univ., Fargo, ND, [2]Napasol North America, Fargo, ND

Abstract Body:

Low moisture foods have been implicated in several outbreaks due to *Salmonella* and *E. coli* O157:H7 contamination. While numerous thermal inactivation studies have been conducted for *Salmonella* on nuts, studies on other seeds and grains are minimal. We wanted to determine the efficacy of vacuum steam pasteurization (VSP) for inactivation of pathogens on whole and milled flaxseed. We also wanted to determine if VSP impacted the water activity (a_w) and microbial counts during storage. For the inactivation studies, whole flaxseed was separately inoculated with *Salmonella* PT30, *E. coli* O157:H7 and *Enterococcus faecium*, and milled flaxseed was inoculated with only *Salmonella* PT30 directly with bacterial lawns grown on brain heart infusion agar plates. Following equilibration of a_w to the initial a_w, 25g of inoculated samples were pasteurized for varying times (0.5-5.0 min) at 75°C, 85°C, 95°C, and 105°C in triplicate. On average, reductions of 5.5 ± 1.2, 5.7 ± 0.4, and 5.3 ± 0.5 log CFU/g were observed for *Salmonella, E. coli* O157:H7 and *E. faecium* respectively at 75°C after treatment for 1 minute on whole flaxseed whereas a reduction of 5.6 ± 1.3 log CFU/g was observed for *Salmonella* on milled flaxseed when treated for 30 seconds at 85C. For the shelf life study, whole and milled flaxseed were treated at 75°C, 90°C, and 105°C for 3 minutes and for 9 minutes at 90°C in triplicate. A_w and plate counts for total aerobes, *B. cereus*, yeast and mold were measured for 4 months. Over this period, microbial counts on the treated samples at all conditions remained lower than unpasteurized samples, and a decrease in a_w of the treated products were observed. Vacuum steam pasteurization can be effectively used for inactivation of pathogens on low moisture seeds with a positive impact on microbial shelf life and a_w, and *E. faecium* may be used as a surrogate when evaluating vacuum steam pasteurization.

Author Disclosure Block:

M.K. Shah: None. **B. Eklund:** None. **G. Asa:** D. Employee; Self; Napasol North america. **K. Graber:** D. Employee; Self; Napasol North America. **J. Sherwood:** None. **T.M. Bergholz:** None.

Poster Board Number:

SUNDAY-140

Publishing Title:

Cross-Reaction of *Legionella* Loop-Mediated Isothermal Amplification (LAMP) and PCR Primers with Other Clinically and Environmentally Important Bacterial Species: A Caution for DNA Based Assays for Pathogen Detection

Author Block:

J. L. Baron[1], J. L. Jacobs[2], J. J. Martinson[1], J. E. Stout[2]; [1]Univ. of Pittsburgh, Pittsburgh, PA, [2]Special Pathogens Lab., Pittsburgh, PA

Abstract Body:

Many molecular detection assays including PCR, qPCR, and Loop-mediated isothermal amplification (LAMP), have been developed to identify microorganisms, which can be highly beneficial for rapidly detecting and diagnosing contamination or infection with pathogenic microbes, such as *Legionella* species. However, comparing results of the gold standard method of microbiological culturing and these new tests shows discordance between the two, with molecular assays exhibiting positivity when culture shows little or no *Legionella* spp. In our attempt to develop LAMP and PCR primers specific for the detection of *Legionella* spp. targeting the 16S rRNA gene we noted numerous cross-reactions with other clinically and environmentally relevant pathogens in each assay. This non-overlapping pattern of cross-reaction was explained phylogenetically based on the relationship of observed sequence similarity of these organisms to *Legionella* spp. We believe that findings of *Legionella* spp. PCR or qPCR positivity in environmental water samples that contain little or no culturable *Legionella* species may be due to unwanted primer cross-reaction with other species of commonly isolated waterborne pathogens. We suggest that other protein-encoding genes be chosen for genus level identification instead and/or that primer specificity be exhaustively tested, especially in their design for pathogen detection.

Author Disclosure Block:

J.L. Baron: None. **J.L. Jacobs:** None. **J.J. Martinson:** None. **J.E. Stout:** None.

Poster Board Number:

SUNDAY-141

Publishing Title:

Comparison Of Pcr And Lamp Techniques To Detect Ompw In Viable But Nonculturable *vibrio Cholerae* O1 In Water Samples

Author Block:

P. Chamanrokh, A. Huq, R. Colwell; Maryland Pathogen Res. Inst., College Park, MD

Abstract Body:

Background: *Vibrio cholerae* can enter to the viable but nonculturable (VBNC) state which is difficult to detect by conventional bacteriological culture methods (Colwell et al., 1994; Chaiyanan et al., 2007). VBNC cells cannot be considered dead but rather a potential hazard to public health (Huq et al., 2000). Loop-mediated isothermal amplification (LAMP) is a gene amplification method in which all reactions are conducted under isothermal conditions without requiring for a thermocycler. (Mazaheri assadi et al., 2015). In this study, ompW gene in VBNC *V. cholerae* O1 employing LAMP and to compare results with PCR for specificity and sensitivity. **Methods:** VBNC cells of *V. cholerae* were prepared in four laboratory microcosms with 1% Instant Ocean (IO) and 60 samples collected at five locations in Maryland. The spiked samples incubated at 4˚C and 25˚C for one year. Samples were examined by culture and also using direct fluorescent antibody-direct viable count (DFA-DVC) (Huq et al., 2012). Chromosomal DNA was extracted from coccoid VBNC cells to detect presence of ompW, using PCR and LAMP and employing specific primers.**Results:** PCR and LAMP showed 100% specificity. PCR was positive at a concentration of 10 copies of targeted DNA. However, LAMP was positive with 5 copies, i.e., twice sensitive than PCR. All microcosm samples entered into VBNC stage determined by plating on non-selective LB agar and selective TCBS agar. All cells of *V. cholerae* O1 were coccoid and however, viable as determined by DFA-DVC. The ompW gene was detected in VBNC coccoid forms of *V. cholerae* O1 in the samples incubated for one year at two different temperatures 4 and 25°C and 49 (76.5%) out of 64 samples were positive by PCR while 60 (93.75%) were positive by LAMP.**Conclusion:** LAMP has higher specificity, is more sensitive, and accurate than PCR in detecting ompW gene in VBNC forms of *Vibrio cholerae* O1.

Author Disclosure Block:

P. Chamanrokh: None. **A. Huq:** None. **R. Colwell:** None.

Poster Board Number:

SUNDAY-142

Publishing Title:

Building a Freshwater Bacterial Flora Database for Remote Sensing Applications

Author Block:

D. Zimmerman[1], L. A. Moore[1], J. A. Concha[2], N. Raqueño[2], M. A. B. Herman[1], **F. Ontiveros**[1]; [1]St. John Fisher Coll., Rochester, NY, [2]RIT, Rochester, NY

Abstract Body:

The identification and classification of microbial flora in bodies of fresh water has the potential of enhancing our understanding of this ecosystem and improving water management and bioremediation. This effort may be facilitated by the use of remote sensing technologies. For the last 3 years our undergraduate students have collected water samples in the Lake Ontario Rochester Embayment and Irondequoit Bay with the goal of constructing a database of bacterial species and water parameters (e.g. organic matter and chlorophyl content). Such a database is necessary to establish potential correlations between the presence of certain bacterial species and water parameters that can be measured using satellite imagery collected by the Landsat 8 OLI and TIRS sensors. In the past we reported initial efforts at mapping the distribution of bacterial species using 16S rRNA. Here we present our results for the summer of 2015 and present a compounded analysis of 3 consecutive summers. Of approximately 450 bacterial isolates, we have cultured and identified more than 40 different species spanning over 20 genera. Several fish and human pathogens were identified, and antibiotic-resistance profiles determined. Year to year variation of the flora's composition at individual locations has emerged as the main challenge in establishing reproducible patterns that may be linked to satellite measurements. We discuss this variability and potential solutions.

Author Disclosure Block:

D. Zimmerman: None. **L.A. Moore:** None. **J.A. Concha:** None. **N. Raqueño:** None. **M.A.B. Herman:** None. **F. Ontiveros:** None.

Poster Board Number:

SUNDAY-143

Publishing Title:

Droplet Digital PCR as an Efficient Tool for the Detection of *Salmonella* in Sediments

Author Block:

G. Singh, T. Stenstrom; Durban Univ. of Technology, Durban, South Africa

Abstract Body:

Background: *Salmonella* is one of the major causes of intestinal diseases globally and etiological agent of more severe systemic illnesses such as paratyphoid and typhoid fevers. Despite the advances in microbial identification and detection techniques like quantitative PCR (qPCR), *Salmonella* quantification in complex environmental samples remains a challenge due to the presence of PCR inhibitors. Sediments are the indicators of sources of microbial contamination and presents a clear picture of microbial load than aquatic resources. The relatively new droplet digital PCR (ddPCR) has emerged as a direct quantitative method which is highly tolerant to PCR inhibitors and relinquishes the necessity for calibration/standard curve. However, only limited information is available on the application of ddPCR to detect *Salmonella* in sediments.**Methods:** This study examined the efficacy of ddPCR assay for quantitative enumeration of *Salmonella* in sediments retrieved from the four sites of Palmeit river near the Quarry road informal settlements, Durban. ddPCR performance was evaluated simultaneously with qPCR targeting *ttr* gene in spiked water samples as well as sediment samples.**Results:** ddPCR significantly improved the analytical sensitivity over that of qPCR, in the sediment samples and improved the detection of low concentrations of *Salmonella* even in presence of PCR inhibitors. The expected copy numbers measured from both the methods (qPCR and ddPCR) showed good R^2 values of 0.999 and 0.994, respectively. The Palmeit river site within the informal settlements exhibits *Salmonella* in the range of 255±37 and 818±30 *Salmonell*a/gram ($p \leq 0.0001$) in qPCR and ddPCR respectively. The significantly higher numbers encountered with ddPCR at all the four sites of Palmeit river, may be due to the partitioning strategy where the PCR reaction is split into 20,000 droplets, ideally each droplet contains 1 or less copies of targeted DNA and efficiently reducing the effect of PCR inhibitors.**Conclusions:** The improved detection of *Salmonella* in sediments makes droplet digital PCR a viable quantification method and a promising technology for the quantification of *Salmonella* in multifarious environmental samples.

Author Disclosure Block:

G. Singh: None. **T. Stenstrom:** None.

Poster Board Number:

SUNDAY-144

Publishing Title:

Validation Of A Method For Isolation Of *clostridium Difficile* From Environmental Samples

Author Block:

C. Beato-Melendez, N. Goodyear; Univ. of Massachusetts Lowell, Lowell, MA

Abstract Body:

Community-acquired *C. difficile* infections are increasing in incidence and severity. While healthcare facilities strive to eliminate environmental *C. difficile*, the incidence and significance of *C. difficile* in home, workplace, and other public environments is unclear. Most isolation protocols are designed for use with stool specimens, therefore effective methods for environmental samples must be validated. In this study, 3 initial enrichment broths, 2 spore enrichment techniques, and 2 agars were compared for their ability to recover *C. difficile* from spiked and environmental samples.For spiked samples, *C. difficile* (ATCC 43255) was inoculated to TSB and incubated (37°C, 48h). Fresh TSB, BHIB and chopped meat broth (CMB) were inoculated (100 µL) and incubated (37°C, 48h). Broths were subjected to no treatment, absolute alcohol shock or heat shock (65°C, 20 min). Post-treatment, 100 µL was inoculated to Columbia CNA (CNA) and *C. difficile* selective (CCFA) agars and incubated anaerobically (37°C, 48h). Colonies were counted and the identification confirmed with a commercial kit. While CMB provided the best overall recovery, TSB with alcohol shock and CCFA was equivalent (see Table). TSB is less costly than CMB therefore we selected TSB, alcohol shock and CCFA as our protocol. In addition, we used blood agar to ensure recovery of susceptible strains.

Enrichment Broth	Agar	No Treatment	Alcohol Shock	Heat Shock
BHIB	CCFA	16	19	22
	CNA	4	4	8
CMB	CCFA	24	24	21
	CNA	23	21	19
TSB	CCFA	21	24	18
	CNA	6	8	9

The protocol was tested environmentally in 2 public restrooms at UMass Lowell and 2 homes. Swiffer cloths were used to sample toilet seats and the floor by the toilet. Swiffers were immersed in 25 mL PBS for 10 min; half the PBS was inoculated directly to TSB and half was spiked with stock *C. difficile* and inoculated. All TSBs were processed as described above. All

spiked samples were positive on all media. All home samples and 1 university restroom were negative, but 1 university restroom floor sample was positive for *C. difficile* (nontoxigenic). This pilot study demonstrates that TSB as an initial enrichment broth followed by alcohol shock and plating to CCFA and BA is a lower cost, effective method of isolating *C. difficile* from environmental sites.

Author Disclosure Block:

C. Beato-Melendez: None. **N. Goodyear:** None.

Poster Board Number:

SUNDAY-145

Publishing Title:

Use of DNA Aptamers as Alternative Ligands for Estimation of Infectious Human Norovirus Particles

Author Block:

M. Moore, B. Mertens, B. Bobay, L-A. Jaykus; North Carolina State Univ., Raleigh, NC

Abstract Body:

Background: Study of human norovirus is hindered by the lack of an *in vitro* cultivation method. Because of this, discrimination of infectious virus particles is not possible. Binding assays with histo-blood group antigens (HBGAs) in conjunction with RT-qPCR have been used for infectivity discrimination based on the hypothesis that loss of binding correlates with loss of infectivity. HBGAs are expensive, hard to purify, and not broadly reactive; however broadly reactive norovirus antibodies and ssDNA aptamers have been identified. The purpose of this study is to determine if a broadly reactive norovirus aptamer can be used to evaluate capsid integrity, serving as a proxy for infectivity discrimination.**Methods:** Virus-like particles (VLPs) of norovirus GII.4 Sydney (SYV) were subjected to various time-temperature combinations resulting in differing degrees of degradation. The binding of aptamer M6-2, HBGA, and antibody NS14 was then assessed by plate-based assay. Binding to completely denatured VLPs was also measured to determine the degree of target sequence-dependent recognition. The structure of aptamer M6-2 was predicted using the MC-Fold/MC-Sym webserver and its binding to the SYV capsid predicted using HADDOCK.**Results:** Aptamer M6-2 binding to heat treated VLPs was more similar to that of HBGAs than antibody NS14. For example, treatment of VLPs at 75^{o}C for one min resulted in 98.1 ± 1.9% and 79.9 ± 10.2% signal loss for HBGA and M6-2, respectively; while only 24.7 ± 9.7% of signal was lost for NS14. M6-2 and HBGA displayed small amounts of capsid protein sequence-dependent binding (2.0 ± 1.3% and 0.5 ± 1.2%, respectively), while NS14 displayed significantly more (26.4 ± 3.9%). These data suggest that binding to M6-2 and HBGA is highly dependent on capsid integrity, making it a good proxy for infectivity. Predicted docking of M6-2 to SYV revealed binding interactions in the P2 and N terminal P1 subdomains in residues that are close to those involved in HBGA binding; whereas NS14 binds the C terminal P1 subdomain.**Conclusions:** Taken together, the binding, structural prediction, and docking analyses suggest that nucleic acid aptamers may serve as favorable alternative ligands to HBGAs for estimating viral infectivity.

Author Disclosure Block:

M. Moore: None. **B. Mertens:** None. **B. Bobay:** None. **L. Jaykus:** None.

Poster Board Number:

SUNDAY-146

Publishing Title:

Optimization of Species Directed Isolation Method Using Magnetic Nanoparticles

Author Block:

E. I. Hussein, A. A. Aljabali, O. I. Aljumaili; Yarmouk Univ., Irbid, Jordan

Abstract Body:

Nanoparticles hold great potential in broad range of applications from biological detection to drug delivery and targeting in the medical field. The research work presented in here will focus on the bacterial detection using magnetic nanoparticles labeled with specific antibodies. *Serratia marcescens* is a gram-negative bacillus that has been recognised as a cause of hospital-acquired infection. According to that we developed a simple and reliable method to capture the bacteria specifically and with high precision. The nanoparticles were labelled with specific antibody that is specifically bind to *Serratia marcescens* as a model and proof-of-concept. Although various diagnostic approaches for pathogen detection have been reported in the literature, most are too expensive, lengthy or limited in specificity for clinical use. Nanoparticle with unique and fascinating properties offers improved accuracy in the specific detection over current laboratory methods. Here, this work presents novel magnetic nanoparticles-antibody probes capable of rapid and specific profiling of pathogens directly in clinical/food samples. A nanoparticle modified with antibodies that is targeted toward the desired species was designed to detect *Serratia marcescens* using simple magnetic devices. Ultimately, antibody labelled magnetic nanoparticle platform will allow both universal and specific detection of various clinically/food relevant bacterial species, with sensitivity down to single bacteria. Furthermore, the generic platform described could be used to rapidly identify and phenotype pathogens for a variety of applications.

Author Disclosure Block:

E.I. Hussein: None. **A.A. Aljabali:** None. **O.I. Aljumaili:** None.

Poster Board Number:

SUNDAY-147

Publishing Title:

How Accurate Is my Metagenomic Analysis? Depends on Diversity of 16s Rrna Gene Targets in Pcr

Author Block:

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Abstract Body:

Metagenomic analyses are crucial to study the diversity, activity and dynamics of uncultivable microbes. An integral part in the analyses is the polymerase chain reaction (PCR), which can also present a bottleneck, such as when amplifying parts of 16S rRNA genes. Often in agarose electrophoresis gels we see smeared DNA bands of wrong size, which are considered to be non-specific PCR errors and are eliminated. However, if these amplicons are not errors, then a part of the sample's true diversity is lost. Therefore, we explored if band smearing after PCR is in fact caused by imperfectly paired strands of the amplified DNA. Using synthetic oligonucleotides that mimic 16S rRNA in PCR, we determined that the amount of smear in agarose gels was proportional to DNA sequence heterogeneity of the 16S rRNA variable regions. Since in denaturing alkaline gels, lack of smear showed that amplified DNA had a uniform size, we suspected that two separate groups of structures had formed - correctly and imperfectly paired DNA strands. This was confirmed, by isolating and sequencing the two groups of structures using a newly developed electroelution procedure and characterizing the pairing of the sequenced DNA strands using a bioinformatics approach. When amplifying highly heterogeneous target DNA, such as 16S rRNA, imperfect pairing of the amplified DNA can lead to band smearing in agarose gels, which is not an indicator of low specificity of the PCR. Since the smear in agarose gels is only a structural part of the correctly amplified DNA, it carries important information about the richness and diversity of the analysed microbial communities. Incorrect handling of 16S rRNA samples, e.g. eliminating smearing by increasing PCR amplification stringency or excision of amplified DNA, thus leads to an underestimation of the richness and diversity of microbial species.

Author Disclosure Block:

J. Zrimec: None. **T. Rijavec:** None. **A. Lapanje:** None.

Poster Board Number:

SUNDAY-148

Publishing Title:

Evaluation of Hygienic Hand Washing Practices of the University Community of Moncton Campus

Author Block:

A. C. Diallo, E. DAKO, P. AUDET, R. Baudouin; Université de Moncton, Moncton, NB, Canada

Abstract Body:

Background: Infectious diseases of bacterial or viral origin are often the cause of a high rate of absenteeism of students and staff at the university. This has been inferred that it leads to a reduction in their academic and/or work performance. **Objective:** The objective of this study was to estimate the general knowledge of hygienic practices of the students and the staff the Université de Moncton and to carry out microbiological samplings of the environmental surfaces of the campus. **Methods:** A campaign to promote hand washing and general hygiene practices was undertaken on the campus. Surveys, performed on university students and staff before and after the campaign, were used to assess the impact of this campaign on their knowledge about hand washing and general hygiene practices. Environmental sampling was conducted before and after the campaign for the presence of certain pathogenic microorganisms i.e. total coliforms, *E. coli*, *Staphylococcus aureus*, and Methicillin-resistant *S. aureus* (MRSA). Care was taken to ensure the sampling was done on the surfaces that come into direct contact with the hands in both the toilet facilities and classrooms. The sampling methods used in this work are those described in the The Compendium of Analytical Methods (Health Canada, 2010). **Results:** The second survey scores are slightly higher than the first survey, The ANOVA (α=0.05), shows there is no significant difference between the scores of the two surveys ($p > 0.05$) except for the scale of questionnaires regarding general knowledge of hygienic practices, for which $p = 0.03$. The statistical results show that the general knowledge of the procedures have been improved, but the behavior of the respondents and their emphasis on hygienic practices were not significantly improved after the awareness campaign. Sampling and microbiological analyzes meanwhile, confirmed the presence of all bacteria except MRSA. After the awareness campaign, the average number of microorganisms has generally declined, especially the total count of *S. aureus* on sampled surfaces. **Conclusion:** This study generated results promising enough to warrant further study on other university campuses. These results highlight the necessity of having reminders regarding hygienic practices and proper handwashing on campus and the value of having awareness campaigns at regular frequencies.

Author Disclosure Block:

A.C. Diallo: None. **E. Dako:** None. **P. Audet:** None. **R. Baudouin:** None.

Poster Board Number:

SUNDAY-149

Publishing Title:

Bacteria on Paper Currency: Methods, Survival and Implications

Author Block:

R. Wolff, A. Stewart-Akers; South Univ., Columbia, SC

Abstract Body:

Background: Paper currency is probably the most handled fomite where transmission of pathogenic microbes occurs in the general public. Because of very limited data on U.S. currency contamination this study was undertaken to examine the methods and biology used, including almost eight years of sampling dollar bills as a classroom project.**Methods:** Sampling of currency, collected from many business locations, includes direct contact pressing one half of a folded bill onto the media surface of a petri plate. This method was compared to using a moistened swab for bacterial recovery. As only ¼ of a bill is sampled onto each type of media in surveillance studies, all four surfaces were sampled to determine sampling efficiency. Bacterial survival was also studied by spraying *E. coli* and *Staphylococcus aureus* onto the bills and sampling over time.**Results:** Direct contact was more effective than swabbing paper currency, resulting in bacterial recovery from 100% greater numbers on Nutrient Agar to more than 1000%, with only 11% showing a lower recovery. When all four quarter surfaces were sampled, 42/45 (93.3%) had mannitol fermenters (on MSA) on the first surface sampled if they were present on another surface. 10 bills had a surface where no MF grew, and all but one had only several total fermenters on the bill. All surfaces of all bills had non-mannitol fermenters. Survival of *E. coli* on the dried bills was less than 48 hours while *Staphylococcus aureus* survived over 3 weeks. Over 300 bacterial species have been identified via 435 pyrosequencing on local currency.**Conclusions:** The simple method of pressing dollar bills onto agar media with direct contact is both a simple and effective procedure for sampling the microbes and pathogens found contaminating paper currency in circulation in the U.S. The method has demonstrated in previous studies both seasonal and weather changes, allows monitoring of specific organisms, and longer term changes in abundance (decline in numbers of MRSA over the past two years). This is an underutilized and underappreciated technique that could be highly valuable for BioSurveillance of disease organisms and even some biothreats.

Author Disclosure Block:

R. Wolff: None. **A. Stewart-Akers:** None.

Poster Board Number:

SUNDAY-150

Publishing Title:

Synthesis of Metal Nanoparticles Using *Stenotrophomonas maltophilia* and *Bacillus subtilis* Isolated from the Natural Habitats of Uae

Author Block:

G. Ganesh, P. Srinivasan, N. Sood, T. Gokhale; Birla Inst. of Technology and Sci. Pilani, Dubai, United Arab Emirates

Abstract Body:

Biological synthesis of nanoparticles is an ecofriendly process that makes use of biological systems such as bacteria, fungi and also plants whereas physical and chemical methods which are routinely used produce toxic substances to the environment. A number of bacteria were screened from the garden soil of Dubai International Academic City, Dubai, UAE for their potential to synthesize metal nanoparticles amongst which two isolates exhibited a high potential to synthesize copper nanoparticles. These isolates were identified using 16s rRNA sequencing as *Stenotrophomonas maltophilia* and *Bacillus subtilis* and could tolerate more than 2mM of Copper Sulphate. The isolates were also able to tolerate high concentrations of different metals. Therefore, we investigated the ability of the two isolates to synthesize extracellular nanoparticles using UV Visible spectrophotometer, whereas the intracellular accumulation of metal nanoparticles was studied using ICP-OES. *S.maltophilia* and *B.subtilis* were grown in presence of 2mM Copper Sulphate, 5mM Lead Nitrate, 2mM Zinc Sulphate, 2mM Sodium Tungstate, 1.7mM Molybdenum Trioxide, 5mM Barium Chloride, 0.2mM Cadmium Sulphate, 5mM Manganese Chloride, 0.2mM Cobalt Chloride in sterile Luria Bertani broth. Spectrophotometric analysis of culture supernatants and media controls exhibited a shift in absorption wavelength from the control to the supernatants of *S.maltophilia* and *B.subtilis*, eg: in presence of zinc, a shift in absorption was observed from 200nm for control to 290nm for *S.maltophilia* and 270nm for *B.subtilis*. This indicates the presence of nanoparticles in the culture supernatant. The ICP-OES analysis of bacterial cells showed *S.maltophilia* and *B.subtilis* accumulated 23650ppm and 596ppm of copper, 234.9ppm and 238.7ppm of lead, 46.1ppm and 28.83ppm of Barium, 74.7ppm and 23.9ppm of manganese respectively. The particle size analysis of the culture supernatant of *S.maltophilia* grown in presence of 34mM Molybdenum Trioxide and 2mM Lead Nitrate showed the presence of 74nm size molybdenum and 160nm size lead nanoparticles. We have demonstrated for the first time synthesis of molybdenum trioxide nanoparticles using bacteria. This work is the first report of such study on bacterial synthesis of nanoparticles from the natural isolates of UAE.

Author Disclosure Block:

G. Ganesh: None. **P. Srinivasan:** None. **N. Sood:** None. **T. Gokhale:** None.

Poster Board Number:

SUNDAY-151

Publishing Title:

Elucidation Of Anti-Leptospiral Efficacy Of Indian Herbal Preparations In Mouse Model

Author Block:

R. Anandan, A. Lakshmipriya, V. Nithyakalyani, C. Banurekha, S. Rajkumar; Dr MGR Janaki Coll. of Arts and Sci. for, Chennai, India

Abstract Body:

Background: Bacteria belonging to the genera *Leptospira* can cause disease in humans and animals (Leptospirosis). Leptospirosis can cause a wide range of symptoms and can be asymptomatic as well. If untreated can lead to kidney damage, meningitis, liver failure, respiratory distress, and even death. Leptospirosis is most common in temperate and tropical countries like India and workers who work outdoors or with animals are particularly predisposed. Antibiotics are the standard treatment strategy. In this study, three Indian herbs were screened for anti-leptosprial efficacy *in vitro* and *in vivo* using a mouse model. **Methods:** Aqueous and organic solvent extracts of three Indian herbs, *Hemisdesmus Indicus*, *Phyllanthus niruri*, and *Alium sativum* were tested *in vitro* for anti-leptospiral effcicacy against *Leptospira gripphotyphosa* and examined dark field microscopically. Aqueous herbal extracts were tested for *in vivo* toxicity in Swiss Webster mice. Mice were infected intraperitoneally with *L. gripphotyphosa* and treated orally with aqueous extracts of the three Indian herbs for 7 consecutive days from day 0. Blood samples were drawn and tested for leptospira with microscopic slide agglutination test (MSAT). Mice kidney and liver were histopathologically examined for specific changes and reversal in response to treatment. **Results:** Aqueous extracts of *H. indicus* exhibited greater anti-leptospiral activity *in vitro* compared to *P. niruri* and *A. sativum*. This superior anti-leptospiral activity of *H. indicus* was more evident in the hepato- and renal-protective effects, as evidenced by histopathological examination of the treated animals. Mice tolerated these Indian herbal preparation to the maximum dose of 2000 mg/Kg body weight without moribundity or histological changes. Further phytochemical studies are in progress to identify the active anti-leptospiral molecules.**Conclusions:** Tropical countries like India, hold a treasure trove of innumerable herbs and native medications that offer tremendous potential to treat a variety of diseases. More Governmental and non-Governmental resources need to be channelized to support research and development efforts to exploit these herbs for public health benefits.

Author Disclosure Block:

R. Anandan: None. **A. Lakshmipriya:** None. **V. Nithyakalyani:** None. **C. Banurekha:** None. **S. Rajkumar:** None.

Poster Board Number:

SUNDAY-152

Publishing Title:

Optimization of a Quick Protocol for Extracting DNA from Environmental Water Samples to Use in Pcr Assays

Author Block:

A. G. Burruss, V. Casas, S. Maloy; San Diego State Univ., San Diego, CA

Abstract Body:

Microbial metagenomic techniques have vastly improved what we know about the bacteria in our environments. Using metagenomic data we now better understand the microbial landscape of our environments and the processes contained therein. Most notably, the process of horizontal gene transfer (HGT) between bacteria and mobile genetic elements such as plasmids and phage. In the case of phage carrying exotoxin genes, the transfer of these genes can lead to the evolution of novel pathogens. Tracking these events at the genetic level therefore becomes a critical component in understanding how and at what frequency these pathogens evolve in our environments. In an effort to study these events in "real time" we found that one major hurdle was the number and volumes of environmental samples we would need to gather and process for DNA to screen by PCR. We needed a DNA extraction method that used a low volume of sample, yielded PCR ready DNA, and was quick enough to process large numbers of samples at one time. We optimized a commercial DNA extraction card kit to extract PCR ready DNA from water samples using small volumes of the sample, an efficient extraction time per sample, and allowing for DNA extraction from large numbers of samples. DNA extraction from the cards was optimized based on volume of sample added to the card, drying time in between sample addition, drying source, and the number of card discs added to a PCR. We added from one to five milliliters of sample water at one ml increments to five separate PCR cards. We also compared the effectiveness of drying them in incubators as opposed to air drying under a biosafety cabinet. To test the quality of DNA extracted from the cards, we ran them in a 16S rDNA PCR assay and compared results. The results showed that adding three ml of sample water in one ml increments, with drying time in a BSC in between, and one DNA card disc per reaction, yielded the best results in a 16S PCR. Overall, DNA extraction from water samples using this alternative protocol was simple, yielded PCR quality DNA, and most favorably, reduced total extraction time per sample. We intend to apply this efficient extraction technique to monitor HGT between phage and bacteria across time in small scale, aquatic mesocosms.

Author Disclosure Block:

A.G. Burruss: None. **V. Casas:** None. **S. Maloy:** None.

Poster Board Number:

SUNDAY-153

Publishing Title:

Biodiversity and Enrichment of Organic Sulfur-Driven Iron-Respiring Microorganisms from Marine Sediments

Author Block:

A. M. Zwolinski[1], S. L. Seston[1], N. H. Szeinbaum[2], S. Ganesh[2], F. J. Stewart[2], M. Taillefert[2], T. J. DiChristina[2]; [1]Alverno Coll., Milwaukee, WI, [2]Georgia Inst. of Technology, Atlanta, GA

Abstract Body:

Anaerobic iron respiration may have been one of the very first forms of microbial metabolism to evolve early on in the history of life; yet up until recent years, little has been known about the mechanisms that drive such ancient respiration processes. The biodiversity of microbial communities capable of iron-reduction in anoxic environments has been previously overlooked and the significance of this activity is not fully understood. To better understand the phylogenetic and metabolic diversity of iron-reducing bacteria (FeRB), sediment samples from the salt marsh ecosystem of Skidaway Island (GA), were obtained and used to test the hypothesis that organic sulfur (S) compounds can act as electron shuttles for FeRB to extracellular insoluble Fe(III) oxides. The chemical composition of multiple core samples collected from Skidaway Island were analyzed, and core sediments exhibiting overlapping peaks of ferrous iron (Fe^{2+}) and organic sulfur compounds (thiols) were used for subsequent microbial community analysis and enrichment of organic S-driven FeRB. Organic S-driven FeRB were enriched in cultures by providing an environment without oxygen (N_2 headspace) with the following conditions i) lactate or acetate as electron donors, ii) various thiols as electron shuttles and iii) solid Fe(III) oxide as electron acceptor. Enrichment of FeRB was also performed in the presence of rifamycin to select for *Archaea*. Microbial community structure in core sediments and enrichment cultures was analyzed via deep sequencing of the 16S rRNA hypervariable V4 region with bacterial and archaeal specific primer sets. Results indicate that thiols increase FeRB iron activity in enrichments and impact the community structure of enriched microorganisms. Molybdenum-containing controls suggest that sulfide-catalyzed electron shuttling is not a dominant mechanism for microbial Fe(III) reduction in marine sediments.

Author Disclosure Block:

A.M. Zwolinski: None. **S.L. Seston:** None. **N.H. Szeinbaum:** None. **S. Ganesh:** None. **F.J. Stewart:** None. **M. Taillefert:** None. **T.J. DiChristina:** None.

Poster Board Number:

SUNDAY-154

Publishing Title:

Groundwater Microbial Communities Across Contamination Gradients At A Nuclear Waste Site

Author Block:

P. Zhang[1], A. Rocha[2], Z. He[1], J. Zhou[1]; [1]Univ. of Oklahoma, Norman, OK, [2]Oak Ridge Natl. Lab., Oak Ridge, TN

Abstract Body:

Nuclear waste contamination of aquifers poses an extensive range of environmental conditions such as U(VI), nitrate, pH and other heavy metals and the contaminated groundwater presents a great human-health concern, thus understanding how the groundwater microbial communities function under different environmental conditions is of high importance for predicting the fate of U(VI) in the environment. In this study, we profiled microbial communities using a functional gene microarray (GeoChip 5.0), which infers functional roles of microbial community members. Groundwater samples were collected from 69 wells at the Oak Ridge site, representing an extensive range of contamination, e.g., U(VI) from 0.002 to 9 mg/L, nitrate from 0.1 to 10,000 mg/L, pH from 3.4 to 10.5, Al from 0 to 120 mg/L. The results showed that microbial communities were distinct among wells differing in groundwater geochemistry characteristics. Canonical correspondence analysis and Mantel tests showed that groundwater U(VI), nitrate, pH, metals (such as Al, Ca), dissolved organic carbon and sulfate were key factors shaping the microbial functional communities. In particular, substantial variations were apparent in stress, C, N, and S cycling genes and genes involved in metal reduction and resistance, and abundances of these genes correlated with their functional roles. However, while high contamination decreased both functional and phylogenetic diversity, the impact was more severe on phylogenetic diversity than community metabolic potential based on GeoChip and 16S rRNA gene sequencing results. This study improves our understanding of microbial communities across a large-scale contamination gradient at a typical nuclear waste site.Acknowledgments: ENIGMA (http://enigma.lbl.gov) at LBNL supported by Office of Biological and Environmental Research US Dept of Energy Contract No: DE-AC02-05CH11231

Author Disclosure Block:

P. Zhang: None. **A. Rocha:** None. **Z. He:** None. **J. Zhou:** None.

Poster Board Number:

SUNDAY-155

Publishing Title:

Methylphosphonate Degradation May Contribute To Methane Production In The Oxic Water Column Of Lake Matano

Author Block:

M. Yao, J. Maresca; Univ. of Delaware, Newark, DE

Abstract Body:

Methane is an important greenhouse gas, and global methane emissions from lakes account for 6 - 16 % of total natural methane emissions. The oxic water column of ultra-oligotrophic Lake Matano is a net methane source: the methane concentration is 0.003 mmol L^{-1}, which is supersaturated compared to the atmosphere. The active methanogenesis in the anoxic bottom waters and sediments does not completely explain the high methane concentration in the oxic surface waters. Here, we demonstrate that 4 out of 7 heterotrophic bacteria isolated from the surface water of Lake Matano can produce methane when using methylphosphononic acid as the only phosphorus source. To determine how phosphate influences methane production, we added 0.2 mM phosphate into the medium with methylphosphonic acid. After adding phosphate, methane production by strain LM-Y was completely inhibited, while the other three strains LM-1, LM-5 and LM6-1 were inhibited 40 - 50%. Metagenomic analysis results also demonstrate that the C-P lyase pathway, a multienzyme complex responsible for phosphonate degradation, is enriched in the surface water. The C-P lyase pathway has broad substrate specificity, and methane is produced when methylphosphonate is the substrate. We further demonstrate that our isolates encode *phnJ*, a component of the C-P lyase complex, and that this gene is expressed when the isolates use methylphosphonic acid as a phosphorus source. Therefore, our results show that methane produced by methylphosphonate degradation may contribute to the high methane concentration in the oxic surface water of Lake Matano.

Author Disclosure Block:

M. Yao: None. **J. Maresca:** None.

Poster Board Number:

SUNDAY-157

Publishing Title:

Coupled Quantitative Proteomic and Genomic Strategies to Elucidate Shifts in Carbon Utilization of Filamentous Ascomycete Fungi

Author Block:

S. Purvine[1], C. A. Zeiner[2], E. M. Zink[1], S. Wu[3], L. Paša-Tolić[1], D. L. Chaput[4], C. M. Santelli[5], C. M. Hansel[6]; [1]PNNL - Battelle, Richland, WA, [2]Boston Univ., Boston, MA, [3]Univ. of Oklahoma, Norman, OK, [4]Smithsonian Inst., Washington D.C., DC, [5]Smithsonian Inst., Washington, DC, [6]Woods Hole Oceanographic Inst., Woods Hole, MA

Abstract Body:

Proteomics information can be used to identify and quantify gene products expressed under different cellular conditions. Using bottom-up proteomics coupled with isotopic labeling such as Isobaric tags for Relative and Absolute Quantification (iTRAQ), multiple experimental conditions can be compared in a single Liquid Chromatography Mass Spectrometry (LC-MS/MS) experiment, with pooled samples allowing comparison across multiple experiments. Furthermore, using initial genomic sequence information from the Joint Genome Institute (JGI), organisms previously not well characterized can be studied. We applied the proteomics pipeline capability housed at the Environmental Molecular Sciences Laboratory (EMSL) at Pacific Northwest National Laboratory (PNNL) to a multi-genome microbial system, specifically to the secretomes of four poorly characterized, cellulose-degrading Ascomycete fungi over a three-week time course in conjunction with a joint proposal with JGI for genome sequencing. This work employed: iTRAQ labelling of multiple time points from multiple secreted protein sources; LC-MS/MS analysis using Thermo Velos Orbitrap with higher-energy collisional dissociation (HCD); data extraction of reporter ion abundances using in house software (MASIC); limit of detection assessment (2 sigma of median abundance as LOD); significant differential expression analysis (+/- 2 sigma from mean log ratio across any two compared time points); and BLAST analysis to assign annotations to extracellular enzymes utilized for carbon degradation by each fungus. We present the methods applied to these samples and demonstrate striking differences in the regulation of extracellular enzymes across the four species over time. This work highlights the utility of quantitative iTRAQ proteomics coupled to genome sequencing in directly comparing metabolic processes across diverse microbial species.

Author Disclosure Block:

S. Purvine: None. **C.A. Zeiner:** None. **E.M. Zink:** None. **S. Wu:** None. **L. Paša-Tolić:** None. **D.L. Chaput:** None. **C.M. Santelli:** None. **C.M. Hansel:** None.

Poster Board Number:

SUNDAY-158

Publishing Title:

Overexpression of the Phosphoketolase Pathway Increases Carbon Conversion Efficiency in an Obligate *Methanotrophic bacterium*

Author Block:

C. A. Henard, Holly K Smith, Loretta Lutackas, Michael T Guarnieri; Natl. Renewable Energy Lab., Golden, CO

Abstract Body:

Microbial conversion of methane to value-added chemicals offers a path to mitigate GHG emissions and valorize this abundant, squandered gas. Preliminary techno-economic analysis has identified carbon conversion efficiency (CCE) as a key cost driver in these processes, underscoring the need for rational methanotrophic bacterial strain engineering. The phosphoketolase pathway functions in a subset of microbes, including several methylotrophs, as an alternative route to produce acetyl-CoA, bypassing the CO_2 lost in the Embden-Meyerhof-Parnas pathway through pyruvate decarboxylation. Based on its ability to limit CO_2 loss, the phosphoketolase pathway can be leveraged for carbon efficient biocatalysis. Here, we show that the promising industrial methane biocatalyst, *Methylomicrobium buryatense*, encodes two phosphoketolase isoforms that are highly expressed in methane-grown cells. Phosphoketolase overexpression increased the CCE of *M. buryatense* by decreasing methane consumption while maintaining similar growth compared to wild-type in stirred-tank bioreactors. Further analysis of carbon partitioning indicated that the increased CCE observed in this engineered strain was partially due to decreased flux through the C1 dissimilatory pathway. Based on these data and that phosphoketolase orthologues are found in most alpha- and gammaproteobacterial methylotroph genomes sequenced to date, these enzymes can be used to increase CCE in methane biocatalytic processes and play an important role in C1 metabolism.

Author Disclosure Block:

C.A. Henard: None.

Poster Board Number:

SUNDAY-159

Publishing Title:

Exploring Anaerobic 4-Hydroxyproline Metabolism in Bacteria

Author Block:

Y. Huang[1], A. Martinez-del Campo[1], S. H. Kopf[2], Y. Wei[3], J. R. Leadbetter[4], D. K. Newman[4], E. P. Balskus[1]; [1]Harvard Univ., Cambridge, MA, [2]Princeton Univ., Princeton, NJ, [3]Metabolic Engineering Res. Lab., Singapore, Singapore, [4]California Inst. of Technology, Pasadena, CA

Abstract Body:

4-Hydroxyproline is one of the most abundant post-translationally modified amino acid and is highly abundant in collagen, a structural protein in animals, and proteins in the plant cell wall. A pathway for anaerobic metabolism of 4-hydroxyproline was first described in Stickland fermentation where 4-hydroxyproline is used by Clostridial species as an electron acceptor (Stickland, L.H., 1934, Biochem. J., **28**:1746-1759). The enzymes and intermediates involved in this pathway have not been previously identified. We designed a chemically defined medium to enrich for microbes that can reduce 4-hydroxyproline anaerobically for energy generation. Sediment samples collected from marshes and swamps in Woods Hole, MA were used to inoculate the enrichment cultures and 4-hydroxyproline concentrations were monitored over time by HPLC. In all enrichment cultures, 4-hydroxyproline concentration decreased over time. To better understand the pathway involved in anaerobic 4-hydroxyproline metabolism, we designed and optimized degenerate primers to amplify a novel 4-hydroxyproline dehydratase strictly found in facultative and obligate anaerobes. We will present our work on the identification of this dehydratase gene using degenerate primers and the characterization of bacterial communities in the enrichment cultures.

Author Disclosure Block:

Y. Huang: None. **A. Martinez-del Campo:** None. **S.H. Kopf:** None. **Y. Wei:** None. **J.R. Leadbetter:** None. **D.K. Newman:** None. **E.P. Balskus:** None.

Poster Board Number:

SUNDAY-160

Publishing Title:

Presence of the Anaerobic Benzoyl-CoA Degradation Pathway in Animal Samples

Author Block:

K. Fullerton, A. W. Porter, L. Y. Young; Rutgers Univ., New Brunswick, NJ

Abstract Body:

Under anaerobic conditions, aromatic compounds are metabolized to a common intermediate, benzoyl-CoA, which is further degraded to enter central metabolism. This pathway has been shown to be widely distributed in environmental samples, including freshwater pond sediment, digester sludge, and agricultural soil, but its presence in animal systems has not been evaluated. Due to the ubiquitous nature of the benzoyl-CoA pathway in the environment and the presence of aromatic residues in animal feedstocks, we predict that this pathway is present in the anaerobic gut environment of animals. Methanogenic enrichment cultures were established from bovine rumen fluid and were amended with lignoaromatic monomers or the aromatic amino acids. HPLC analysis shows loss of substrate within two weeks with no apparent accumulation of intermediates in the enrichments, establishing that aromatics are rapidly metabolized in the rumen. DGGE fingerprints of tyrosine and tryptophan enriched cultures show distinct community profiles with different dominant banding patterns. Culture-independent studies were performed using fecal samples from a variety of domesticated farm animals to understand the prevalence of the benzoyl-CoA pathway in a wider scope. The presence of methanogens in these samples implies that these environments are highly reducing and that oxygen-dependent methods of aromatic catabolism are not likely to be utilized. However, these fecal samples show no amplification with common molecular probes of the benzoyl-CoA pathway, including the benzoate reductase and the BamA hydrolase, indicating that this pathway may not be abundant in the gut or that existing probes do not account for the added microbial diversity seen in the gut environment. These results suggest that aromatic metabolism in the gut is more complex than initially believed, and further investigations into its role in global carbon cycling is needed.

Author Disclosure Block:

K. Fullerton: None. **A.W. Porter:** None. **L.Y. Young:** None.

Poster Board Number:

SUNDAY-161

Publishing Title:

Entomopathogenic Activity of *Lysinibacillus sphaericus* on the Life Cycle of *Aedes aegypti* ≪and≫ Expression of the Hemolysin ≪and≫ Chitinase Genes

Author Block:

P. Rojas Pinzón, J. Dussán Garzón, Microbiological Research Center(CIMIC); Univ. de los Andes, Bogotá, Colombia

Abstract Body:

Lysinibacillus sphaericus (Ls) has been used for the biological control of *Culex* <and> *Anopheles* but not for *Aedes*, the main vectors of tropical diseases. Some strains of *Ls* used for this purpose were isolated in Colombia, a country with reports of dengue, Chykungunya <and> the most recent Zika virus. Due to the genome sequencing of the native strains III(3)7 <and> OT4b.25 genes of pathogenic importance as hemolysins (*hly*) <and> chitinases (*cda*) were annotated. The objective of this study was to monitor the effect of the OT4b.25, III(3)7 <and> 2362 (WHO reference) strains on the mosquito life cycle and determining the expression of chitin-binding protein <and> hemolysin genes on third instar larvae.The larvicidal bioassays were tested following the WHO guidelines for mosquito. The number of hatched eggs <and> live larvae were recorded every 24 h until 72 h. Triplicates for each stage were performed <and> the results were analyzed with ANOVA <and> Tukey test. The expression of a chitin-binding domain <and> hemolysin genes were determinated through RTPCR in the strain III(3)7, after 24h <and> 48h of exposure to instar 3 larvae. The samples were run in triplicate <and> the results were analyzed with a t-test.The bioassays with each stage revealed that native strains retard hatching <and> once the larvae emerge, they die. Likewise, the first larval stages (instar 1-3) are more sensitive to the three *L. sphaericus* strains, which presented 98% of mortality at 48h. With the instar 4 the mortality was 58% at 72h. The expression of a chitin-binding domain was greater at 24 h (ΔRn = 13, 9± 0, 16) than at 48 h (ΔRn = 12, 34± 0, 22) <and> the hemolysin genes are expressed more at 48 h (ΔRn= 17, 45± 0, 26) than at 24 h (ΔRn = 12, 89 ± 0, 58). Although, there are only significant differences in the hemolysin expression.We found that the native strains of *L. sphaericus* have entomopathogenic activity on the *Ae. aegypti* life cycle <and> the expression of chitin-binding domain <and> hemolysin genes may be another important factors in the pathogenicity of this bacteria.

Author Disclosure Block:

P. Rojas Pinzón: None. **J. Dussán Garzón:** None.

Poster Board Number:

SUNDAY-162

Publishing Title:

Conidiation by *Isaria fumosorosea* Under Oxidant Pulses Relates to Initial Substrate

Author Block:

F. R. Muñiz Paredes[1], P. M. Garza-López[2], G. Viniegra-González[1], O. Loera Corral[1]; [1]Univ. Autónoma Metropolitana, Iztapalapa, Mexico, [2]Univ. Autónoma del Estado de Hidalgo, Pachuca de Soto, Mexico

Abstract Body:

Background: Conidia are the main propagules for pest control by entomopathogenic fungi. Hyperoxidant atmospheres (26% O_2) improved conidia production for *Metarhizium anisopliae* and *Isaria fumosorosea* (Tlecuitl-Beristain *et al.* 2010; Miranda-Hernández *et al.* 2014) on agar cultures. However the effect of 26% O_2 pulses remains unclear in solid-state cultures (SSC), the preferred method for large scale production. We determined the effect of 26% O_2 pulses on conidia production in SSC with *I. fumosorosea* strains ARSEF 3302 and CNRCB1, measuring residual O_2 and CO_2 accumulation as indicatives of metabolic activity.**Methods:** SSC were carried out in bottles with 1 or 5 g of initial dry substrate (gds), and inoculated with $1x10^6$ conidia gds^{-1} At 60 h, cotton caps were replaced by rubber seals and 26% O_2 pulses were administrated. Conidia were quantified periodically. Determination of O_2 and CO_2 was carried out by gas chromatography for treatments with 26% O_2 pulses. Bottles maintained with cotton caps throughout culture were used as controls. Variables were determined by triplicate.**Results:** The effect of O_2 pulses on conidia production depended on the initial amount of substrate, since with 1 gds, conidia production increased compared to control in both strains. In contrast, 26% O_2 pulses with 5 gds decreased conidia production. For both strains with 1 gds, O_2 levels were maintained between 20 to 25% and CO_2 remained lower than 7%. Oxidant pulses may increase reactive oxygen species that trigger differentiation of hyphae to conidia. In cultures with 5 gds, O_2 levels were depleted after first pulse and CO_2 accumulated up to 26%, and then cultures decreased respiration leading to conidial inhibition. Even when respiration reassumed at later stages, conidiation did not recover.**Conclusion:** Oxidant pulses improved conidia production in SSC, although amount of initial substrate has to be optimized and considered as a criterion for scaling up SSC with oxidant pulses.

Author Disclosure Block:

F.R. Muñiz Paredes: None. **P.M. Garza-López:** None. **G. Viniegra-González:** None. **O. Loera Corral:** None.

Poster Board Number:

SUNDAY-163

Publishing Title:

Newly Isolated Member of the Genus *Shewanella* Couples Anaerobic Acetate Oxidation to Metal Reduction

Author Block:

N. Szeinbaum, H. Lin, M. Taillefert, T. DiChristina; Georgia Inst. of Technology, Atlanta, GA

Abstract Body:

Background: Mn-linked redox transformations drive a variety of environmental processes in marine and freshwater environments. Mn(III) is a likely significant yet overlooked terminal electron acceptor in modern and ancient metal-rich anaerobic environments. Acetate widely fuels heterotrophic microbial activity in the modern biosphere, and is one of the primordial forms of organic carbon. The microorganisms responsible for coupling anaerobic acetate oxidation to Mn(III) reduction, however, have yet to be identified. **Methods:** Enrichment cultures were initially inoculated with Mn(II)-rich sediments from a perennial creek bank on Skidaway, Georgia. Cultures were incubated in serum bottles under a 100% N_2 atmosphere with acetate and Mn(III)-pyrophosphate as sole electron donor and acceptor, respectively, and maintained by periodic transfers to fresh medium. Bacterial 16S rDNA amplicons were sequenced with Ion Torrent technology. Purified colonies were obtained by standard agar plating techniques. Whole genome sequences were obtained using an Illumina sequencing platform and analyzed using CLC genomic workbench V8, RAST and BioCyc and NCBI databases. Mn(III) and Fe(III) reduction were monitored spectrophotometrically. Acetate concentrations were measured by HPLC. **Results:** After seven successive transfers spanning two years, *Shewanella* (90% 16S rDNA amplicon abundance) dominated the resulting Mn(III)-reducing microbial community. Isolated strain (MN-01) displayed 99% 16S rDNA sequence similarity to *S. haliotis*. MN-01 coupled anaerobic lactate oxidation to Mn and Fe reduction. However, unlike other metal-reducing members of the *Shewanella* genus, strain MN-01 also coupled anaerobic acetate oxidation to Mn(III)-PP and Fe(III)-citrate reduction. Comparative genomic analyses of strain MN-01 and strains within and outside of the genus revealed that AOMR metabolism may utilize the tetrahydrofolate branch of the Woods-Ljungdahl pathway and the extracellular electron transfer conduit of metal-reducing bacteria.**Conclusions:** Strain MN-01 is the first reported *Shewanella* strain to couple anaerobic acetate oxidation to metal reduction thus extending their known ecological niche. These findings highlight a potentially significant link between the reductive acetyl-coenzyme A pathway and metal reduction.

Author Disclosure Block:

N. Szeinbaum: None. **H. Lin:** None. **M. Taillefert:** None. **T. DiChristina:** None.

Poster Board Number:

SUNDAY-164

Publishing Title:

Spatial and Temporal Variation in Enzymatic Activities of St. Claire River and Lake Sediments via API ZYM Method

Author Block:

D. Patel, R. Gismondi, M. Fenner; Univ. of Michigan-Dearborn, Dearborn, MI

Abstract Body:

Background: Spatial and temporal variations in enzymatic activities of lake and river sediments were determined using API ZYM assay. API ZYM is a semi-quantitative micromethod designed to determine activities of 19 different enzymes. **Methods:** Three sites were selected on St Claire River (SC1, SC2, and SC3) and three sites on Lake St. Clair (LC1, LC2, LC3). A 1:10 sediment : water extract was prepared; and 30 l of the extract was dispensed in each of the 20 cupules. Each of the 19 cupules contained substrate for the 19 enzymes and and one cupule served as a control. Once incubated one drop of ZYM A and ZYM B were added to the cupules and results were collected after 10 mins. **Results:** Results were analyzed with a variety of tests. T-test was used to compare activities between river and lake. There was no significant distinction between the river and the lake except for lipase and leucine aminopeptidase, of which St. Claire river sediments had more enzymatic activities. ANOVA test was used to compare lake and river site sediments for enzymatic acitvities. Leucine aminopeptidase and valine aminopeptidase activities were significantly among the three sites. SC3 had highest activities of leucine, valine aminopeptidase and β-glucuronidase. Leucine aminopeptidase activity was significantly different in each of the three lake sites and with the highest activity in LC3. **Conclusion:** The research results give an important insight on different contaminants lake and river water are being exposed to and how these contaminants influence the diversity of the microbial community.

Author Disclosure Block:

D. Patel: None. **R. Gismondi:** None. **M. Fenner:** None.

Poster Board Number:

SUNDAY-165

Publishing Title:

***Varunavibrio sulfuroxidans* gen. Nov., Sp. Nov., A Novel Facultatively Chemoautotrophic Alphaproteobacterium from a Shallow-Water Hydrothermal Vent in the Tyrrhenian Sea**

Author Block:

S. Patwardhan, C. Vetriani; Rutgers Univ., New Brunswick, NJ

Abstract Body:

A mesophilic, anaerobic, chemolithoautotrophic bacterium, designated strain $TC8^T$, was isolated from the surface sediment of a shallow-water hydrothermal vent located in the Tyrrhenian Sea. The cells were Gram-negative, vibrio-shaped with one or more polar flagella. Cell size was approximately 1-1.5μm in length and 0.6 μm in width. Strain $TC8^T$ grew between 20 and 35°C (optimum 30°C), 0.5 and 4.50g NaCl l^{-1}(optimum 15-20 g l^{-1}) and pH 4.5 and 8.5(optimum pH 6.0-7.0). Generation time under optimal conditions was 8 hours. Strain $TC8^T$ was a facultative chemolithoautotroph, facultative chemoorganautotroph as well as a facultative chemoorganoheterotroph and growth occurred with sulfur, thiosulfate, sulfate, tryptone, peptone, and casamino acids as the energy source, CO_2 as the carbon source, and nitrate, 5%(w/v) oxygen and ferric ions as electron acceptors. Growth was not inhibited by the presence of acetate, lactate, D- (+) -glucose, sucrose, casamino acids, tryptone, peptone,yeast extract and all of them could be used as sole sources of carbon too. Reduced growth occurred in the presence of formate. $TC8^T$ grew on selenate and arsenate as electron acceptors but the growth rate was very slow.The G + C content of the genomic DNA was 59.9mol%. Phylogenetic analysis of the 16S rRNA gene of strain $TC8^T$ showed that this organism formed a divergent lineage within the family '*Rhodospirillaceae* ', which branched separately from the two closest clades representing the genera *Magnetovibrio*, *Magnetospira*, both of which are magnetotactic bacteria. However, $TC8^T$did not show magnetosome formation in the growth conditions under which it was tested for. Strain $TC8^T$ contained several unique fatty acids in comparison to the two closest relatives. Based on phylogenetic, physiological and chemotaxonomic characteristics, it is proposed that the organism represents a novel genus within the family *Rhodospirillaceae, Varunavibrio sulfuroxidans,*gen. nov., sp. nov.

Author Disclosure Block:

S. Patwardhan: None. **C. Vetriani:** None.

Poster Board Number:

SUNDAY-166

Publishing Title:

Sample Acquisition and Preservation on Mobile Ecogenomic Sensors in Support of Microbial Studies

Author Block:

C. M. Preston[1], D. Pargett[1], K. Yamahara[1], S. Jensen[1], B. Roman[1], A. Romano[2], J. Cardwell[2], B. Hobson[1], B. Kieft[1], J. Ryan[1], Y. Zhang[1], J. Birch[1], E. DeLong[3], J. Bellingham[4], C. Scholin[1]; [1]MBARI, Moss Landing, CA, [2]Univ. of Hawaii, Honolulu, HI, [3]Univ. of Hawaii, Moss Landing, CA, [4]WHOI, Woods Hole, MA

Abstract Body:

Twenty-five years of near-monthly observations at Station ALOHA suggest that certain microbial processes are under-sampled, especially those operating on diel to weekly time scales, due to short-lived but ecologically significant aperiodic excursions from the mean state. There is strong evidence that short-lived (<5 days), stochastic events contribute significantly to integrative ecosystem properties such as nutrient loading, total primary production and carbon export. Since most oceanographic and planktonic microbial processes remain undescribed, new approaches are required to improve our understanding of the metabolic balance in the sea.One approach for expanding observational frequencies and resolution of microbial processes involves implementation of automated sensing and sampling systems. To achieve this goal, we are developing a cohort of long-range autonomous underwater vehicles known as MiVEGAS (Multiple Vehicle EcoGenomic Automated Sampler). MiVEGAS represents the merger of the 3rd generation Environmental Sample Processor (3G ESP) and Long-Range AUV (LRAUV), that were developed at MBARI. The biological material recovered using MiVEGAS will capture the native state of organisms, which in turn will enable a wide range of laboratory based -omic analyses.Here, we report results on testing the MiVEGAS prototype to collect and preserve material for subsequent analyses that include qPCR, tag sequencing, and metatranscriptomics. We show the abundance and diversity of microbial genes and transcripts from samples collected and preserved using the 3G ESP sampling strategies are statistically similar to those processed using more standard field sampling procedures. Initial field trials in Monterey Bay, CA, illustrate autonomous feature finding and adaptive sampling. Material recovered from native samples was used to survey the genetic diversity using SSU tag sequencing. Those same samples were also subjected to qPCR as a means to look for the presence of exogenous DNA indicative of anchovy and to access changes in the abundance and expression of wide range of microbial genes. The MiVEGAS fleet is projected to be operational by 2017.

Author Disclosure Block:

C.M. Preston: None. **D. Pargett:** None. **K. Yamahara:** None. **S. Jensen:** None. **B. Roman:** None. **A. Romano:** None. **J. Cardwell:** None. **B. Hobson:** None. **B. Kieft:** None. **J. Ryan:** None. **Y. Zhang:** None. **J. Birch:** None. **E. DeLong:** None. **J. Bellingham:** None. **C. Scholin:** None.

Poster Board Number:

SUNDAY-167

Publishing Title:

Fluorescence Activated Cell Sorting Reveals Active Microbial Community Structure In Ancient Permafrost

Author Block:

A. Burkert, R. Mackelprang; California State Univ., Northridge, Northridge, CA

Abstract Body:

Permafrost microbial communities must contend with extreme conditions such as water stress, subzero temperatures, high salinity, and low nutrient availability. Previous studies have used metagenomic and 16S rRNA gene sequencing to characterize community structure. However, freezing temperatures may preserve DNA from dead organisms for extended periods of time (1). Because metagenomic and 16S rRNA gene sequencing does not distinguish between live, dead, and dormant cells, it is difficult to determine which organisms are viable and active.In order to circumvent this problem, we combined Live/Dead differential fluorescent staining with flow cytometry to sort cells before sequencing. Samples, ranging in age from 12kyr to 35kyr, were collected from the Cold Regions Research and Engineering Laboratory permafrost tunnel near Fairbanks, Alaska. Cells were separated from soil matrix using a Nycodenz density cushion and stained using SYTO 9 and SYTOX 13 which differ in their ability to penetrate the membrane of live cells. After staining, cells were sorted using a BD Calibur fluorescent cell sorter. DNA was extracted from live, dead, and dormant pools and 16S rRNA genes were sequenced to characterize community structure.This approach revealed the ratios of live, dead, and dormant cells in permafrost and how the ratios changed with increasing age. Microbial community structure changed, likely due to adaptations to the harsh environment. Further, we identified the taxa more likely to persist in permafrost. These data suggest that permafrost age is strongly selective for traits that enable bacteria and archaea to survive over geologic time.

Author Disclosure Block:

A. Burkert: None. **R. Mackelprang:** None.

Poster Board Number:

SUNDAY-168

Publishing Title:

Quantification of Total and Active Gut Microbiota in Healthy Subjects

Author Block:

S. Tamburini, J. C. Clemente; Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract Body:

Advances in high-throughput sequencing and computational methods have allowed us to characterize the wide variety of bacteria that are harbored in the human gut. However, sequencing alone cannot determine whether the bacterial DNA observed in a sample originates from a viable, damaged, or dead bacterial cell. Here we develop a method that combines sonication, fluorescence-activated cell sorting (FACS), and viability fluorescent markers to absolutely quantify and characterize at a single cell level the bacterial viability of the gut microbiota of healthy subjects. Viability was assessed based on membrane integrity while cells with high or low activity were identified on the basis of their DNA content. Sonication was able to disaggregate bacterial cell clusters without damaging the bacterial membrane. Through our FACS analysis we estimate that a gram of gut microbiota from healthy subjects contains on average $5.0x10^{10}$ bacterial cells and distinctive subpopulations of high nucleic acid content (HNA), low nucleic acid content (LNA), viable, damaged and dead bacterial cells. Viable bacteria (61%) and bacteria with HNA (75%) are the largest subpopulations in the gut microbiota with $3.4x10^{9}$ cells/g and $3.7x10^{10}$ cells/g, respectively. Our results highlight the variability in physiologically distinct bacterial cell subpopulations, suggesting that microbiome studies should take into account these factors in order to better characterize bacterial composition.

Author Disclosure Block:

S. Tamburini: None. **J.C. Clemente:** None.

Poster Board Number:

SUNDAY-169

Publishing Title:

Surface-Attached Phototrophic Communities Assemble i*n situ* with Narrow-Spectrum Led Illumination

Author Block:

S. M. Kearney[1], S. H. Kopf[2], K. Hanselmann[3], D. K. Newmann[4], J. R. Leadbetter[4]; [1]Massachusetts Inst. of Technology, Cambridge, MA, [2]Univ. of Colorado - Boulder, Boulder, CO, [3]Swiss Federal Inst. of Technology (ETH), Zuerich, Switzerland, [4]California Inst. of Technology, Pasadena, CA

Abstract Body:

Background: Chlorophyll-based phototrophy spans multiple, phylogenetically diverse bacterial groups. The phylogenetic dispersion of this functional trait reflects its evolutionary success as a metabolic strategy for organisms living in the light. Each wavelength of the solar spectrum represents a potential niche for phototrophy.Isolation of specific phototrophic microorganisms in the laboratory can best be achieved by using radiation at different wavelengths aimed at characteristic photon-absorbing pigments in combination with various electron donors. This strategy has led to the successful enrichment of numerous phototrophic microorganisms with absorption characteristics that span the spectrum from blue to the infrared. **Methods:** We expanded on this classical approach by developing an inexpensive, reusable, submersible battery-powered narrow-spectrum LED illumination chamber with a 3D printed, environmentally exposed microscope slide holder. The method allows phototrophs to grow on glass slides exposed directly to the chemical environment of their natural habitat while illuminated in situ with selected LED light sources. **Results:** We deployed several devices to enrich for phototrophic communities in situ at Trunk river basin, an organic-rich, sulfidic brackish habitat. LED/wavelength-specific biofilm growth could be observed within several hours to a few days. Phototrophic organisms present in the formed biofilm communities could then be directly differentiated and were characterized in detail based on their topographical, spectral, and morphological features. **Conclusions:** This approach proved to be a reliable strategy for selectively enrichingphototrophs and provides a simple and effective means to conduct culture-independent profiling of phototrophic communities as they are present in the environment.

Author Disclosure Block:

S.M. Kearney: None. **S.H. Kopf:** None. **K. Hanselmann:** None. **D.K. Newmann:** None. **J.R. Leadbetter:** None.

Poster Board Number:

SUNDAY-170

Publishing Title:

Soil Printing as a High Throughput Method to Isolate and Culture Environmental Microorganisms

Author Block:

B. R. Ringeisen[1], P. A. Fulmer[1], L. A. Fitzgerald[1], M. Montgomery[1], J. Bethancourt[1], J. P. Goetz[1], A. Li[1], P. K. Wu[2]; [1]Naval Res. Lab., Washington, DC, [2]Southern Oregon Univ., Ashland, OR

Abstract Body:

Background: Culturing environmental microorganisms is one of the biggest challenges facing microbial ecologists and microbiologists today. High throughput, nanoporous membrane and microarray approaches have recently emerged to help improve culturing success rates; however, laboratory culture rates remain far behind metagenomics approaches with respect to characterizing environmental biodiversity. **Methods:** High throughput biological laser printing, or BioLP, was employed as a novel method to isolate near-neighbor consortia and microbial isolates directly from soil samples without the need to vortex or sonicate to dislodge microorganisms from their solid-phase microenvironment prior to culture. BioLP is an orifice-free printing technique that uses laser pulses to initiate the transfer of micron-scale portions of soil from a transparent support ribbon while maintaining the viability of printed microorganisms attached to the soil. Culturing and subsequent metagenomics analysis was used to characterize the soil printing process. **Results:** Printing soil microniches to culture microtiter plates resulted in isolation of microorganisms and microbial consortia in a single step. Significant colony morphology was observed upon streaking microtiter plate cultures. Printing to ten 96 well plates resulted in over 500 pure culture isolates from an agricultural soil sample. Metagenomics was used to analyze the difference when utilizing different methods to culture the sample (dilution, batch or soil printing). Soil printing resulted in the highest number and diversity at the bacteria genus level, closely followed by dilution. Batch culture reduced the diversity by 12%. **Conclusions:** The results indicate that soil printing exceeds manual dilution and batch culture with respect to the biodiversity of culturable bacteria from soil. Additionally, BioLP is a high throughput (hundreds of soil portions isolated per second) and unique approach that isolates microorganisms while still attached to soil, enabling near-neighbor relationships and consortia to be explored.

Author Disclosure Block:

B.R. Ringeisen: None. **P.A. Fulmer:** None. **L.A. Fitzgerald:** None. **M. Montgomery:** None. **J. Bethancourt:** None. **J.P. Goetz:** None. **A. Li:** None. **P.K. Wu:** None.

Poster Board Number:

SUNDAY-171

Publishing Title:

Optimization Rk2(Rp4) Conjugal Transfer System for *B. subtilis*

Author Block:

T. Yokoi; Shinshu Univ., Nagano, Japan

Abstract Body:

Bacillus subtilis is one of the most widely used bacteria in the Industrial Biotechnology. It is well known that *B. subtilis* and the closely related species show non pathogenic and endotoxin free. Their fermentation method has been well optimized for producing various enzymes, antibiotics, and chemicals. However, the genetic manipulation system including transformation method has not been maintained in the most B. subtilis strains except the well characterized laboratory strain. Natural isolates strains of B. subtilis are often show difficulty of transformation due to their lack of natural competency.In this meeting, we will present the in vivo heterogeneous gene transfer system between E. coli and *B. subtilis* by using RP4 conjugation system. We used the mobility plasmid pUB307 Tn5, a conjugative plasmid carrying RP1 tra genes. pUB307 could be mobilized other plasmids containing transfer origin(oriT) sequence. We constructed the series of shuttle vector containing various lengths of transfer origin (oriT) to assess the effect on heterogeneous DNA transfer. The plasmid containing a Wild Type oriT showed well conjugation efficiency between E.coli and B.subtilis. In contrast, other plasmids containing partial deleted oriT showed the smaller frequency. To optimize the inter-genus conjugation, we examined the time required for DNA conjugation. Conjugation for 1 hour gave enough number of the trans conjugants. Finally our optimized conjugation method showed more effective DNA transfer than the natural transformation usually used.

Author Disclosure Block:

T. Yokoi: None.

Poster Board Number:

SUNDAY-172

Publishing Title:

Identification Of Spacecraft-Associated Microorganisms Using Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight Mass Spectrometry

Author Block:

W. W. Schubert, H. Aronson, P. A. Vaishampayan; Jet Propulsion Lab., Pasadena, CA

Abstract Body:

Microbes collected from robotic spacecraft and cleanroom assembly areas are archived in JPL's microbial culture collection. The microbial isolates that have been identified by 16SrRNA gene sequence (2318) most frequently belong to the following major genera: *Bacillus* (64%), *Staphylococcus* (18%) and *Paenibacillus* (3%) with other genera composing the remaining 15%. As an alternate, rapid and more sensitive approach, a subgroup of these isolates were identified by Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF) and compared to their 16S rRNA gene sequence identifications. An in-house database was created using bacterial isolates with known 16S rRNA identifications collected from Phoenix Mars lander hardware and was used to validate the correlation between 16S rRNA-based identifications and MALDI-TOF identifications. Similar gene sequences at the 99% homology level were grouped into operational taxonomic units (OTUs), and MALDI-TOF spectral profiles (MSP) were created for one representative isolate from each OTU and added to the in-house database. Fifty-three unique OTUs were produced from 147 Phoenix isolates based on 16S rRNA gene sequencing. Using MALDI-TOF based real time classification (RTC), 74% of Phoenix isolates matched their 16S rRNA gene identifications. RTC analysis was able to detect sub-species variations that were not possible by 16S rRNA sequencing. Creation of a more comprehensive database is required in order to correctly identify the cleanroom microbial isolates.

Author Disclosure Block:

W.W. Schubert: None. **H. Aronson:** None. **P.A. Vaishampayan:** None.

Poster Board Number:

SUNDAY-173

Publishing Title:

Novel Workflows for Advanced Analysis of Human Microbiota Communities by Sequencing and Qpcr

Author Block:

A. Vlassov, S. Larocca, 78744, M. Li, 78744, R. Setterquist; Thermo Fisher Scientific, Austin, TX

Abstract Body:

Background: Recent studies had a shocking finding that human body is populated by 10(14) bacteria and other members of the microbiota community, that outnumber human cells 10-fold. Microbes have multiple beneficial functions, and interestingly deviations from the "normal" microbial compositions have been linked with many human diseases. Although our understanding of the microbiome is in the nascent stages, it's clear that we need to treat it as a sophisticated system, much like the circulatory and immune systems, playing multiple roles within the human body. Our team's goal is to address the urgent needs of the microbiome community and provide the complete arsenal of tools to the scientists exploring microbiota through nucleic acids analysis. **Methods:** We developed tools and a workflow enabling: (1) sample collection; (2) storage; (3) isolation of microbial DNA with novel PureLink Microbiome DNA Purification Kit; (4) initial DNA analysis by NanoDrop spectrophotometer, Qubit fluorometer, and agarose gel electrophoresis; (5) qPCR with TaqMan assays, and next gen sequencing.**Results:** The PureLink kit enables fast purification of inhibitor-free microbial and host DNA from a wide variety of sample types, including stool, urine, saliva, swabs, transport/growth media, and combines efficient triple lysis approach and unique inhibitor removal technology. Investigation of the "healthy" microbial communities in the gut will be exemplified by 16S Sequencing of stool-derived microbial DNA. Utility of the kit and downstream assays for rapid detection of *C.difficile* and other pathogens will be demonstrated as well, with 2h sample-to-answer workflow.**Conclusions:** Microbiome is a hot topic of research, growing exponentially due to the fact that it has high potential to revolutionize medicine. We developed the complete workflow solution for microbiome research, starting from sample collection and storage, to DNA isolation and downstream analysis.

Author Disclosure Block:

A. Vlassov: None. **S. Larocca:** None. **M. Li:** None. **R. Setterquist:** None.

Poster Board Number:

SUNDAY-174

Publishing Title:

Reclassification of *Clostridium cocleatum, Clostridium ramosum, Clostridium spiroforme* and *Clostridium saccharogumia* to a Novel Genus, "*flintia*" Gen. Nov

Author Block:

L. Saavedra[1], S. Finegold[2], P. A. Lawson[1]; [1]Univ. of Oklahoma, Norman, OK, [2]UCLA Sch. of Med., Los Angeles, CA

Abstract Body:

Background: The recent proposal to restrict the genus *Clostridium* to *C. butyricum* (rRNA cluster I) and relatives indicates that many species will no longer be considered as true members of this genus (1). *Clostridium cocleatum, Clostridium ramosum, Clostridium spiroforme* and *Clostridium saccharogumia* are located in clostridial rRNA cluster XVIII and form a robust phylogenetic group based on 16S gene sequencing. In this study, in addition to molecular analysis *Clostridium cocleatum, Clostridium ramosum, Clostridium spiroforme* and *Clostridium saccharogumia* were subjected to morphological, phenotypic, biochemical and chemotaxonomic investigations. **Materials and Methods/Results:** *C. cocleatum, C. ramosum, C. spiroforme* and *C. saccharogumia* were recovered from children with late-onset autism. For chemotaxonomic data, fatty acids were analyzed using the MIDI system (2). The profiles showed that *C. cocleatum, C. spiroforme, C. ramosum* and *C. saccharogumia*'s dominant fatty acids were $C_{14:0}$, $C_{16:0}$ and $C_{18:1\ w7c}$. Whole-cell sugar analysis (3) also held concordance between all organisms for the presence of galactose, while analysis of the peptidoglycan (4) showed that *meso*-2,6-diaminopimelic acid was present as the diagnostic diamino acid. The biochemical profiles derived from API test systems were consistent with being closely related species. Negative staining and thick sectioning performed at the University of Oklahoma provided highly defined visual images that complimented morphological descriptions of cellular parameters provided in Bergey's Manual of Systematic Bacteriology. **Conclusion:** According to these findings, it is proposed to create a novel genus, "*Flintia"* to accommodate *Clostridium cocleatum, Clostridium ramosum, Clostridium spiroforme* and *Clostridium saccharogumia* as "*Flintia* cocleatum gen. nov. comb. nov.", "*Flintia ramosum* comb. nov.", "*Flintia saccharogumia* comb. nov." and "*Flintia spiroforme* comb. nov". The genus is named in honor of the British microbiologist Harry Flint for his many contributions to anaerobic microbiology of the human gut.

Author Disclosure Block:

L. Saavedra: None. **S. Finegold:** None. **P.A. Lawson:** None.

Poster Board Number:

SUNDAY-175

Publishing Title:

Use of a Global Survey to Direct the Implementation of Alternative/Rapid Microbiological Methods Supporting Microbiological Quality Across Multiple Pharmaceutical Manufacturing Sites

Author Block:

D. Gessell-Lee, M. Luebke; Baxter Hlth.care Corp., Round Lake, IL

Abstract Body:

Background: Current pharmaceutical microbiology laboratories are continually challenged to produce faster, more accurate data using century-old, labor intensive techniques. To address this need, a variety of Alternative/Rapid Microbiological Methods (ARMMs) have been developed to support microbiological quality and facilitate improved product and process control. The application of ARMMs in the pharmaceutical industry, however, may be hindered by a range of challenges; a globally harmonized validation approach is essential for successful implementation. In an effort to purposefully direct the purchase and implementation of new methodologies within the microbiology laboratories of a large pharmaceutical company, a global survey was conducted. Our objective was to identify the highest priority needs across a global landscape, assess these needs to guide appropriate ARMM choices, and subsequently identify and remediate potential barriers to successful ARMM implementation.**Methods:** An online survey tool was utilized to develop and distribute a global questionnaire evaluating potential applications and barriers preventing the use of ARMM technology within microbiology laboratories. The data were analyzed to identify and prioritize the ARMM applications providing the greatest impact to pharmaceutical microbiology operation efficiencies.**Results:** The survey results directly influenced global ARMM implementation. Two microbiological tests, sterility and microbial identification, were identified as candidates for ARMM applications. As a result, a qualitative ATP-based system for finished product sterility and a MALDI TOF-based microbial identification system were successfully validated in several laboratories. A number of real-world industrial challenges and lessons learned were uncovered during the evaluation and validation of the two ARMM platforms.**Conclusions:** The pragmatic application of a global microbiological methods survey was instrumental in the successful implementation of two ARMMs within a large pharmaceutical company. The identification and remediation of a number of technical and non-technical challenges enabled a harmonized validation approach across multiple manufacturing sites and regulatory landscapes.

Author Disclosure Block:

D. Gessell-Lee: D. Employee; Self; Baxter Healthcare Corporation. **M. Luebke:** D. Employee; Self; Baxter Healthcare Corporation.

Poster Board Number:

SUNDAY-176

Publishing Title:

***In-Silico* Analysis of Bacterial Polar Lipid Synthesis as a Tool for Chemotaxonomy**

Author Block:

N. Patel, K. Sankaranarayanan, P. A. Lawson; Univ. of Oklahoma, Norman, OK

Abstract Body:

Background: The characterization of novel microorganisms traditionally requires a polyphasic approach that includes phylogenetic, biochemical and chemotaxonomic investigations. Chemotaxonomic traits such as polar lipids help augment the data obtained from physiological tests to achieve a complete profile in microbial taxonomy. However, the labor intensive nature of these analyses coupled the lack of curated databases makes reproducibility and comparative analysis of results difficult. Alternatively, the ease of generating complete/draft genome assemblies allows for rapid *in silico* characterization of functional potential for novel microbial strains. Embracing this *in silico* approach to screen for genes associated with polar lipid synthesis can thus be a powerful tool for microbial systematics.**Methods:** This study focused on the biosynthetic pathways for four common polar lipids found in bacterial membranes: Diphosphatidylglycerol (DPG), Phosphatidylglycerol (PG), Phosphatidylethanolamine (PE), and Phosphatidylserine (PS). Fifty organisms with sequenced genomes and published polar lipid data (TLC/Mass Spectrometry) were selected from across six bacterial phyla (Firmicutes, Bacteroidetes, Actinobacteria, Acidobacteria, Spirochaetes and Proteobacteria). For each organism, genomic annotations derived through the KEGG automatic annotation server (KAAS) were compared to published polar lipid data and patterns of concordance/discordance were documented.**Results:** For polar lipids PE and PS, strong concordance between the genomic and *in vitro* characterizations was observed across all phyla examined. In contrast, the lipid DPG showed phylum specific trends, with a strong concordance among Firmicutes, and discordance among Actinobacteria and Acidobacteria. Finally, the lipid PG showed discordance for one of two genes required for its synthesis, across most organisms examined.**Conclusion:** Collectively, these results highlight the need for (a) standardized *in vitro* polar lipid characterization, and (b) experimental characterization of polar lipid biosynthesis pathways in a phylogenetically diverse range of organisms. Addressing these challenges are critical to evaluating the predictive accuracy of *in silico* models, and their broader application as a tool for chemotaxonomy.

Author Disclosure Block:

N. Patel: None. **K. Sankaranarayanan:** None. **P.A. Lawson:** None.

Poster Board Number:

SUNDAY-177

Publishing Title:

An Improved Method for Inferring Accurate Co-Occurrence Networks from Microbial Metagenomes Based on Random Matric Theory and Machine Learning

Author Block:

Z. Shi, Y. Xu, Y. Qin, Z. He, J. Zhou; Univ. of Oklahoma, Norman, OK

Abstract Body:

The emerging metagenomics technologies have advanced the characterization of interactive patterns among the microorganisms, and provided opportunities better understanding microbial responses to perturbations and even improving the predictive capability of ecosystem models. Because of its straight-forward calculation procedure and high processing speed, co-occurrence network inference has become a widely adopted method for efficiently identifying networks from large and complex microbial community systems. While this method is neither sufficient to determine the causality between given pair of variables, nor does it reflect true microbial interactions, it allows to examine meaningful co-occurrence patterns in large data sets without prior knowledge of the relationships. However, determining a threshold or cutoff value for real world studies to exclude weak and random co-occurrence patterns is problematic, but necessary in order to obtain accurate and reliable networks. Most studies have selected thresholds empirically, so the constructed networks are inevitably subject to artificial deviations and inaccurate structure. We previously proposed a random matrix theory (RMT)-based approach for detecting such thresholds automatically and objectively, but this method still had issues with detection limit and slow processing speed. Here we developed an improved RMT method to determine appropriate thresholds based on Nearest Neighbor Spacing Distribution (NNSD) characterization and Support Vector Machine (SVM) learning, to further extend the capability of the method . Results showed that the improved method successfully determined the thresholds of systems for which the previous method failed. Meanwhile, the original RMT method and the improved version were consistent with each other on the threshold values from the systems that can be determined by the both methods. In addition, the processing speed was increased many fold in our test runs. Based on these results, we concluded that the RMT-based method is reliable, robust and consistent in tackling the mathematical challenge of refining microbial interactions. In addition, the improvements described in this study increase our ability to accurately determine co-occurrence networks from microbial metagenomes with minimal randomness and noise.

Author Disclosure Block:

Z. Shi: None. **Y. Xu:** None. **Y. Qin:** None. **Z. He:** None. **J. Zhou:** None.

Poster Board Number:

SUNDAY-178

Publishing Title:

Development of a Biosensor Using *Photobacterium* spps for the Detection of Environmental Pollutants

Author Block:

T. Gokhale, S. Biswal, A. Thapa, 345055, N. Sood; BITS Pilani Dubai Campus, Dubai, United Arab Emirates

Abstract Body:

Bioluminescence is a natural phenomenon in which energy is spent to emit light. It is exhibited by many organisms. These organisms emit light through a enzymatic reaction between the substrate Luciferin, which is a pigment and an enzyme Luciferase. The *Lux* operon in bacteria controls the process of luminescence, which follows the quorum sensing mechanism. We used the idea of bioluminescence of develop a photobiosensor to detect the presence of environmental pollutants. *Photobacterium spps* was isolated from the squid using BOSS media and identified using the 16s rRNA analysis. The effect of environmental pollutants was studied in *Photobacterium spps* by analyzing the luminescence exhibited by the bacterium in presence and absence of the pollutant. The luminescence was recorded using Perkin Elmer Victor 320. Metals were selected as environmental pollutants for the study, as metal contamination is a major concern in many parts of the world and the concentration of heavy metals have increased in soil and water beyond the safe limit. The effect of varying concentrations of different metals such as cadmium (0-1mg/ml), barium (0-150mg/ml), zinc (0-0.05mg/ml), cobalt (0-1.25mg/ml) and copper (0-1.5mg/ml) was studied on the luminescence of *Photobacterium spps*. A linear decrease in the luminescence was observed with the gradual increase in the concentration of the heavy metals. This signifies the ability of the bacterium to sense the presence of the pollutant and estimate the quantity. This luminescence property of *Photobacterium spps*. can be exploited for the development of the photobiosensor. *Photobacterium* is a non-pathogenic organism and hence can be safely used for the development of biosensors. Biosensors are analytical devices that are constituted of a biological element like an enzyme, an analyte, which is generally a substrate for the enzyme and a transducer that converts the chemical signal or light into an easily measurable signal. The *Photobacterium* species isolated in this work can be efficiently used in developing the biosensor where the biological element can be the whole bacterial cells. An optical biosensor is designed using fiber optic technique and photodiodes to detect the luminescence and convert it into a measurable signal.

Author Disclosure Block:

T. Gokhale: None. **S. Biswal:** None. **A. Thapa:** None. **N. Sood:** None.

Poster Board Number:

SUNDAY-179

Publishing Title:

Culturing the Unculturables: Strategies for Closing the Sequenced vs Cultured Gap

Author Block:

P. A. Fulmer[1], L. A. Fitzgerald[1], J. Goetz[2], A. Li[2], J. Bethencourt[3], P. K. Wu[4], D. Haridas[1], L. J. Hamdan[1], B. R. Ringeisen[1]; [1]Naval Res. Lab., Washington, DC, [2]Thomas Jefferson High Sch. for Sci. and Technology, Alexandria, VA, [3]Florida Intl. Univ., Miami, FL, [4]Southern Oregon Univ., Ashland, OR

Abstract Body:

Background: With the advancement of next-generation sequencing technologies, the vast diversity of environmental microbes has become apparent. While sequencing technologies have advanced at a rapid pace the ability to culture these microbes has lagged behind, despite the fact that these environments have proved to be extremely rich depositories of functional molecules and synthetic pathways. **Methods:** A variety of techniques were employed to increase the speed with which previously uncultured microbes can be isolated from three environments: 1) topsoil from an active corn field; 2) marine sediments; 3) marine water column. A variety of growth condition variables were employed, including media formulation, carbon source, and temperature. Cultures were produced by traditional plate streaking (water column), followed by colony isolation as well as high-throughput biological laser printing methods (soil and sediment). Candidates for further study were identified by sequencing of 16s rRNA followed by additional analysis of *rpoB* and *gyrB* sequences.**Results:** All environments tested resulted in the growth and subsequent identification of novel microbes. Due to the large number of isolates generated (~750), a relatively conservative threshold of ≤90% 16s rRNA identity to cultured microbial sequences in the BLAST database was used to identify candidates for further study. Topsoil (15), marine sediments (25), and marine water column samples (16) resulted in the identification of unique, culturable isolates. Subsequent *rpoB* and *gyrB* sequencing, and phenotypic assays were employed to further characterize novel isolates.**Conclusion:** These results show that a variety of methods, both traditional and high-throughput, can be successfully utilized to increase the diversity of cultured microbes, even in environments which, to date, have been heavily studied. High-throughput culture methods of unique, rare, or difficult to sample environments in particular show themselves to be extremely rich in culturable, but yet to be cultured microbes.

Author Disclosure Block:

P.A. Fulmer: None. **L.A. Fitzgerald:** None. **J. Goetz:** None. **A. Li:** None. **J. Bethencourt:** None. **P.K. Wu:** None. **D. Haridas:** None. **L.J. Hamdan:** None. **B.R. Ringeisen:** None.

Poster Board Number:

SUNDAY-180

Publishing Title:

Heterotrophic Bacterial Production Measured on Soil Microaggregates Sampled Using a Biological Laser Printer

Author Block:

M. T. MONTGOMERY, P. A. Fulmer, J. Gaston, N. McAuliff, J. Compton, B. Ringeisen; NAVAL RESEARCH LABORATORY, WASHINGTON, DC

Abstract Body:

Background: Although routinely measured in aquatic systems over the last 30 years, heterotrophic bacterial production (^{3}H-leucine method) has only more recently been measured in terrestrial ecosystems to elucidate the role of soil biogeochemical processes in global carbon cycles and climate change. Studying bacterial metabolism at the microaggregate scale (100-300 μm) may unmask important microscale biogeochemical relationships amongst bacterial processes and the surrounding soil. **Methods:** Biological laser printer technology was used to sample soil core sections (*ca.* one mm thick) nondestructively into *ca.* 100-300 μm microaggregates. Bacterial production was measured on collections of five aggregates each (180 samples over 5 cm depth profile) by suspending aggregates into filtered rainwater with ^{3}H-leucine and incubated for 4 h. Some aggregates samples were sacrificed to measure enzymatic activity (*e.g.* phosphatase using MUF-PO_4) fluorometrically.**Results:** Bacterial production varied by over two orders of magnitude amongst groups of five microaggregates collected over millimeter scale core slices (range, 4-149 fg C microaggregate^{-1} d^{-1}). As expected, variability amongst adjacent microaggregates groups generally decreased with depth. Corresponding enzyme activity (using fluorescent MUF substrate analogs) also generally decreased with depth and showed variation amongst adjacent samples but less than the two orders of magnitude variation seen with bacterial production (phosphatase: 1.1-6.8 fmoles microaggregate^{-1} d^{-1}). Some of this lack of variation may have been due to lesser sensitivity of fluorometric assay relative to the radioassay.**Conclusions:** Variation in bacterial production at the microaggregate scale was greater than that seen at larger scale (ca. 100 μg) sampling of the same core. Smaller sample size dramatically reduced radiotracer quench due to soil particles. This is the first demonstration of using a biological laser printer for fine scale sampling of soil core slices and subsequent measurement of bacterial metabolism.

Author Disclosure Block:

M.T. Montgomery: None. **P.A. Fulmer:** None. **J. Gaston:** None. **N. McAuliff:** None. **J. Compton:** None. **B. Ringeisen:** None.

Poster Board Number:

SUNDAY-181

Publishing Title:

Metabolomics on the Differential Secondary Metabolite Profiles Induced by Epigenetic Modifiers in Fungal Endophytes, a Case Study

Author Block:

V. Gonzalez-Menendez, F. Muñoz, C. Toro, I. Perez-Victoria, J. Martin, F. Reyes, O. Genilloud, **J. R. Tormo**; Fundacion MEDINA, Granada, Spain

Abstract Body:

Background: Fungal endophytes are known to produce a wide variety of secondary metabolites (SMs) involved in their adaptation and survival within higher plants. Plant-microbe interaction may influence the expression of some biosynthetic pathways, otherwise cryptic in these fungi when grown in laboratory conditions. Epigenetic small-molecule modifiers of Histone Deacetylase (HDAC) and DNA methyltransferase (DNMT) activities have been successfully used to perturb the fungal secondary biosynthetic mechanisms, which has led to the induction of the expression of silent metabolite pathways. Adding epigenetic elicitors in fungal endophyte fermentations may induce the expression of biosynthetic pathways which may occur naturally in plant-microbe interaction. **Methods:** The systematic addition of small-molecule epigenetic elicitors was evaluated by uHPLC profiling, and the most interesting cases were further analyzed by LC/MS metabolomic approaches for the characterization of the metabolites that resulted increased in production under these fermentation conditions. In addition, strains that produced differential SMs in presence of the small elicitors were studied in detail and their principal induced components purified, identified and quantified as a proof of concept of the OMICs methodology.**Results:** The effects of 7 epigenetic modifiers is described for a case study group of 13 fungal endophytes fermented during 7 and 14 days to evaluate the possible activation of silent biosynthetic pathways compared to their growth in standard conditions. Addition in both, the inoculum and the production medium, were evaluated. The effects of the elicitors during *inocula* had not been previously reported, so we intended to evaluate any potential differences in the metabolite profiles generated in these different scenarios. The increase in production titers of global SMs profiles in some of the strains was slightly higher when the elicitors were present from that inoculum stage.**Conclusions:** The described approach on the use of epigenetic modifiers in fungi ensured a successful systematic generation of new SMs. Current scale-up, purification and chemical characterization of selected strains/conditions are being carried out for the most promising new metabolites induced.

Author Disclosure Block:

V. Gonzalez-Menendez: None. **F. Muñoz:** None. **C. Toro:** None. **I. Perez-Victoria:** None. **J. Martin:** None. **F. Reyes:** None. **O. Genilloud:** None. **J.R. Tormo:** None.

Poster Board Number:

SUNDAY-182

Publishing Title:

Lineage Tracking for Probing Heritable Phenotypes at Single-Cell Resolution

Author Block:

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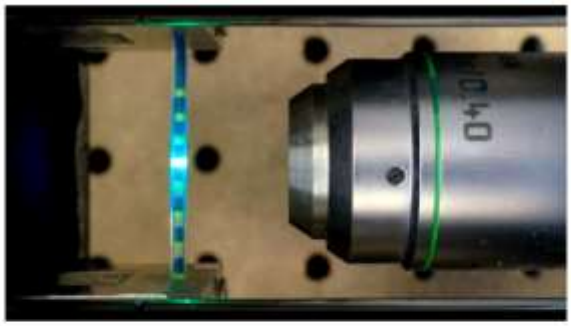
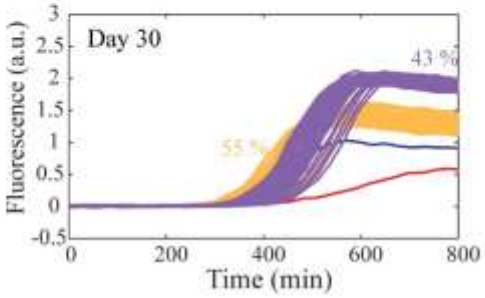
Abstract Body:

Determining the phenotype and genotype of single cells is central to understand microbial evolution. DNA sequencing technologies allow the detection of mutants at high resolution, but similar approaches for phenotypes analysis are still lacking. We show that a drop-based millifluidic system (image top picture, Baraban 2010) enables the detection of heritable phenotypic changes in evolving bacterial populations. At time intervals cells were sampled and individually compartmentalized in 100 nL drops. Growth of their progeny through 15 generations was monitored using a fluorescent protein reporter. The amplification of heritable changes over multiple generations yields clusters of narrowly defined phenotypes reflecting variation relevant for evolution (image bottom graph). To demonstrate the utility of this approach, we follow the evolution of *Escherichia coli* populations during starvation (Zambrano 1993). Phenotypic diversity was observed to rapidly increase upon starvation with the emergence of heritable phenotypes. Mutations corresponding to each class were identified by DNA sequencing. This scalable lineage-tracking technology opens the door to large-scale phenotyping methods.





Author Disclosure Block:

D. Cottinet: D. Employee; Self; Millidrop Instruments. **J. Bibette:** None. **J. Baudry:** None. **J.G.M. de Visser:** None.

Poster Board Number:

SUNDAY-183

Publishing Title:

Separation and Isolation of Bacterial Cells Using Microfabricated Devices

Author Block:

E. D. Goluch, N. Tandogan; Northeastern Univ., Boston, MA

Abstract Body:

Lab-on-a-chip technology offers a promising solution for separating individual bacterial cells from complex mixtures. We developed a passive sub-microfluidic device designed specifically for automatic *in situ* separation and cultivation of species. The device contains multiple isolation chambers with size specific constrictions, and a main entrance for bacteria to enter into the device. Each isolation chamber has an access hole that is sealed with a polycarbonate membrane to facilitate chemical communication with the environment while preventing contamination. The first bacterial cell that enters the constriction becomes trapped. It divides in the constriction and its progeny eventually reach the isolation chamber. The cells proliferate in the presence of nutrients and growth factors diffusing into the isolation chamber from the environment and form a pure culture.For the preliminary studies, water samples from the Charles River in Boston, Massachusetts were collected in sterile 50 mL tubes. The devices were placed into the bottles for four weeks, and taken out to collect samples for plating and identifying the species. Our results show successful separation of species into different isolation chambers. This preliminary study showed promising results of our passive size-based isolation technique. Using a range of constriction sizes, a diverse distribution of species can be obtained in the isolation chambers.

Author Disclosure Block:

E.D. Goluch: None. **N. Tandogan:** None.

Poster Board Number:

SUNDAY-184

Publishing Title:

Nanopore Technology for Full Length 16s Rrna Sequencing of Mixed Microbial Communities

Author Block:

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Abstract Body:

Current high-throughput platforms for 16S rRNA sequencing are limited by the size of the fragment that can be successfully sequenced. This amplicon length limitation not only necessitates choice of the target region of the gene can be sequenced, but also limits taxonomic resolution of the sequenced gene product. Newer platforms (e.g. Oxford Nanopore's MinION™) allow for sequencing of larger DNA fragments, albeit at a higher raw error rate. Nonetheless, the rapid developments in error correction and bioinformatics tools for high-error reads suggests that robust full length 16S rRNA sequencing should be soon be feasible.Here, we describe results of a study using MinION™ device for sequencing of full-length 16S rRNA genes from mock community constructed using 15 reference organisms from three independent experiments. Raw reads were aligned to reference sequences using four alignment algorithms, specifically designed for error prone reads followed by estimation of mismatch, insertion, and deletion rates. We also assessed the error rate as impacted by data filtering (i.e. base quality, mapping quality), error correction and read polishing approaches. Finally, we determined the effect of data processing steps on the ability to reconstruct the structure of the theoretical mock community.All 15 reference sequences were detected for all three independent sequencing runs with 2D read numbers varying between 48,279 to 11,177. All alignment algorithms mapped in excess of 98% of the 2D pass reads to the reference sequences. Assessment of aligned vs unaligned reads for each algorithm indicated that base quality was not an optimal data filtering approach. In contrast, filtering of aligned reads based on the mapping quality generated by the BWA allowed for a significant reduction in overall error rate. However, data filtering based on mapping quality also resulted in loss of significant amount of raw data (>70%). The error rates for quality-filtered data ranged from 6.5% for BWA (ont2D algorithm) to 14.7 for GraphMap (gotoh algorithm). In keeping with the consistent error rates, the relative abundance of each mock community member and multiple diversity indices, though deviating from the theoretical, were highly reproducible across the three sequencing runs.

Author Disclosure Block:

S.T. Calus: None. **U.Z. Ijaz:** None. **A.J. Pinto:** None.

Poster Board Number:

SUNDAY-185

Publishing Title:

A Microfluidic Screening Platform for Electroporation-Mediated Genetic Engineering

Author Block:

P. A. Garcia, Z. Ge, J. L. Moran, C. R. Buie; Massachusetts Inst. of Technology, Cambridge, MA

Abstract Body:

There are millions of species of bacteria on the planet, yet we can only genetically engineer a few dozen. The potential to solve many of mankind's most pressing challenges, including the needs for alternative fuels, enhancing oil recovery, and even treating cancer, could involve engineering the numerous bacteria currently beyond our reach. New tools are needed to unlock bacteria's true potential to solve many challenges of interest to mankind. In this work we have designed, fabricated, and tested a new microfluidic assay to quantify the critical electric field needed for electroporation in a single experiment.The microfluidic platform was designed with a converging microchannel that produces a linear electric field gradient when an electric pulse is applied, inducing electroporation in certain portions of the channel. The assay is performed in the presence of green nucleic acid stain (SYTOX®) that cannot permeate cell membranes unless there is membrane disruption. Electroporation-induced membrane disruption in regions of high electric field strength allows for intracellular delivery of the SYTOX® and a $\geq$ 500-fold fluorescence enhancement upon intracellular DNA binding. Optical detection of the transition zone was captured under fluorescence microscopy and correlated with computational models of the electric field distribution to determine critical electroporation conditions.The critical electric field threshold for *Corynebacterium glutamicum* ($OD_{600} \approx 0.5$ in BHI) electroporation in 0.01×PBS ($\sigma \approx 0.02$ S/m) is estimated as 4.13±0.34 kV/cm with a single exponentially decaying pulse ($t = 1.0$ ms; $\tau = 5$ ms). This technique has several advantages compared to the traditional trial-and-error experimental approach. First, the assay samples a continuum of electric field strengths in a single experiment, minimizing the experimental time required. Second, the assay uses low sample volume compared to conventional cuvettes, making it economical. Last, the optical confirmation allows for nearly real-time evaluation of electroporation conditions as opposed to waiting for next-day cultures. We envision this assay as a convenient tool to develop electroporation protocols for genetic transformation of previously intractable or difficult-to-transfect microorganisms.

Author Disclosure Block:

P.A. Garcia: None. **Z. Ge:** None. **J.L. Moran:** None. **C.R. Buie:** None.

Poster Board Number:

SUNDAY-186

Publishing Title:

A New Scalable Approach For Phenotypical Classification Of Microbial Communities

Author Block:

E. Asgari, K. Garakani, M. Mofrad; Univ. of California, Berkeley, Berkeley, CA

Abstract Body:

Background:A major challenge in microbial informatics is the classification of microbial communities of different phenotypes. Typical classifications based on community profiling involve reference alignment of billions of metagenomic sequences, which requires extensive data processing. Thus, using a reference-free method, which only requires a low fraction of data for characterization, would be extremely beneficial.**Method:**We introduce a new reference-free technique for classification based on k-mer sequence analysis of 16S rRNA. Using a random sampling component before estimation of k-mers, we reduce the processing data size dramatically (by 10^5 folds). Key steps in data extraction from each sample are shown in Figure 1. Support vector machine is used for classification of different conditions on k-mer distributions.

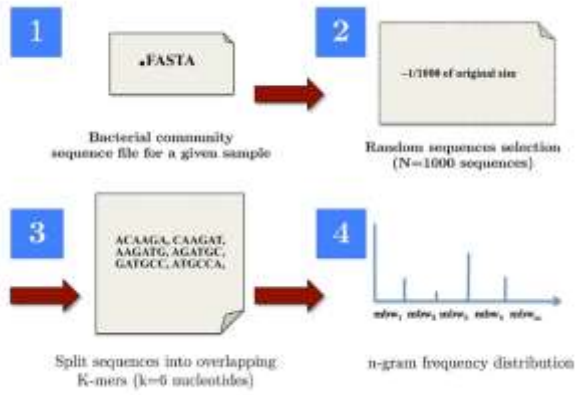

Figure 1 Key steps involved in feature extraction from each sample.**Results:**We employ this new approach to classify phenotypic microbial community differences in various settings. Specifically, we applied this approach in the classification of microbial communities across different body sites (1108 samples related to 5 major body sites), in the characterization of oral microbiomes associated with healthy and diseased individuals (20 samples, 10 healthy and 10 diseased), and in the classification of microbial communities longitudinally during the development of infants (63 samples obtain throughout 5 years). For these data sets, classification accuracies of 98.60% ± 1.8%, 100% ± 0.0%, and 99.9% ± 1.9% were obtained, respectively.**Conclusion:**Our findings support the use of the proposed reference-free data

analysis approach as a scalable approach for the rapid yet accurate analysis of 16S metagenomic data. The web tool and visualization system will be on: llp.berkeley.edu/mb.

Author Disclosure Block:

E. Asgari: None. **K. Garakani:** None. **M. Mofrad:** None.

Poster Board Number:

SUNDAY-187

Publishing Title:

Role of Synergistic Phage-antibiotic Combinations in Preventing Antibiotic Resistance

Author Block:

A. Jo, J. Kim, L-S. Jung, J. Ahn; Kangwon Natl. Univ., Chuncheon, Korea, Republic of

Abstract Body:

Background: The emergence of antibiotic-resistant *Staphylococcus aureus* has long been of great concern in hospitals because the nosocomial infections with methicillin-resistant *S. aureus* (MRSA) are easily transmitted between patients and furthermore become difficult to treat with common antibiotics Therefore, this study was designed to assess the role of phage-antibiotic synergy (PAS) in reducing antibiotic resistance. **Methods:** The antimicrobial activity of ciprofloxacin, phages, and combination (ciprofloxacin and phages) against *Staphylococcus aureus* was evaluated for 60 h at 37ºC as measured by disk diffusion susceptibility, biofilm-forming ability, bacterial spreading ability, and mutant frequency.**Results:** In the disk diffusion assay, the clear zone was noticeably increased to 10% in the presence of phages. The phage plaque size was increased with being close to the ciprofloxacin disk. The initial numbers of *S. aureus* treated with ciprofloxacin, phages, and combination were significantly reduced by 3.47, 4.62, and 5.75 log CFU/mL, respectively, at the early 12 h of incubation. The combination treatment was most effectively inhibited the growth of *S. aureus*, showing more than 4 log reduction in 18 h of incubation at 37ºC. Compared to the control, the most significant reduction in biofilm formation by *S. aureus* was observed at the combination treatment (3.91 log), followed by phages alone (3.13 log) and ciprofloxacin alone (1.04 log). Ciprofloxacin-treated *S. aureus* cells became resistant to both ciprofloxacin and phage, showing the mutant frequencies of 27% and 25%, respectively, while no antibiotic- and phage-resistant *S. aureus* cells were observed at the combined treatment of ciprofloxacin and phages.**Conclusions:** The most significant finding in this study was that PAS can be a potential alternative to effectively control antibiotic-resistant *S. aureus* with the least frequency of phage or antibiotic resistance. Therefore, these results provide useful information for a better understanding of PAS effect on the growth of *S. aureus* in association with mutation frequency.

Author Disclosure Block:

A. Jo: None. **J. Kim:** None. **L. Jung:** None. **J. Ahn:** None.

Poster Board Number:

SUNDAY-188

Publishing Title:

Antimicrobial Activity of the Essential Oils of Ziziphora Clinopodioides Lam. from Armenia

Author Block:

N. SAHAKYAN[1], M. Petrosyan[1], A. Avetisyan[2], A. Markosian[2], S. Aloyan[2], A. Trchounian[1]; [1]Yerevan State Univ., Yerevan, Armenia, [2]Nairian, Yerevan, Armenia

Abstract Body:

The aim of this study was to analyze the chemical composition of essential oils from Z. clinopodioides (Lamiaceae) collected in Armenia and to test their biological activities. The essential oils were obtained by steam distillation in a Clevenger-type apparatus, a HP GC-MS setup was used to determine their chemical composition.The 34 names of different substances, generally terpenoids (monoterpenes) have been identified in the essential oil of Z. clinopodioides. The main component was pulegone ((R)-5-Methyl-2-(1-methylethylidine) cyclohexanone) which is used in flavoring agents, perfumery, and in aromatherapy. The concentration reached 42.1%. The concentrations of other components were 9.7 % (Isomenthone) and lower - 2.4 % (Menthone); 8.22 % (1,8- Cineol); 7.35 % (Piperitenone); 5.9 % (D- Neomenthol); 3.9 % (DL-Menthol); 3.56 % (Sabinene).According to the agar diffusion method the essential oil of Z. clinopodioides has also antimicrobial activity against Gram-positive, Gram-negative bacteria (including ampicillin- resistant E.coli dhpα-pUC18) and fungi. The highest antibacterial activity was established against St. aureus MDC 5233 (Microbial Depository Center, Armbiotechnology Scientific and Production Center, Armenia) (Laboratory control strain) (the minimal inhibitory concentration (MIC) was 3.125 µl/mL). The highest antifungal activity was established against Candida guillermondii WDCM with MIC value of 6.25 µl/mL.The obtained data indicate that essential oils can be useful natural agents for cosmetic applications and food dietary supplements.This study was done in the frame of Basic research support from State Committee on Science, Ministry of Education and Science of Armenia.

Author Disclosure Block:

N. Sahakyan: None. **M. Petrosyan:** None. **A. Avetisyan:** None. **A. Markosian:** None. **S. Aloyan:** None. **A. Trchounian:** None.

Poster Board Number:

SUNDAY-189

Publishing Title:

Essential Oils of Basil Plants as Antimicrobial Agents

Author Block:

M. PETROSYAN[1], A. Avetisyan[2], A. Markosian[2], N. Sahakyan[1], A. Babayan[1], S. Aloyan[2], A. Trchounian[1]; [1]Yerevan State Univ., Yerevan, Armenia, [2]Nairian, Yerevan, Armenia

Abstract Body:

The aim of the investigation was to study the antimicrobial activity of *Ocimum basilicum* var. *purpureum*, *O. basilicum* var. *thyrsiflora*, and *O. x citriodorum* essential oils. Antimicrobial activity was determined by the agar diffusion method. Gram-positive (*Bacillus subtilis* WT-A; *Staphylococcus aureus* MDC 5233) and Gram-negative (*E. coli* VKPM-M17; *Pseudomonas aeruginosa* GRP3 and ampicillin-resistant *E. coli* dhpα-pUC18) bacteria and yeasts (*Candida albicans* WT-174 and *Debariomyces hansenii* WT) were tested.The gas chromatography-mass spectrometry setup was used to reveal the chemical composition of essential oils. *O. basilicum* var. *purpureum* essential oil contained 57.3% of methyl-chavicol; *O. basilicum* var. *thyrsiflora* oil had 68.0% of linalool and the main constituents of *O. x citriodorum* oil were nerol (23.0%) and citral (20.7%).Gram-positive bacteria were more sensitive to all essential oils than Gram-negatives. The minimal inhibitory concentration (MIC) of *O. x citriodorum* essential oil was 3.125 mg/mL against *B. subtilis* and *St. aureus*. The same MIC was recorded for *O. basilicum* var. *thyrsiflora* essential oil against *St. aureus*, and *O. basilicum* var. *purpureum* essential oil against *B. subtilis*. The MIC of *O. basilicum* var. *thyrsiflora* essential oil against *B. subtilis* and the MIC of *O. basilicum* var. *purpureum* against *St. aureus* were 6.25 mg/mL. The resistant *E. coli* was also sensitive to the essential oils: the MIC values of *O. x citriodorum* and *O. basilicum* var. *purpureum* against that bacterium were 6.25 mg/ml, while *O. basilicum* var. *thyrsiflora* displayed MIC of 12.5 mg/mL. The action of the essential oils was bactericidal. Tested fungi were the most sensitive: MIC of *O. x citriodorum* against *D. hansenii* and *C. guillermondii* were 1.56 and 3.125 mg/mL, respectively.The obtained data show that basil essential oils can be useful natural agents for cosmetics, medicine and food as antimicrobial agents.This study was done in the frame of research support from Science State Committee, Ministry of Education and Science of Armenia.

Author Disclosure Block:

M. Petrosyan: None. **A. Avetisyan:** None. **A. Markosian:** None. **N. Sahakyan:** None. **A. Babayan:** None. **S. Aloyan:** None. **A. Trchounian:** None.

Poster Board Number:

SUNDAY-190

Publishing Title:

Antimicrobial Mechanisms of Benzyl Alcohol and Pentanol on *Methylobacterium* Isolated from Bathrooms

Author Block:

T. Yano[1], Y. Miyahara[1], N. Morii[2], T. Okano[2], H. Kubota[1]; [1]Safety Sci. Res., Ichikai, Tochigi, Japan, [2]Household Products Res., Wakayama, Japan

Abstract Body:

The genus *Methylobacterium* tolerates various stresses including desiccation and hygiene agents such as benzalkonium chloride (BAC), and its infection is an important public health issue. We previously demonstrated that some *Methylobacterium* strains survived in 5.0% BAC after a 5-min exposure, indicating that cleaners with more immediacy and versatility are required. Fluorescence *in situ* hybridization assays of 14 pink biofilms in Japanese bathrooms have indicated that the genus *Methylobacterium* is predominant. Therefore, we searched for effective antimicrobial agents available for use in bathrooms. We isolated *Methylobacterium* strains from bathrooms, and screened agents with high antimicrobial potencies. While screening various alcohols, we found that the combined use of BAC with particular alcohols at non-lethal concentrations in terms of their solitary uses significantly reduced bacterial viability after only a 5-min exposure. These effects were also verified in biofilms on fiber-reinforced plastics, a general material of bath tubs, floors, and walls. Among the alcohols tested, Raman spectroscopic analyses showed that pentanol (PeA) and benzyl alcohol (BzA) accelerated the cellular accumulation of BAC. *In vivo* fluorescent spectroscopic assays and *in vitro* morphological assays with giant vesicles (GV) indicated that PeA rarely attacked membrane structures, while BzA increased membrane fluidity and destabilized structures. Other *in vivo* fluorescent spectroscopic assays indicated that PeA and BzA inactivated bacterial membrane proteins, including the efflux pump for BAC transportation. These results suggest that the inactivation of membrane proteins by PeA and BzA led to cellular accumulation, whereas BzA only also enhanced the penetration of BAC by membrane fluidization at non-lethal concentrations. These mechanisms may be exploited to develop a new theory that facilitates the search for the best compounds for combined use.

Author Disclosure Block:

T. Yano: None. **Y. Miyahara:** None. **N. Morii:** None. **T. Okano:** None. **H. Kubota:** None.

Poster Board Number:

SUNDAY-191

Publishing Title:

Transfer of Antibiotic Resistance between Bacteria within Quagga Mussels

Author Block:

A. D. KAPPELL, M. J. Walsh, K. Paavola, T. Metcalf, J. S. Maki, K. R. Hristova; Marquette Univ., Milwaukee, WI

Abstract Body:

The filter-feeding quagga mussel (*Dreissena bugensis*) has been invading the Great Lakes since the 1990's. Biological and chemical contaminants can accumulate in the tissues of filter-feeding organisms allowing the use of quagga mussels as indicators of freshwater quality and antibiotic resistant bacteria. In addition to the use of quagga mussels as indicator or sentinel organisms, the possibility of mussels as point sources and "hot spots" for the proliferation of antibiotic resistance and exchange was explored. The ability of quagga mussels to accumulate bacteria and allow exchange of antibiotic resistance in their tissue in a controlled setting was determined utilizing a selectable and differential *Escherichia coli* strain. Quagga mussels were placed in artificial lake water (ALW) containing *E. coli* CV601gfp, a strain bearing selection of kanamycin and rifampicin with constitutive expression of GFP. Mussel tissue and water were plated to quantify the presence of GFP expressing *E. coli* during exposure and post-exposure depuration. The tissue and water were also plated on media with the additional antibiotic of ampicillin, tetracycline, streptomycin, chloramphenicol, and gentamycin. Preliminary results indicate a correlation of bacteria in mussel tissue with water. The *E. coli* strain appeared to establish itself within the tissue of the mussel during depuration. After the 48 hr initial exposure and 3 hours of depuration in fresh ALW showed the remaining population of *E. coli* CV601gfp within the mussel tissue had acquired resistances to the 5 antibiotics in 4% to 18% of isolates of the 171±54 CFU/g mussel tissue. Plasmid extraction and agarose gel electrophoresis indicated the acquisition of plasmids in a number of the isolates. Additional exposure experiments in the lab and urban water ways of Milwaukee indicated a correlation between the presence of fecal indicator bacteria in mussel tissue and the waterways determined by qPCR. While these results indicate that quagga mussels could be used as an indicator for biological contamination, quagga mussels may also represent a point source of bacterial contamination and antibiotic resistance of waterways.

Author Disclosure Block:

A.D. Kappell: None. **M.J. Walsh:** None. **K. Paavola:** None. **T. Metcalf:** None. **J.S. Maki:** None. **K.R. Hristova:** None.

Poster Board Number:

SUNDAY-192

Publishing Title:

Targeted Amplification of Antibiotics Resistant Genes Associated with the International Space Station Environment

Author Block:

A. Checinska[1], K. G. Frey[2], C. L. Redden[2], J. Thissen[2], J. E. Allen[3], C. Jaing[3], K. Wheeler[4], **K. Venkateswaran**[1]; [1]Jet Propulsion Lab., California Institue of Technology, Pasadena, CA, [2]Naval Med. Res. Ctr.-Frederick, Fort Detrick, MD, [3]Lawrence Livermore Natl. Lab., Livermore, CA, [4]Allosource, Centennial, CO

Abstract Body:

The International Space Station (ISS) is a closed environment which is shaped by microgravity, radiation, and limited human presence. The microbial diversity associated with the ISS environmental surfaces utilizing cultivation-dependent and –independent approaches was already documented. In this report, targeted amplification technique, a novel method, was used to measure the microbial lineages that are resistant to known antibiotics.Bacterial strains isolated from ISS surfaces were tested for their resistance to nine antibiotics using conventional disc method and Vitek 2 system. Most of the *Staphylococcus aureus* strains were resistant to penicillin. Five strains were specifically resistant to erythromycin and the *ermA* gene was also detected. The nine-erythromycin sensitive *S. aureus* strains exhibited spontaneous mutation when rifampin was tested. Some of the *S. aureus* strains tolerated gentamycin and tobramycin but cefazolin, cefoxitin, ciprofloxacin and oxacillin inhibited the growth of the *S. aureus*. Whole genome sequencing (WGS) of 21 ISS strains, exhibiting resistance to various antibiotics, was carried out. The antibiotic resistant genes deduced from the WGS were compared with the resistomes generated directly from the gene pool of the environmental samples.Using a targeted amplification panel consisting of over 500 antimicrobial resistance genes, we were able to confirm the results of the phenotypic assays. Specifically, the presence of multiple β-lactamase genes was observed. The class A gene, *tem-1,* was present in multiple ISS locations from both flights. A second class A gene, *ctx-M-14,* was also found in multiple sites of ISS. In addition, presence of *mec-A* gene was confirmed in several sampling locations from both ISS flights. Finally, the existence of the *ermA* gene was established. These results suggest widespread and consistent distribution of multiple antibiotic resistance genes throughout the ISS. The resistome data generated via molecular methods will be extremely crucial in determining the microbial significance to the crew health and the ISS maintenance.

Author Disclosure Block:

A. Checinska: None. **K.G. Frey:** None. **C.L. Redden:** None. **J. Thissen:** None. **J.E. Allen:** None. **C. Jaing:** None. **K. Wheeler:** None. **K. Venkateswaran:** None.

Poster Board Number:

SUNDAY-193

Publishing Title:

Bisthiazolidine (Btz) Inhibitors of Ndm-1: The Importance of the Thiol Substituent

Author Block:

A. J. Vila[1], M. M. Gonzalez[1], A. Rossi[1], L. I. Llarrull[1], V. Castillo[2], G. Mahler[2], M. F. Mojica[3], R. Bonomo[3], J. Spencer[4], L. Castellano[2], V. Villamil[2], C. Saiz[2]; [1]IBR - Univ. of Rosario, Rosario, Argentina, [2]Univ. of the Republic, Montevideo, Uruguay, [3]Cleveland VAMC, Cleveland, OH, [4]Univ. of Bristol, Bristol, United Kingdom

Abstract Body:

Background: Carbapenems are recognized as "last-resort" β-lactam drugs. However, the appearance of highly resistant bacteria producing metallo-β-lactamases (MBLs), such NDM-1 (New Delhi Metallo-β-lactamase-1), challenge the therapeutic primacy of carbapenems in the clinical setting. NDM-1 and other B1 MBLs are able to hydrolyze almost all β-lactam classes and are not inhibited by available serine-β-lactamase inhibitors. Clinically useful MBL inhibitors have not yet been introduced into the clinic. Recently, the synthesis and biological characterization of the first series of BTZ heterocycles against the MBLs from B1 subclass (NDM-1, VIM-2 and VIM-24) was reported. From the crystal structures between the MBLs with BTZs, we found that the thiol moiety present in the BTZ scaffold is intercalated between both Zn(II) ions and forms a stable ternary complex. Our goal is to explore the importance of the thiol interaction with NDM-1 in a novel series of derivatives. **Methods:** BTZ derivatives were synthesized wherein the thiol group was replaced with fluoroalkyl, carboxylate, sulfonic acid, tetrazole groups or methylated as a thiomethyl derivative. MBLs were purified to homogeneity. Inhibition constants were determined by following imipenem hydrolysis at 300 nm absorbance The inhibition constants were determined by data fitting to the Competitive Inhibition Model.**Results:** The inhibition assays against NDM-1 revealed that in all cases the substitution of the thiol moiety is essential for the inhibitory activity, highlighting the importance of thiol groups as the main driving force for the inhibition observed in MBLs. Instead, insertion of substituents in the bridging position did not affect the inhibition constants in the low micromolar range observed for the first series of BTZs.**Conclusions:** We report the essential nature of thiol groups in BTZs for MBL. These compounds represent a versatile and promising scaffold for the investigation and development of molecules with a diverse set of moieties capable of inhibiting MBLs.

Author Disclosure Block:

A.J. Vila: None. **M.M. Gonzalez:** None. **A. Rossi:** None. **L.I. Llarrull:** None. **V. Castillo:** None. **G. Mahler:** None. **M.F. Mojica:** None. **R. Bonomo:** E. Grant Investigator; Self;

AstraZeneca, Merck and Wockhardt. **J. Spencer:** None. **L. Castellano:** None. **V. Villamil:** None. **C. Saiz:** None.

Poster Board Number:

SUNDAY-194

Publishing Title:

Effect of Engineered Nanoparticles on the Operation of Wastewater Treatment Plant

Author Block:

S. Eduok, B. Martin, R. Villa, B. Jefferson, F. Coulon; Cranfield Univ., Bedfordshire, United Kingdom

Abstract Body:

The effect of mixed engineered nanoparticles (ENPs) consisting of silver oxide, (20 nm), titanium dioxide, (30 - 40 nm) and zinc oxide, (20 nm) compared with bulk metal salts and unspiked control was evaluated using 3 parallel pilot-scale wastewater treatment plants. ENPs induced 2 times higher specific oxygen uptake rate relative to the control and the heterotrophic biomass retained 98% and 80% removal rate for ammonia and COD respectively. Ammonia- and nitrite oxidizing bacteria e.g. *Nitrosomonas, Nitrobacter* and *Nitrospira* in ENP spiked sludge were inhibited with 1.4 times reduced floc size, whereas the sludge volume index was unaffected. SEM evidenced selective damage to some microbes and pyrosequencing indicated a temporal shift in microbial community structure and diversity with *Acidovorax*, *Rhodoferax, Comamonas*, *Methanocorpusculum* and *Methanosarcina* identified as nano-tolerant species in the activated sludge. Based on ether-linked isoprenoid concentration, abundance of methanogens was 1.4 times lower in ENP spiked digester, whereas pyrosequencing indicated 80% decrease in abundance and diversity of methanogens compared to the control and metal salt spiked digester. *Methanosarcina acetivorans* and *M. barkeri* were nano-tolerant with a factor of 6 and 11 times increase in relative abundance respectively. ENPs exerted 1.2 times lower hydrogen sulfide and 2.4 times higher methane production than the control and metal salt spiked digester. Exposure of wastewater microorganisms to ENPs conferred competitive advantage that enhanced growth of *Fusobacteria, Actinobacteria, Acidovorax, Rhodoferax, Comamonas M. acetivorans* and *M. barkeri* with a Trojan horse-like effect on microbial abundance and diversity.Keywords: Engineered nanoparticles, activated sludge, anaerobic digester, inhibitory effect, nanotolerant Archaea

Author Disclosure Block:

S. Eduok: None. **B. Martin:** None. **R. Villa:** None. **B. Jefferson:** None. **F. Coulon:** None.

Poster Board Number:

SUNDAY-195

Publishing Title:

A Chimeric Protease Inhibitor Secreted By *escherichia Coli* With The Potential For Use In Biofuel Fermentations And Therapeutic Bacterial Delivery Vectors

Author Block:

D. Quintero, D. Bermudes; California State Univ., Northridge, Northridge, CA

Abstract Body:

Naturally occurring sunflower trypsin inhibitor (SFTI) is a 14 amino acid bicyclic peptide that contains an internal disulfide bond and is cyclized by covalently binding the N-terminal and C-terminal amino acids. SFTI is a member of the Bowman-Birk family of protease inhibitors that can inhibit both trypsin and chymotrypsin. SFTI is also capable of inhibiting the tumor protease matriptase, which is expressed in a number of tumor cells and contributes to tumor cell metastasis. Although the N- to C-terminal cyclization of SFTI contributes to it's stability, SFTI lacking this covalent linkage is known to be functional. We constructed chimeric forms of SFTI with N-terminal secretion signals from the *Escherichia coli* OmpA and the *Pseudomonas aeruginosa* ToxA as well as a fusion with the C-terminus of *E. coli* YebF. Using a culture-based protease inhibitor assay, we detected small amounts of protease inhibition in the OmpA and ToxA signal sequence fusions. Co-expression of these N-terminal fusion with the ColE3 lysis protein increased the amount of protease inhibition only for the OmpA fusion. A substantially higher degree of protease inhibition was detected from the YebF fusion, which radiated more than a centimeter from a single bacterial colony. In order to assess the potential of the secreted form of SFTI to protect the ability of a cytotoxic protein to kill tumor cells, we performed a trypsin challenge on the *Pseudomonas* ToxA and its ability to kill MDA-MD-468 breast cancer cells. ToxA was able to kill greater than 90% of tumor cells based on the tetrazolium salt (MTT) reduction cytotoxicity assay, but lost substantial activity when treated with trypsin. Trypsin-challenged ToxA cytotoxicity was restored when culture supernatant of YebF:SFTI was included in the challenge. These data suggest that tumor-targeting bacterial vectors such as VNP20009 might be useful for delivering protease inhibitors with antitumor effects. Furthermore, secretion of protease inhibitors might be useful in other applications such as biofuel fermentations that may have the efficiency of exoenzymes diminished by the presence of proteases.

Author Disclosure Block:

D. Quintero: K. Shareholder (excluding diversitied mutual funds); Self; Magna Therapeutics, unincorporated. **D. Bermudes:** K. Shareholder (excluding diversitied mutual funds); Self; Aviex Technologies, LLC.; Magna Therapeutics, unincorporated.

Poster Board Number:

SUNDAY-196

Publishing Title:

Linking Biosynthetic Clusters with Secondary Metabolites across Phylogenetically Diverse Soil Isolates

Author Block:

L. P. Silva[1], M. Hadjithomas[1], N. Ivanova[1], R. Lau[1], U. Karaoz[2], E. Brodie[2], N. Kyrpides[1], B. P. Bowen[1], T. Northen[1]; [1]Joint Genome Inst., Walnut Creek, CA, [2]Lawrence Berkeley Natl. Lab, Berkeley, CA

Abstract Body:

Actinobacteria are widely known for their ability to secrete bioactive secondary metabolites into their surroundings, and many of these natural products have proven critical in a diversity of applications from agriculture to human health. Given their widespread application, there is great interest in discovering new secondary metabolites as well as improving methods for predicting natural products from annotated genomes. The recent development of new HILIC stationary phases now enable characterization of polar metabolites, which are less explored than commonly detected non-polar molecules. We selected 40 phylogenetically diverse isolates (Actinobacteria and Proteobacteria) for both prediction of biosynthetic clusters and testing secondary metabolite production using LC-MS/MS. These isolates were chosen from a set of 699 isolates with known full-length 16s rRNA sequences, collected from two field sites (Hopland, CA and Moab, UT). Isolates were cultured in 1/10 R2A media in quadruplicate, and then extracted with methanol for LC-MS/MS analysis. Samples were analyzed using both pHILIC and C18 chromatographies. Mass spectrometry was performed using a QExactive mass spectrometer, in positive and negative ionization mode. Data were analyzed using Metabolite Atlas and Pactolus software. DNA was extracted from each isolate and genomes sequenced on an Illumina HiSeq 2500 platform. Genomes were assembled with SPADES, and antiSMASH was used to identify biosynthetic clusters (BCs). BCs were searched with IMG-ABC and correlated to secondary metabolites for each isolate. Approximately 500 compounds were putatively identified from each isolate using Pactolus software. The MS/MS spectra of compounds with significant differences (consumption or production) from the control media were manually validated and compared to the predicted BCs. In many cases, the metabolites identified by Pactolus were supported by the predicted BCs for each genome in IMG-ABC. However, there are numerous examples of identified metabolites without predicted BCs, and the combination of these two approaches is promising for the improvement of secondary metabolite prediction algorithms.

Author Disclosure Block:

L.P. Silva: None. **M. Hadjithomas:** None. **N. Ivanova:** None. **R. Lau:** None. **U. Karaoz:** None. **E. Brodie:** None. **N. Kyrpides:** None. **B.P. Bowen:** None. **T. Northen:** None.

Poster Board Number:

SUNDAY-197

Publishing Title:

Assessment of Natural Compounds Against Micro-biota Isolated from Peptic Ulcer

Author Block:

A. Rehman[1], A. N. Sabri[1], S. Hasnain[2]; [1]Univ. of the Punjab, Lahore, Pakistan, [2]Women Univ. Multan, Multan, Pakistan

Abstract Body:

Background: *Helicobacter pylori* is a close relative of *Campylobacter* spp., characterized as a gram-negative, flagellated bacterium. This pathogen is capable to colonize peptic mucosa and causes peptic ulcer in human population. The elimination of *H. pylori* infection needs a long therapeutic course having multiple antibiotics administered for 7-14 days.The day by day increase in antibiotic resistance emphasizes on the establishment of novel therapeutics against *H. pylori*. The current study was designed to encompass major issues associated with H. pylori infection including detection of antimicrobial susceptibility, EPS (exo-poly-saccharide) production and formation of complex bacterial biofilms among H. pylori and associated micro-biota in gastric ulcer. In order to address issue of novel therapeutics, phytochemical based compounds were employed to eliminate pathogen in-vitro. **Methods:** Normal saline was used to crush and homogenize peptic biopsy samples which were later spread on Columbia blood agar. In microbiological medium, serum to support growth and selective antibiotics were added. All selected isolates were identified by biochemical identification scheme. The inhibitory effect of Honey and ethanolic/methanolic extracts of Black Caraway (*Nigella sativa* seeds) on isolated pathogens was observed by microtiter plate assay. The biofilm formation capacity of isolates was determined by standard motility, EPS production and adherence detection methods. **Results:** Overall 15 different microbes were isolated from 5 peptic biopsies among which one isolate was confirmed as *Helicobacter pylori*. Both honey and Black Caraway (*Nigella sativa)* were observed to have significant inhibitory effect on all isolates at potentially lower concentrations such as 2mg/ml and 1mg/ml respectively. Among all extracts the methanolic extracts of Black caraway (*Nigella sativa)* had observable significant impact on the adherence, EPS production and biofilm development. A variety of structures were found to block urease enzyme in computational studies.**Conclusions:** Natural compounds have huge potential to provide mankind with novel therapies against pathogens. Further investigation of curative potential of natural compounds and phytochemicals can lead to establishment of novel therapeutics against peptic ulcer.

Author Disclosure Block:

A. Rehman: None. **A.N. Sabri:** None. **S. Hasnain:** None.

Poster Board Number:

SUNDAY-198

Publishing Title:

Fitness Center Equipment Made with Copper-Alloys Hold Decreased Bacterial Loads Compared to Control Surfaces

Author Block:

Z. Ibrahim, P. Hooke, S. Hinsa-Leasure; Grinnell Coll., Grinnell, IA

Abstract Body:

With a rise in antibiotic resistance and an increase in community-acquired infections, it is important to find ways to decrease human exposure to pathogenic microorganisms. Athletic centers in particular, contain many high-touch surfaces and have been shown to house pathogenic bacteria including MRSA. One way to decrease exposure to pathogens in this environment is to employ antimicrobial surfaces. In 2008, the Environmental Protection Agency registered 300 different copper-alloys as antimicrobial materials. The aim of our study was to investigate the antimicrobial effects of copper-alloys on high-touch athletic center equipment including grips on barbells, kettle bells, dumbbells, and grip attachments for weight machines. We hypothesized there would be decreased bacterial loads on the 16 copper-alloy surfaces tested compared to the bacterial loads found on standard equipment. Samples were collected from the athletic center equipment at Grinnell College, by swabbing surfaces with a sterile pre-moistened wipe. Copper-alloy equipment was switched in and out of the facility on a regular basis throughout the study to decrease differences due to season or student usage. Bacteria were removed from the swabs through vortexing and plated on trypticase soy agar with 5% Sheep blood (TSAII). We found a statistically significant decrease in bacterial loads on all copper-alloy surfaces ($P<0.05$) compared to loads on standard equipment with an overall average decrease in bacterial load of just over 90%. The results support our hypothesis and previous studies conducted in hospital settings that have demonstrated the antimicrobial properties of copper-alloys. We propose the use of copper-alloy material in strategic locations will lead to a decrease in community-acquired infections from that environment. On-going and future studies aim to identity the most common bacteria, determine their antibiotic resistance profiles, and investigate possible halo effects from the copper-alloy surfaces.

Author Disclosure Block:

Z. Ibrahim: None. **P. Hooke:** None. **S. Hinsa-Leasure:** None.

Poster Board Number:

SUNDAY-199

Publishing Title:

Characterization of Ctx-M-151, a Novel Extended-Spectrum β-Lactamase from *Enterobacteriacae*

Author Block:

A. Yoshizumi[1], T. Saga[2], K. Aoki[1], A. Ohno[1], Y. Ishii[1], K. Tateda[1]; [1]Toho Univ., Ohta-ku, Tokyo 143-8540, Japan, [2]Akita Univ. Graduate Sch. of Med. and Faculty of Med., Akita city, Akita, Japan

Abstract Body:

Background: CTX-M extended-spectrum beta-lactamases (ESBLs) are a major cause of β-lactam resistance among Gram-negative bacteria worldwide. In our previous study, we identified the novel CTX-M gene, *bla* $_{\text{CTX-M-151}}$ on the chromosome of an unknown species belonging to *Enterobacteriaceae* isolated from piglet feces. CTX-M151 showed 68.3% sequence similarity to CTX-M-14 belonging to CTX-M group 9. In this study, we characterized the kinetic properties of CTX-M-151.**Methods:** The entire *bla* $_{\text{CTX-M-151}}$ was PCR-amplified from the total DNA extracted from the isolate. The PCR product was subcloned into a pET 9a+ expression vector and overexpressed in *Escherichia coli* BL21 (DE3)/pLysS using 1mM IPTG. CTX-M-151 was purified by cation exchange chromatography, HiTrap SP FF column. The kinetic properties of the purified CTX-M-151 were calculated for cefotaxime, ceftazidime (CAZ), cefepime, piperacillin and nitrocefin by measuring the initial hydrolysis rates using a UV2550 spectrophotometer. K_i value for clavulanic acid.(CVA) was determined using nitrocefin as the competitor substrate.**Results:** CTX-M-151 hydrolyzed all tested β-lactams except for CAZ. CTX-M-151 had K_i value for CAZ of 5784μM. Compared to CTX-M-9, CTX-M-151 showed similar hydrolytic efficiencies (k_{cat}/K_m) for nitrocefin ; 3.0 x 10^7 $M^{-1}s^{-1}$ vs. 1.8 x 10^7 $M^{-1}s^{-1}$ for CTX-M-9. The k_{cat} of CAZ for CTX-M-151 and CTX-M-9 could not be determined when 20000 μM of CAZ was used while scanning for 15 min. Using nitrocefin as a substrate demonstrated that CVA was competitive inhibitors of CTX-M-151 and CTX-M-9 with K_i values for CVA of 0.78 μM and 0.17 μM ± 10%, respectively.**Conclusions:** CTX-M-151 hydrolyzed all tested β-lactams except for CAZ. The combination of amino acid substitutions observed in CTX-M-151 result in a similar catalytic efficiency when compared to CTX-M-9for nitrocefin, cefalotin. The K_i values of CVA for CTX-M-151 was about 5 times higher than those observed for CTX-M-9.

Author Disclosure Block:

A. Yoshizumi: None. **T. Saga:** None. **K. Aoki:** None. **A. Ohno:** None. **Y. Ishii:** None. **K. Tateda:** None.

Poster Board Number:

SUNDAY-200

Publishing Title:

Melipona Beecheii Honey and Its Compounds Against Virulence Factors of *Helicobacter pylori*

Author Block:

M. Lara Torres, G. Varguez Cruz, J. Ramón Sierra, E. Ortiz Vázquez, D. Magaña Ortiz, J. Ruiz Ruiz; Inst. Tecnológico de Mérida, Mérida, Mexico

Abstract Body:

Background: Helicobacter pylori is the dominant species of the human gastric microbiome, the colonization of this bacteria causes a persistent inflammatory response & plays an important carcinogenic role. Usually H. pylori infections are treated with a triple therapy, including antibiotics & proton pump inhibitors. In most cases, this treatment could fail for H. pylori eradication and cause resistance to the antibiotics employed. Research related to natural products has demonstrated significant progress in the discovery of new compounds with antimicrobial activity. In this sense, oral administration of honey to treat & protect against gastrointestinal disorders such as gastritis, duodenitis & gastric ulceration caused by bacteria & rotavirus has been reported. The goal of this study was to evaluate the effect of honey produced by Melipona beecheii on expression of virulence genes of Helicobacter pylori.**Methods:** Methods: The strain of H. pylori (ATCC 49503) was grown under microaerophilic conditions (5% O2, 10% CO2, and 85% N2) at 37 °C for 3 days. The antimicrobial activity & minimum inhibitory concentration (MIC) were determined by agar diffusion method in Brucella agar supplemented with 10% fetal bovine serum. Chromosomal DNA extraction was realized using CTAB method. The detection of presence the housekeeping & virulence genes was performed using PCR standard protocols with specific primers.**Results:** The MIC of honey against Helicobacter pylori was 10% (v/v). Chromosomal DNA extraction allowed the detection of housekeeping (hsp60 and sigma80) & virulence (vacA & ureA) genes.**Conclusions:** Honey from M. beecheii inhibits the growth of H. pylori at concentrations of 10% (v/v). The presence of phenolic compounds & proteins in honey it could be responsible of the antimicrobial effect against H. pylori; then they could be related to repression virulence of genes in the microorganism. In future studies, both components will be isolated & evaluated to test its antibacterial effect on H. pylori.

Author Disclosure Block:

M. Lara Torres: None. **G. Varguez Cruz:** None. **J. Ramón Sierra:** None. **E. Ortiz Vázquez:** None. **D. Magaña Ortiz:** None. **J. Ruiz Ruiz:** None.

Poster Board Number:

SUNDAY-203

Publishing Title:

Prevalence of Antibiotic Resistant *E. coli* in Drinking Water Sources in Tamale Metropolis of Ghana

Author Block:

F. Adzitey, C. K. S. Saba; Univ. for Dev. Studies, Tamale, Ghana

Abstract Body:

Background: The use of antibiotics for therapeutic purpose is unavoidable despite concerns about multidrug resistance pathogens. The presence of antibiotic resistant *E. coli* in drinking water is a threat to public health. This study determined the prevalence of resistant *E. coli* in in drinking water samples collected from Tamale Metropolis of Ghana. **Methods**: Antibiotic susceptibility test was performed using the disc diffusion method and the results interpreted using the Clinical and Laboratory Standards Institute guidelines. Fifty six (56) *E. coli* were tested against 9 different antibiotics. **Results**: Overall, 37.90% of the *E. coli* isolates were resistant, 12.90% were intermediate and 49.21% were susceptible. Resistance to vancomycin (94.64%) and erythromycin (85.71%) was high. A relatively higher percentage of the *E. coli* isolates exhibited intermediate resistance to amoxycillin/clavulanic acid (50%). The *E. coli* isolates also exhibited 24 antibiotic resistant patterns with the pattern E-VA (erythromycin-vancomycin) and SXT-E-VA-TE (trimethoprim/sulfamethoxazole-erythromycin-vancomycin-tetracycline) being the commonest (each exhibited by nine different isolates). Multiple antibiotic index (MAR index) ranged from 0.11 to 0.56. Resistance to five (MAR index of 0.56) and four (MAR index of 0.44) different antibiotics were exhibited by 8 and 21 isolates, respectively. **Conclusions**: This study revealed that *E. coli* from drinking water sources in Tamale, Metropolis are resistant to some antibiotics. Therefore, the use of antibiotics in animal production and for human treatments in the Metropolis need to be checked and curbed to prevent more isolates from becoming resistant.

Author Disclosure Block:

F. Adzitey: None. **C.K.S. Saba:** None.

Poster Board Number:

SUNDAY-204

Publishing Title:

Evaluation of Bacteriological Quality of Street Vended Guinea Fowl Meat Sold in Nkalagu Motor Park, Ishielu Local Government Area of Ebonyi State, Nigeria

Author Block:

W. I. Egwurochi, D. 0. Uchendu, V. U. Olugbue, B. C. Anyaegbunam, E. I. Okonkwo; Akanu Ibiam Federal Polytechnic, Unwna-Afikpo, Ebonyi State, Nigeria

Abstract Body:

Many studies have been done on street vended meats such as bush meat but little has been done of the guinea fowl meat. This study evaluated the bacteriological quality of Street Vended Guinea fowl Meat sold in Nkalagu Motor Park in Ishielu Local Government Area of Ebonyi State, Nigeria. Guinea fowl meat samples were randomly purchased from three different hawkers at the motor park, labeled accordingly and taken to the Microbiology Laboratory of the Department of Science Laboratory Technology, Akanu Ibiam Federal Polytechnic for bacteriological analysis. Each sample was pounded in a sterile porcelain mortar. 1g of the pounded meat was homogenized in 9ml of distilled water until a serial dilution of 10^{-6} was obtained. Dilutions 10^{-6} was spread plated on MacConkey and nutrient agar plates, incubated at 37°C for 24 hours. Colony forming units were counted to determine the microbial load. Based on the colonial, morphological and biochemical characteristics, nine genera of isolates - *Staphylococcus* sp., *Salmonella* sp., *Escherichia coli*, *Bacillus* sp., *Pseudomonas* sp., *Enterobacter aerogenes*, *Streptococcus* sp., *Salmonella* sp., *Proteus* sp., were identified. From the results, there is need for observance of good personal hygiene by the sellers, potable water should be provided to avoid the use of contaminated water in the processing of these product, buyers should be prevented from touching the meats during bargain; there is need for proper covering of the products as it is being carried around in the motor park.**Results**

ϖ Table 1: Aerobic and Coliform plate count of isolates

sample	Aerobic Count (cfu/g)	Coliform count (cfu/g)
A	$3.21x10^{-6}$	$3.41x10^{-6}$
B	$4.10x10^{-6}$	$4.30x10^{-6}$
C	$3.50x10^{-6}$	$3.32x10^{-6}$

Table 2:Biochemical and microscopic characteristics of isolates

	Catalase indole urase citrate oxidase motility coagulase gram VP	G. M S L	isolated organism
Sample A	+ + - - - - - - - + - - - - + + - -	+ + + + + - + +	*Staphylococcus* sp. *E. coli.*

	+ + - + - + - + + + - - - + + - - - + - - + - - - + +	- - + - + - + +	*Salmonella* sp. *Pseudomonas sp.* *Enterobacter aerogenes*
Sample B	+ - - + - - - + + + - + + - - ND - + + - - - - + + - + + - - - + + - - - + + - + - + - + + + + - - - - - - -	+ + - + + - + + + + - - + + - + + - + +	*Enterobacter aerogenes* *Klebsiella* sp. *Staphylococcus* sp. *Pseudomonas* sp. *Bacillus* sp *E. coli.*
sample C	- - - + - - - - + + - - - + + - - - + - - - - + + - - + + - - - - - - - + + + - - + + - - + - - + - - - + +	+ - + + + + - - - - + - + - + + + - + - + + + +	*Staphylococcus* sp. Pseudomonas sp. *Salmonella* sp. *E. coli* *Proteus* sp. *Enterobacter aerogenes*

Author Disclosure Block:

W.I. Egwurochi: None. **D.0. Uchendu:** None. **V.U. Olugbue:** None. **B.C. Anyaegbunam:** None. **E.I. Okonkwo:** None.

Poster Board Number:

SUNDAY-205

Publishing Title:

Microbial Evaluation and Identification of Aflatoxin Producing Fungi in Dried Stored Maize Samples Sold in Open Markets in Benin City, Nigeria

Author Block:

S. E. OMONIGHO, S. V. Obatusin; Univ. of Benin, Benin City, Benin City, Nigeria

Abstract Body:

Maize (*Zea mays*) is an annual monoecious crop and the most important cereal in the world after wheat and rice with regard to cultivation areas and total production. Despite the various improvements in the drying and storage of maize, the growth of moulds is usually a common occurrence in stored grains. The grain is vulnerable to biodeterioration by mycotoxigenic fungi which include *Aspergillus*, *Fusarium* and *Penicillium*. In this study, the microbial load of dried stored maize samples from three different open markets in Benin City was evaluated, and the aflatoxin-producing *Aspergillus* species identified. The pH and moisture contents of samples were also determined. Four fungal species; *Rhizopus* sp., *Fusarium* sp., *Aspergillus flavus*, and *Penicillium sp*., as well as three bacterial species; *Staphylococcus aureus*, *Bacillus* sp. and *Pseudomonas* sp. were isolated and identified using standard cultural and morphological techniques. The mean total bacterial count (cfu/g) ranged from $3.80 \pm 0.20 \times 10^3$ (Oba market) to $5.20 \pm 0.20 \times 10^3$ (New Benin market), while the mean total fungal count ranged from $3.70 \pm 0.29 \times 10^4$ (Oba market) to $6.47 \pm 0.38 \times 10^4$ (New Benin market). The mean pH ranged from 5.90 (Oba market) to 6.70 (New Benin market) while the mean moisture content ranged from 16.10 % (Oba market) to 18.20 % (New Benin market). *Aspergillus niger* was identified as the potential aflatoxin-producing specie, and it had 100.00 % occurrence in all samples. The study showed that stored maize samples had higher fungal load than bacteria, and were contaminated with *Aspergillus flavus*; a potential aflatoxin-producing fungus. It is then necessary to observe strict pre- and post-harvest practices in order to reduce microbial contamination of stored grains.

Author Disclosure Block:

S.E. Omonigho: None. **S.V. Obatusin:** None.

Poster Board Number:

SUNDAY-206

Publishing Title:

Mechanism for the Antibacterial Action of Theaflavin 3,3'-Di-O-Gallate on *Bacillus* Coagulans

Author Block:

J. Sato[1], A. Horiuchi[1], M. Nakayama[2], T. Sonoda[1], M. Hasumi[1], T. Miyamoto[3]; [1]Kao Corp., Ichikai, Haga, Japan, [2]Kao Corp., Sumida-ku, Tokyo, Japan, [3]Kyushu Univ., Fukuoka, Japan

Abstract Body:

Background: Tea polyphenols, such as catechins and teaflavins, have high antibacterial activity against various microorganisms including spore-forming bacteria. *Bacillus coagulans* is one of the most important targets of sterilization in beverage manufacturing, especially for tea drink, because of the high heat-resistance of the spores and high resistance against antibacterial action of catechin in vegetative cells of some strains of the bacterium. However, the antibacterial mechanism of tea polyphenols against B. coagulans has not been fully elucidated. To study the mechanism for antibacterial action of tea polyphenols, we investigated the interaction of theaflavin 3,3'-di-O-gallate (TFDG) to cell surface of *B. coagulans*. **Methods:** To examine the antibacterial effect against *B. coagulans*, minimum bactericidal concentration (MBC) of TFDG was determined in phosphate buffer for each time (1, 4, and 24 h). Changes in cell membrane fluidity were measured by using fluorescent reagent, Laurdan after the treatment of the cells with and without TFDG. Effects of TFDG on transporter of *B. coagulans* were investigated by measuring the uptake of 2-deoxyglucose. To examine the interaction between cell surface proteins and TFDG, two-dimensional electrophoresis patterns of the cellular proteins were compared between the cells treated with and without TFDG.**Results:** Viable count of *B. coagulans* decreased after the treatment with TFDG at 125 mg/L for 1h. Cell membrane fluidity and the activity of glucose transporter decreased after the treatment with TFDG. The 2D-PAGE patterns of cellular proteins of *B. coagulans* showed that some spots disappeared or showed markedly decreased intensity after the treatment with TFDG. Four cell surface proteins were identified among these proteins.**Conclusions:** TFDG showed bactericidal effect on *B. coagulans* at 125 mg/L. After the treatment with TFDG, membrane fluidity and the activity of glucose transporter of the cells decreased. Together with the changes in the 2D-PAGE pattern of the cellular proteins by the treatment with TFDG, it seems that TFDG interacts with the cytoplasmic membrane and the membrane proteins and affects their functions leading to death.

Author Disclosure Block:

J. Sato: None. **A. Horiuchi:** None. **M. Nakayama:** None. **T. Sonoda:** None. **M. Hasumi:** None. **T. Miyamoto:** None.

Poster Board Number:

SUNDAY-207

Publishing Title:

Natural Plant Products Inhibits Growth and Alters Expression of Virulence Genes in Enteroaggregative and Enterohemorrhagic *Escherichia coli*

Author Block:

A. García[1], S. Garcia[1], J. Merino[1], P. Feng[2], N. L. Heredia[1]; [1]Univ. de Nuevo León, San Nicolás, N.L., Mexico, [2]Food and Drug Admin., College Park, MD

Abstract Body:

Background: Most Escherichia coli strains are innocuous to human beings; however, some can cause diseases. Physical, chemical and therapeutic treatments have effectively controlled pathogens to some extent either in foods or human and animal infections; however, current trends also involve the use of natural antimicrobials. Although the ability of pathogenic *E. coli* to cause infections is enhanced by mechanisms that allows for colonization and persistence in different environments. little is known about the effect of natural antimicrobials on the expression of genes involved in virulence of *E. coli.* **Methods:** In this study, the effect of extracts from Mexican oregano (*Lippia berlandieri*) and Mexican logwood (*Haematoxylon brassiletto,* Hb) and their principal antimicrobial components, carvacrol and brazilin, respectively was evaluated on the growth and expression of virulence genes in enterohemorrhagic *E. coli* (EHEC) O157:H7, enteroaggregative *E. coli* (EAEC) strain 042 and Shiga toxin producing EAEC strain of O104:H4 serotype. Citral and rifaximin were used as positive controls. Minimum bactericidal concentrations (MBC) were determined in microtiter plates using various concentrations of extracts. When appropriate, the expression of *rpoS* and the virulence genes *stx2, aggR, pic,* was determined.**Results:** All of the compounds affected the survival of all three strains. The MBC ranged from 0.07 to 0.09 mg/ml for rifaximin; 0.08 to 0.1 mg/ml for carvacrol; 0.12 to 0.2 mg/ml for citral; 0.3 to 0.4 mg/ml for oregano 2.2 to 2.3 mg/ml for brazilin, and 7.2 to 8.1 mg/ml for Hb. Most compounds decreased expression of the *stx2* gene in both *E. coli* O157:H7 and *E. coli* O104:H4. The expression levels of *Pic* and *rpoS* in *E. coli* O104:H4 varied depending on compound; however, in *E. coli* 042 all compounds suppressed both genes.**Conclusions:** These antimicrobials show great potential to control pathogenic *E. coli* by affecting growth and virulence gene expression.

Author Disclosure Block:

A. García: None. **S. Garcia:** None. **J. Merino:** None. **P. Feng:** None. **N.L. Heredia:** None.

Poster Board Number:

SUNDAY-208

Publishing Title:

The Effects of Vietnamese Star Anise Fruits Essential Oil Vapour on Growth Rate of Fungi *In Vitro* and *In Vivo*

Author Block:

T. Dao[1], H. Tran[2], M. Eeckhout[3], F. Devlieghere[4]; [1]Ho Chi Minh city Univ. of Food Industry, Ho Chi Minh City, Viet Nam, [2]Cai Lan Oils & Fats Industries Company, Ho Chi Minh City, Viet Nam, [3]Lab. for Applied Mycology, Univ. Ghent, Gent City, Belgium, [4]Lab. of Food Microbiol. and Food Preservation, Univ. Ghent, Gent City, Belgium

Abstract Body:

Background: Moulds growth rapidly and can cause serious damage at post-harvest storage of agricultural products. In addition, the products can be contaminated by mycotoxins including aflatoxins, ochratoxins, and fumonisins. Therefore, it is necessary to control mould contamination of post-harvested products. One of the solutions that would be effective is the use of essential oil as natural preservative.**Methods:** In this study, the composition of Star Anise fruit (*Illicium verum* Hook.f) essential oil was identified. The main compound of *Illicium verum* Hook.f is (E)-anethole (89%). This essential oil has been considered as a gaseous biological control agent able to inhibit the growth of three strains *Aspergillus flavus*, *Penicillium digitatum* and *Penicillium italicum in vitro* and *in vivo*. The inhibition effect *in vitro* was tested in potato dextrose agar medium (PDA), *in vivo* effects were examined in Sweet orange (*Citrus sinensis*). Inhibition capacity has been evaluated through the effect on the mycelium growth**Results:** showed that at 25^0C, 0,99 a_w, 120 µl.ml^{-1} concentration of essential oil, the growth of three strains have completely inhibited development. Significant inhibition of these three strains was also observed *in vivo* although the antifungal activity of *Illicium verum* Hook.f. vaporized essential oil showed less efficacy in Sweet orange.**Conclusion:** The essential oil of Star Anise fruits is able to control the growth of three studied strains. Essential oil-based fungicides can be effectively used for bio-preservation of agricultural products.

Author Disclosure Block:

T. Dao: None. **H. Tran:** None. **M. Eeckhout:** None. **F. Devlieghere:** None.

Poster Board Number:

SUNDAY-209

Publishing Title:

The Anti-Noroviral Property of Curcumin in *In Vitro* and *In Vivo* Models

Author Block:

G-j. Lee[1], H. You[1], M. Yang[1], S-J. Lee[2], G. Ko[1]; [1]Seoul Natl. Univ., Seoul, Korea, Republic of, [2]Korea Univ., Seoul, Korea, Republic of

Abstract Body:

Background: Human norovirus (HuNoV) is recognized as the leading cause of foodborne outbreak and is responsible for more than 90% of all nonbacterial gastroenteritis. Conventional intervention methods to reduce HuNoV contamination in foods and food preparation processes include physical (i.e. heating or radiation) or chemical methods (i.e. sodium hypochlorite and titanium dioxide), and have several disadvantages for direct application to foods such as potential toxicity and changes in food quality. Natural substances such as phytochemicals that possess antiviral effect on HuNoV have received attention as a potential substitute with low-toxicity and additional bioactive functions. Curcumin is a well-known phytochemical, derived from turmeric, with diverse biological functions such as antioxidant, anticancer, antiobesity, antibacterial, and antiviral activities. However, anti-noroviral property of curcumin has not been reported yet. In this study, we investigated the effectiveness of curcumin in *in vitro* and *in vivo* models.**Methods:** We firstly investigated the effect of curcumin on infectivity in aspects of inactivating viral particles using MNV-1 surrogate model and inhibiting viral replication using HG23 HuNoV replicon model. Curcumin was treated to MNV-1 (1.0×10^6 PFU/ml at 4°C at various concentrations (0.25, 0.5, 0.75, 1.0, 2.0 mg/ml) and time points (short-term, 10, 30, 60, 120 min; long-term, 1, 3, 7, and 14 day), and MNV survival in RAW264.7 cells was measured by plaque assay.**Results:** Curcumin showed anti-noroviral activity in a dose- and time-dependent manner. HuNoV replication assay was performed based on quantifying replicon level by qRT-PCR after curcumin treatment to HG23 Norwalk virus replicon bearing cells. Curcumin did not show the notable inhibitory effect on viral replication. In order to investigate *in vivo* anti-noroviral effect of curcumin, C57BL/6 mice were administered 10mg/mice/day of curcumin for 5 wks after persistent infection of MNV. Considering all the results from *in vitro* and *in vivo* study, curcumin showed the anti-noroviral properties in inactivating viral particles and inhibiting their entry to host cells.**Conclusions:** Curcumin could be a potential candidate for reducing norovirus infection in food processing industry.

Author Disclosure Block:

G. Lee: None. **H. You:** None. **M. Yang:** None. **S. Lee:** None. **G. Ko:** None.

Poster Board Number: SUNDAY-210

Publishing Title:

Comparison of Microbiological Characteristics of Chill Tank Water and Carcasses Treated with Chlorine or Peroxyacetic Acid Antimicrobials Applied During Poultry Processing

Author Block:

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Abstract Body:

Presence of the pathogens *Campylobacter* and *Salmonella* on poultry carcasses post-processing is a serious problem due to the burden of health issues resulting from associated human illnesses. Immersion chilling represents a risk for microbiological cross-contamination of pathogens and spoilage organisms on carcasses. To mitigate this risk, chlorine- and acid-based antimicrobials are often used in chill tanks. Traditionally, chlorine has been used although efficacy is greatly decreased by high organic loads and pH levels above 7.0. Peroxyacetic acid (PAA), a combination of acetic acid and hydrogen peroxide, is approved as an antimicrobial for use in poultry chill tanks in North America. The effectiveness of PAA was compared to chlorine in poultry chill tanks (PAA was used in a commercial setting that traditionally had employed a chlorine antimicrobial treatment). In this trial, broiler carcasses and chill tank water were sampled for *Salmonella* spp., *Campylobacter* spp. and indicator organisms at various stages of processing over a number of processing days. In all, 150 carcasses were sampled at various stages of processing over seven processing days that were immersed in chilled either with 30 ppm of PAA or 30 ppm chlorine. PAA reduced the rate of *Salmonella*- and *Campylobacter*-positive carcasses exiting the chiller by 100% (P=0.0005) and 74% (P=0.0358) (45 min contact time), compared to chlorine. Aerobic colony counts (ACC), coliform and *E. coli* levels were also reduced on carcasses by 1.15-log (P<0.0001), 2.21-log (P<0.0001), and 2.62-log (P=0.0027), respectively, with PAA compared to chlorine treatment. The risk of cross-contamination during immersion chilling was also evaluated by examining chill tank water. Chill tank water maintained at 30 ppm PAA had significantly decreased occurrences of *Salmonella* (P=0.0158) and *Campylobacter* (P<0.0001) compared to 30 ppm chlorine use in the same chill tank as measured throughout processing days. *E. coli* levels were also significantly reduced in occurrence (P=0.0438) with PAA use as were coliform (P=0.0217) and ACC (P=0.0011), compared to chlorine use. These results suggest that PAA is a more effective antimicrobial in poultry chill tank applications than chlorine.

Author Disclosure Block:

J. Boulter-Bitzer: None.

Poster Board Number:

SUNDAY-211

Publishing Title:

Determination of Antagonism between Nslab Strains and *Lactobacillus wasatchensis* Wdc04 Using the Agar-Flip Method

Author Block:

C. Oberg, M. Walker, M. Culumber, 84408-2506; Weber State Univ., Ogden, UT

Abstract Body:

Lactobacillus wasatchensis WDC04, a new obligatory heterofermentative nonstarter lactic acid bacteria (NSLAB), was recently isolated from "gassy" Cheddar cheese. Evidence indicates WDC04 may be an important cause of late gassy defect in aged cheese. One way to control WDC04 may be to incorporate other NSLAB strains into cheese that inhibit its growth. Experiments were performed to determine if inhibition occurs between common NSLABs and WDC04 utilizing the agar-flip method. A lawn of WDC04 was swabbed on MRS agar with 1.5% ribose (MRS-R) and incubated anaerobically at 25°C for 2 d or 4 d. Agar was then aseptically flipped over and individual NSLAB challenge cultures swabbed on the exposed surface. Plates were incubated anaerobically at 30°C or 37°C (for *Lb. helveticus*) for 5 d. Growth of NSLAB cultures was compared to their growth on MRS-R plates without a WDC04 lawn (controls). In a second experiment, the same procedure was utilized except the media contained 4% NaCl and pH 5.2 to mimic the cheese environment. In a third experiment, MRS-R (4% NaCl, pH 5.2) was also used but the NSLAB cultures were initially swabbed as the lawn, incubated, then WDC04 was streaked on the opposite side of agar. In the first and second experiments, *Lb. curvatus* WSU1 showed the greatest inhibition by WDC04 while *Lb. paracasei* Lila and *Lb. rhamnosus* 7469 were the least inhibited. All challenge NSLAB strains showed decreased levels of growth compared to their control plates. In both experiments, most NSLAB cultures showed more inhibition by WDC04 grown for 4 d compared to 2 d before the culture challenge. Results from the third experiment suggest some NSLAB strains can affect growth of WCD04 under cheese-like conditions with *Lb. casei* F19, *Lb. paracasei* Lila, and *Lb. rhamnosus* 7469 exhibiting inhibition. Since there was no direct contact between WDC04 and each NSLAB challenge strain, any inhibition was due to secretion of inhibitory compounds. Examining the antagonism between NSLABs and WDC04 allows for the selection of NSLAB strains that could inhibit this problematic bacterium during cheese ripening.

Author Disclosure Block:

C. Oberg: None. **M. Walker:** None. **M. Culumber:** None.

Poster Board Number:

SUNDAY-212

Publishing Title:

Inhibition of *Bacillus cereus* Growth and Toxin Related Genes by *Bacillus* sp. Isolated from Korean Traditional Fermented Soybean Paste

Author Block:

H. Choi, J. Eom, B. Seo, J. Kim, G. Song; Natl. Academy of Agricultural Sci., Wanju, Jeollabuk, Korea, Republic of

Abstract Body:

Background: *Bacillus subtilis* HJ18-4 is isolated from buckwheat *sokseongjang*, a traditional Korean traditional fermented soybean paste, and it have functions of producing several enzymes and extensive antibacterial spectrums against food-borne pathogens including Bacillus cereus**Methods:** In this study, we investigated the antibacterial efficacy and regulation of toxin gene(groEL, nheA, nheC, and entFM) expression in *B. cereus* by B. subtilis HJ18-4 with q-PCR and western blotting analysis.**Results:** As a result, The survival of B. cereus in the presence of 0.5% and 1% *B. subtilis* HJ18-4 decreased by approximately 6.87 and 5.65 log CFU/mL, respectively, when compared to coculture with *B. subtilis* KACC12680 and in the control (8.23 log CFU/mL) at 24 h postincubation. Transcript expression of toxin-related genes groEL, nheA, nheC, and entFM was significantly lower in *B. cereus* cocultured with *B. subtilis* HJ18-4 than in the control and coculture with *B. subtilis* KACC12680. We tested whether the *B. subtilis* HJ18-4 strain containing antibacterial activity against *B. cereus* in broth could have the same effect as antimicrobial in the fermented soybean product. Consistent with the result of Figure 1, the growth of *B. cereus* in fermented soybean product was inhibited by *B. subtilis* HJ18-4. The growth of *B. cereus* in coculture ratios of 5 : 5 and 1 : 9 *B. cereus* KACC10004 to *B. subtilis* HJ 18-4 was reduced by *B. subtilis* HJ18-4 inhibits *B. cereus* approximately 2.5- and 3-fold, respectively, in comparison to the control at 24 h.**Conclusions:** In conclusion, *B. subtilis* HJ18-4 is able to prevent pathogen growth on fermented soybean paste and we predict also that antibacterial substance from HJ18-4 can be candidate of novel natural food preservatives and alternative antibiotics.

Author Disclosure Block:

H. Choi: None. **J. Eom:** None. **B. Seo:** None. **J. Kim:** None. **G. Song:** None.

Poster Board Number:

SUNDAY-213

Publishing Title:

Assessing the Effect of Gums on the Growth, Viability and ß-Galactosidase Activity of *Lactobacillus* spp. in Milk Drink During Refrigerated Storage

Author Block:

B. D. Karlton-Senaye[1], L. Williams[1], R. Tahergorabi[2], V. Giddings[2], S. Ibrahim[2]; [1]NCAT& SU, Kannapolis, NC, [2]NCAT& SU, Greensboro, NC

Abstract Body:

Recently, there has been a growing interest in the consumption of functional foods containing probiotic microorganisms. The composition of gums could support the growth of probiotics. Therefore, the aim of our study was to examine the growth, viability and ß-galactosidase activity of *Lactobacilli* in milk containing gums during 28 day refrigerated storage at 4°C. Our results showed that the addition of xanthan led to the highest growth of *L. rhamnosus* GGB101 (8.81±0.01logCFU/mL) and *L. rhamnosus* GGB103 (8.32±0.01 log CFU/mL) in milk. Carrageenan-maltodextrin promoted the highest growth (8.30±0.23log CFU/mL) of *L. reuteri* DSM 20016 in the medium and was found to support significant growth of *Lactobacillus* strains in both milk and medium. The population of *Lactobacillus rhamnosus* GGB101 and *Lactobacillus rhamnosus* GGB103 was maintained, whereas the population of *Lactobacillus reuteri* DSM20016 and *Lactobacillus reuteri* SD2112 significantly decreased during refrigerated storage. The highest viable number of *Lactobacillus rhamnosus* GGB103 (8.76±0.03 log CFU mL_1) was obtained in the product containing carrageenan-maltodextrin. The addition of guar-locust bean-carrageenan led to 20-fold increase in the level of b-galactosidase activity for *Lactobacillus rhamnosus* GGB101 (1208±2.12 Miller units mL_1) compared to the control (61±2.83 Miller units mL_1). Our results suggest that carrageenan-maltodextrin, xanthan and carrageenan could serve as functional ingredients for the enhanced growth, viability and ß-galactosidase activity of *Lactobacillus* to improve management of lactose intolerance and thereby promote human health.

Author Disclosure Block:

B.D. Karlton-Senaye: None. **L. Williams:** None. **R. Tahergorabi:** None. **V. Giddings:** None. **S. Ibrahim:** None.

Poster Board Number:

SUNDAY-214

Publishing Title:

Determination of Treatments to Reduce Late Gassy Defect in Cheese Due to *Lactobacillus wasatchensis* Wdc04 Contamination

Author Block:

I. Bowen, C. Oberg, M. Culumber; Weber State Univ., Ogden, UT

Abstract Body:

Lactobacillus wasatchensis WDC04 is a newly discovered obligately facultative lactic acid bacterium isolated from gassy Cheddar cheese. Previous research has associated WDC04 with late gassy defect in aged cheese, which causes serious commercial losses from bloated cheese packages and textual defects. Experiments were performed to determine its salt tolerance at pH 5.2 and 6.5, resistance to HTST pasteurization, and pH growth range. Understanding the growth characteristics of *Lb. wasatchensis* WDC04 could allow some manipulation of the cheese environment to control it. MRS with 1.5% ribose (MRS-R) was prepared at either pH 5.2 or 6.5 with salt concentrations ranging from 0.0%-10.0%. Two ml of the MRS-R test media was added to each well in a 24 micro-well plate and a pre-inoculated absorbance reading was taken at 600 nm. Immediately after, 100 microliters of WDC04 was inoculated into each well, and the plate incubated at 25^{o}C for 3 d (pH 6.5 MRS-R) or 2 d (MRS-R pH 5.2). Plates were then placed in a Teacon Infinite 2000 with absorbance readings (A_{600}) taken every 4 h for 24 h. Results showed WDC04 grew best at 3.0% salt (pH 6.5) and 2.0% salt (pH 5.2) but showed some growth up to 6% at either pH. Further testing was performed using a narrower salt range (5.25%-6.75%) at pH 6.5 to determine if a salt concentration used in cheese could suppress WDC04 growth. The same protocol was followed with the micro-well plate incubating for 3 d before absorbance readings were taken. Above 6.0% salt WDC04 was inhibited and ceased to grow. Using the same methodology, the pH range (2-8) for WDC04 was also determined. Results confirmed WDC04 grows best at pH 5-6 (cheese pH) but not below pH 4 or above pH 7. A heat shock experiment was performed to model HTST pasteurization. WDC04 was grown in MRS-R broth for 2 d and diluted, then heat shocked in a hot water bath at 72^{o}C for 15 sec and plated. With an initial count of 10^{8} CFU/ml, results showed a decrease of 10^{5} CFU/ml in survival of WDC04, indicating WDC04 could be contaminating the cheese by surviving pasteurization if it is at high levels in the milk. These results suggest using a higher salt/moisture ratio in cheese and increasing pre-pasteurization sanitation to remove biofilms would decrease the likelihood of *Lb. wasatchensis* WDC04 in ripening cheese, thus, reducing the possibility of late gassy defect.

Author Disclosure Block:

I. Bowen: None. **C. Oberg:** None. **M. Culumber:** None.

Poster Board Number:

SUNDAY-

Publishing Title:

The Effect of Different Fiber Sources on the Growth of *Lactobacillus acidophilus* and *Lactobacillus rhamnosus*

Author Block:

A. B. Santos, F. B. Santos, A. A. Santos, C. Gillen; Adventist Univ. of Hlth.Sci., Orlando, FL

Abstract Body:

Background: Probiotics and prebiotics have been recognized as important tools, which can be used to modulate intestinal health. The best known probiotics are those in the genera *Lactobacillus* and *Bifidobacterium*. The most common prebiotics are fibers, which have the potential to stimulate the growth of specific intestinal bacteria. Gaining insight on how fibers can affect the growth of probiotics is of great importance. Therefore, the present study aims to evaluate different sources of fibers for their capacity of functioning as prebiotics by enhancing *in vitro* bacterial growth. **Methods:** The supplementation of media with different fibers commonly used in fiber supplement products might stimulate bacterial growth. *Lactobacillus acidophilus* (ATCC® 4356™) and *Lactobacillus rhamnosus* (ATCC® 53103™) were used to prepare the bacterial inoculum, which was grown at 37°C for 48 hours in an atmosphere of 5% CO_2, yielding ca. 10^7 CFU/mL. *Lactobacillus* culture media, MRS (de Man, Rogosa and Sharpe) broth, was supplemented with four different fiber sources to evaluate the effect of these nutrients on bacterial growth. Fibers were mixed in the media at a concentration of 1% (Kuo, 2012). The fibers used were inulin, fructoligosaccharide (FOS), β-glucan, and psyllium (hulls). **Results:** Based on the results, the hypothesis that supplementation of culture media with different fibers stimulates bacterial growth was proven correct. For *L. acidophilus*, supplementation with psyllium resulted in the highest growth both at 24 and 48 h (7.7 log/mL, p < 0.05, and 7.8, p < 0.0001; respectively). *L. rhamnosus* behaved somewhat differently when compared to *L. acidophilus*. In the beginning and at the end of the experiment, the *L. rhamnosus* control growth was significantly lower than the supplemented bacteria. Supplementation with inulin and FOS increased growth of *L. rhamnosus* throughout the trial, even though psyllium produced the highest counts at 24 h (9.1 log/mL, p < 0.05). **Conclusions:** In conclusion, fiber supplementation influenced the two bacterial species differently. Therefore, the results show that the use of a single fiber supplement will not stimulate all the different bacterial species humans harbor in the intestinal tract. Finally, more research is needed to better understand the interaction between probiotic bacteria and the different prebiotics.

Author Disclosure Block:

A.B. Santos: None. **F.B. Santos:** None. **A.A. Santos:** None. **C. Gillen:** None.

Poster Board Number:

SUNDAY-216

Publishing Title:

Survival of Lyophilized, Probiotic *Lactobacillus plantarum* and *Pediococcus acidilactici* in Refrigerated, Acidified Cucumbers

Author Block:

S. Cauley; North Carolina State Univ., Raleigh, NC

Abstract Body:

Non-pasteurized, refrigerated, acidified cucumbers represent a significant percentage of pickled cucumber products consumed in the US. Addition of probiotics would offer a non-dairy, low-calorie, health-promoting product. This study explored methods for addition of commercially available, lyophilized, probiotic cultures that would allow for high, long-term survival in this product. Cucumbers or a model system using cucumber juice (CJ) were acidified to pH 3.65 ± 0.05 with acetic acid and inoculated to ~8 $\log_{10}$ CFU/mL. Microbial counts during refrigerated storage were obtained by plating samples on MRS followed by anaerobic incubation at 30 °C for 48 h. Direct addition of 7 lyophilized probiotics, *Lactobacillus plantarum* or *Pediococcus acidilactici*, to acidified cucumbers resulted in cell death ranging from 2.3 to 5.5 $\log_{10}$ CFU/mL after 1 mo at 4 °C. Therefore, further experiments were conducted to determine the effect of sodium benzoate concentration, pre-adaptation of cultures, and modification of shelf-life temperature on survival. Sodium benzoate is a commonly used pickled cucumber preservative, known to inhibit growth of lactic acid bacteria. Although there were differences between strains in survival in the acidic pickling brine ($P < 0.05$), sodium benzoate (up to 9 mM) had no detrimental effect on survival after 2.5 h at 4 ºC ($P > 0.05$). Pre-adaptation of cells in CJ for 2 or 18 h at 4 ºC prior to inoculation demonstrated ~1 $\log_{10}$ CFU/mL improvement in survival. Acidification of the CJ pre-adaptation solution to pH 4.0 with acetic acid showed a 1 $\log_{10}$ of CFU/mL reduction in survival. Preliminary investigation of storage temperature modifications, with and without trehalose supplementation (1.5%), showed that by incubating the inoculated acidified CJ model system for 24 h at 21 ºC or 24 h at 21 ºC followed by 24 h at 14 ºC prior to storage in 4 ºC shows further improvement of survival by ~2.5 $\log_{10}$ CFU/mL. Experimentation to date demonstrates that survival can be improved by hydration in CJ prior to addition and allowing acclimation in the acidified cucumber environment at a temperature permissive of growth prior to 4 ºC storage.

Author Disclosure Block:

S. Cauley: None.

Poster Board Number:

SUNDAY-217

Publishing Title:

Partial Characterization of β-Galactosidase Extracted from *Pediococcus* sp. as Potential Source of Probiotics for Treating Lactose Intolerance

Author Block:

R. Alpay[1], E. M. Pangan[1], M. K. Devanadera[2], M. Santiago[1]; [1]Faculty of Pharmacy, Manila, Philippines, [2]Res. Ctr. for Natural and Applied Sci., Manila, Philippines

Abstract Body:

Background: Lactose intolerance is prevalent among people especially the elderly. Lactose intolerance occurs when the body is incapable of producing β-galactosidase – the enzyme that hydrolyzes lactose. Improper digestion of lactose causes a lot of symptoms like bloating, diarrhea, and abdominal pain. This study aims characterize β-galactosidase from *Pediococcus* sp. as a potential source of probiotics for treating lactose intolerance. **Methods:** The strains used were *Pediococcus pentosaceus* K3A2-2, *Pediococcus acidilactici* K2A2-5, K2A2-1, and K2A1-1. The crude enzyme was extracted via sonication for 15 minutes in 3 minutes intervals at maximum volume. Microplate based Bradford assay was performed to measure the protein content of the crude extract and ONPG assay for the enzyme activity. The enzyme activities were measured according to three parameters: substrate, substrate concentration and pH. MRS Broth supplied with 1% glucose or lactose was used to compare which substrate gave the highest value of enzyme activity. MRS Broths at different pH (4.8, 6.8 and 8.8) and different substrate concentrations (1%, 5%, and 10%) were used to determine which value gives the highest enzyme activity.**Results:** K3A2-2 had the highest enzyme activity of $3.6x10^{-4}$ U/ml in MRS Broth supplied with 1% glucose while K2A2-5, K2A2-1, and K2A1-1 had the highest value of $4.3x10^{-4}$ U/ml, $4.9x10^{-4}$ U/ml, and $4.2x10^{-4}$ U/ml respectively in MRS Broth supplied with 1% lactose. K3A2-2, K2A2-5, K2A2-1, and K2A1-1, the highest activity of $2.4x10^{-3}$ U/ml, $2.6x10^{-3}$ U/ml, $2.4x10^{-3}$ U/ml, and $2.5x10^{-3}$ U/ml were obtained from media supplied with 10% lactose. K3A2-2 has the highest activity of $2.7x10^{-3}$ U/ml at pH 8.8 while K2A2-5, K2A2-1, and K2A1-1 had the highest activity of $5.1x10^{-3}$ U/ml at pH 4.8, $2.8x10^{-3}$ U/ml and $2.7x10^{-3}$ U/ml at pH 8.8 respectively.**Conclusion:** The best conditions for optimum production of β-galactosidase in *Pediococcus* species were 10% lactose as substrate at pH 8.8. *Pediococcus* species could be a potential source of probiotic that could help treat lactose intolerance. However, the enzyme activity is quite low thus optimization parameters such as incubation temperature and time must still be done on the bacterial strains to achieve maximum yield.

Author Disclosure Block:

R. Alpay: None. **E.M. Pangan:** None. **M.K. Devanadera:** None. **M. Santiago:** None.

Poster Board Number:

SUNDAY-218

Publishing Title:

Effects of Lactulose on Growth Kinetics, Fermentation, Antioxidant Activity, and Short-Chain Fatty Acid Production of Probiotics

Author Block:

E. Puspitasari, C. K. Yeung, M. Yeung; California Polytechnic State Univ. San Luis Obispo, San Luis Obispo, CA

Abstract Body:

Prebiotics, such as fructooligosaccharide (FOS), are non-digestible oligosaccharides that selectively stimulate the growth of beneficial bacteria in the human intestine. Previous studies suggest that lactulose, a derivative of lactose, has prebiotic potential. Our study was aimed to characterize the prebiotic activities of lactulose. In addition to stimulating the growth of probiotics, we hypothesized that lactulose would enhance probiotics' antioxidant activity and short-chain fatty acid (SCFA) production, thereby alleviating gastrointestinal disorders and strengthening intestinal structure. Hence, our objectives were to determine 1) growth kinetics in aerobic condition; 2) pH and titratable acidity after fermentation; 3) antioxidant capacity; and 4) SCFA profiles of probiotics when prebiotics were given as the main carbohydrate source. Ten probiotics and two non-probiotic strains, representing genera *Lactobacillus*, *Bifidobacterium*, *Bacillus*, and *Escherichia* were assembled. Basal media containing dextrose was modified to include 2% FOS or lactulose. Except SCFA analysis, all experiments were done in at least triplicates. In aerobic condition, most strains cultured with prebiotics did not grow optimally compared to dextrose, except for two *Bifidobacterium* spp. In anaerobic condition, the non-probiotic strains were able to catabolize both prebiotics, but displayed weaker lactic acid production (0.07±0.05%) relative to *Lactobacillus* spp (0.44±0.08%) and *Bifidobacterium* spp (0.14±0.05%). All strains yielded significantly lower pH from lactulose fermentation than FOS ($p<0.01$), as seen in *Lactobacillus paracasei* (pH 4.30±0.33 vs pH 5.92±0.15) and *Bifidobacterium infantis* (pH 4.35±0.44 vs pH 5.20±0.41). Antioxidant activity of spent medium was measured with Trolox as the reference standard. Lactulose fermentation yielded 0.18±0.24 mM Trolox equivalent, whereas dextrose fermentation yielded 0.20±0.20 mM. Preliminary SCFA analysis showed that *Lactobacillus casei* and *Lactobacillus acidophilus* produced higher levels of propionate when lactulose was fermented, supporting our hypothesis. In conclusion, lactulose is a promising prebiotic ingredient that can be incorporated in functional food products.

Author Disclosure Block:

E. Puspitasari: None. **C.K. Yeung:** None. **M. Yeung:** None.

Poster Board Number:

SUNDAY-219

Publishing Title:

Method Validation for Bacterial and Viral Analysis of Geoducks

Author Block:

V. A. Balta, A. L. Kossik, N. K. Beck, J. S. Meschke; Univ. of Washington, Seattle, WA

Abstract Body:

Background: The shellfish industry in Western Washington is an integral part of the State economy; with products being distributed both domestically and internationally. Oysters and geoducks are widely harvested in Washington, and ensuring growing areas with low levels of bacterial and viral contamination is essential for protecting consumers from disease. Methods for assessing viral and bacterial contamination in oysters have been established; however, similar testing procedures for geoducks have not been validated. Our study adapted viral and bacterial testing methods for oysters to better suit geoduck meat. **Methods:** To validate our methods, four trials were performed with MS2 coliphage and five trials were performed with *E. coli* CN-13 (EC) seeded into geoduck meat harvested in Washington. Each MS2 trial contained four seeding levels for which we calculated PFU/100g and percent recovery using the double agar layer method. The EC trials contained three seeding levels with the most probable number (MPN) and percent recovery calculated for each. MPN and percent recovery were assessed using the APHA's 5-tube fermentation method. Fermentation on seeded geoduck meat was first performed in Lauryl tryptose broth (LST) and then passaged into EC broth and EC-MUG medium. In addition, six unseeded field samples were processed using these methods to evaluate ability of the method to detect naturally occurring levels of *E. coli* and male-specific coliphage (MSC). **Results:** The average percent recoveries for MS2 trials ranged from 90.4% to 153.7%, with higher recoveries occurring at more concentrated seeding levels. Recoveries of over 100% are likely due to MS2 aggregation. The average percent recoveries for *EC* trials were greater than 100%, with higher recoveries also occurring at more concentrated seeding levels. Recoveries of over 100% are likely due to *EC* aggregation and potential bacterial growth in geoduck meat. All geoduck meat used in the *EC* trials was shown to have prior levels fecal coliforms but tested negative for *EC*. In unseeded geoduck, MSC levels ranged from 9.55 to 139.39 PFU/100g and *EC* levels ranged from .20 to 79.0 MPN/g. **Conclusion:** Our findings suggest that, with modifications, current oyster viral and bacterial testing methods can be applied successfully to geoduck meat. The percent recoveries indicate that both methods provide good assessment of bacterial and viral contamination in geoducks.

Author Disclosure Block:

V.A. Balta: None. **A.L. Kossik:** None. **N.K. Beck:** None. **J.S. Meschke:** None.

Poster Board Number:

SUNDAY-220

Publishing Title:

Evaluation of Modified Charcoal-Cefoperazone-Deoxycholate Agar Supplemented with Tazobactam for the Isolation of *Campylobacter* from Chicken Carcass Rinse

Author Block:

J. Chon[1], K. Sung[1], K. Seo[2], S. Khan[1]; [1]Natl. Ctr. for Toxicological Res., US Food and Drug Admin., Jefferson, AR, [2]Konkuk Univ., Seoul, Korea, Republic of

Abstract Body:

Tazobactam, an extended-spectrum β-lactamase (ESBL) inhibitor, is an effective supplement for quantitative isolation of *Campylobacter* from chicken cecum inhibiting ESBL-producing *E. coli*, while supporting *Campylobacter* growth (Smith et al., 2015; Int. J. Food Microbiol. 210:131-135). However, no studies have evaluated tazobactam-supplemented media for the qualitative detection of *Campylobacter* in raw chicken meat samples. We evaluated tazobactam-supplemented, modified charcoal-cefoperazone-deoxycholate agar (T-mCCDA) for the qualitative detection of *Campylobacter* in chicken carcass rinse. First, using 25 strains each of ESBL-producing *E. coli* and *Campylobacter*, we found 4 mg/L of tazobactam to be optimal for mCCDA. Smith et al. (2015) observed inhibition of ESBL-producing *E. coli* at concentrations as low as 1 mg/L tazobactam when they evaluated T-mCCDA using three ESBL-producing *E. coli* strains. However, some ESBL-producing *E. coli* strains (4 of 25, 16%) were still able to grow in the presence of 2 mg of tazobactam. Next, a total of 120 whole chickens from retails were rinsed with buffered peptone water (BPW), and 25 mL of BPW was mixed with 2× blood-free Bolton broth (25 mL) followed by incubation at 42°C for 48 h. A loopful of the culture was inoculated on normal or 4 mg/L T-mCCDA and incubated at 42°C for 48 h microaerobically. Suspected *Campylobacter* colonies were confirmed by colony PCR. The modified media (56.7%) showed a better ($p<0.05$) *Campylobacter* isolation rate than normal selective media (30.8%). Moreover, selectivity of the modified media was superior ($p<0.05$) to normal media, with only one modified media plate contaminated with non-*Campylobacter* (0.8%) compared to 83.3% of the normal plates being contaminated with non-*Campylobacter* species. This study is a follow -up of previously published investigation using mCCDA supplemented with polymyxin B (P-mCCDA) as an ESBL-inhibiting agent (Chon et al., 2012; Appl. Environ. Microbiol. 78:1624-1626). However, in this study the T-mCCDA showed higher selectivity than that observed using P-mCCDA. Our results suggested that tazobactam is a suitable supplement for mCCDA for qualitative detection of *Campylobacter* from chicken carcass rinse.

Author Disclosure Block:

J. Chon: None. **K. Sung:** None. **K. Seo:** None. **S. Khan:** None.

Poster Board Number:

SUNDAY-221

Publishing Title:

Comparison of the Liquid versus Lyophilized Inoculation Approaches in Oregano Artificially Contaminated for *Salmonella* Recovery

Author Block:

J. Jean-Gilles Beaubrun[1], **N. Addy**[2], L. Ewing[1], G. Gopinath[1], D. E. Hanes[1]; [1]US FDA, Laurel, MD, [2]US FDA, ORISE, Laurel, MD

Abstract Body:

Many studies have demonstrated the effectiveness of oregano essential oils and phenolic compounds in inhibiting the growth of pathogenic microorganisms. One of the concerns with artificial contamination is that during the inoculation process some of the phenolic compounds such as carvacrol and thymol may be released. This study was designed to evaluate two methods to inoculate oregano for use in developing methods for the isolation of *Salmonella.* Dry oregano samples (25g) were artificially contaminated with *Salmonella* serovar Montevideo using either a 10^3 cfu cell suspension for liquid inoculation or 10^3 cfu of *Salmonella* lyophilized in dry milk. Samples were aged for 2 weeks at room temperature prior to analysis. Oregano samples were pre-enriched overnight at 37^{o}C in modified Buffered Peptone water (mBPW) with 2% (vol/vol) corn oil; previous work has shown that addition of corn oil sequesters the phenolic compounds allowing recovery of *Salmonella.* Samples were transferred to selective enrichment broths, Rappaport-Vassiliadis and tetrathionate, and plated on Xylose-Lysine-Tergitol 4 agar following 24 h of pre-enrichment. Typical *Salmonella* colonies were identified as described in the FDA Bacteriological Analytical Manual (BAM), and confirmed using Vitek® 2 Compact. The results showed a significant (P= <0.050) increase in the survival of *Salmonella* from the samples inoculated with the lyophilized cells; an average of 220 CFU of *Salmonella* were recovered from liquid inoculum samples compared to 316 CFU recovered from samples inoculated with lyophilized *Salmonella.* These results suggest the artificial inoculation with lyophilized *Salmonella* increases survival in oregano and may mimic the natural contamination route of oregano and other spices. This study demonstrated that using a dry, lyophilized form of inoculation *Salmonella* increase survivability and is crucial for successfully mimicking natural contamination of oregano samples used to compare and improve detection methods.

Author Disclosure Block:

J. Jean-Gilles Beaubrun: None. **N. Addy:** None. **L. Ewing:** None. **G. Gopinath:** None. **D.E. Hanes:** None.

Poster Board Number:

SUNDAY-222

Publishing Title:

Use of Date Palm (*Phoenix dactylifera l.*) to Develop a Medium for Cultivation of Lactic Acid Bacteria

Author Block:

A. A. Ayad[1], R. Gyawali[1], D. Gad El-Rab[2], R. Tahergorabi[1], A. Shahbazi[1], S. A. Ibrahim[1];
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Abstract Body:

Lactic acid bacteria (LAB) have many applications in food industry to develop fermented food products. The standard medium DeMan Rogosa Sharp (MRS) to grow LAB is expensive and the industry is always looking for an alternative low cost medium. There are several agricultural by-products that can be utilized to develop medium for the growth of LAB. Date fruit industry produces large amount of useless by-products. Therefore, the objective of this study was to develop date palm medium for the cultivation of LAB using date by-products. The date palm extract (DPE) was prepared by pressing fresh date fruits for one week. The extract was then mixed with deionized distilled water (DDW) at ratio 1:2 (w/v), centrifuged (4696 x *g* for 25 min) at 4°C, and then supernatant was autoclaved at 110°C for 15 min. The DPE was mixed with buffer solution prepared as MRS (Tween 80 1mL, L-Cysteine.HCL (1g), Na_2HPO_4 (2g), $NH_4C_6H_5O_7$ (2g) $C_2H_3NaO_2$ (5g), $CaCl_2$ (0.15g), K_2HPO_4 (2g), $MgSO_4.7H_2O$ (0.2g) and $MnSO_4.5H_2O$ (0.05g)) to form a date palm medium (DPM). DPM was then mixed with different concentrations of sterile phytone peptone solution (PP) (0, 0.2, 0.4, 0.6, and 0.8 %, w/v). Lactobacilli MRS was used as a standard growth medium. Three *Lactobacillus reuteri* (DSM 20016, CF2-7F, and SD 2112) strains were individually inoculated into batches of MRS, and DPMs at initial inoculum level of approximately 2.5 log CFU/ml. Inoculated media were then incubated at 37ºC for 18 h. Bacterial growth was monitored by measuring the optical density readings (O.D 610 nm) for up to 18 h. At the end of the incubation period, final populations of each individual strain were verified by enumeration on MRS agar. Our results showed that in DPM (control), bacterial population reached to 3.18±0.5 log CFU/mL. Addition of low concentration of phytone peptone did not support the bacterial growth. However, when DPM medium was supplemented with 0.8% of phytone peptone, bacterial populations reached to 6.94±0.1 log CFU/mL which was similar to the population found in standard MRS medium (7.90±0.24 log CFU/mL). These results showed that there was no significant ($P > 0.05$) difference in the growth of LAB in MRS and developed DPM medium. Therefore, our findings suggest that date by-products could be used as an alternative low cost medium to develop a growth medium for LAB.

Author Disclosure Block:

A.A. Ayad: None. **R. Gyawali:** None. **D. Gad El-Rab:** None. **R. Tahergorabi:** None. **A. Shahbazi:** None. **S.A. Ibrahim:** None.

Poster Board Number:

SUNDAY-223

Publishing Title:

Purification of Bile Salt Hydrolase from Philippine Cocowater Kefir Lactic Acid Bacteria

Author Block:

E. Pangan, R. Alpay, M. Devanadera, M. Santiago; Univ. of Santo Tomas, Manila City, Philippines

Abstract Body:

Bile salt hydrolase (BSH) is an enzyme produced by lactic acid bacteria that holds promise in lowering cholesterol levels, consequently decreasing the risk of cardiovascular diseases, with its presence having been correlated to hypocholesterolemic effects of food rich in probiotic bacteria, such as in kefir. In this study, lactic acid bacteria (LAB) isolates from cocowater kefir were screened for BSH activity, followed by the extraction and purification of the enzyme. The isolates were screened by observing the precipitation of deconjugated bile salts in a specific media. Purification was done via ammonium salt precipitation and size exclusion gel chromatography using Sephadex G-100. The crude and purified extracts were then assayed for protein content, using Bradford's assay, and for BSH activity. Determination of BSH activity was done by measuring the amount of glycine liberated from the deconjugation of the bile salt glycocholic acid. Four isolates, preliminary identified as bacteria isolates I002, I004, J006 and K001 upon isolation from cocowater kefir, were subjected to the purification process and the improvement of enzyme activity was analyzed. Additionally, genomic DNA extraction was performed on the four isolates for identification of the BSH-producing bacteria. The initial enzyme activities of the crude enzyme extracts from the isolates were found to be $1.33x10^{-4}$ U/mg, $1.28x10^{-4}$ U/mg, $1.25x10^{-4}$ U/mg and $1.13x10^{-4}$ U/mg, respectively. After salt precipitation, the enzyme activities increased to $4.35x10^{-4}$ U/mg, $5.41x10^{-4}$ U/mg, $4.25x10^{-4}$ U/mg and $5.20x10^{-4}$ U/mg, respectively. Finally, after gel chromatography, the eluates were assayed, and peak enzyme activities were determined to be at $7.23x10^{-4}$ U/mg from I002, $1.98x10^{-3}$ U/mg from I004, $3.55x10^{-4}$ U/mg from J006 and $2.50x10^{-4}$ U/mg from K001. From the results gathered, enzyme activity was observed to increase with every purification step for bacteria isolates I002 and I004, with the gel chromatography filtrates producing the highest enzyme activity per milligram of protein. The decrease in activity observed in isolates J006 and K001 could possibly be due to the elution conditions, thus requiring adjustments for the two isolates. Additionally, it was determined that bacteria isolate I004 expressed the highest enzyme activity, suggesting that the isolate could potentially be a good source of BSH.

Author Disclosure Block:

E. Pangan: None. **R. Alpay:** None. **M. Devanadera:** None. **M. Santiago:** None.

Poster Board Number:

SUNDAY-224

Publishing Title:

Characterization of the Bacteiocin-Like Peptide (*blp*) Gene Cluster in *Streptococcus thermophiles* Strain B59671

Author Block:

J. A. Renye, Jr., G. A. Somkuti; USDA, Agricultural Res. Service, Wyndmoor, PA

Abstract Body:

Streptococcus thermophilus is a food-grade bacterium commonly used in the production of fermented dairy foods, including cheese and yogurt. Some strains have been shown to possess a *blp* gene cluster encoding for the production of antimicrobial peptides called thermophilins; however their production is largely dependent on the artificial induction of a quorum sensing (QS) system that regulates their expression. *S. thermophilus* strain B59671 has been shown to naturally produce thermophilin 110, a broad spectrum bacteriocin with anti-listerial activity, which is encoded within the *blp* locus. In this study, chromosome walking was used to sequence the *blp* gene cluster in strain B59671. Sequence analysis of the 10.7 kb gene cluster revealed 14 open readings, including two genes, *blpU* and *blpK*, that potentially encoded antimicrobial peptides. Deletion of *blpK* from the B59671 chromosome did not result in a loss of antimicrobial activity, suggesting that *blpU* encoded the bacteriocin; however, repeated attempts did not yield a *blpU* mutant. Both *blpU* and *blpK* were overexpressed in *E. coli*, and only purified BlpU was shown to have antimicrobial activity, confirming it is a functional bacteriocin. Real-time PCR analysis was used to compare the expression of *blp* components in strains B5971 and LMD-9, the latter being a sequenced strain which requires synthetic induction of the QS system to allow for thermophilin production (Fontaine *et al*., 2007). Results showed that *blpC*, which encodes the QS induction peptide, and *blpU* were expressed 22-fold and 49-fold higher in strain B59671, respectively. The lower level of BlpU expression in LMD-9 may explain why it was not previously identified as a bacteriocin in this strain. Studies are continuing to determine why the QS system is expressed at levels which allow it to function properly in strain B59671, resulting in the natural production of thermophilin 110.

Author Disclosure Block:

J.A. Renye: None. **G.A. Somkuti:** None.

Poster Board Number:

SUNDAY-225

Publishing Title:

Regional Similarity and Consistent Patterns of Local Variation in Beach Sand Bacterial Communities Throughout the Northern Hemisphere

Author Block:

C. Staley, M. J. Sadowsky; Univ. of Minnesota, Saint Paul, MN

Abstract Body:

Recent characterization of bacterial community structure (BCS) in beach sands has revealed patterns of biogeography similar to those observed in aquatic environments. Studies to date, however, have focused mainly on subtidal sediments from marine beaches. Here, we introduce the Beach Sands of the World investigation of bacterial diversity, using Illumina-based sequencing of the V5+V6 regions of 16S rRNA gene, at 11 beaches representing the Great Lakes, the Gulf of Mexico, and the Pacific Ocean. Alpha diversity differed significantly among regions ($P < 0.0001$), while within-region diversity was more similar. Beta diversity also differed by region ($P < 0.001$), where freshwater sands had significantly higher abundances of taxa within the *Actinobacteria*, *Betaproteobacteria*, and *Verrucomicrobia* than marine environments. In contrast, marine sands harbored greater abundances of *Gammaproteobacteria* and *Planctomycetes*, and those from the Gulf of Mexico had more *Deltaproteobacteria* and *Firmicutes*. Marine beaches had significantly different phylogenetic BCS ($P \leq 0.018$), but freshwater and Gulf beaches were more similar. Furthermore, regionally-distinct patterns in taxonomic variation were observed in backshore sands, which had BCS distinct from nearshore sands ($P < 0.001$). Sample depth minimally influenced BCS. Results of this study reveal distinct sand community structures globally, but moderate regional similarity, and suggest local variation is primarily related to distance from the shoreline. This study offers a novel comparison of bacterial communities in freshwater and marine beach sands and provides an important basis for future comparisons and analysis to elucidate factors affecting microbial ecology in this under-explored environment.

Author Disclosure Block:

C. Staley: None. **M.J. Sadowsky:** None.

Poster Board Number:

SUNDAY-226

Publishing Title:

A High-Throughput Sequencing-Based Approach For Determining Sources Of Fecal Bacteria In The Lake Superior Watershed

Author Block:

C. M. Brown[1], C. Staley[1], P. Wang[1], C. L. Chun[2], M. J. Sadowsky[1]; [1]Univ. of Minnesota - Twin Cities, St. Paul, MN, [2]Univ. of Minnesota - Duluth, Duluth, MN

Abstract Body:

While several methods have been developed that aim to determine sources of fecal bacteria in freshwater systems, most techniques utilize animal or human-specific molecular markers to identify single indicator organisms (SIO) present in animal and human feces. However, developing and validating sensitive and specific SIO molecular markers to detect different sources of fecal contamination can be difficult and time-consuming. To circumvent this, a high-throughput sequencing (HTS)-based method to determine sources of fecal bacteria in a freshwater system was developed. This HTS-based method compared the total bacterial community structures (BCS) of fourteen different types of animals and wastewater effluent (n=273) to the total BCS in environmental samples (n=202) from the freshwater Lake Superior watershed, via sequencing of the 16S rRNA gene. Analysis from HTS of the 16S rRNA gene of nearly 500 fecal and environmental samples revealed that bacteria present in environmental and fecal samples are significantly different (p-value: <0.001), thus allowing phylogenetic differentiation between the bacterial taxa in the two matrices. While there were common fecal-associated phyla across fecal samples, animal-specific, or unique relative abundances of phyla allowed for differentiation between different animals (all comparisons had p-values <0.05). The statistical program SourceTracker, which calculates the amount that a source(s) contributed to contamination in a particular environment, revealed the predominant fecal pollution sources across sampled environmental sites were from fowl, and effluent from a nearby wastewater treatment plant. To corroborate SourceTracker results, host-specific SIO molecular markers were used to detect fecal contamination sources. The detection of fecal bacteria and general waterfowl by host-specific SIO molecular markers agreed with the predicted fecal contamination sources in the SourceTracker results. The results of this study may allow for an alternative method of determining sources of fecal bacteria that organizations tasked with pollution control can utilize in protecting freshwater resources.

Author Disclosure Block:

C.M. Brown: None. **C. Staley:** None. **P. Wang:** None. **C.L. Chun:** None. **M.J. Sadowsky:** None.

Poster Board Number:

SUNDAY-227

Publishing Title:

Microbial Source Tracking in the Urbanized Tropical Watershed: Multiple Lines of Evidence to Confirm Sewage as the Cause of Water Quality Impairment

Author Block:

M. KIRS[1], V. Kisand[2], R. A. Caffaro Filho[3], M. Wong[1], V. J. Harwood[4], B. Yoneyama[1], P. Moravcik[1], R. S. Fujioka[1]; [1]Univ. of Hawaii, Honolulu, HI, [2]Univ. of Tartu, Tartu, Estonia, [3]Federal Univ. of Alagoas, Maceió, Brazil, [4]Univ. of South Florida, Tampa, FL

Abstract Body:

Indicator bacteria, which are conventionally used to evaluate recreational water quality, can originate from various non-human enteric and extra-enteric sources, hence they are not always indicative of human health risk nor do they provide information on the sources of contamination. In this study we utilized traditional (enterococci and *E. coli*) and alternative indicator organisms (*Clostridium perfringens,* coliphage), molecular markers (human-associated *Bacteroides* and human polyomaviruses), and microbial community analysis tools to evaluate human sewage-related impacts in the Manoa watershed (Hawaii). The elevated concentrations of enterococci (mean $2.8x10^3$ - $4.8x10^3$ CFU $100mL^{-1}$) and *C. perfringens* (mean $5.9x10^1$ - $1.4x10^2$ CFU $100mL^{-1}$) indicated impairment of the urbanized section. The standard threshold value triggering recreational water quality violation notifications in Hawaii was exceeded in 33 - 75% of samples from the urbanized section of Manoa Stream, but was not exceeded in samples from an upstream forested site. Indicator bacteria concentrations were correlated with rainfall measurements, while human molecular markers were not, indicating chronic sewage-related inputs of contamination. Presence of human sewage was confirmed by human-associated *Bacteroides* and human polyomavirus markers in the urbanized section of the watershed (83.3 - 100% and 41.7 - 66.7% positive samples respectively) and was further confirmed by microbial community analyses which suggested that an average 2.4 - 3.4% of the total bacterial population in this section was associated with sewage. Microbial community profiles were significantly related to rainfall (R^2=0.44, P<0.001), pH (R^2=0.31, P=0.006), salinity (R^2=0.26, P=0.038), and conductivity (R^2=0.27, P=0.031). Microbial diversity decreased in the impaired section. Leaking sewer systems, cesspools, and illegal cross-connections are implicated in the impairment. Collectively our data suggest that information derived from the analysis of microbial communities complements current marker-based microbial source tracking techniques and environmental monitoring programs.

Author Disclosure Block:

M. Kirs: None. **V. Kisand:** None. **R.A. Caffaro Filho:** None. **M. Wong:** None. **V.J. Harwood:** None. **B. Yoneyama:** None. **P. Moravcik:** None. **R.S. Fujioka:** None.

Poster Board Number:

SUNDAY-229

Publishing Title:

Bacterial Community Composition and Structure in an Urban River

Author Block:

A. M. IBEKWE[1], M. Jincai[2]; [1]USDA-ARS, RIVERSIDE, CA, [2]Jilin Univ., Changchun 130021, China

Abstract Body:

Microbial communities in rivers are diverse and dynamic in composition due to different environmental factors. Here we used 454 pyrosequencing to determine the total bacterial community composition, and bacterial communities that are potentially of fecal origin, and of relevance to water quality assessment. The results were analyzed using UniFrac coupled with principal coordinate analysis (PCoA) to compare diversity, abundance, and community composition. Detrended corresponding analysis (DCA) and canonical correspondence analysis (CCA) were used to correlate bacterial composition in streams and creeks to different environmental parameters impacting bacterial communities in the sediment and surface water within the watershed. From all the sampling points, bacteria were numerically dominated by the phyla *Proteobacteria, Bacteroidetes, Acidobacteria,* and *Actinobacteria*—accounting for the majority of taxa detected. Overall results, using the β diversity measures, UniFrac, coupled with PCoA and DCA showed that bacterial composition in sediment and surface water was significantly different (P =0.001). Also, there were differences in bacterial community composition between agricultural runoff and urban runoff based on parsimony tests using 454 pyrosequencing data. Fecal indicator bacteria in surface water along different creeks and channels were significantly correlated with pH (P = 0.01), NO_2 (P = 0.03), and NH_3N (P = 0.005); and in the sediment with NO_3 (P =0.015). Our results suggest that microbial community compositions were influenced by several environmental factors, and these factors were also major drivers in fecal indicator bacterial composition in the watershed.

Author Disclosure Block:

A.M. Ibekwe: None. **M. Jincai:** None.

Poster Board Number:

SUNDAY-230

Publishing Title:

Metagenomic Analysis of Microbiomes in an Urban Fresh Water Lake In Seattle, Washington

Author Block:

Z. Li[1], T. Liu[2], L. Wang[3]; [1]Washington State Dept of Hlth., Shoreline, WA, [2]Washington Univ., St. Louis, MO, [3]Ohio Dept. of Agriculture, Reynoldsburg, OH

Abstract Body:

Lakes in metropolitan areas are an important part of the urban ecosystems. Urban lakes not only host recreational activities, they also play a crucial role in stormwater runoff and groundwater recharge. Microbiomes of lake water could be influenced by a variety of factors, including industrial and residential activities as well as wildlife inhabitation. In addition, many microorganisms could degrade pollutants and recycle nutrients in the lakes, which is beneficial to improve water quality. Thus, analyzing microbiomes in urban lakes could provide useful information for pollution surveillance and bioremediation. In this study, we analyzed the microbiomes from the Haller Lake located in Seattle metropolitan area, which is also in close proximity to a hazardous waste recycling facility. Water samples from the late were collected in October, 2015. Bacterial cells were collected through filtration of the lake water using 0.2 micrometer filters. Bacterial DNA was then extracted using Qiagen DNeasy Blood & Tissue Kit and sequenced using Illumina MiSeq sequencer via shotgun metagenomics method. Sequencing data was subsequently analyzed using BaseSpace and metagenomics analysis server (MG-RAST). The dominant phyla of the microbiomes in the lake water were identified as *Proteobacteria*, *Actinobacteria* and *Bacteroidetes*. Functional abundance analysis indicated the existence of several bacterial genes which are related to heavy metal resistance including Cobalt-Zinc-Cadmium resistance and copper homeostasis. In addition, several antibiotics resistance genes, such as fluoroquinolones, erythromycin and methicillin resistance genes, and multidrug resistance efflux pumps, were also detected in these microbiomes. In addition, our results also indicate that metagenomic analysis using next generation sequencing techniques could be potentially used as an indicator for urban lake water quality.

Author Disclosure Block:

Z. Li: None. **T. Liu:** None. **L. Wang:** None.

Poster Board Number:

SUNDAY-231

Publishing Title:

A Two Year Study on the Virome of a Singapore Reservoir

Author Block:

M. Q. X. Tay[1], L. Loh[1], S. H. Te[2], K. Y. H. Gin[2], J. R. Thompson[1]; [1]SMART, Singapore, Singapore, [2]Natl. Univ. of Singapore, Singapore, Singapore

Abstract Body:

Viruses and phage are able to shape the microbial community in different environments through "kill the winner" predation. Despite the important role phage play in freshwater systems, the field of freshwater virology is largely underdeveloped. The specific questions we have addressed in this study include (1) What is the diversity of freshwater phage in a Singapore reservoir? (2) How does the diversity of phage vary on monthly and annual frequency and at different sites within the reservoir? (3) Is there evidence that cyanophage that may associate with algal blooms? In this study we have characterized the virome of a freshwater reservior in Singapore, using next generation sequencing (NGS). NGS allows for the highthroughput sequencing of the viral community without proir knowledge of what signature genes to look out for. DNA was extracted from the virus-containing fraction (<0.22um) of 34 samples collected 10 cm below the surface monthly over 20 months. DNA was sequenced by Illumina MiSeq (250+250 paired end reads) using the Nextera XL protocol for library preparation generating 1.3 to 15 million reads per library. De novo assembly (CLC workbench) produced 188,491 contigs with N50 score of 2,466 bp and largest contig of 163,843 bp. The principle coordinate analysis read count to contig of individual libraries revealed that the temporal variability excceded spatial variability in the reservoir. A spike in the relative abundance of reads recruiting to 23 de-novo assembled contigs corresponded to a bloom of the cyanobacterium *Microcystis* and may represent novel *Microcystis*-associated phage. These results provide the first overview of viral diversity in a Singapore reservoir and motivate ongoing work to understand how phage influence the dynamics of bloom-forming algae in the reservoir.

Author Disclosure Block:

M.Q.X. Tay: None. **L. Loh:** None. **S.H. Te:** None. **K.Y.H. Gin:** None. **J.R. Thompson:** None.

Poster Board Number:

SUNDAY-232

Publishing Title:

Microbial Source Tracking by Dna Sequence Analysis of the *E. coli* Based on Partial Sequence of Beta-galactosidase Gene

Author Block:

M. Noda, K. Okuda, K. Nanba; Fukushima Univ., Fukushima, Japan

Abstract Body:

Coliforms and *E. coli* are key regulatory indicators of healthfulness of freshwater environment. In lake *Hibara* in Fukushima the number of coliforms was often beyond the environment standard (1000 MPN/100 ml) since 1989(Japanese Ministry of the Environment). The villages in the catchment of Lake *Hibara* have small population with high percentage of sewered population. As unpopulated mountainous area is wider, non-human source is suspected. Eight feces were collected from the road within the catchment. The animals might be fox, marten, bear and so on. Twenty five *E. coli* strains were isolated with BGLB and Colilert from the 8 feces. Rectum contents were collected from wild animals of other part of Fukushima-pref. including 4 wild boar, 4 masked palm civet and a raccoon dog. Thirty seven strains of *E. coli* were isolated with BGLB and Colilert from the 9 rectum contents. DNA extracts from 62 isolates were partially sequenced (240bp) for *lacZ*. The isolates *E. coli* from wild animal feces formed 7 groups and those from rectum contents formed 10 groups by *lacZ* partial sequence. In the previous study, 29 isolates of *E. coli* from the environment, the lake and tributary rivers formed 10 groups. Three groups from 14 wild animal groups (feces + rectum content) could be found from human isolates. All these three groups were also found in the isolates from the environment. Fifteen *E. coli* isolates of 29 from the environment belonged to one of the three groups that are common in wild animal and human. The result shows that partial sequence of *lacZ* does not give a clue to identify the source.

Author Disclosure Block:

M. Noda: None. **K. Okuda:** None. **K. Nanba:** None.

Poster Board Number:

SUNDAY-233

Publishing Title:

Examining Sources of Fecal Contamination in Private Groundwater Wells Across an Ontario Rural Township with Next-Generation Sequencing

Author Block:

P. Naphtali, H. Schellhorn; McMaster Univ., Hamilton, ON, Canada

Abstract Body:

Source water protection requires monitoring the presence of waterborne pathogens and fecal indicator bacteria over time, and when necessary, determining potential contamination sources. Standard methods for monitoring fecal contamination includes quantifying fecal indicators like *E. coli* using selective and differential methodology. Such assays however cannot provide conclusive identification of contamination sources. Advances in culture-independent methods, including next-generation sequencing (NGS), utilize the 16S gene as a target, enabling the characterization of entire microbial communities in many environments. With NGS, many bacterial taxa containing waterborne pathogens can be detected. Furthermore, the microbiotas of drinking water and fecal sources can be compared with one another with NGS to measure the contributions of anthropogenic inputs including sewage and agricultural runoff to water pollution. This information can complement the standard methods for quantifying fecal pollution and tracking sources of fecal contamination. To evaluate this possibility, twenty groundwater well samples, two septage samples, and manure from four farm animals were collected from a rural Ontario township between March and November 2015, and the microbial community profile determined by 16S sequencing. These sequences were then matched against the 16S Greengenes database and processed with the Quantitative Insights into Microbial Ecology (QIIME) pipeline[1]. Groundwater microbiotas are typically comprised of members within the Proteobacteria and Actinobacteria phyla, while members of the Bacteroidetes and Firmicutes phyla predominate in manure and sewage. With the substantial difference in microbiota composition, the probability of a groundwater site being polluted by a fecal contaminant increases with increased similarity between groundwater and fecal microbiotas. Sequences belonging to pathogens such as *Clostridium* are also detected in the groundwater wells, suggestive of sewage contamination. The information gained by analyzing the microbial community profiles with metagenomics, when combined with standard plate counts, can aid end-users in quantifying fecal contamination and mitigating the possibility of a waterborne outbreak.

Author Disclosure Block:

P. Naphtali: None. **H. Schellhorn:** None.

Poster Board Number:

SUNDAY-234

Publishing Title:

Analysis of the Microbiome and Antibiotic Sensitivities of Fecal Bacteria in the Sparkill Creek

Author Block:

A. Panchyshyn, B. Maharaj, M. Scrudato, B. Connors; Dominican Coll. of Blauvelt, Orangeburg, NY

Abstract Body:

Microbial contamination of numerous rivers and their tributaries has been the subject of both funded and unfunded studies carried out by citizen science groups in the US. In 2010, the NYS Department of Environmental Conservation added the Sparkill Creek to its list of impaired waters due to the high concentration of fecal indicator bacteria. The source of contamination has yet to be identified, but several years of research have shown that the problem continues to exist and the levels of the indicator organism have not been diminished. This research aimed to determine the diversity of bacteria present at selected sites along the Sparkill Creek using 16S rDNA pyrosequencing, in addition to an analysis of the antibiotic resistances of the bacterial isolates. By determining the species of bacteria, it was hypothesized that the origin of contamination could be deduced and its source could be mitigated. Resistance to antibiotics, especially towards multiple drugs, poses a serious health hazard. Data show that 100% of these isolates were resistant to streptomycin and ampicillin, while 16% were resistant to vancomycin (19% showed intermediate resistance). Microbiome sequencing from three of the six sites has revealed that 36.7% of all bacteria belong to Proteobacteria, 34.8% to Bacteriodetes, and 25.6% to the Firmicutes. Success of this research is expected to help the community understand the problem before it and allow for the establishment of a healthy environment and community.

Author Disclosure Block:

A. Panchyshyn: None. **B. Maharaj:** None. **M. Scrudato:** None. **B. Connors:** None.

Poster Board Number:

SUNDAY-235

Publishing Title:

Characterization of the Microbiological Quality of Non-potable Water Use Activities

Author Block:

C. L. Haines[1], S. Ishii[2], M. A. Borchardt[3], **T. M. LaPara**[1]; [1]Univ. of Minnesota, Minneapolis, MN, [2]Univ. of Minnesota, St. Paul, MN, [3]United States Dept. of Agriculture, Marshfield, WI

Abstract Body:

Many cities and water managers in Minnesota are implementing new stormwater and rainwater use systems, with the aim to reduce the demand for high quality potable water, to increase infiltration to groundwater, and to better manage stormwater. However, many of these water reuse systems could adversely affect public health due to the elevated presence of pathogens in storm water and rainwater. In this study, we examined rainwater collected and used for toilet flushing and stormwater used for the irrigation of athletic fields. The bacterial content of each system was investigated using cultured-based techniques for the presence of total coliforms, Enterococcus spp. and E. coli. Bacterial community composition was characterized by deep sequencing (Illumina MiSeq) of PCR-amplified 16S rRNA gene fragments. Finally, the quantities of numerous pathogens were simultaneously determined using a microfluidic qPCR technique. Both rainwater and stormwater samples had significantly higher levels of cultivable fecal indicator bacteria and genes from pathogenic organisms compared to tap water and well water samples (used as a negative controls). These results suggest that there is an elevated risk to public health associated with these creative uses of non-potable water; on-going work is being performed to quantify the extent of this increased risk.

Author Disclosure Block:

C.L. Haines: None. **S. Ishii:** None. **M.A. Borchardt:** None. **T.M. LaPara:** None.

Poster Board Number:

SUNDAY-236

Publishing Title:

Microbial Source Tracking of Fecal Bacteria in Surface Waters in Gunpower Creek Watershed in Kentucky

Author Block:

M. Wang[1], M. Scott[2], M. Jacobs[3], **Z. Zhou**[1]; [1]Purdue Univ., West Lafayette, IN, [2]Sanitation District No. 1 of Northern Kentucky, Frankfort, KY, [3]Boone County Conservation District, Burlington, KY

Abstract Body:

Microbial source tracking (MST) is a technique to detect dominant sources of fecal contamination in environmental waters. Fecal pollution poses a health risk to humans via pathogens and MST has been developed during the last two decades to monitor fecal pollution. As it is unrealistic to monitor all pathogens in surface waters, cultivable fecal indicator bacteria (FIB), such as *E. coli*, have been traditionally used to evaluate fecal pollution. However, FIB are not always correlated well with pathogens, and cultivable microorganisms typical only represent less than 1% of microorganisms in the total microbial community, which could result in an inaccurate estimation of fecal pollution of surface waters. Instead, molecular culture-independent and library-independent method, such as real-time PCR, can be used to trace host-specific gene markers in humans and animals in the total microbial community. In this study, we collected surface water samples from five location in Gunpower Creek Watershed in Kentucky and targeted gene markers of human, pig, cow, dog, and horse using qPCR. The results indicated that there was fecal contamination derived from pigs at all five locations, and most of the fecal contamination sources one site were from pigs (65%), but there were still othercontamination sources at Gunpowder creek. The results suggest that microbial source tracking tools could provide useful information to identify fecal pollution sources and provide guidance for better water management strategies.

Author Disclosure Block:

M. Wang: None. **M. Scott:** None. **M. Jacobs:** None. **Z. Zhou:** None.

Poster Board Number:

SUNDAY-237

Publishing Title:

Fecal Contamination Sources in Storm Water from a Ms4 Regulated Town

Author Block:

D. Rothenheber, S. Jones, E. Urquhart, J. Guimond; Univ. of New Hampshire, Durham, NH

Abstract Body:

Storm water often transports bacterial contamination to municipal separate storm sewer systems (MS4s), which is then discharged into local oceans, rivers, etc. without treatment. In the popular tourist towns of southern Maine, untreated MS4 storm water is often discharged onto recreational beaches where it flows into the ocean. Previous work in 2014 showed the fecal indicator bacteria (FIB) enterococci were consistently above regulatory limits in three storm drains at the two most popular swimming beaches. This contamination affected downstream ocean water quality and was most pronounced during wet weather. FIB provide indications of fecal contamination, however the source of the contamination can't be determined from their measurement. In this study we monitored three storm drains (n=46) and the downstream ocean (n=51) each week from late May through September 2015, to determine the seasonality of FIB contamination and dynamics of different sources that may be present. Samples were collected for enterococci enumeration and PCR-based microbial source tracking (MST) to determine general mammal (Bac32) and specific human (HF183) sources. MST PCR showed that Bac32 was detected in all storm drain samples, with 60% of those samples also showing positive detection of HF183. Downstream ocean samples were 90% positive for Bac32 with only 18% of samples positive for HF183. Significant rainfall events were sparse in 2015, with only four events over 1" in 24-48hrs. The largest rainfall (2.8") resulted in enterococci concentrations above regulatory limits in all storm drains and ocean samples, and both Bac32 and HF183 were detected in 3/3 storm drains. One storm drain also showed a significantly higher geometric mean of FIB concentration compared to others, with greater detection of human contamination. These results have provided detailed data to inform work with the EPA to eliminate storm water contamination that impairs beach water quality. Current work is being conducted to implement a qPCR method to quantify mammal and human fecal sources.

Author Disclosure Block:

D. Rothenheber: None. **S. Jones:** None. **E. Urquhart:** None. **J. Guimond:** None.

Poster Board Number:

SUNDAY-238

Publishing Title:

Validation of Host_Associated *Bacteroidales* Gene Marker Assays for Microbial Source Tracking in Urban Tropical Environment of Singapore

Author Block:

J-P. Nshimyimana[1], C. Cruz[2], J. Thompson[1], S. Wuertz[2]; [1]Singapore-MIT Alliance for Res. and Technology, Singapore, Singapore, [2]Singapore Ctr. for Environmental Life Sci. Engineering, Singapore, Singapore

Abstract Body:

Background: Microbial source tracking (MST) is a widely applied tool in many temperate regions of Europe and North America as well as Australia, New Zealand and Japan, but has not seen much use yet in tropical urban environments. This study applied qPCR to validate the use of host-associated *Bacteroidales* marker assays based on the 16S rRNA gene to identify human fecal pollution in the urban tropical environment of Singapore.**Methodology:** We collected a total of 285 animal and human stool as well as sewage samples. Animals tested included cats, dogs, rabbits, chicken, birds, monkeys, and wild boars. Following DNA extraction, samples were analysed by qPCR using seven assays targeting human-associated *Bacteroidales* (HF183-SYBR, HF183, BacHum, BacH and *B. thetaiotaomicron* (B. theta)), dog-associated *Bacteroidales* (BacCan), and total *Bacteroidales* (BacUni). Results were used to compare the sensitivity and specificity of assays.**Results:** The five human-associated assays (HF183-SYBR; HF183, BacHum, BacH and B. theta) and the total *Bacteroidales* assay BacUni had 100% sensitivity for sewage. The overall sensitivity for human stool samples ranged from 50 to 70% and B. theta and BacHum displayed the highest human stool sensitivity at 68.6% and 65.7%, respectively. In addition, these two assays had the highest specificity (98.6% for B. theta and 91.4% for BacHum). The HF183 assay recently recommended for MST in California, USA, had a specificity of 90% and sensitivity of 60% and 100%, respectively, for human stool and sewage samples. BacCan assay sensitivity and specificity for dog fecal samples were 80% and 97.3%, respectively.**Conclusion:** We confirmed the applicability of previously developed human-associated (B.theta and BacHum), dog-associated (BacCan) and total (BacUni) *Bacteroidales* gene markers for MST inthe tropical urban environment of Singapore. Such validation studies are needed before applying any existing host-associated *Bacteroidales* assays under new environmental conditions and at different geographical locations. Source tracking could be improved by identifying a specific human-associated gene marker for Singapore.

Author Disclosure Block:

J. Nshimyimana: None. **C. Cruz:** None. **J. Thompson:** None. **S. Wuertz:** None.

Poster Board Number:

SUNDAY-239

Publishing Title:

***lachnospiraceae* as Host-Specific Markers of Fecal Pollution**

Author Block:

S. McLellan, S. Feng; Univ. of Wisconsin-Milwaukee, Milwaukee, WI

Abstract Body:

Human and animal microbiome studies offer new opportunities to select alternative indicators for fecal pollution that are host specific and more informative than general indicators that are ubiquitously present in humans and animals. Although *Lachnospiraceae* is a major member of the microbial community in humans and many animals, this group has not been examined in detail for potential indicators. Using next generation sequencing data of sewage, dogs, cats, cattle, deer, pigs, and chickens, we chose V6 16S rRNA gene markers for populations of *Lachnospiraceae* that were found in sewage but not other animals. Overall, there were 14 different *Lachnospiraceae* V6 16S rRNA sequences that were potentially human indicators, which comprised 23% of all *Lachnospiraceae* in sewage samples. Most of these markers sequences where exclusively present in sewage and not in the animals we tested animals, however, several marker gene sequences appeared to be host preferred, that is they were highly abundant and present in one or two other hosts at low levels. We designed assays for Lachno 2, the second most abundant *Lachnospiraceae* (human preferred, but present in some domestic pets), Lachno 3, 11 and 12 (human specific), and Lachno 15 and 17 (found in humans, but also pigs), Combinations of these markers were tested against host sources and environmental water samples. In addition to PCR applications, direct sequencing approaches could make use of the distinctive host patterns within the family *Lachnospiraceae* for fecal pollution source identification.

Author Disclosure Block:

S. McLellan: None. **S. Feng:** None.

Poster Board Number:

SUNDAY-240

Publishing Title:

Nutritional Niche of *E. coli* in Marine Sediment

Author Block:

Y. Yu, S. Lau; HongKong Univ. of Sci. and technology, Hong Kong, Hong Kong

Abstract Body:

Escherichia coli, one of the constitutive microflora in mammalian guts, was originally thought to be able to survive only in the enteric environment. Hence, the occurrence of *E. coli* in water and food has been used as an indicator of fecal contamination. However, recent reports indicate that *E. coli* can be found in many different types of external habitats (e.g. seawater, sediment and soil) independent of fecal input. Environmentally adapted *E.coli* has been reported in marine sediment. Marine sediment is suggested as a potential reservoir for the microorganism as it confers protection, nutritional entrainment and provision of surfaces for biofilms formation. Such expansion of the *E.coli* population may have a tremendous impact on both water quality monitoring and environmental management. The nutrients and physical stresses associated with a habitat are the most fundamental factors delimiting the ecological niche of a species. Heretofore, the nutritional requirement for *E.coli* to colonize external habitats remains largely unexplored. This study investigates the nutritional basis of *E.coli* to establish a niche in the marine sediment. Eight selected *E.coli* isolates from various environmental and fecal sources were cultured in a minimal medium (M9-Glucose) and in interstitial water extracted from sediment of a tidal flat in Hong Kong.RNA extracted from the culture was subjected to transcriptomic analysis for the identification genes and metabolic pathways involved in the utilization of nutrient associated with the interstitial water. Many genes that appeared to be differentially expressed in the two media were associated with B vitamins, suggesting that the vitamins may play a key role in growth of *E.coli* in interstitial water. B vitamins are crucial to amino acid synthesis and carbon resupply to the TCA cycle, and also are essential cofactors for the functioning of a variety of enzymes. Our findings have important implications to further investigations of how nutrient exploitation as a selection pressure for the establishment of *E.coli* populations in marine sediment.

Author Disclosure Block:

Y. Yu: None. **S. Lau:** None.

Poster Board Number:

SUNDAY-241

Publishing Title:

Use of Boxa1r Primer and PCR to Differentiate *Escherichia coli* isolates in the Salt Marsh Periwinkle*, Littorina irrorata,* from Human and Non-Human Sources

Author Block:

M. C. Scalise, C. L. Rocha; The Citadel, Charleston, SC

Abstract Body:

Increased urban development of tidal creek systems has led to an increase in water quality degradation, namely through the presence of fecal coliform bacteria and *Escherichia coli.* Although a number of efforts have been implemented to reduce contamination of these systems, fecal input into fresh and salt water communities continues to pose a significant health risk as a result of failure to identify its non-point sources. The objective of this study was to investigate the use of repetitive extragenic palindromic polymerase chain reaction (rep-PCR) coupled with BOXA1R primer in differentiating human and non-human *E. coli* strains isolated from the Salt Marsh Periwinkle, *Littorina irrorata*. Samples of the periwinkles were collected from three different tidal creeks around Charleston, South Carolina. Two tidal creeks represented urban tidal creeks and one represented forested tidal creeks. The DNA of 48 *E.coli* isolates, obtained from homogenized periwinkle whole tissue, were subjected to PCR and analyzed by agarose gel electrophoresis. The DNA fingerprints obtained were analyzed by using the Jaccard index and the algorithm for Unweighted Pair Group Method with Arithmetic Mean (UPGMA). A dendogram constructed using Jaccard similarity coefficients separated the human isolates from the nonhuman isolates, revealing a characteristic structure of human *E. coli* populations among 53% of isolates found in one of the urban sites. Our results show that bacterial DNA isolated from the periwinkle can be sourced as human or nonhuman when rep-PCR with BOXA1R primer is performed. These results further support the use of this technique for monitoring the source of fecal pollution and, consequently, the presence of human-specific enteric pathogens in the environment.

Author Disclosure Block:

M.C. Scalise: None. **C.L. Rocha:** None.

Poster Board Number:

SUNDAY-242

Publishing Title:

Genomic Characterization of *Escherichia* Isolates from a Watershed

Author Block:

M. Hughes, L. Ragon, K. Lee, M. Pikaart, G. F. Peaslee, A. A. Best; Hope Coll., Holland, MI

Abstract Body:

Extensive work has characterized the extent of variation seen in isolates of *Escherichia coli* from host-associated environments around the world. These studies fall into two broad categories: global studies of the *E. coli* species through the analysis of fully sequenced genomes, and targeted studies of particular pathogen sub-types through the acquisition hundreds of draft genome sequences of clinical isolates. Both types lead to insights into the genus of *Escherichia*, however, they do not assess naturally occurring, non-host-associated populations in a systematic, in-depth manner. Thus, questions arise as to the extent of habitats for *Escherichia* strains and the extent to which there are non-host-associated reservoirs that provide opportunity for genetic exchange among diverse strains in the genus. We have a begun to perform a deep examination of *Escherichia* isolates derived from a non-host-associated environment - a watershed. The origin of *Escherichia* in watersheds include point sources associated with sewage treatment, livestock farming, animals and fowl; and non-point sources through sediment runoff from urban and agricultural environments. *Escherichia* is consistently found in aqueous environments, beach sands, and in soil, and the existence of environmental clades of *Escherichia* that fall outside of the traditional *E. coli* group is established. A limited set of the environmental clade genomes exist in public databases, yet thousands of *Escherichia* genomes from clinical, commensal, and host-associated sources are available. We have produced 56 draft genome sequences of *Escherichia* isolates as part of a longitudinal survey of microbial communities in a watershed with high sediment pollution, representing a significant increase of genomic information from non-clinical sources. We have placed each of these isolates in context with sequenced isolates using core-genome phylogenetic approaches, revealing that all of the isolates routinely obtained from this watershed are members of the traditional *E. coli* clade and represent the major subtypes. We have assessed potential for virulence and antibiotic resistance of these isolates through computational screens. These data serve as a resource for detailed examination of genome dynamics in *Escherichia* and evaluation of existing molecular identification markers used to monitor water quality in recreational bodies of water.

Author Disclosure Block:

M. Hughes: None. **L. Ragon:** None. **K. Lee:** None. **M. Pikaart:** None. **G.F. Peaslee:** None. **A.A. Best:** None.

Poster Board Number:

SUNDAY-244

Publishing Title:

Role of Detection of Stool CRE Colonization in Indian Scenario -Search and Thou Shall Find

Author Block:

A. Ghafur[1], V. P. R. Lakshmi[1], R. Arjun[1], R. Raj[1], C. Ravi[1], A. Tarigopula[2]; [1]apollo speciality Hosp.,, Chennai, India, [2]apollo Hosp.s, Chennai, India

Abstract Body:

Background: Increasing prevalence of Carbapenem Resistant *Enterobacteriacea*(CRE) is a global and regional challenge. Stool screening to identify carriers of these bacteria will help augment contact isolation precautions and in selecting empirical antibiotic regimen when these patients develop sepsis. Aim of our study was to screen rectal swabs of oncology patients undergoing Bone marrow transplant, to identify CRE colonizers and to explore incidence of subsequent gram-negative bacteremia in these patients.**Methods:** We did retrospective analysis of oncology patients who had rectal swab screening for CRE over the last 6 months (July -Dec 2015). Xpert® Carba-R test was used to screen rectal swabs for the rapid detection and differentiation of the *bla*KPC, *bla*NDM, *bla*VIM, *bla*OXA-48, and *bla*IMP. Case records of these patients were analysed to identify *Enterobacteriaceae* blood stream isolates, subsequent to the rectal screening. **Results:**Stool screening was done in 43 patients. Carba R PCR was positive in 22. (NDM 15, VIM2, NDM+OXA 48 +KPC -1,NDM+VIM-2,NDM+OXA 48-2]. Of the 43 patients, 8 had blood culture positivity for *Enterobacteriaceae*, subsequent to the stool screening study.(4 carba sensitive and 4 resistant). Out of the 22 CRE colonized patients, 4 had CSE(carbapenem sensitive *Enterobacteriaceae*) infection and 3 had CRE. Of the 21 stool Carba R negative patients, only 1 patient had CRE blood stream infection. **Conclusions:** Stool/rectal swab screening for CRE colonisers, will help strengthen infection control measures. This may also guide clinicians in selecting the empirical antibiotic.

Author Disclosure Block:

A. Ghafur: None. **V.P.R. Lakshmi:** None. **R. Arjun:** None. **R. Raj:** None. **C. Ravi:** None. **A. Tarigopula:** None.

Poster Board Number:

SUNDAY-245

Publishing Title:

The Use of Biorad'S ßCarba Assay in Conjunction with Cepheid'S Xpert Carba-R to Confirm Suspect Carbapenemase-Producing *Enterobacteriaceae* (CPE)

Author Block:

B. M. Willey[1], X. Trimi[1], P. Rahman[1], D. N. Grohn[1], D. Boyd[2], G. Ricci[3], O. Leung[1], S. Dhunna[1], P. Lo[1], T. Mazzulli[4], **S. M. Poutanen**[4]; [1]Mount Sinai Hosp., Toronto, ON, Canada, [2]Natl. Microbiol. Lab., Winnipeg, MB, Canada, [3]William Osler Hlth.System, Brampton, ON, Canada, [4]Univ. Hlth.Network, Mount Sinai Hosp., Univ. of Toronto, Toronto, ON, Canada

Abstract Body:

Background: Timely detection and confirmatory testing of CPE is important. Using a diverse collection of retrospective isolates, we verified Bio-Rad's ßCARBA assay, a rapid CPE assay based on colour changes reflecting hydrolysis of a chromogenic carbapenem in conjunction with Xpert CARBA-R assay, a research-use-only (RUO) PCR test that claims to detect IMP1, KPC, NDM, OXA48-like and VIM CPE genes.**Methods:** 259 species-diverse isolates, highly-characterized by PCR/sequencing, were used to evaluate the ßCARBA assay comprising 221 CPE (108 class A: 99 KPC, 4 SME, 2 IMI-1, 2 GES, 1 NMC-A; 80 class B: 73 NDM, 6 VIM, 1 IMP7; 26 class D; OXA48; OXA181, OXA232, OXA244; 7 class B+D: NDM+OXA181, NDM+OXA232) and 38 non-CPE (derepressed/ plasmid-mediated ampC, ESBL, ompC/ompF-mutants, ompK35/ompK36-mutants, cphA, OXA252). All 335 ßCARBA tests were inoculated with 1µL-loopful of growth from around ERT and incubated at 37ºC. Each tube was read at 30min independently by 5 blinded readers for colour-changes from yellow (negative) to orange, red or purple (positive). Consensus reads were analyzed. 228 of the above isolates (194 CPE; 34 non- CPE) were tested by the Xpert CARBA-R assay following manufacturer's instructions using isolates instead of specimens.**Results:** Overall, ßCARBA was 98.2% sensitive (95%CI: 95.8-99.3) and 100% specific (95%CI: 92.2-100) detecting 274/279 CPE in 335 tests. Detection included 100% of OXA48-like CPE (CSBA: 33/33; MHA: 21/21). False-negatives included 1/1 NMC *Enterobacter cloacae* and 1/2 GES *Klebsiella oxytoca*. The CARBA-R assay detected all KPC, NDM, VIM, and OXA48-like genes (13/13 OXA48, 1/1 OXA244, 9/9 OXA181, and 5/5 OXA232). Not surprisingly, CARBA-R was negative for SME and NMC CPE genes not claimed by the test.**Conclusions:** ßCARBA is a rapid low-complexity assay that detects CPE with high sensitivity and specificity with results in 30min using 1µL-loopful of organism. The CARBA-R RUO assay was simple to perform and provided confirmatory results within 1h for the claimed CPE genes KPC, NDM, VIM and OXA48 for which it was 100% sensitive.

Author Disclosure Block:

B.M. Willey: None. **X. Trimi:** None. **P. Rahman:** None. **D.N. Grohn:** None. **D. Boyd:** None. **G. Ricci:** None. **O. Leung:** None. **S. Dhunna:** None. **P. Lo:** None. **T. Mazzulli:** None. **S.M. Poutanen:** None.

Poster Board Number:

SUNDAY-246

Publishing Title:

The Importance of Inanimate Surfaces for the Transmission of Carbapenemase Producing *Klebsiella pneumoniae* (Cpkp) in a Tertiary Care Hospital in Izmir,Turkey

Author Block:

Z. Gulay, S. Alpcavus, A. Sari, 35340, V. Oguz, M. Bicmen; Dokuz Eylul Sch. Of Med., Izmir, Turkey

Abstract Body:

Background: Background: The prevalance of CPKp at the 9 Eylul University Hospital, increased from nil to 33.5% between 2007 and December 2015. This was mostly due to OXA-48 producers in years 2007-2014 but this situation has been changed after NDM- producing Kp was introduced in the hospital in May 2014 and most (95 %)of the carbapenem resistance is now due to this carbapenemase. Although cross transmission is usually via contaminated hands, inanimate surfaces are also implicated as potential sources. Aim: To investigate the contamination of inanimate surfaces by CPKp **Methods:** Carbapenemases produced by carbapenem resistant K.pneumoniae and their PFGE patterns are routinely investigated. Enzyme types are determined by PCR. Enviromental cultures from inanimate surfaces are performed during an outbreak or whenever the prevalance of CPKp increases in a certain hospital unit. Swabs are directly plated to selective EMB agar and also placed in enrichment broths and subcultured after overnight incubation. Species identification by Vitek 2.0 and carbapenem disk diffusion is performed from all gram negative isolates which is followed by PCR for carbapenemases and PFGE analysis.**Results:** During May 2014- December 2015, 269 enviromental sampling were done from patient rooms such as monitors, desks, ventilation units, bed rails and also from the nurse stations, dressing carts in surgical units, medication trays and the items on these mobile units. A total of 23 CPKp (21 NDM producer; 2 OXA-48 producer) was identified. These were recovered not only from the immediate patient enviroment but from common places such as medication closets, surgical carts and surgical bands. Cultures taken from the Emergency Department, Medical ICU, Surgical departments such as neurosurgery, cardovascular surgery and orthopaedics and Haemotology department were positive for NDM- Kp. The PFGE patterns were identical to the patients' hospitalized in that department almost everytime but in two occasions (emergency and haemotology units) . These two shared the most common pattern (A1a)found in the surgical units and this situation was linked to the cardiovascular surgeons who performed central line catheterization.**Conclusions:** This study shows the dissemination of NDM producing Kp in the hospital enviroment.

Author Disclosure Block:

Z. Gulay: None. **S. Alpcavus:** None. **A. Sari:** None. **V. Oguz:** None. **M. Bicmen:** None.

Poster Board Number:

SUNDAY-247

Publishing Title:

Antimicrobial Susceptibility and Resistomes of Carbapenem Resistant Enterobacteriaceae

Author Block:

W. C. Rutter[1], G. C. Lee[2], D. S. Burgess[1]; [1]Univ. of Kentucky, Lexington, KY, [2]Univ. of Texas Hlth.Sci. Ctr., San Antonio, TX

Abstract Body:

Background: Carbapenem resistant Enterobacteriaceae (CRE) is increasingly becoming a threat for human health. This pilot investigation sought to characterize the resistance patterns of CRE and to identify genotypic resistance mechanisms to predict resistant phenotypes.**Methods:** CRE isolates were obtained from the University of Kentucky Medical Center clinical microbiology laboratory. MICs were obtained via Phoenix II ® and E-test methods. Whole genome sequencing (WGS) was performed on 8 isolates. Multi-locus sequence type (MLST) was determined from WGS data using the *K. pneumoniae* and *E. cloacae* MLST databases. The resistome was assembled by identifying antimicrobial resistance determinants related to the phenotypically derived antibiogram.**Results:** Eighty-eight CRE isolates were obtained. *Klebsiella* (47%) and *Enterobacter* (41%) species were most common. The most active β-lactam agent was meropenem, with 30% of isolates demonstrating susceptible MICs. No isolate was susceptible to piperacillin-tazobactam, while 20% retained susceptible MICs for cefepime. Amikacin was active against 91% of isolates. Eight isolates (3 *K. pneumoniae,* 4 *E. cloacae*, and 1 *C. amalonaticus*) were selected for WGS. The *K. pneumoniae* isolates were all ST-258. One *E. cloacae isolate* was ST-484; 3 were unknown. All isolates harbored multiple genes encoding for β-lactamase genes (range 4 to 10). The most prevalent β-lactamase identified was *bla*OXA-9 (7 isolates). All *K. pneumoniae* isolates harbored *bla*KPC-3. Among *E. cloacae* isolates, 2 harbored *bla*VIM-2 and 3 harbored *bla*KPC-2. Notably, the concordance of amikacin-resistance conferring genes with susceptibility data were poorly predictive. The majority of amikacin susceptible isolates (88%) possessed genes associated with conferring aminoglycoside resistance, suggesting that expression analysis may be required. The aac(6′)-Ib-cr cassette that mediates resistance to both aminoglycosides and fluoroquinolones was identified in all 8 isolates.**Conclusions:** Despite carbapenemase production, isolates may display phenotypic susceptibility to meropenem and other β-lactams. Amikacin remains a viable option in many of these organisms. This pilot study demonstrates the potential utility of WGS to define the diversity and distribution of resistance mechanisms among CRE.

Author Disclosure Block:

W.C. Rutter: None. **G.C. Lee:** None. **D.S. Burgess:** None.

Poster Board Number:

SUNDAY-248

Publishing Title:

Factors Responsible for Colistin Resistance in *Klebsiella pneumoniae*

Author Block:

F. Can[1], E. Nurtop[1], N. Atac[1], S. Menekse[2], N. Lack[1], O. Kurt Azap[3], S. Simsek Yavuz[4], F. Yoruk[5], S. Karahan[1], T. Demir[1], D. Karaaslan[1], A. Azap[5], O. Ergonul[1]; [1]Koc Univ., Istanbul, Turkey, [2]Kosuyolu Hosp., Istanbul, Turkey, [3]Baskent Univ., Ankara, Turkey, [4]Istanbul Univ., Istanbul, Turkey, [5]Ankara Univ., Ankara, Turkey

Abstract Body:

Background: Alterations in *mgrb*, overexpression of *phoQ,pmrK*, and *pmrCAB*, and *mcr-1* plasmid are molecular mechanisms of colistin resistance (CR). We aimed to describe the factors contributing the CR. **Methods:** A total of 70 patients infected with CR K.pneumoniae were included. Genotyping and MLST were performed, carbapenemase type was detected. The overexpression of *phoQ*, *pmrK*, and *pmrCAB* were tested. Alterations in *mgrB* and presence of *mcr-1* were explored. **Results:** The mean age of 70 patients was 57±19 and 39% of patients were female, 80% of the patients stayed in ICU. Bacteremia was found in 28% of the patients. The mortality rate was 65%. The colistin MIC's was between 3-256 mg/l. Carbapenemase production was detected in 87% of the isolates. Rep-PCR and MLST revealed 3 large clones belonging to ST101. Intact *mgrB* gene was found in 25(36%) isolates, altered mgrB gene by IS or point mutations was detected in the 34 (49%) and 3 (4%) of the isolates, respectively. The overexpression of *pmrCAB* was shown in 4 of the isolates with intact mgrB. All of the isolates were found to be negative for *mcr-1*. Colistin was used in 40 (56%) patients and the mean duration of therapy was 17±18 days. Colistin use and length of therapy were found to be similar in isolates with intact and altered *mgrB* ($p>0.05$). **Conclusions:** Isolates with high numbers of intact *mgrB* and lack of *mcr-1* suggested that there might be unknown mechanisms that contribute to the colistin resistance. Alteration of *mgrB* was not associated with exposure to colistin.

Author Disclosure Block:

F. Can: None. **E. Nurtop:** None. **N. Atac:** None. **S. Menekse:** None. **N. Lack:** None. **O. Kurt Azap:** None. **S. Simsek Yavuz:** None. **F. Yoruk:** None. **S. Karahan:** None. **T. Demir:** None. **D. Karaaslan:** None. **A. Azap:** None. **O. Ergonul:** None.

Poster Board Number:

SUNDAY-249

Publishing Title:

***Klebsiella pneumoniae* Strains Producing Carbapenemases (Cpkp) and Resistance to Colistin (Co): A Four-Year Study Updated to December 2015**

Author Block:

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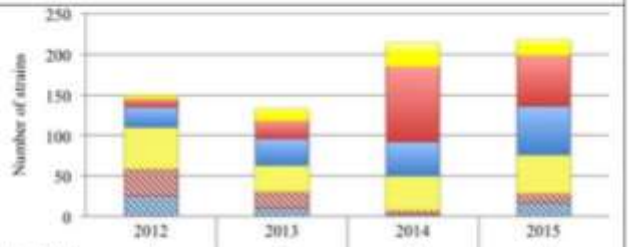
Abstract Body:

Background: to characterize the spread of CPKP in a tertiary level hospital using ongoing active surveillance with rectal swab (SRS) cultures and analyze the presence of CPKP in the clinical samples (CS) as well as the evolution of Co-sensitive (CoS) to Co-resistant strains (CoR).**Methods:** This study was performed from Jan 1, 2012 to Dec 31 2015. In 2012, an active monitoring was conducted in the Intensive Care Dept (IC); in autumn 2013 it was extended to the Surgery Dept (SurD), and since mid-2014 to the Medical Dept (MedD). Only the first strain from each patient (pt) was included. KPC, OXA-48, VIM and NDM were detected by a validated in-house PCR and multilocus sequence typing (MLST) was used.**Results:** Among 27324 pts 714 consecutive non-replicated strains of CPKP were collected: 149 strains in 2012 (39 [26.2%] from SRS), 133 in 2013 (70 [52.6%] from SRS), 214 in 2014 (164 [76.6%] from SRS) and 218 in 2015 (142 [65,1%] from SRS). The percentage of SRS+ increased from 2012 to 2015 (chi square for trend $p<0.0001$, Figure 1). CoR were 10.7%, 25.5%, 21.9% and 15.1% ($p=0.003$) and CoR isolated from SRS were higher in 2013, 2014 and 2015 compared with 2012 (chi square for trend $p=0.03$). Among pts with a CoS strain and with a follow-up available, 12 in 2012, 18 in 2013, 20 in 2014 and 20 in 2015 switched to a CoR. ST-258, ST-512 and ST-745 were the more prevalent types.**Conclusions:** This 4-year study highlights the clinical relevance of antimicrobial resistance as well as the drug-selection pressure of colistin use. The active surveillance increased the level of CPKP isolated by SRS but the results reported in 2015 suggest the need to maintain a high attention.

Figure 1. Carbapenemase producing Klebsiella pneumoniae strains isolated from surveillance rectal swabs and from clinical samples in 2012, 2013, 2014 and 2015
IC: intensive care. CS: clinical samples. SRS: surveillance rectal swabs



	2012	2013	2014	2015
SRS Medical dept.	4	15	29	19
SRS Surgical dept.	10	22	93	63
SRS IC dept.	25	33	42	60
CS Medical dept.	51	32	42	47
CS Surgical dept.	33	20	5	12
CS IC dept.	26	11	3	17

Author Disclosure Block:

S.G. Parisi: None. **A. Bartolini:** None. **E. Santacatterina:** None. **A. Berto:** None. **E. Franchin:** None. **N. Menegotto:** None. **E. De Canale:** None. **T. Tommasini:** None. **M. Basso:** None. **S. Stefani:** None. **G. Palù:** None.

Poster Board Number:

SUNDAY-251

Publishing Title:

Epidemiology, Clinical Characteristics & Outcomes of Carbapenemase-Producing *Enterobacteriaceae* (Cpe) Infections in a Carbapenemase-Diverse Setting

Author Block:

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Abstract Body:

Background: CPE infections are a major global concern. Selecting an optimal therapy for these infections is a challenge due to the diversity of CPE infections. This study described the clinical characteristics, treatment & outcomes of CPE infections in Singapore. **Methods:** A retrospective cohort study of adult inpatients with clinically significant CPE infections, which had reduced susceptibility to ≥1 carbapenem, was conducted between Jan 2013 - June 2015 in a large Singapore tertiary hospital. Carbapenemase production was confirmed with PCR. MIC was assessed using CLSI broth dilution. **Results:** 80 cases (47 KPC, 7 $OXA_{48\text{-}like}$, 22 NDM, 1 IMP, 3 co-producers) were included. CPE were isolated from blood (30%), urine (25%), respiratory (24%), abdominal (18%) & wound (16%) cultures. Main CPE implicated were *Klebsiella spp.* (48%), *Enterobacter spp.* (20%), *E. coli* (15%). 21% were extensively drug-resistant (XDR). CPE was most susceptible to tigecycline (93%), polymyxin B (88%), amikacin (73%) & levofloxacin (45%). 94% had carbapenem MICs of ≥ 8 mg/L. Median (range) patient age was 66 (18 - 89) years [55% male, 50% medical patients]. Most cases had previous hospitalizations (88%) or invasive devices/procedures (85%). The median Charlson & SAPS scores were 4 (0 - 10) & 36 (12 - 69). 70 cases received treatment (39% monotherapy, 61% combination). The most common combinations were carbapenems with polymyxin B &/or tigecycline (30%), or with aminoglycosides (14%). The 30-day in-hospital all-cause mortality rate was 30%. The median time to death was 14 (1 - 152) days. Non-survivors were older ($p<0.05$), while other clinical characteristics were similar between groups, including carbapenemase type & culture source. Neither combination use, therapy type (e.g. carbapenem-based regimens) nor XDR status was associated with mortality. Mortality rates were similar in patients receiving monotherapy & combination (31% vs 29%). However, only 1/5 (20%) patients receiving therapy guided by *in vitro* combination test died. **Conclusions:** CPE infections were diverse & treated with varied non-standardized antibiotic combinations. There was no single therapy which was associated with better outcome. Combination testing-guided therapy may be considered.

Author Disclosure Block:

J. Teo: None. **C. Ng:** None. **Y. Cai:** None. **W. Lee:** None. **T. Lim:** None. **T. Koh:** None. **T. Tan:** None. **A. Kwa:** None.

Poster Board Number:

SUNDAY-252

Publishing Title:

A Risk Factor Study for Carbapenem Resistant *Klebsiella pneumoniae* Bloodstream Infection in an Endemic Area

Author Block:

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Abstract Body:

Background: Carbapenem resistant *Klebsiella pneumoniae* (CRKP) is an emerging pathogen that is endemic in New York City. This study sought to describe factors associated with CRKP bloodstream infection.**Methods:** A case control study design included patients at three hospitals in Bronx, NY. We identified 187 case patients that had a first episode of CRKP bloodstream infection between January 1, 2009 and April 30, 2014. Patients who had a blood culture drawn that did not grow CRKP and had antibiotics started within 48 hours of the blood culture were eligible for selection as control patients. We randomly selected 567 control patients. Data on comorbidities, antibiotic use, prior cultures, and other potential risk factors were electronically abstracted from the medical record using a local tool called Clinical Looking Glass. A multivariate logistic regression model for the outcome of CRKP bloodstream infection was built using a backwards stepwise process.**Results:** The patients had a median age of 65 years (IQR 54, 78). A total of 276 (37%) patients had been admitted from a skilled nursing facility (SNF). The variables in the logistic regression model for CRKP bloodstream infection are shown in the table below. A culture with a carbapenem resistant enterobacteriaceae (CRE) in the prior 6 months and admission from a SNF were most strongly associated with CRKP bloodstream infection. There was a significant interaction between admission from a SNF and having a central line during the 30 days before the blood culture (p= 0.049).**Conclusions:** A prior culture with a CRE organism was strongly associated with CRKP bloodstream infection. Admission from a SNF may alter the association of risk factors with multi drug resistant infections.

Multivariate Associations with CRKP Bloodstream Infection

Variable	Odds Ratio (95% CI)	P-Value
Culture with CRE in Prior 6 Months	7.7 (4.2, 14)	<0.001
Admission from SNF (No Central Line)	6.9 (3.8, 12)	<0.001
Admission from SNF (Central Line)	3.0 (1.7, 5.4)	<0.001
Central Line in Prior 30 Days (Not from SNF)	3.0 (1.5, 6.0)	0.001
Central Line in Prior 30 Days (From SNF)	1.3 (0.74, 2.4)	0.34
Antibiotic Received in Prior 30 Days	1.8 (1.1, 3.0)	0.02

Mechanical Ventilation in Prior 30 Days	1.8 (1.1, 2.9)	0.01
SNF*Central Line Interaction Term	-	0.049

Author Disclosure Block:

G. Weston: None. **E. Bellin:** None. **B. Ostrowsky:** None.

Poster Board Number:

SUNDAY-253

Publishing Title:

Intra-Abdominal Infections (Iai) Caused by Carbapenem-Resistant Enterobacteriaceae (Cre): Clinical and Microbiological Characteristics & Outcomes

Author Block:

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Abstract Body:

Background: Infections caused by CRE pose a major challenge. While several studies have described the characteristics of CRE infections, studies of specific infection types are limited. The objective of this study is to describe the clinical characteristics & outcomes of patients with CRE IAI.**Methods:**A retrospective cohort study was conducted in a large tertiary Singapore hospital for all inpatients with CRE IAIs (defined as resistant to at least 1 carbapenem) from January 2013 - June 2015. Patient demographics, clinical & microbiological data, & antibiotic (abx) use data were collected. The outcome measured was death from date of 1st positive culture to 30 days from end of CRE IAI treatment. Characteristics of survivor (SV) and non-survivor (NSV) subgroups were compared.**Results:**A total of 41 patients were included. The most common CRE IAI was hepatobilliary infection (59%). 24 patients (59%) had concurrent bacteremia. *K. pneumoniae* was most common (42%), followed by *E. coli* (39%). 88% were resistant to all carbapenems (meropenem MIC_{50}: 8mg/L, MIC_{90}: >=32mg/L); however, most were susceptible to polymyxin B (93%) & tigecycline (98%). Half (46%) of empirical regimens were inadequate. Post-antibiogram, almost half of the patients (42%) were prescribed abx combinations - 13 (32%) received 2-abx combination; 4 (10%) received combinations with ≥3 abx. The most common combinations were polymyxin B-based (24%). 10 (24%) patients had adequate source control. Overall, 19 patients (46%) died from CRE-IAI within 30 days treatment. Clinical response and microbiological clearance was documented in 22 (54%) & 6 (15%) patients respectively. Comparing the SV & NSV subgroups, median SOFA score was higher in the NSV subgroup [8 (5 - 13) in SV, 4 (2 - 4) in NSV, $p < 0.001$]. The proportion of patients with adequate source control is smaller in the NSV subgroup (24% in SV, 0% in NSV, $p < 0.001$). Post-antibiogram use of polymyxin B was higher in the non-survivor subgroups (53% in SV, 17% in NSV, $p = 0.017$), reflecting physicians' inclination for polymyxin B as a last-resort option in mainly in the critically-ill.**Conclusions:**CRE IAIs are associated with high mortality. Other than optimizing antibiotic choice & doses, adequate source control is a key modifiable area in improving patient survival in CRE IAIs.

Author Disclosure Block:

Y. Chen: None. **Y. Cai:** None. **L. Loo:** None. **J. Teo:** None. **W. Lee:** None. **T. Lim:** None. **T. Koh:** None. **T. Tan:** None. **A. Kwa:** None.

Poster Board Number:

SUNDAY-254

Publishing Title:

Impact of Infectious Disease Consultation in Carbapenem-Resistant *Klebsiella pneumoniae* (Crkp) Infections

Author Block:

A. Jain, T. Bias; Drexel Univ. Coll. of Med., Philadelphia, PA

Abstract Body:

Background: Carbapenem-Resistant *Klebsiella pneumoniae* (CRKP) infections remain a serious public health problem given its unfavorable impact on mortality and shortage of treatment options. Infectious Disease (ID) consultation has been shown to increase adherence to evidence based treatment for select infections with implications for improved quality measures, reduced in hospital mortality and earlier discharge. Our study aimed to assess the impact of ID consultation on the management and outcomes of patients with documented CRKP infections. **Methods:** A retrospective, observational study was conducted to evaluate adult patients (age ≥ 18 years) who had a documented CRKP infection between January 2009 and September 2014. Demographics and disease specific information were collected. Patients were stratified into 3 cohorts: no ID consultation (NIDC), Early ID consultation (EIDC) and Late ID consultation (LIDC). Primary outcomes included 30-day and infection-related mortality. Secondary outcomes included microbiological cure, overall length of stay, and intensive care unit length of stay. **Results:** One hundred and four patients met criteria for inclusion; 9 in the NIDC group, 59 in the EIDC and 38 in the LIDC group. There was no statistically significant difference in 30 day all-cause mortality (19% vs. 44% vs. 33%, p= 0.122) across all groups, however a difference was observed in infection related mortality (13% vs. 41% vs. 33%, p = 0.025). In patients with Charlson Comorbidity Index (CCI) scores greater than 5, infection related mortality was still higher in the EIDC group in comparison to LIDC and NIDC groups respectively (50% vs. 13% vs 40%), emphasizing the high baseline severity of illness. Source of infection was a confounder in respect to mortality endpoints as all indications were pooled together. **Conclusions:** Interestingly ID consultation did not show improvement in mortality endpoints. Given the rise in multidrug resistance and lack of rapid diagnostic tests, ID consultation is still imperative for selection of antimicrobials, infection control measures, and adherence to guidelines.

Author Disclosure Block:

A. Jain: None. **T. Bias:** None.

Poster Board Number:

SUNDAY-255

Publishing Title:

Efficacy of Nebulized Colistin in Treatment of Patient with Nosocomial Respiratory Infections from Multidrug-Resistant Gram Negative Organisms

Author Block:

C. Quarshie, V. Sundareshan, A. Botchway; Southern Illinois Univ Sch., Springfield, IL

Abstract Body:

Background: Nebulized colistin (Polymyxin E), a bactericidal antibiotic, is used in the treatment of infections with a wide range of resistant aerobic gram negative (GN) organisms including Pseudomonas aeruginosa (P. aeruginosa), and Acinetobacter baumannii (A. baumanii). This study aims to evaluate the patterns and efficacy of use of nebulized colistin either alone or in combination with other antimicrobial agents in the treatment of respiratory infections in patients with resistant P. aeruginosa, and A. baumannii.**Methods:** We conducted a retrospective chart review of patients greater than 18 years of age diagnosed with healthcare associated pneumonia (HCAP), or ventilator associated pneumonia (VAP) secondary to MDR P.aeruginosa or A.baumanii admitted from January 2009 to January 2014 at Memorial Medical Center, Springfield, IL. The respiratory infection was diagnosed based on clinical signs, leukocytosis, and chest x ray showing infiltrate or consolidation, along with a microbiological identification of multi-drug resistant P.aeruginosa, and A. baumannii. The clinical cure rate was defined as complete resolution of all signs and symptoms of pneumonia, with improvement of chest x-ray by the 7 days. Logistic regression was used to analyze the results.**Results:** A total of 32 charts were reviewed from January 2009 to January 2014. There were 19 patients with infections with A. baumanii and there were 12 patient with P. aeruginosa who received nebulized colistin. The clinical cure rate for the P. aeruginosa was 50% and 50% for A.baumanii. The clinical cure rate of P. aeruginosa was not different from A. baumanii, OR=0.82 (CI 0.07-9.91), p = 0.88 with nebulized colistin used alone or in combination with other antibiotics. 5 patients with infection from P. aeruginosa without resolution on chest Xray and 6 patients with A. baumanii that did not show any changes in the chest Xray. There is no baseline resistance noted for P. aeruginosa and A. baumanii for colistin in our hospital. **Conclusions:** The cure rate with use of nebulized colistin was similar with P. aeruginosa and A. baumaniipatients indicating that it is equally difficult to treat infections with both organisms and when used for treatment of respiratory infections, patients should be closely monitored for clinical progress.

Author Disclosure Block:

C. Quarshie: None. **V. Sundareshan:** None. **A. Botchway:** None.

Poster Board Number:

SUNDAY-256

Publishing Title:

Incidence and Antimicrobial Resistance of Eskape Organisms in a Second Level Care Hospital in Mexico

Author Block:

M. D. Alcántar-Curiel[1], E. Toledano-Tableros[2], S. Giono-Cerezo[2], M. Jarillo-Quijada[1], J. Fernández-Vázquez[1], C. Gayosso-Vázquez[1], M. López Alvarez[3], J. Santos-Preciado[1]; [1]Univ. Natl. Autónoma de México, México D.F., Mexico, [2]Escuela Natl. de Ciencias Biológicas, IPN, México D.F., Mexico, [3]Hosp. Regional Gen. Ignacio Zaragoza, México D.F., Mexico

Abstract Body:

Background: The ESKAPE pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae-[Kpn], Acinetobacter baumannii-[Aba], Pseudomonas aeruginosa-[Psa], Enterobacter* species-*[Eclo]*) are responsible for a substantial percentage of nosocomial infections and represent the vast majority of isolates resistant to antimicrobial agents. The aim of this study was to assess the incidence and epidemiology of ESKAPE pathogens in patients who acquired a nosocomial infection at a second level care hospital in Mexico City. **Methods:** ESKAPE isolates were collected from February-July/2015. Carbapenem susceptibility was determined by disk diffusion method, using CLSI/2015 interpretative criteria. Metallo-β-lactamases-(MBL) phenotype was confirmed by modified-Hodge test and PCR assay. The ESBL and AmpC production was determined by double disc test. Clonality among *Psa* and *Aba* isolates was determined by PFGE. **Results:** Of the 1,480 specimens cultured from patients with suspected infections, only 272 isolates (18%) were positive; of these, 132 (48.5 %) were ESKAPE species isolated mainly from severely ill patients: *Psa* (32%) followed by *Aba* (28%) were the most frequent isolates. These bacteria were most frequently isolated from blood (51%) and surgical wounds (20%). Carbapenem resistance was 90% *Aba*, 87% *Psa*, 67% *Eclo* and 23% for *Kpn*. The carbapenem resistance was associated with MBL production in 84% *Aba*, 60% *Psa*, and 67% for *Kpn* and *Eclo*, but not with KPC or NDM-1 type. The cephalosporin resistance was associated with ESBL production in 85% *Kpn* and 17% *Eclo*, and with AmpC production in 53% *Psa* and 7% *Aba*. As for clonality, *Psa* showed variability and *Aba* showed dissemination of one clone. **Conclusions:** These results are worrisome because the emergence of the ESKAPE group, traditionally problematic in tertiary referral hospitals, reflects a growing problem in a second level hospital. This is undoubtedly due to the widespread use of cephalosporin and carbapenems, generally the last drugs choice for the treatment of multidrug-resistant isolates in nosocomial infections.

Author Disclosure Block:

M.D. Alcántar-Curiel: None. **E. Toledano-Tableros:** None. **S. Giono-Cerezo:** None. **M. Jarillo-Quijada:** None. **J. Fernández-Vázquez:** None. **C. Gayosso-Vázquez:** None. **M. López Alvarez:** None. **J. Santos-Preciado:** None.

Poster Board Number:

SUNDAY-257

Publishing Title:

Phenotypic and Molecular Characterization of Beta-Lactam Resistance in Gram-Negative Bacilli from Pediatric Urology Patients

Author Block:

E. Powell, J. Schaffzin, M. Beckman, J. Muldoon, D. Haslam, **J. Mortensen**; Cincinnati Children's Hosp., Cincinnati, OH

Abstract Body:

Background: Antibiotic Stewardship Programs (ASPs) are a key partner of clinical microbiology laboratories and patient services, particularly those services that care for patients with long-term antibiotic exposure. In an effort to better understand patterns of antibiotic resistance in one such group, this study sought to determine the prevalence of beta-lactam resistance mechanisms in Gram-negative bacilli from pediatric urology patients.**Methods:** All Gram-negative bacilli isolated from the urine cultures of patients seen by the Urology service during a one-week period were identified by MALDI-ToF and antimicrobial susceptibilities were determined using VITEK2 GN-69 and XN-06 cards. DNA was isolated using the NucliSENS easyMAG extractor and the presence of various beta-lactamases—including penicillinases, AmpCs, extended spectrum beta-lactamases (ESBL), and carbapenemases—was determined using the Check-points system CT 103XL Check-MDR panel.**Results:** 39 Gram-negative bacilli were isolated: *Eschericia coli* (25), *Klebsiella pneumoniae* (3), *Pseudomonas aeruginosa* (3), *Proteus mirabilis* (3), *Enterobacter* species (2), *Serratia liquifacens* (1), *Pantoea* species (1), and *Klebsiella oxytoca* (1). The genes coding for penicillinases were detected in 14 isolates: 2 *K. pneumoniae* isolates with wild-type SHV, 11 *E. coli* isolates with wild-type TEM, and 1 *K. pneumoniae* isolate with both wild-type SHV and wild-type TEM. ACT/MIR AmpC genes were detected in both *Enterobacter* species isolates. An ESBL gene was detected in 2 *E. coli* and 1 *K. pneumoniae* and was identified as CTX-M-1, group 15-like in all three isolates. No carbapenemase genes were detected. MIC results were consistent with these genotypic findings.**Conclusion:** During this one-week period ESBLs were relatively rare (7.69%) in Gram-negative bacilli from pediatric urology patients. As part of our ASP support of clinical care, the association between antibiotic use and beta-lactamase gene prevalence will be monitored. Ongoing collaboration between ASP, the Clinical Microbiology Laboratory, and patient services will be essential to monitor for antibiotic resistance and guide appropriate and effective therapy for vulnerable patients.

Author Disclosure Block:

E. Powell: None. **J. Schaffzin:** None. **M. Beckman:** None. **J. Muldoon:** None. **D. Haslam:** None. **J. Mortensen:** None.

Poster Board Number:

SUNDAY-258

Publishing Title:

Complete Sequence of an Inchi2 Plasmid Carrying Two Copies Of *Bla*$_{\text{ctx-M-2}}$ From *Escherichia coli* Sequence Type 410 Isolate

Author Block:

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Abstract Body:

Background: CTX-M-2 is one of the common groups of extended-spectrum-β-lactamases (ESBL) in the world especially in Japan, Southern Europe and South America. The CTX-M-2-prodcing plasmid pYD786-1 was identified in an *Escherichia coli* ST410 strain isolated from the urine of an inpatient at a U.S. hospital. **Methods:** pYD786-1 was sequenced on a PacBio RSII sequencing instrument. Assembly and annotation was conducted and comparison with previously sequenced genomes. **Results:** pYD786-1 is a 227-kb IncHI2 plasmid. Its backbone is composed of replication, partition, conjugation regions and toxin-antitoxin systems as discovered in IncHI2 plasmids of *Salmonella enterica* from Hong Kong and Canada. There is also a multidrug resistance mosaic region (MRR) generated by several transposition and recombination events. The MRR is composed of a Tn*21*-derivative transposon, followed by IS*Ecp1* transposition unit and Tn*5393*::IS*1133* element. Insertion of IS*1R* truncated the *tnpR* of Tn*21* and IS*Ecp1*. Tn*21* here apparently underwent multiple insertions involving In*2*, IS*1326* and IS*1353*, thus creating a nested complex structure. Furthermore, the In*2*-derivative integron captured an IS*CR1*-*bla*$_{\text{CTX-M-2}}$ module embedded between two 3' conserved segments (3′-CS). Uniquely, however, the 3′-CS-*bla*$_{\text{CTX-M-2}}$-IS*CR1*-3′-CS module is duplicated, generating two copies of *bla*$_{\text{CTX-M-2}}$ in tandem thus forming an unusual complex class 1 integron. **Conclusions:** An IncHI2 plasmid carrying two copies of *bla*$_{\text{CTX-M-2}}$ was identified, which had undergone multiple transposition and recombination events, forming a multidrug resistance plasmid.

Author Disclosure Block:

Q. Guo: None. **C.L. McElheny:** None. **M. Wang:** None. **Y. Doi:** None.

Poster Board Number:

SUNDAY-259

Publishing Title:

Distribution of β-Lactamases among Gram-Negative Pathogens from the Middle East and Turkey

Author Block:

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Abstract Body:

Background: The Study for Monitoring Antimicrobial Resistance Trends (SMART) tracks *in vitro* activity of antimicrobials used to treat intra-abdominal and urinary tract infections. In this analysis, we identified β-lactamases (BL) carried by *Enterobacteriaceae* (*Ebac*) collected from patients in 5 countries in the Middle East and Turkey from 2010-2014. **Methods:** Eighteen sites each collected up to 150 consecutive, non-duplicate Gram-negative isolates per study year. Susceptibility and extended-spectrum β-lactamase (ESBL) phenotypes were determined by broth microdilution. All ertapenem (ETP) non-susceptible (NS) isolates and a randomly selected ≥50% of ESBL-phenotype positive (ESBLp+) *Escherichia coli*, *Klebsiella pneumoniae*, *K. oxytoca*, and *Proteus mirabilis* collected from each country were molecularly characterized for genes encoding ESBLs, carbapenemases (Cpases) and AmpC BL.**Results:** 5960 *Ebac* were collected in Israel, Jordan, Lebanon, Saudi Arabia, United Arab Emirates (UAE) and Turkey. The activities of tested β-lactam agents differed among countries, with 91.4% (UAE) to 97.9% (Lebanon) of collected isolates susceptible *in vitro* to ETP and 33.6% (UAE) to 76.0% (Israel) of isolates susceptible to ceftazidime. 1297 isolates (793 *E. coli*, 414 *K. pneumoniae*, 35 *Enterobacter cloacae*, and 55 isolates of 10 other species of *Ebac*) were molecularly characterized. BL types detected and overall percentages of ESBLp+ and ETP-NS isolates are shown by country (Table).

	Country[a] (n)						
Phenotype/ BL detected	ISR	JOR	LBN	SAU	UAE	TUR	Total
ESBLp+, ETP-S characterized[b]	168	148	101	115	144	336	1012
ESBL	160	146	96	100	135	316	953
ESBL+AmpC		1	3	9	5		18
AmpC	1				2		3
No ESBL or AmpC	7	1	2	6	2	20	38
ETP-NS characterized[c]	35[d]	31	13	46	40	120	285
KPC	20				1		21
OXA-48-like		11	9	31	5	86	142

MBL		7		3	20	4	34
MBL+OXA-48-like		1			2	2	5
No Cpase detected	15	12	4	12	12	28	83
Total collected	1273	646	607	981	467	1986	5960
% ESBLp+	27.4	51.1	37.7	28.0	70.8	38.2	38.4
% ETP-NS	2.9	4.8	2.1	4.7	8.6	6.0	4.8

[a]ISR, Israel; JOR, Jordan; LBN, Lebanon; SAU, Saudi Arabia; UAE; United Arab Emirates; TUR, Turkey.[b]Co-carry original-spectrum BL (OSBL), including TEM-1, SHV-1, and SHV-11.[c]Co-carry additional ESBL, AmpC, and OSBL.[d]Two collected isolates were not characterized.**Conclusions:** Country-specific variations in ESBLp+ and ETP-NS rates and in Cpase prevalence were observed and can be used to inform treatment choices.

Author Disclosure Block:

K. Kazmierczak: M. Independent Contractor; Self; IHMA, Inc. **S. Lob:** M. Independent Contractor; Self; IHMA, Inc. **R. Badal:** M. Independent Contractor; Self; IHMA, Inc. **D. Sahm:** M. Independent Contractor; Self; IHMA, Inc..

Poster Board Number:

SUNDAY-260

Publishing Title:

Evolution of the Carbapenem-Resistance in *Acinetobacter baumannii* Isolated in Chilean Hospitals

Author Block:

A. F. Opazo-Capurro, C. Lima, H. Bello-Toledo, M. Dominguez-Yevenes, G. Gonzalez-Rocha; Univ. of Concepcion, Concepcion, Region del Bio-Bio, Chile

Abstract Body:

Background: *A. baumannii* (Aba) is an important nosocomial pathogen, often multidrug- or pandrug-resistant, and responsible for several outbreaks worldwide. Nowadays, is commonly resistant to imipenem and meropenem, which are one of the most important remaining treatment options against this bacillus. In Chile, according to the Chilean Society for Infectious Diseases, the percentage of carbapenem-susceptible Aba isolates was nearly 30% in 2012. Due to a lack of data regarding the mechanisms of carbapenems-resistance in Chilean isolates, the aim of this study was to investigate the genetic determinants associated to this resistance. **Methods:** Seventy-seven strains collected from hospitals in 9 Chilean cities, were analyzed. They were grouped in 3 periods: 1990-99, 2000-09 and 2010-15. The isolates were identified by multiplex-PCR. Susceptibility testing to carbapenems was performed by disk diffusion. The genes encoding OXA-type carbapenemases (OTCs) were detected by multiplex-PCR. The association of *bla*$_{\text{OXA-51-like}}$ with the IS*Aba1* element was identified by standard PCR and sequencing.**Results:** All isolates (26) collected during 1990-99 were susceptible to both carbapenems. They did not harbor any OTCs genes, except for the *bla*$_{\text{OXA-51-like}}$ gene not associated to IS*Aba1*. However, 21 over 30 isolates from the second period were resistant to carbapenems; where 11 carried the *bla*$_{\text{OXA-58-like}}$ gene and 7 harbored the *bla*$_{\text{OXA-23-like}}$ gene. Finally, 21 collected during 2010-15 were resistant to both carbapenems; where 3 harbored the *bla*$_{\text{OXA-58-like}}$ gene, 11 carried the *bla*$_{\text{OXA-23-like}}$ gene and 10 were positive for the IS*Aba1-bla*$_{\text{OXA-51-like}}$ arrangement.**Conclusions:** This study shows the dynamic process of carbapenem-resistance in Aba Chilean isolates, indicating that the first OTC gene acquired was *bla*$_{\text{OXA-58-like}}$. Interestingly, this gene was rapidly replaced during the next years by the *bla*$_{\text{OXA-23-like}}$ gene and by the IS*Aba1-bla*$_{\text{OXA-51-like}}$ association. These mechanisms could confer higher carbapenem-resistance levels than *bla*$_{\text{OXA-58-like}}$, reflecting an alarming situation in the country, especially considering colistin-resistant isolates have already emerged and the potential of every single isolate of becoming resistant due to the overexpression of the intrinsic *bla*$_{\text{OXA-51-like}}$ gene.

Author Disclosure Block:

A.F. Opazo-Capurro: None. **C. Lima:** None. **H. Bello-Toledo:** None. **M. Dominguez-Yevenes:** None. **G. Gonzalez-Rocha:** None.

Poster Board Number:

SUNDAY-261

Publishing Title:

Dissemination of Incx3 Type Plasmid Encoding Ndm-5 in *Escherichia coli* from Companion Animals in China

Author Block:

j. sun, r-s. yang, x-p. liao, y-h. liu; South China Agricultural Univ., guangzhou, China

Abstract Body:

Objectives: The emergence and worldwide spread of carbapenemase-producing *Enterobacteriaceae* has become a major public health threat. In the present study, we investigate the dissemination of carbapenemase-producing *Enterobacteriaceae* in companion animals in China.**Methods**: 174 rectal swabs were obtained from healthy or diseased dogs and cats from Harbin (20), Yangzhou (20), Chongqing (67), Wuhan (13), Chengdu (24) and Guangzhou city (30), during the July to November in 2015. Each swabs was isolated from an individual animal were direct streaked on a MacConkey agar plate containing 1 mg/L meropenem without culture. Carbapenem-non-susceptible isolates were screened the carbapenemase genes, including bla_{KPC}, bla_{IMP}, bla_{VIM}, bla_{OXA-48} and bla_{NDM} were screened and sequenced. For carbapenemase genes-positive isolates, antibiotic susceptibilities were assessed and molecular typing was performed using PFGE. Plasmid transfer to *E. coli* recipients was investigated using filter mating experiments. The genetic location of bla_{NDM} was determined by analysis of PFGE profiles of *S1* nuclease-digested genomic DNA and Southern blot hybridization. The genetic context analysis of the bla_{NDM} gene was applied by PCR mapping and primer walking.**Results**: A total of 6 bla_{NDM}-positive *E. coli* were collected from three disparate cities: Yangzhou (1), Chongqing (3), and Guangzhou (2). All isolates belonged to bla_{NDM-5}, which were resistant to carbapenems, cephalosporins, tetracycline, fluoroquinolones, and two isolates among them were also resistant to colistin. Plasmids carrying bla_{NDM-5} were successfully transferred to *E. coli* recipient EC600. *XbaI*-PFGE revealed that only two strains isolates from Guangzhou were the clonal spread. S1-PFGE and Southern blot analysis showed that bla_{NDM-5} gene was located on IncX3-type plasmids with same size ranging from 33.3 to 54.7 kb. PCR mapping and sequencing demonstrated that all isolates contained a common gene environment around bla_{NDM-5} (*IS*Aba125-*IS*5-bla_{NDM-5}-bla_{MBL}-*trpF- dsbC-IS*26).**Conclusions**: We describe the emergence of *E. coli* harboring IncX3 Type Plasmid Encoding NDM-5 from Companion Animals in China. We suggest that isolates producing New Delhi metallo-β-lactamase are currently circulating among companion animals and may act as a reservoir of gene transmission between humans and animals.

Author Disclosure Block:

J. sun: None. **R. yang:** None. **X. liao:** None. **Y. liu:** None.

Poster Board Number:

SUNDAY-262

Publishing Title:

The Importance of ST258 and IncFII$_{K2}$ Plasmids among a Global Collection of *Klebsiella pneumoniae* with *bla*$_{KPCs}$

Author Block:

G. Peirano[1], P. Bradford[2], K. Kazmierczak[3], L. Chen[4], B. Kreiswirth[4], **J. Pitout**[1]; [1]Univ. of Calgary, Calgary, AB, Canada, [2]AstraZeneca Pharmaceuticals, Waltham, MA, [3]Intl. Hlth.Management Associates, Schaumburg, IL, [4]Rutgers Univ., Newark, NJ

Abstract Body:

Background: A study was designed to characterize 470 KPC-producing *K. pneumoniae* from a global surveillance study obtained from the urinary tract, skin structures, intra-abdominal, and respiratory tract specimens during the years 2012-14. **Methods:** Multilocus sequence typing was used to define STs and PCR typing was used to determine the presence of *bla*$_{KPCs}$ on IncFII$_{K2}$, IncFII$_{K1}$ and IncI$_2$ types of plasmids. **Results:** The majority of *K. pneumoniae* (58%) belonged to ST258; clade I was associated with *bla*$_{KPC-2}$ on both IncFII$_{K2}$, and IncFII$_{K1}$ plasmids and was present in Argentina, Greece, Romania and USA. ST258 clade II was associated with *bla*$_{KPC-3}$ on IncFII$_{K2}$ plasmids and was present in Italy, Germany, USA, Brazil and Israel. ST11 (18%) was present in Argentina, Brazil, Venezuela, China and Taiwan and was associated with *bla*$_{KPC-2}$ on IncFII$_{K1}$ and IncFII$_{K2}$ plasmids. The remaining *K. pneumoniae* STs (24%) had a global distribution and was associated with both *bla*$_{KPC-2}$ and *bla*$_{KPC-3}$ on IncFII$_{K1}$ plasmids. **Conclusions:** There was a strong affiliation between ST258 and IncFII$_{K2}$ (pKpQIL-like) plasmids and non-ST258 with IncFII$_{K1}$ plasmids among a global collection of *K. pneumoniae* that harbours *bla*$_{KPCs}$. This study highlights the importance of surveillance programs using molecular techniques in providing insight into characteristics and global distribution of STs and their respective plasmids.

Author Disclosure Block:

G. Peirano: None. **P. Bradford:** D. Employee; Self; Astra Zeneca. **K. Kazmierczak:** None. **L. Chen:** None. **B. Kreiswirth:** None. **J. Pitout:** None.

Poster Board Number:

SUNDAY-263

Publishing Title:

Ges Carbapenemases in *Enterobacteriaceae* and *Pseudomonas aeruginosa* in the UK

Author Block:

K. L. Hopkins, J. Findlay, D. Meunier, D. Godoy, J. Turton, R. Hill, N. Woodford; Publ. Hlth.England, London, United Kingdom

Abstract Body:

We sought GES carbapenemases in Enterobacteriaceae and *P. aeruginosa* submitted to the national reference lab with raised carbapenem MICs in the absence of other carbapenemases, and characterised positive isolates to understand their epidemiology.MICs were determined by BSAC agar dilution. *bla*GES PCR amplicons were sequenced to define variants. For GES-positive *P. aeruginosa* isolates, MLST data were inferred from MLVA profiles. For GES-positive Enterobacteriaceae, plasmid DNA was transformed into *E. coli* DH5α and whole genomes of clinical isolates and GES transformants were sequenced. Plasmid contigs were subtracted from the DH5α genome and assembled using SPAdes. WGS data were analysed to determine bacterial identification, MLST, total plasmid *rep* types and resistance gene complements.*bla*GES alleles were found in 76 UK isolates (2 in 2012, 6 in 2013, 14 in 2014, 54 in 2015). GES-carbapenemase positive *P. aeruginosa* (n=42) had GES-5 and were submitted by 7 labs from 21 patients and 2 environmental samples. Two labs accounted for most submissions: NE-1 (n=26) and London-1 (n=10). Markers of GES-5 in *P. aeruginosa* antibiograms were MICs (mg/L) of imipenem (IPM) and meropenem (MEM) >32, IPM-EDTA ≥16, and ceftazidime (CAZ) 8-16. Typing of 34 isolates found all belonged to ST235. Most (33/34) GES-positive Enterobacteriaceae also had GES-5 (23 *K. oxytoca*, 6 *E. cloacae*, 4 *K. pneumoniae*) but 1 *Raoultella terrigena* had GES-24. These were submitted from 6 labs, from 15 patients and 12 wastewater samples; lab London-2 accounted for 18 *K. oxytoca* isolates. β-lactam MICs varied (IPM/MEM 0.5 - >32, IPM-EDTA 0.5 - >16, CAZ 8 - >256) with the underlying carbapenem resistance mechanism wrongly inferred as ESBL and impermeability in some antibiograms. MLST profiles were unique to each lab. *bla*GES-5 was located on IncQ (8.3 - 9kb), IncP-6 (25.4 kb) and untypable (76.4 kb) plasmids.GES-positive isolates are currently limited to a few scattered centres. Whilst a characteristic antibiogram and ST235 aids identification of GES-positive *P. aeruginosa*, the lack of specific antibiogram markers and strain variability makes interpretative reading less reliable for detection of GES in Enterobacteriaceae. GES enzymes are not reliably detected by carbapenemase activity assays or covered by commercial molecular assays, thereby complicating their detection.

Author Disclosure Block:

K.L. Hopkins: None. **J. Findlay:** None. **D. Meunier:** None. **D. Godoy:** None. **J. Turton:** None. **R. Hill:** None. **N. Woodford:** None.

Poster Board Number:

SUNDAY-264

Publishing Title:

Tracking KPC-Producing *Klebsiella pneumoniae* in a Large Healthcare Facility Using Whole Genome Sequencing

Author Block:

F. Onmus-Leone, P. T. Mc Gann, E. Snesrud, R. Maybank, A. C. Ong, R. J. Clifford, M. K. Hinkle, E. P. Lesho; WRAIR, Silver Spring, MD

Abstract Body:

Klebsiella-producing carbapenemase (KPC), encoded by bla_{KPC}, is the most common carbapenemase gene in North America. It confers resistance to all β-lactams, including carbapenems, and had become endemic in some areas of the U.S.A. In this study, we sought to unravel the epidemiology of KPC-producing *Klebsiella pneumoniae* from a large healthcare facility in the eastern U.S.A.Short and long-read whole genome sequencing (WGS) was performed on 31 bla_{KPC} -positive *K. pneumoniae* clinical samples from 17 patients using the Illumina Miseq and PacBio RSII platforms, respectively. The samples were collected between 2009 and 2015 at a 274-bed hospital that serves over 1 million beneficiaries annually.The majority of isolates (54.8%) were cultured from groin and rectal surveillance swabs, followed by urine (29%), tissue (6.4%), blood (2.4%) and respiratory (1.2%) samples. Isolates from the same patients were genetically identical, but no two patients shared the same isolate, indicating that nosocomial transmission was not a factor in the spread of KPC-producing *K. pneumoniae* in the hospital. *In silico* multi-locus sequence type (MLST) revealed six ST's circulating within the community, with ST-258 and ST-340 predominating (12 and 10 isolates respectively). Only bla_{KPC-2} (N=10) and bla_{KPC-3} (N=21) variants were identified; All ST-340 isolates carried bla_{KPC-3}, whereas one-third of ST-258 isolates carried bla_{KPC-2}. Every isolate carried from 1 to 8 plasmids representing the major incompatibility (Inc) groups, including IncA/C2, FIB, FII, H1B, L/M, R, and IncX3. However, bla_{KPC} was harbored on IncF1B or IncFII in 50% of the isolates.Epidemiological data indicates that multiple strains are circulating within the community, but there was no evidence for nosocomial transmission in the healthcare facility. The majority ofisolates belonged to ST-258 and ST-340, two prominent members of the globally distributed clonal complex 11. This diversity was reflected in the plasmids harboring bla_{KPC}, which spanned a wide range of sizes and Inc types.

Author Disclosure Block:

F. Onmus-Leone: None. **P.T. Mc Gann:** None. **E. Snesrud:** None. **R. Maybank:** None. **A.C. Ong:** None. **R.J. Clifford:** None. **M.K. Hinkle:** None. **E.P. Lesho:** None.

Poster Board Number:

SUNDAY-265

Publishing Title:

Increasing B Lactam and Carbapenem Resistance in Mdr Enterobacteriaceae Isolates from Human Health Care Associated Infections in Egyptian Hospitals Between 2000-2011

Author Block:

E. Newire[1], S. F. Ahmed[2], N. Woodford[3], A. P. Roberts[1]; [1]UCL Eastman Dental Inst.. Univ. Coll. London, London, United Kingdom, [2]US. NAMRU-3, Cairo, Egypt, [3]Publ. Hlth.England, London, United Kingdom

Abstract Body:

Co-production of class-D carbapenemase OXA-48 and Extended Spectrum β-Lactamase-(ESBL) CTX-M-15 in *Escherichia coli* and *Klebsiella pneumoniae* confer broad beta-lactam resistance and is increasingly reported. The objectives of this study were to phenotypically and genotypically characterize two groups of Enterobacteriaceae isolates collected from Egypt.One hundred and sixty-nine ESBL producing Enterobacteriaceae from health care associated infections were collected from 10 Egyptian hospitals in two surveillance studies (Group 1: 65 *K. pneumoniae* collected between 2000-2003, and Group 2: 41 *E. coli* and 63 *K. pneumoniae* isolates collected from 2009-2011). ESBLs and carbapenemase production was assessed with double disc-synergy and E-test. $bla_{\text{OXA-48}}$, $bla_{\text{CTX-M-15}}$, and bla_{VIM} genes were identified by PCR and DNA sequencing.Phenotypic tests confirmed all isolates as ESBL producers with co-carriage of the Non-metallo-carbapenamse $bla_{\text{OXA-48}}$ and ESBL $bla_{\text{CTX-M-15}}$ observed in 11% (n=7) of isolates in Group 1 increasing to 92% (n=96) in group 2. Metallo-carbapenamse bla_{VIM} was detected 34% (n=22) in group 1 isolates increasing to 77% (n=80) in group 2. Additionally 8% (n=5) of isolates from group 1 and 24% (n=25) from group 2 also produced MBL. KPC was produced by 8% (n=5) of group 1 isolates increasing to 10% (n=10) in group 2.We have shown in this study that different carbapenem-hydrolysing β lacatamses of three ambler classes are carried and expressed among MDR enterobacteriaecae isolates from Egypt and their prevalence increased from 2000 to 2011.

Author Disclosure Block:

E. Newire: None. **S.F. Ahmed:** None. **N. Woodford:** None. **A.P. Roberts:** None.

Poster Board Number:

SUNDAY-266

Publishing Title:

Detection of Plasmid-borne Ndm-1 Gene in *Enterobacteriaceae* Isolated from In-patients and Out-patients Visiting Selected Health Facilities in Cross River State, Nigeria

Author Block:

A. E. Asuquo, U. I. Ulom, E. O. Ibeneme, S. J. Utsalo; Univ. of Calabar, Calabar, Nigeria

Abstract Body:

Background: *Enterobacteriacae* expressing the carbapenemase NDM-1 gene are emerging worldwide. This plasmid-mediated gene codes for resistance to carbapenems, which are regarded as the last resort for the treatment of multidrug resistant bacterial infections. This study aimed at detecting the presence of NDM-1 gene on plasmids of clinical and community enterobacterial isolates in Calabar, Nigeria. The performance of modified Hodges test (MHT) and re-modified Hodges test (rMHT) in the phenotypic detection of carbapenemase production were compared. **Methods:** A total of 79 *Enterobacteriaceae* were isolated from meropenem-supplemented agar (1µg/mL) inoculated with urine and stool samples of out-patients and in-patients attending selected health facilities. They include *Klebsiella pneumoniae* (32), *Escherichia coli* (25), *Proteus vulgaris* (13), *Proteus mirabilis* (1), *Providencia stuartii* (4) and *Serratia rubidaea* (4). The susceptibility of the isolates to antimicrobial agents was performed by the disc diffusion method. Phenotypic detection of carbapenemase production was determined using MHT and rMHT. Plasmids from isolates were analyzed by PCR using specific primers for NDM-1.**Results:** Of the 79 enterobacterial isolates, 86% (68/79) were resistant to at least one of 10 antibiotics tested and about 40% (27/68) of these were resistant to more than half of the antibiotics tested. The phenotypic test for carbapenemase production revealed that 33% (26/79) and 19% (15/79) of isolates showed correlating positive results and conflicting results respectively for MHT and rMHT. Two *Klebsiella pneumoniae* isolates from two separate urine samples harboured the NDM-1 gene; both isolates were sensitive only to tigecycline and were MHT and rMHT positive. Both patients from whom the urine specimens were obtained had previously visited India for medical treatment.**Conclusion:** To the best of our knowledge, this is the first report of the NDM-1 gene in Nigeria. It further underscores the origin of the gene and its rapid spread. This has grave public health implications for Nigeria as India remains a major medical tourism destination for Nigerians.

Author Disclosure Block:

A.E. Asuquo: None. **U.I. Ulom:** None. **E.O. Ibeneme:** None. **S.J. Utsalo:** None.

Poster Board Number:

SUNDAY-267

Publishing Title:

Higher Risk of Esbl-producing *Escherichia coli* Infection by the Large-scale Chicken Farming Style in Vietnam

Author Block:

B. T. K. Ngan[1], B. T. M. Huong[1], I. Hirai[2], S. Ueda[2], N. Q. Anh[1], L. D. Tuyen[1], Y. Yamamoto[3]; [1]Natl. Inst. of Nutrition, Hanoi, Viet Nam, [2]Univ. of the Ryukyus, Okinawa, Japan, [3]Osaka Prefectural Inst. of Publ. Hlth., Osaka, Japan

Abstract Body:

Background: CTX-M type of extended-spectrum β-lactamase (ESBL) is dominant antibiotic resistant mechanism of bacteria in nosocomial and community-acquired infections. Large-scale farm (LSF) and medium-scale family farm (MSFF) are two main poultry husbandry styles in Vietnam. In this study, we examined whether there was any difference of ESBL-producing *Escherichia coli* in the two poultry farming styles**Methods:** A total of 149 fecal swabs were collected from 24 human individuals and 38 chickens of one LSF and from 51 human and 36 chickens of 3 MSFF between June, 2013 and June, 2014 in a Vietnamese rural area. One typical *E. coli* isolate of each sample, which had been screened with 1μg/ml of cefotaxime, was subjected to antibiotic resistance test and to molecular characterization. Genetic relatedness was assessed by pulsed-field gel electrophoresis (PFGE). Genotypes of $bla_{\text{CTX-M}}$ was determined by sequencing analysis. Statistical significance was calculated by Student's *t*-test**Results:** Prevalence of ESBL-producing *E. coli* in the LSF human (100%) was significantly higher than one of the MSFF (65%). A significant difference ($p < 0.01$) was obtained in prevalence of ESBL-producing *E. coli* from chicken samples between the two farming styles (79% of the LSF and 42% of the MSFF). In isolates from human samples, $bla_{\text{CTX-M-27}}$ (56.6%) was predominant, whereas majority in isolates from chicken samples was $bla_{\text{CTX-M-14}}$ (46.9%). PFGE results suggested certain clonal distributions in the isolates from the LSF chicken**Conclusions:** Higher prevalence and antibiotics resistance of *E. coli* isolates producing ESBL were observed in both of human and chicken of the LSF. These results indicate relatively higher risk of ESBL-producing bacterial infection in large-scale farming style. Further studies are needed to elucidate transmission and distribution mechanism of ESBL-producing bacteria between chicken and human

Author Disclosure Block:

B.T.K. Ngan: None. **B.T.M. Huong:** None. **I. Hirai:** None. **S. Ueda:** None. **N.Q. Anh:** None. **L.D. Tuyen:** None. **Y. Yamamoto:** None.

Poster Board Number:

SUNDAY-268

Publishing Title:

Geographic Distribution of Carbapenem-resistant Gram-negative Infections in Adult Patients in US Hospitals

Author Block:

B. Cai[1], R. Echols[1], G. Magee[2], J. Arjona Ferreira[1], M. Ariyasu[3], T. Sawada[3], T. Nagata[3]; [1]Shionogi Inc., Florham Park, NJ, [2]Premier Res. Service, Inc., Charlotte, NC, [3]Shionogi & Co., Ltd., Osaka, Japan

Abstract Body:

Background: Carbapenem-resistant (CR) Gram-negative infections, especially KPC- producing Enterobacteriaceae, have received much attention; however, CR non-fermenters, *A. baumannii* and *P. aeruginosa,* may have a greater clinical impact. In the US, there may be regional differences in the frequency of infections due to each of these CR pathogens that need to be explored. **Methods:** Hospitalized patients with infections due to laboratory-confirmed Gram-negative pathogens from specified infection sites between 2009 and 2013 were identified from electronic health records of Premier Healthcare Database. Based on the total number of infections for each pathogen and infection site (respiratory, blood, urine, other) in each geographic region defined by one of nine US Census Bureau Divisions, the proportion of CR infections was calculated. This study describes the geographic distribution of CR *A. baumannii, P. aeruginosa, K. pneumoniae,* and *E. coli* among adult patients in US hospitals by regions. **Results:** From 2009 to 2013, 13,262 (4.5%) of 292,742 Gram-negative infections were caused by CR pathogens. Of these CR infections, 82.3% were caused by *A. baumannii* and *P. aeruginosa*, while 17.7% were caused by *K. pneumoniae* and *E. coli*. Carbapenem resistance among *A. baumannii* and *P. aeruginosa* was 44.8% and 14.2%, respectively. *P. aeruginosa* accounted for 60.3% of all CR infections while *A. baumannii* accounted for 22%.Differences among nine regions were noted: Middle Atlantic region had the highest proportion of CR infections due to *K. pneumoniae* and *E. coli* (33.1%) whereas four regions (East South Central, Mountain, New England, and West South Central) had less than 10% CR infections due to these two pathogens. Among CR infections in the New England region, 85% were caused by *P. aeruginosa*.Respiratory tract is the most common site of CR infection for *A. baumannii* (40.3%) and *P. aeruginosa* (40.1%) while urine is the most common site of CR infection for *K. pneumoniae* (48.7%) and *E. coli* (57.3%). **Conclusions:** The vast majority of CR infections in the US are caused by non-fermenters *A. baumannii* and *P. aeruginosa.* There was larger variability in CR infections due to *K. pneumoniae* and *E. coli* in different US geographic regions than non-fermenters.

Author Disclosure Block:

B. Cai: None. **R. Echols:** None. **G. Magee:** None. **J. Arjona Ferreira:** None. **M. Ariyasu:** None. **T. Sawada:** None. **T. Nagata:** None.

Poster Board Number:

SUNDAY-269

Publishing Title:

Phenotypic And Multiplex Pcr Detection Of Esbl Resistant Genes In Gram Negative Bacilli Isolated From Calabar, Nigeria

Author Block:

E. O. Ibeneme[1], A-A. O. Eyo[1], E. J. Okoi[2], S. M. Obeten[2], A. E. Asuquo[1], S. J. Utsalo[1]; [1]Univ. of Calabar, Calabar, Nigeria, [2]Dr. Lawrence Henshaw Mem. Specialist Hosp., Calabar, Nigeria

Abstract Body:

Background: The occurrence and spread of plasmid-borne extended-spectrum β-lactamases (ESBLs) among Gram negative bacilli (GNB) is a growing concern worldwide.This study compared phenotypic and multiplex PCR protocols in the detection of ESBL genes carried on plasmids of Gram negative bacilli obtained from chronic cough patients attending a tuberculosis referral hospital in Calabar, Nigeria. **Methods:** A total of 180 GNB were screened for ESBL production using five ESBL indicator antibiotics (cefpodoxime, ceftriaxone, ceftazidime, cefotaxime and aztreonam) and further confirmed using the combined disc test (CDT) and double-disc synergy test (DDST). Plasmids from isolates which were positive after screening for ESBL production, were subjected to multiplex PCR using gene-specific primers to detect the presence of ESBL genes (*bla*$_{\text{TEM}}$, *bla*$_{\text{SHV}}$) and groups of CTX-M-type genes (*bla*$_{\text{CTX-M-1,2,8,9,25}}$).**Results:** Of the 180 isolates, 31% (55/180) were resistant to more than half of the antibiotics tested. A total of 136 (75.6%) isolates were resistant to at least one of the ESBL indicator antibiotics (EIAs) of which 48 (35.3%) were resistant to all five EIAs. The phenotypic methods (CDT and DDST) detected ESBL production in 11 (8.1%) and 46 (33.8%) isolates respectively compared with 87% (118/136) using multiplex PCR. About 11% (15/136) and 23% (31/136) of isolates showed correlating positive and conflicting CDT and DDST test results respectively. A total of 98 (72.1%) isolates harboured single ESBL genes: *bla*$_{\text{TEM}}$ in 94 isolates, *bla*$_{\text{CTX-M-1}}$ in 2 isolates, while one isolate each had *bla*$_{\text{CTX-M-2}}$ and *bla*$_{\text{CTX-M-9}}$. Eighteen (13.2%) isolates harboured two ESBL genes: 5 with *bla*$_{\text{TEM}}$ and *bla*$_{\text{SHV}}$, 12 with *bla*$_{\text{TEM}}$ and a *bla*$_{\text{CTX-M}}$ type, one with *bla*$_{\text{SHV}}$ and *bla*$_{\text{CTX-M-1}}$. Two (1.5%) isolates harboured all 3 ESBL gene types.**Conclusions:** Multiplex PCR enables quick and cost-effective detection of ESBL genes and eliminates the limitations of phenotypic methods.

Author Disclosure Block:

E.O. Ibeneme: None. **A.O. Eyo:** None. **E.J. Okoi:** None. **S.M. Obeten:** None. **A.E. Asuquo:** None. **S.J. Utsalo:** None.

Poster Board Number:

SUNDAY-270

Publishing Title:

First Report of New Delhi Metallo-β-Lactamase-4 (Ndm-4) and New Delhi Metallo-β-Lactamase-5 (Ndm-5) in Egypt

Author Block:

H. O. Khalifa, A. M. Soliman, A. M. Ahmed, T. Shimamoto, **T. Shimamoto**; Hiroshima Univ., Higashi-Hiroshima, Japan

Abstract Body:

Carbapenemase-producing Enterobacteriaceae are a serious problem worldwide. Among the newly emerging carbapenemases, New Delhi metallo-β-lactamase (NDM) is among the antimicrobial resistance factors causing greatest concern because of its rapid global spread and frequent association with other resistance genes. NDM-4 and NDM-5 differ from NDM-1 by one and two amino acid substitutions, respectively, and have stronger carbapenemase activity than NDM-1. The prevalence of both genes in the Gram-negative bacteria is unclear, especially in developing countries. We report the first detection of NDM-4- and NDM-5-producing organisms in Egypt.A total of 136 nonduplicate Gram-negative isolates were recovered from clinical samples from different hospitals in Egypt. PCR and sequencing identified one *Klebsiella pneumoniae* strain (KPE127) carrying $bla_{\text{NDM-4}}$ and two isolates (*K. pneumoniae* KPB140 and *Escherichia coli* ECW169) carrying $bla_{\text{NDM-5}}$. Interestingly, NDM-4- and NDM-5-producing *K. pneumoniae* were isolated from a 6-month-old child suffering pneumonia after the transposition of the great arteries with an atrial septostomy and intracranial hemorrhage. NDM-5-producing *E. coli* ECW169 was isolated from a wound pus swab sample from a 65-year-old patient.The three strains were resistant to different classes of antibiotics. PCR and DNA sequencing confirmed that the three isolates also carried other resistance genes.The genetic environments of the $bla_{\text{NDM-4}}$ and $bla_{\text{NDM-5}}$ genes were analyzed with PCR mapping. We identified an insertion sequence, IS*Aba125*, upstream and the bleomycin-resistance gene, ble_{MBL}, downstream. Multilocus sequence typing showed that *K. pneumoniae* KPE127 and *K. pneumoniae* KPB140 belonged to ST45, corresponding to the ST of an NDM-1-positive *K. pneumoniae* identified in Turkey. *Escherichia coli* ECW169 belonged to ST5018, unlike any other known NDM-5-producing *E. coli*.This study confirms the presence of NDM-4- and NDM-5-producing bacteria in Africa, after their recent identification in Cameroon and Algeria. The worldwide dissemination ofNDM-4 and NDM-5 producers is alarming, particularly because their carbapenemase activity exceeds that of NDM-1. Therefore, screening for NDM producers in developing countries is essential if we are to prevent their future epidemic spread.

Author Disclosure Block:

H.O. Khalifa: None. **A.M. Soliman:** None. **A.M. Ahmed:** None. **T. Shimamoto:** None. **T. Shimamoto:** None.

Poster Board Number:

SUNDAY-271

Publishing Title:

β-Lactamases Conferring Resistance to Third Generation Cephalosporins in *Escherichia coli* from Pets in Argentina

Author Block:

V. Rumi, E. Gentilini, J. Di Conza, **G. Gutkind**; UBA, BA, Argentina

Abstract Body:

Background: The spread of antimicrobial resistance and the emergence of multidrug-resistant (MDR) pathogens increased in the last decade. The production of extended spectrum β-lactamases (ESBL) is a common cause of resistance to third generation cephalosporins (TGC) and it is considered a concern problem both in human and veterinary medicine. Genes encoding resistance to TGC are usually found in transmissible plasmids and are often associated to other resistance determinants. The aim of this study was to determine the resistance profile of *E. coli* isolated from pet and to characterize the resistance mechanisms to TGC.**Methods:** A total of 54 *E. coli* isolates were collected from clinical samples in dogs and cats. Resistance was determined by the disk diffusion method according to CLSI VET01-A4 and VET01-S2 recommendations. Screening for ESBL and plasmid AmpC β-lactamases (AmpCp) were conducted by double disk synergy tests. ESBL and AmpCp determinants were investigated by PCR. Clonal relatedness was investigated by REP/ERIC-PCR. The main phylogenetic groups in *E. coli* isolates were assessed by PCR according to Clermont *et al*.**Results:** Of the 54 strains, 31 (57%) were resistant to AMP and 20/31 (64%) isolates were also resistant to TGC. No carbapenem resistance was observed. Seven isolates were characterized as ESBL producers, while 13/20 as AmpC carriers. Most of these isolates belong to the phylogenetic group D (14/20) and to a lesser extent B2 (4/20) and B1 (2/20). In ESBL-producing isolates were detected the $bla_{\text{CTX-M-2}}$ group (4), $bla_{\text{CTX-M-1}}$ group (2) and $bla_{\text{CTX-M-9}}$ group (1) genes. Thirteen AmpC-producing strains were positive for bla_{CMY}-like genes and all of them were clustered in a single electropherotype. A significant proportion of AMP resistant strains were also resistant to quinolones [NA: 80.6% (25), CIP: 67.7% (21) and LEV: 61.3% (19)] and TMS [64.5% (20)]. Finally, 58.1% (18) isolates showed a profile of MDR. **Conclusions:** AmpCp constitute the main TGC resistance mechanism in *E. coli* isolated from pets in our region. Cefotaximases belonging to three genetically different groups were also found being CTX-M-2 group the commonest amongst *E. coli*. It is important to highlight the high level of resistance to different families of antimicrobial (commonly used in pets) associated with AMP resistance.

Author Disclosure Block:

V. Rumi: None. **E. Gentilini:** None. **J. Di Conza:** None. **G. Gutkind:** None.

Poster Board Number:

SUNDAY-272

Publishing Title:

Characterization of Multi-Resistant *K. pneumoniae* Isolates from Romania, Region Cluj

Author Block:

M. Tompa[1], R. G. Otto[2], J. D. D. Pitout[3], G. Peirano[3], **W. H. F. Goessens**[2]; [1]Central laboratory of the Regional Inst. of Gastroenterology and Hepatology "Prof. Dr. O. Fodor", Cluj, Romania, [2]Erasmus Univ. Med. Ctr. Rotterdam, Rotterdam, Netherlands, [3]Univ. of Calgary, Calgary, AB, Canada

Abstract Body:

Background: Antimicrobial resistance is increasing worldwide and especially the increasing resistance to the last resort antibiotics i.e. carbapanems is alarming. Also in Romania a limited number of studies reported the presence of carbapenemase-producing Enterobacteriaceae. Based on these findings we prospectively collected in 2013 in the Cluj region of Romania, Enterobacteriaceae resistant to imipenem and/or meropenem. These isolates are characterized by PCR for the detection of carbapenemase-encoding genes. **Methods:** 77 *K. pneumoniae* isolates collected during 2013 at the clinic of Gastroenterology and Hepatology at Cluj were included for further characterization. The identification of the isolates was confirmed by MALDI-TOF. Susceptibility determinations were performed in the VITEK 2 system. The presence of bla_{KPC}, bla_{NDM}, $bla_{\text{OXA-48-like}}$, bla_{VIM} *and* bla_{IMP} like genes were determined by PCR.**Results:** Susceptibility determinations revealed 30 isolates with MICs for meropenem (MER) of $\geq$ 16 µg/ml and imipenem (IMP) of 2 - 8 µg/ml (group A), 27 isolates with MICs for MER and IMP of $\geq$ 16 µg/ml (group B), 2 isolates (group C) with MICs for MER of 4 µg/ml and of $\geq$ 16 µg/ml for IMP and 4 isolates (group D) with MICs for MER of 4 µg/ml and MICs of 1-2 µg/ml for IMP. The remaining 14 isolates had MICs for MER of 0.5 - 1 µg/ml and $\leq$ 0.25 µg/ml for IMP. All isolates belonging to group A, B and C were positive for the $bla_{\text{OXA-48-like}}$ gene. In group D only one isolate was $bla_{\text{oxa-48-like}}$ positive. In addition to the OXA-48 gene, two isolates of group B also harbored a NDM-gene.**Conclusion**: In the majority of the *K. pneumoniae* isolates a $bla_{\text{OXA-48-like}}$ gene was demonstrated and correlates with the reduced susceptibility to the carbapenems. The spread in the MICs for MER and IMP probably reflects the presence of additional mechanisms of resistance like efflux pumps and/or reduced permeability of the outer membrane. The present results demonstrate the further spread of the $bla_{\text{OXA-48-like}}$ gene into Eastern-Europe.

Author Disclosure Block:

M. Tompa: None. **R.G. Otto:** None. **J.D.D. Pitout:** None. **G. Peirano:** None. **W.H.F. Goessens:** None.

Poster Board Number:

SUNDAY-273

Publishing Title:

Promises and Pitfalls of Illumina Sequencing for Resistance Plasmid Tracking

Author Block:

A. E. Sheppard[1], N. Stoesser[1], R. Sebra[2], A. Kasarskis[2], A. S. Walker[1], T. E. Peto[1], D. W. Crook[1], A. J. Mathers[3]; [1]Univ. of Oxford, Oxford, United Kingdom, [2]Icahn Sch. of Med. Mount Sinai, New York, NY, [3]Univ. of Virginia, Charlottesville, VA

Abstract Body:

Antibiotic resistance is a major public health concern. In *Enterobacteriaceae*, resistance genes are often located on mobile plasmids. Traditional methods for plasmid investigation are laborious and/or provide limited resolution. Whole genome sequencing is a high throughput, cost effective tool for bacterial genomics, and is attractive for plasmid analysis. However, short read sequencing (e.g. Illumina) is not ideal for plasmid reconstruction due to repeat structures, and plasmids are generally fragmented in *de novo* assemblies. Here we explore various approaches for plasmid analysis using Illumina data, and demonstrate that naïve interpretation can be misleading.We performed whole genome Illumina sequencing on 281 carbapenem resistant *Enterobacteriaceae* isolated from a hospital bla_{KPC} outbreak over five years. This outbreak shows high levels of diversity in species, strains and plasmids carrying the bla_{KPC} resistance gene, providing an ideal framework for testing plasmid analysis methods given frequent resistance gene mobility. We use a combination of *de novo* assembly and mapping approaches to investigate methods for accurately inferring plasmid presence and structure. We also performed long read PacBio sequencing on 23 isolates, providing 97 complete, closed plasmid sequences for comparison with Illumina data, including 22 bla_{KPC} plasmids.We explore various methods for defining plasmid presence and show that due to frequent rearrangements, this is not a straightforward concept. Further, presence does not always correlate with expected plasmid structure and can be misleading for resistance plasmid tracking. For example, for four plasmids where structure could be resolved from Illumina data, bla_{KPC} was contained in the plasmid in only 1/20, 3/10, 3/5 and 45/52 isolates in which the plasmid was present. PacBio sequencing revealed similar patterns for plasmids where structure was unresolvable from Illumina data due to the presence of long repeats.We demonstrate important limitations for resistance plasmid analysis from Illumina data, and highlight how erroneous conclusions can easily be obtained through naïve interpretation. We provide suggestions for how to avoid this while maximising data potential.

Author Disclosure Block:

A.E. Sheppard: None. **N. Stoesser:** None. **R. Sebra:** None. **A. Kasarskis:** None. **A.S. Walker:** None. **T.E. Peto:** None. **D.W. Crook:** None. **A.J. Mathers:** None.

Poster Board Number:

SUNDAY-274

Publishing Title:

Genomic Tracking of Carbapenem-Resistant *Serratia marcescens* in a Hospital Setting

Author Block:

N. Pecora[1], N. Li[1], X. Zhao[1], M. Allard[2], A. Onderdonk[1], L. Bry[1]; [1]Brigham and Women's Hosp., Boston, MA, [2]U.S. Food and Drug Admin., College Park, MD

Abstract Body:

Background: *Serratia* species are ubiquitous in the environment and can be a cause of serious nosocomial infections. Carbapenem resistance has been sporadically reported, due to both intrinsic and horizontally-acquired elements, though relatively few clinical isolates have been sequenced. In this study 11 carbapenem-resistant clinical isolates of *Serratia marcescens* collected over a 3 year period were sequenced in order to identify the underlying causes of carbapenem resistance, other resistance and virulence factors, and potential patient and hospital reservoirs. **Methods:** 11 isolates of *Serratia marcescens* were collected between 2011-2014 from 6 different patients. All strains were resistant to either imipenem or imipenem and meropenem. Sequencing was done on a Miseq (Illumina, 150-300bp reads). Assembly, beta-lactamase, transposon, and plasmid analysis were done on in-house pipelines using a combination of *de novo* (SPAdes) and reference-based (Bowtie-2) aligners. SNP analysis was done using CSI-phylogeny (cge.cbs.dtu.dk). Annotation was done with RAST (rast.nmpdr.org). Multiple alignments were done using the MAUVE aligner.**RESULTS:** The strains formed 4 clusters with 32-77000 SNPs between strains. In some cases, closely related strains occurred years apart, raising concern for an environmental hospital reservoir. Isolates had 0-2 plasmids (representing INC F, R, and L/M groups). In two clusters, carbapenem resistance was due to mobile carbapenemases (SME-4 and KPC-3). All strains harbored a chromosomal AmpC beta-lactamase as well as SHV and OXA-family enzymes. Resistance determinants to aminoglycosides, sulfonamides, and tetracyclines were present in a majority of the strains. Other virulence factors included type VI secretion systems and hemolysins.**Conclusions:** *Serratia marcescens* is an increasingly recognized nosocomial pathogen, whose combination of intrinsic and acquired resistance presents major treatment challenges. In this study, the sequencing of several clinical isolates illuminates the genomic landscape of carbapenem-resistant strains in a hospital setting. This consisted of multiple clusters of organisms, some with enzymes/mechanisms specific to *Serratia* and some with elements, such as KPC-3, likely acquired from other *Enterobacteriaceae*.

Author Disclosure Block:

N. Pecora: None. **N. Li:** None. **X. Zhao:** None. **M. Allard:** None. **A. Onderdonk:** None. **L. Bry:** None.

Poster Board Number:

SUNDAY-275

Publishing Title:

Genomic Evaluation of Nosocomial *Klebsiella pneumoniae* Carbapenemase Producing *Serratia marcescens* (KPC-Sm)

Author Block:

A. J. Mathers[1], N. Stosser[2], J. Carroll[1], J. Ainsworth[1], C. Sifri[1], D. Crook[2], A. E. Sheppard[2]; [1]Univ. of Virginia, Charlottesville, VA, [2]Oxford Univ., Oxford, United Kingdom

Abstract Body:

Background: Carbapenem resistant Enterobacteriaceae are an urgent threat to hospitalized patients. *S. marcescens* is a nosocomial pathogen with a significant degree of inherent resistance to last line drugs such as colistin. The KPC gene (bla_{KPC}) is usually carried on the transposon Tn*4401* within mobile plasmids and acquisition by *S. marcescens* results in highly antibiotic resistant strains. We investigated how KPC-Sm manages plasmid DNA and is transmitted nosocomially over extended time periods.**Methods:**KPC-producing Enterobacteriaceae were prospectively identified in a clinical lab between 8/2007-4/2015. All available isolates were Illumina sequenced and select isolates PacBio sequenced to understand population and plasmid structures in the context of an ongoing multi-species outbreak.**Results:**There were 60 KPC-Sm isolates from 36 patients from the following sources: peri-rectal surveillance (63%), respiratory (12%), blood (10%), urine (8%), and abdominal (7%). Ten (28%) patients were co-colonised/infected with another blaKPC-Gammaproteobacteria in addition to KPC-Sm. The first KPC-Sm was identified in December 2011; by 2014 KPC-Sm was the predominant species (36 KPC-Sm versus 34 KPC-*K. pneumoniae*; KPC-*Citrobacter freundii* as the next most common [n=13]). All KPC-Sm were highly genetically homogeneous (max 61 pairwise SNVs) indicating a stably circulating clone. There was a unique Tn*4401* variant found in all but one patient with KPC-Sm. This variant was only found in other species (*E. coli, Aeromonas* spp., *Enterobacter aerogenes*) in patients who also had KPC-Sm, indicating likely horizontal gene transfer from KPC-Sm. PacBio sequencing revealed a conserved 69 kb bla_{KPC} plasmid in all 4 tested isolates. Illumina data was consistent with this plasmid being present in 97% of KPC-Sm strains.**Conclusion:**We describe sustained transmission of a KPC-Sm clone with a conserved plasmid which evolved to be the predominant species within a multi-species outbreak. KPC-Sm may represent a persistent source of bla_{KPC} in nosocomial settings, as demonstrated here. Understanding the role of *S. marcescens* in the spread of drug resistance genes could be critical to combating carbapenemase producing isolates around the world.

Author Disclosure Block:

A.J. Mathers: None. **N. Stosser:** None. **J. Carroll:** None. **J. Ainsworth:** None. **C. Sifri:** None. **D. Crook:** None. **A.E. Sheppard:** None.

Poster Board Number:

SUNDAY-276

Publishing Title:

Dissemination of *Escherichia coli* Co-Producing Ndm-5 and Mcr-1 in a Chicken Farm

Author Block:

L. Lv, J. Wang, X. Yao, Z. Zeng, **J-H. Liu**; South China Agricultural Univ., Guangzhou, China

Abstract Body:

Background: Recently, reports of carbapenemase-producing bacterium from animal sources are increasing. Until now, all these animal original carbapenemase producers were selected by plates supplied with carbapenems, detection of carbapenemase gene among randomly isolated organisms from animals have not been reported. Plasmid-mediated colistin resistance gene *mcr-1* has been emerged and caused great concern all over the world. Here we report the emergence of *E. coli* co-producing NDM-5 and MCR-1 in one chicken farm in China. **Methods:** In 2015, 21 commensal *E. coli* were recovered from healthy chickens of a farm located in Anhui province of China. MICs of various antimicrobial agents were determined by agar dilution method. The presence of carbapenemase genes and *mcr-1* were determined by PCR. The clonal relatedness of NDM-5 producers was determined by PFGE and multi-locus sequence typing (MLST). Transferability and location of of $bla_{\text{NDM-5}}$ and *mcr-1* genes were investigated by conjugation experiment, S1-PFGE, and hybridization. The genetic context of $bla_{\text{NDM-5}}$ gene was determined by PCR mapping and sequencing. **Results:** Five (23.8%) *E. coli* isolates showed reduced susceptibility to imipenem (MIC≥1 mg/L) and were positive for both $bla_{\text{NDM-5}}$ and *mcr-1*. Three of them had very similar PFGE patterns and belonged to ST10, the other two isolates were both typed as ST5442. All $bla_{\text{NDM-5}}$ genes were located on ~40kb IncX3 plasmids and can be transferred by conjugation. Analysis of the flanking regions of the $bla_{\text{NDM-5}}$ genes revealed that IS*Aba125* was located upstream of $bla_{\text{NDM-5}}$. Downstream of the $bla_{\text{NDM-5}}$ gene, the ble_{MBL}, *trpF*, and *dsbC* genes were observed. **Conclusions:** To the best of our knowledge, this is the first report of carbapenemase gene found in randomly isolated *E. coli* from food animals. The emergence and clonal spread of commensal *E. coli* carrying both $bla_{\text{NDM-5}}$ and *mcr-1* in chicken farm is alarming. Consequently, there is urgently needed to survey the occurrence of carbapenemase genes among bacteria from food animals in order to prevent their potential spread.

Author Disclosure Block:

L. Lv: None. **J. Wang:** None. **X. Yao:** None. **Z. Zeng:** None. **J. Liu:** None.

Poster Board Number:

SUNDAY-277

Publishing Title:

Antimicrobial-loaded Bone Cement Does Not Negatively Influence Sonicate Fluid Culture Positivity for the Diagnosis of Prosthetic Joint Infection

Author Block:

K-H. Park[1], K. Greenwood-Quaintance[2], A. Hanssen[2], M. Abdel[2], R. Patel[2]; [1]Chonnam Natl. Univ. Med. Sch., Gwangu Ju, Korea, Republic of, [2]Mayo Clinic, Rochester, MN

Abstract Body:

Background: Although sonication of removed implants with culture of the resultant "sonicate fluid" has high specificity and sensitivity for the diagnosis of prosthetic joint infection (PJI), the influence, if any, of antimicrobial-loaded bone cement on sonicate fluid culture positivity has not been assessed.**Methods:** We compared sonicate fluid culture results between patients with PJI who had implants with and without antimicrobial-loaded bone cement. Implants were processed according to a previously described protocol, including sonication, vortexing, and concentration. A total of 54 patients were assessed, 18 of whom had implants with antimicrobial-loaded polymethylmethacrylate.**Results:** The sensitivity of sonicate fluid culture of removed implants was 77.8% (14 of 18) in subjects with antimicrobial-loaded cemented prostheses, and 58.3% (21 of 36) in those without antimicrobial-loaded prostheses. Among the 35 sonicate culture positive cases, seven presented within 12 months after implantation, nine presented between 13 and 24 months after implantation and 19 presented beyond 24 months of implantation.**Conclusions:** Antimicrobial-loaded bone cement did not have an obvious negative impact on the microbiologic yield from removed prostheses subjected to sonication culture.

Author Disclosure Block:

K. Park: None. **K. Greenwood-Quaintance:** None. **A. Hanssen:** None. **M. Abdel:** None. **R. Patel:** None.

Poster Board Number:

SUNDAY-278

Publishing Title:

Routine Use of a Multiplex Serological Assay in the Management of Prosthetic Joint Infection

Author Block:

A-L. Roux[1], F. El Sayed[1], T. Bauer[1], B. Combourieux[2], C. Nich[2], V. Sivadon-Tardy[1], C. Lawrence[2], J-L. Herrmann[2], J-L. Gaillard[1], **M. Rottman**[2]; [1]Hosp. Ambroise Paré, APHP, Boulogne Billancourt, France, [2]Hosp. Raymond Poincare, Garches, France

Abstract Body:

The diagnosis of prosthetic joint infections (PJI) is a critical challenge for orthopedic surgeons and infectious disease specialists. The diagnosis of PJI is often delayed because non-invasive assays lack sensitivity and specificity. We evaluated in routine practice the relevance of a novel serological multiplex immunoassay detecting antibodies against staphylococci, *Propionibacterium acnes* and *Streptococcus agalactiae* to assist in the management of PJI patients. The Luminex-based assay measures serum IgG against aa array of recombinant purified antigens from *S. aureus, S. epidermidis, S. lugdunensis, S. agalactiae* and *P. acnes* selected by immunoproteomics to specifically diagnose PJI. We reviewed the cases of patients suspected of PJI who had benefited from a BJI Inoplex™ test in a French reference center for complex bone and joint infections. PJI cases were defined microbiologically (≥2 intraoperative samples yielding the same microorganism) for confrontation of microbiological and immunoassay data. Cases of probable acute hematogenous PJI (AH-PJI) eligible for debridement and implant retention (DAIR) were further studied to document the relevance of the assay in this context.32 patients were eligible for review (patients with prosthesis and signs of infection dating tested with immunoassay results). Of the 32 patients, 20 had a total hip prosthesis, 11 a total knee prosthesis and 1 an ankle prosthesis. Sensitivity and specificity for staphylococci 90% and 90%.. The review of 7 cases of AH-PJI cases showed that the immunoassay is negative in acute settings. However, in one case of *K. pneumonia* AH-PJI, BJI Inoplex™ detected an underlying infection with *S. capitis* confirmed by culture, and in one case of *S. aureus* AH-PJI, BJI Inoplex™ detected *P. acnes* that was confirmed by a PCR hybridization assay.Conclusions: This novel multiplex serological test allows the non-invasive diagnosis of staphylococcal PJI, showing a good correlation with microbiological culture. It allows the detection of occult chronic infections in patients with AH-PJI and could help identify the best candidates for DAIR. Overall it appears to be a promising tool in the management of patients with PJI.

Author Disclosure Block:

A. Roux: None. **F. El Sayed:** None. **T. Bauer:** None. **B. Combourieux:** None. **C. Nich:** None. **V. Sivadon-Tardy:** None. **C. Lawrence:** None. **J. Herrmann:** None. **J. Gaillard:** A.

Board Member; Self; Diaxonhit. C. Consultant; Self; Diaxonhit. K. Shareholder (excluding diversitied mutual funds); Self; Diaxonhit. **M. Rottman:** C. Consultant; Self; Diaxonhit. J. Scientific Advisor (Review Panel or Advisory Committee); Self; Diaxonhit.

Poster Board Number:

SUNDAY-279

Publishing Title:

Molecular Approaches to the Rapid and Accurate Diagnosis of Lymphadenitis, Bone and Joint Infections

Author Block:

K. Ohkusu, T. Matsumoto; Tokyo Med. Univ., Tokyo, Japan

Abstract Body:

Background: The rapid and accurate diagnosis of lymphadenitis, bone and joint infections continues to be a major challenge. The objective of this study was to evaluate molecular approaches including conventional PCR, broad-range PCR amplification followed by direct sequencing to identify genomic DNA of pathogens from patients with clinically suspected lymphadenitis, bone and joint infections. **Methods:** A prospective study was carried out from January 2005 to December 2015, during which time, 85 clinical specimens from 85 patients at 56 hospitals in Japan were analyzed. For detection of bacterial DNA, broad-range PCR of the 16S rDNA was performed using the primers 8UA and 1485B. Sequences obtained from amplified bacterial DNA were compared with those available in the GenBank and/or EzTaxon databases in identification. Specific PCR assays were also used in the study. **Results:** Eighty-five clinical specimens, including 30 pus, 23 joint fluids, 17 bone tissues, 15 lymph node tissues, were analyzed. Of the 85 specimens, 22 specimens were culture positive. In 32 patients with lymphadenitis, we detected the following pathogens from 29 patients (91%): 9 *M. bovis* BCG strains, 4 *B. henselae*, 4 *M. tuberculosis*, 2 *S. aureus* (*mecA*+), 1 *B. cepacia*, 1 *M. avium*, 1 *M. colombiense*, 1 *M. genavense*, 1 *M. haemophilum*, 1 *S. aureus*, 1 *S. aureus* (*mecA*+) & CMV & EBV, 1 *S. hominis*, 1 *S. pyogenes*, and 1 *S. pyogenes* & EBV. In 24 patients with arthritis, we detected the following pathogens from 15 patients (63%): 3 *S. aureus*, 2 *H. influenzae* type b , 2 *S. pyogenes*, 1 *M. bovis* BCG strain, 1 *M. tuberculosis*, 1 *M. wolinskyi*, 1 *N. gonorrhoeae*, 1 *N. meningitidis* Y, 1 *S. epidermidis*, 1 *Streptobacillus moniliformis*, and 1 *S. dysgalactiae* subsp. *equisimilis*. In 22 patients with osteomyelitis, we detected the following pathogens from 19 patients (83%): 15 *M. bovis* BCG strains, 1 *A. israelii*, 1 *M. kansasii*, 1 *Paecilomyces lilacinus*, and 1 *S. aureus* (*mecA*+). In 5 patients with spondylitis, we detected 3 *S. aureus*, and 1 *E. coli*. In 2 patients with discitis, we detected 1 *Helicobacter cinaedi*. **Conclusions:** We confirmed that molecular detection and identification can be a reliable approach in the diagnosis and management of lymphadenitis, bone and joint infections. This is especially true when patients have received antimicrobial therapy prior to specimen collection or when the likely agents are fastidious, slow-growing or uncultivable microorganisms.

Author Disclosure Block:

K. Ohkusu: None. **T. Matsumoto:** None.

Poster Board Number:

SUNDAY-280

Publishing Title:

Comparison of Initial Stream Urine Samples and Cervical Samples for Detection of Human Papillomavirus

Author Block:

Y. Yamagishi[1], K. Izumi[2], N. Miyazaki[1], H. Suematsu[1], H. Mikamo[1]; [1]Aichi Med. Univ., Aichi, Japan, [2]Izumi Ladies Clinic, Gifu, Japan

Abstract Body:

Background: Uterine cervical cancer is a treatable and preventable cancer. Medical efforts to reduce rates of cervical cancer focus on the promotion of human papillomavirus (HPV) vaccination and the promotion of routine cervical cancer screening done by cervical cytology and cervical HPV testing. Urine-based HPV testing would be simple and noninvasive approach to screen for cervical cancer. **Methods:** Two biospecimens (clinician-taken sample from cervix and initial stream urine sample) were provided from a total of 100 healthy women attending for cancer screening provided for HPV testing. We have assessed the HPV detection rates among cervical specimens, unfractionated urine specimens and pellet fraction of urine specimens using HPV test (Anyplex II HPV28 Detection test, Seegene) and also assessed HPV prevalence.**Results:** HPV prevalence was almost similar in unfractionated and pellet fractions of urine samples with 34% and 36%, respectively. Sensitivities of HPV detection for cervical samples and pellet fractions of urine samples were almost identical with 38% and 36%, respectively. HPV detection was almost similar in women detected only 1 HPV (17/38) and women detected over 2 kinds of HPV from cervical specimens (21/38), sensitivities of HPV detection were almost similar with 100% and 95%, respectively.**Conclusions:** Comparing methodologies of collection of samples for HPV detection, there have no differences between cervical specimens and pellet fractions of urine samples. These results suggest that urine could be a good noninvasive tool to monitor HPV infection in women. Additional research in a larger and general screening population would be needed.

Author Disclosure Block:

Y. Yamagishi: None. **K. Izumi:** None. **N. Miyazaki:** None. **H. Suematsu:** None. **H. Mikamo:** None.

Poster Board Number:

SUNDAY-281

Publishing Title:

Design and Construction of Multiplex Late Pcr Assay for Genotyping High Risk Human Papillomaviruses

Author Block:

J. Sanchez, N. Sirianni, L. Wangh; Brandeis Univ., Waltham, MA

Abstract Body:

Background: Chronic infection by one or more of 13 high-risk human papillomavirus (HR-HPV) types is the major cause of cervical cancer and several other anogenital and head-and-neck neoplasias. Cancer risk is determined by HR-HPV type or combination of co-infecting types, with some HR-HPV type combinations exhibiting synergistic effects. FDA-approved methods for HR-HPV genotyping by conventional multiplex symmetric PCR do not identify all HR-HPV types and use consensus amplification primers, which favor detection of the most abundant co-infecting HR-HPV types. We have constructed a multiplex PCR assay that detects and identifies all 13 HR-HPV types in a single closed-tube assay independent of relative HR-HPV type abundance.**Methods:**This HR-HPV assay utilizes LATE-PCR, an advanced form of asymmetric PCR. The multiplex includes HR-HPV type-specific primers/probes, as well as reagents that guarantee the specificity and temperature precision of amplification and probe hybridization. Amplification and detection are carried out in a closed-tube in a Hain Fluorocycler®96 instrument. At endpoint, detection probes hybridize to single-stranded PCR products over a wide temperature range. Each HR-HPV types is identified by a unique combination of probe fluorescent color and probe melting temperature. Targets were tested in the presence of up to 5,000 copies of human genomic DNA in according with international standards for validation of HPV assays (Int. J. Cancer, 516, 2009; J. Clin Microb. 48, 4147, 2010).**Results:**The multiplex assay detected all 13 HR-HPV types, as well as the 15 most common types of viral co-infections. Thus the assay met international HPV assay validation standards for limit of and diagnostic performance for known HR-HPV types. The assay can be run alongside a multiplex LATE-PCR STI assay under development (see Sirianni and Wangh abstract).**Conclusions:**This multiplex LATE-PCR assay promises the same or more complete information of cancer risk as more complex, non-PCR HPV genotyping assays with the added convenience being in a single closed-tube format, ease of use, potential automation, and faster results. Supported by Hain Lifescience.

Author Disclosure Block:

J. Sanchez: None. **N. Sirianni:** None. **L. Wangh:** None.

Poster Board Number:

SUNDAY-282

Publishing Title:

Comparison of the Amplivue® Trichomonas Assay with the Affirm® Vpiii for the Detection of *Trichomonas vaginalis* in Vaginal Specimens

Author Block:

P. A. Granato, B. R. Alkins; Lab. Alliance of Central New York, Liverpool, NY

Abstract Body:

Background: *Trichomonas vaginalis* is the leading cause of vaginitis worldwide and produces an estimated 7.4 million infections in the U.S. annually. The purpose of this study was to evaluate the performance of the AmpliVue Trichomonas Assay (Quidel, San Diego, CA) with the AFFIRM VP III (BDMS, Sparks, MD) for the detection of *T. vaginalis* in vaginal specimens. **Methods:** Vaginal swab specimens from E.R. and outpatients were collected and placed in the AFFIRM VPIII Ambient Temperature Transport System and tested within 24 h of laboratory receipt. The AFFIRM assay was performed and the results were interpreted according to the manufacturer's instructions. Residual specimen from the AFFIRM transport tube was used for AmpliVue testing. The AmpliVue Trichomonas Assay is an isothermal, helicase-dependent amplification assay that was performed according to the manufacturer's instructions. All discordant results were arbitrated by using the Solana® Trichomonas Assay (Quidel) that targets a different region of DNA than the AmpliVue assay. **Results:** A total of 257 specimens were evaluated in this comparative study. One invalid AmpliVue result was obtained and that specimen was removed from the study. Of the remaining 256 specimens, 28 (10.9%) were positive by both methods. Five discordant specimens were obtained and all were positive by the AmpliVue assay but negative by the AFFIRM test. Using AFFIRM as the reference method, the AmpliVue had a sensitivity and specificity of 100% and 98% respectively. Solana arbitration testing was performed on the 5 AmpliVue positive/AFFIRM negative discordant specimens. Three specimens were confirmed as positive by the Solana test and 2 were confirmed as negative. Using Solana as the reference method, the AmpliVue and the AFFIRM tests had resolved sensitivities of 93% and 90% respectively and each had a resolved specificity of 99%. **Conclusions:** The results of this study showed that the AmpliVue Trichomonas Assay performed slightly better than the AFFIRM test when discordant results were arbitrated by an independent molecular method. The assay is easy-to-perform with a specimen time to result of less than 60 minutes and a minimum of hands-on time. The AmpliVue assay does not require specialized equipment and provides a rapid and highly reliable alternative for the molecular diagnosis of *T. vaginalis* infection.

Author Disclosure Block:

P.A. Granato: None. **B.R. Alkins:** None.

Poster Board Number:

SUNDAY-283

Publishing Title:

Rapid Detection of *Trichomonas vaginalis* with the Quidel Solana Trichomonas Assay

Author Block:

L. Fleck, H. Ty, S. Caddle, H. Kong, W. Tang; Biohelix Corp., A Quidel Company, Beverly, MA

Abstract Body:

Background: *Trichomonas vaginalis* (trichomoniasis) is the most common curable, non-viral sexually transmitted disease (STD) with an estimated 7.4 million new cases occurring annually in the U.S. The Solana Trichomonas Assay is developed based on a novel helicase-dependent isothermal amplification method (HDA) and a compact fluorescence analyzer for rapid detection of *Trichomonas vaginalis* DNA from vaginal swabs and urine samples.***Methods:*** We developed a rapid DNA amplification test for the detection of *Trichomonas vaginalis.* Simple sample preparation includes heat lysis and dilution steps followed by Helicase-Dependent Amplification (HDA) and endpoint detection by fluorescent probes. A swab or neat urine specimen is transferred into either a Swab or a Urine Lysis Tube respectively. After heating the Lysis Tube for 5-minutes, the lysed specimen is transferred into a Dilution Tube. 50 µL of diluted sample is added to a Reaction Tube and then placed into the Solana instrument for 25-minutes for amplification and detection. Twelve specimens can run as a batch in less than 35 minutes.***Results:*** Analytical studies determined that the Solana Trichomonas Assay had a limit of detection (LoD) around 306 TVs/mL in swab transport media and 108 TVs/mL in urine. More than twenty *T. vaginalis* reference strains and clinical isolates were inclusive for both the swab and urine assays tested at the 1× LoD level. Both assays do not cross-react or interfere with the 47 organisms that have been evaluated. No substance interference was detected in the swab or urine assay with the 15 and 17 substances that may exist in samples. The clinical performances of Solana Trichomonas Assay are being evaluated in a multi-center clinical study and the initial results are encouraging. Compared to a composite reference method of wet-mount microscopy plus InPouch TV culture, the Solana Trichomonas Assay showed 100% sensitivity and 99.3% specificity on 165 vaginal swabs, and 100% sensitivity and 98.5% specificity on 162 urine specimens.***Conclusion:*** The Solana Trichomonas Assay provides simple and cost-effective method for rapid detection of *Trichomonas vaginalis*. It has significantly higher sensitivity than traditional wet-mount test and can be used for testing of multiple types of clinical specimens including urine and vaginal specimens.

Author Disclosure Block:

L. Fleck: None. **H. Ty:** None. **S. Caddle:** None. **H. Kong:** None. **W. Tang:** None.

Poster Board Number:

SUNDAY-285

Publishing Title:

Performance of the Bd Max™ Vaginal Panel for the Diagnosis of Vaginitis

Author Block:

C. Gaydos[1], S. Beqaj[2], B. Smith[3], J. Lebed[4], J. Schwebke[5], D. Furgerson[6], P. Nyirjesy[7], T. Davis[8], T. Spurrell[9], R. Reagan[10], K. Fife[11]; [1]Johns Hopkins Univ., Baltimore, MD, [2]Pathology Inc., Torrance, CA, [3]Planned Parenthood Gulf Coast, Houston, TX, [4]Planned Parenthood Southeastern Pennsylvania, Philadelphia, PA, [5]Univ. of Alabama at Birmingham, Birmingham, AL, [6]Planned Parenthood Mar Monte, San Jose, CA, [7]Drexel Univ. Coll. of Med., Philadelphia, PA, [8]Sidney and Lois Eskenazi Hosp., Indianapolis, IN, [9]Planned Parenthood of Southern New England, New Haven, CT, [10]Women's Hlth.Care Res. Corp., San Diego, CA, [11]Indiana Univ. – Sch. of medicine, Indianapolis, IN

Abstract Body:

Background: Vaginitis, one of the most common problems in clinical medicine, accounts for millions of office visits per year. The three main categories of vaginitis are bacterial vaginosis (BV), yeast vaginitis (candidiasis) and *T. vaginalis* vaginitis (TV). The BD MAX Vaginal Panel (not available for sale or use in the US) detects TV, BV and different Candida species (*C. albicans*, *C. tropicalis*, *C. parapsilosis*, *C. dubliniensis* as Candida group; *C. glabrata* and *C. krusei* as a separate call). The objective of this prospective clinical trial (sponsored by BD) was to evaluate the performance of the BD MAX Vaginal Panel on the BD MAX System. **Methods:** The BD MAX Vaginal Panel results obtained were compared to target specific, gold standard Reference Methods (RM) for the detection of TV (wet mount/culture), BV (Amsel/Nugent) and *Candida* (culture/sequencing). For each consented woman (N = 1686), one clinician-collected vaginal swab (BD MAX UVE Specimen Collection Kit) was tested with the BD MAX Vaginal Panel. For RM, a total of 4 swabs were collected, one for wet mount; one for InPouch™ TV Culture; one for Nugent score and yeast culture and one for Aptima® TV. **Results:** For TV, the sensitivity was 92.7% with a specificity of 99.3%. For BV, the sensitivity was 90.5% with a specificity of 86.1%. For the *Candida* group, a sensitivity/specificity of 90.9% /94.1% was observed. The specificity for *C. glabrata* and *C. krusei* were 99.7% for both. **Conclusions:** Compared to gold standard RM, the BD MAX Vaginal Panel performed with high sensitivity and specificity for the diagnosis of vaginitis/vaginosis, providing clinicians with a new tool for management of vaginitis. The assay performance supports the appropriateness of target choice and design of the BD MAX Vaginal Panel, and indicates a high clinical relevance.

Author Disclosure Block:

C. Gaydos: E. Grant Investigator; Self; Becton Dickinson, Cepheid, Quidel. J. Scientific Advisor (Review Panel or Advisory Committee); Self; Roche, Eve Medical. L. Speaker's

Bureau; Self; Becton Dickinson, Quidel, Sekisui. **S. Beqaj:** None. **B. Smith:** None. **J. Lebed:** None. **J. Schwebke:** None. **D. Furgerson:** None. **P. Nyirjesy:** None. **T. Davis:** None. **T. Spurrell:** None. **R. Reagan:** None. **K. Fife:** None.

Poster Board Number:

SUNDAY-286

Publishing Title:

Urinary Meatal Swabbing Detects More Sexually Transmitted Infections in Men Than First Catch Urine

Author Block:

M. Chernesky[1], D. Jang[1], J. Gilchrist[1], M. Arias[1], M. Smieja[1], I. Martin[2], B. Weinbaum[3], D. Getman[3]; [1]St. Joseph's Hlth.care/McMaster Univ., Hamilton, ON, Canada, [2]Natl. Microbiol. Lab., Publ. Hlth.Agency of Canada, Winnipeg, MB, Canada, [3]Hologic Inc, San Diego, CA

Abstract Body:

Background: Traditionally, sexually transmitted infections in men; such as *Chlamydia trachomatis* (CT), *Neisseria gonorrhoeae* (NG), *Trichomonas vaginalis* (TV), *Mycoplasma genitalium* (MG), and human papillomavirus (HPV) have required urethral swabs for diagnosis. With nucleic acid amplification tests, first catch urine (FCU), which is non-invasive, can be used instead of the urethral swab. . FCU sampling can be problematic because it requires accurate collection of the first 20-30 ml of urine. Gentle swabbing of the urinary meatus (UM) has been used successfully for CT and NG diagnosis, is easy to self-collect, and is comfortable.**Methods:** During 2015, 356 men (ages 16-25) consented to self-collect a FCU and a UM swab (UMS) under supervision. A subset of patients compared 2 Aptima swab types [Unisex and vaginal for UMS. The order of collection was randomized. Within 48 hours, each sample was tested for CT and NG (Aptima Combo2), TV (Aptima Trichomonas vaginalis), MG (Hologic *Mycoplasma genitalium* analyte specific reagents), and HPV (Aptima HPV test) on Tigris or Panther instruments. Confirmation of MG and TV positives was performed using alternative (Alt) laboratory derived tests. MG positives were tested for macrolide resistance phenotype by 23S sequencing.**Results:** For all organisms, the UMS identified more infections than FCU. Prevalence rates for FCU and UMS were as follows: CT 9.3% and 11.3%; NG 0.84% and 1.4%; TV 1.7% and 7.9%; MG 12.6% and 15.3%; HPV 3.4% and 5.9%, respectively. Both unisex and vaginal swab types yielded equal numbers of infections. Macrolide resistance at 2058 and 2059 positions were identified in 62.5% (21/35) patients tested.**Conclusions:** The highest prevalence rates were recorded for MG (15.3%) followed by CT (11.3%). All TV and MG positives were confirmed with Alt TMA testing. UMS detected more infections, was easy and comfortable to collect, and provides a useful sample for men.

Author Disclosure Block:

M. Chernesky: B. Collaborator; Self; Hologic Study. E. Grant Investigator; Self; Hologic Study. **D. Jang:** None. **J. Gilchrist:** None. **M. Arias:** None. **M. Smieja:** None. **I. Martin:** None. **B. Weinbaum:** D. Employee; Self; Hologic Inc employee. **D. Getman:** D. Employee; Self; Hologic Inc employee.

Poster Board Number:

SUNDAY-287

Publishing Title:

Performance of the Cepheid Xpert® Tv Test for the Detection of *Trichomonas vaginalis* in Women

Author Block:

C. Gaydos[1], J. Coleman[1], T. Davis[2], J. Schwebke[3], J. Marrazzo[4], D. Furgerson[5], S. Taylor, MD[6], R. Lillis[6], B. Smith[7], L. Bachmann[8], R. Ackerman[9], T. Spurrell[10], D. Ferris[11], C-A. Burnham[12], J. Lebed[13], D. Eisenberg[14], P. Kerndt[15], S. Philip[16], J. Jordan[17]; [1]J Hopkins U, Baltimore, MD, [2]IUHPL, Indianapolis, IN, [3]U of AL, Birmingham, AL, [4]U of WA, Seattle, WA, [5]PP Mar Monte, San Jose, CA, [6]LA State U, New Orleans, LA, [7]PP Gulf Coast, Houston, TX, [8]Wake Forest U Hlth.Sci., Winston-Salem, NC, [9]Comprehensive Clin. Trials, W. Palm Beach, FL, [10]PP of Southern New England, New Haven, CT, [11]Georgia Regents U, Augusta, GA, [12]Washington U, St. Louis, MO, [13]PP Southeastern PA, Upper Darby, PA, [14]PP St. Louis, St. Louis, MO, [15]U. of Southern California, Los Angeles, CA, [16]SF Publ. Hlth., San Francisco, CA, [17]George Washington Univ., Washington, DC

Abstract Body:

Background: *Trichomonas vaginalis* (TV) is the etiologic agent of trichomoniasis, a common sexually transmitted infection. The Xpert® TV Assay, performed on the GeneXpert® Instrument Systems, is an *in vitro* qualitative real-time PCR test for automated detection of genomic TV DNA, providing detailed test results in 45 minutes.**Methods:** This multi-center study prospectively collected urine (UR), endocervical swabs (ES) and patient-collected vaginal swabs (PC-VS) from symptomatic and asymptomatic females. All specimens were tested with the Xpert TV Assay, the Gen-Probe® APTIMA® TV Assay, and InPouch culture. The performance was determined relative to a Patient Infected Status (PIS) algorithm- based on the result for each specimen type compared to the combined reference assays. Sequencing was performed on specimens with discrepant results.**Results:** For 1867 participants, 714 were symptomatic and 1153 asymptomatic. Average age was 33.5 yr. Relative to PIS, Xpert TV had a sensitivity and specificity on PC-VS (N = 1791) of 96.4% and 99.6%, respectively; prevalence 10.8%. Xpert TV demonstrated a sensitivity and specificity on ES (N = 1799) of 98.9% and 98.9%, respectively; prevalence 9.8%. The TV Assay demonstrated a sensitivity and specificity on UR (N = 1793) of 98.4% and 99.7%, respectively; prevalence 10.2%. There were no significant differences with respect to symptomatic status. Assay success was 99.9% (5383/5391).**Conclusions:** The Xpert® TV Assay demonstrated very high sensitivity and specificity compared to a PIS and offers a fast result that can be used to treat patients before they leave the clinic.

Author Disclosure Block:

C. Gaydos: E. Grant Investigator; Self; Becton Dickinson, Cepheid, Quidel. J. Scientific Advisor (Review Panel or Advisory Committee); Self; Roche. L. Speaker's Bureau; Self; Becton Dickinson, Quidel, Sekisui. **J. Coleman:** None. **T. Davis:** None. **J. Schwebke:** None. **J. Marrazzo:** None. **D. Furgerson:** None. **S. Taylor:** None. **R. Lillis:** None. **B. Smith:** None. **L. Bachmann:** None. **R. Ackerman:** None. **T. Spurrell:** None. **D. Ferris:** None. **C. Burnham:** None. **J. Lebed:** None. **D. Eisenberg:** None. **P. Kerndt:** None. **S. Philip:** None. **J. Jordan:** None.

Poster Board Number:

SUNDAY-288

Publishing Title:

Validation of Simulated Body Fluid Specimens (SBFS) in Bactec Blood Culture Bottles

Author Block:

E. L. Rank, Ph.D.; Quest Diagnostics, Inc., Teterboro, NJ

Abstract Body:

Background: BD Bactec Blood Culture media (BCM) are not currently validated for the cultivation of pathogens retrieved from body fluids, but they are often submitted by physicians in that capacity. Transportation to the lab can lead to delayed vial entry time points (DVETP), which may affect recovery rates. We systematically tested the ability of Bactec BCM to grow pathogens in simulated body fluid specimens (SBFS) at various DVETPs.**Methods:** One BCM set includes 2 bottles (1 aerobic and 1 anaerobic). Fifteen isolates (8 aerobic, 5 anaerobic, 2 fastidious) and 5 pairs of isolates were each inoculated into 8 BCM sets (128 + 80 + 32 + 80 = 320 bottles, each with 1 mL suspensions of ~10^1 CFU/mL). Twenty BCM sets were negative controls. These 360 bottles were incubated at 35ºC to 37ºC up to 120 hr. Half of the bottles received SBFS and half received PBS as a control for inhibitory substances in SBFS; no inhibitory effects were observed. Of the 8 sets per isolate, 2 were incubated immediately (0 hr); 6 sets were held at room temperature (RT) to replicate DVETP. Two sets each were incubated at 12 hr, 24 hr, and 48 hr time points. Positive bottles were identified using the Vitek system.**Results:** Overall recovery rates were ≥90% for aerobes (99.2%, 127/128), anaerobes (90.0%, 36/40, with an additional 7 anaerobes recovered from aerobic bottles), and paired isolates (91.3%, 73/80); the rate for fastidious organisms was 37.5% (12/32). As the DVETP increased, the overall recovery rates decreased and the average time to recovery increased (Table). All negative controls showed no growth at 120 hrs of incubation.**Conclusions:** This study demonstrates the ability of Bactec BCM to grow pathogens in SBFS for DVETP of up to 48 hours at RT. Fastidious microorganisms were more difficult to recover, probably due to their exacting temperature requirements; at or beyond the 48 hr time point, recovery of fastidious isolates appears minimal. Despite lower recovery of fastidious isolates, it is prudent to accept all specimens up to the 48 hr DVETP for pathogen recovery.

Table: Overall Recovery % Rates and Average Time (hrs) to Recovery for DVETP

DVETP	Recovery %	Aerobes, Average Time	Anaerobes, Average Time	Fastidious, Average Time
0 hr delay	95%	4.8	7.8	16.1
12 hr delay	76.3%	5.7	9.6	17.6

24 hr delay	76.3%	10.1	16.6	33.4
48 hr delay	71.3%	16.4	14.3	0

Author Disclosure Block:

E.L. Rank: None.

Poster Board Number:

SUNDAY-289

Publishing Title:

Ascites Fluid (AF) Cultures: Evaluation of Commercial Blood Cultures versus Direct Agar Plating (Dap)

Author Block:

B. A. Forbes, K. L. Godwin, M. E. Wallace, C. D. Doern, J. L. Forbes; VCU Med. Ctr., Richmond, VA

Abstract Body:

With respect to bacteriologic culture of AF, DAP has been reported as insensitive, with European and US guidelines recommending inoculation directly into blood culture bottles at the bedside. Purchase of a new automated blood culture system (BD BACTEC^{TM} FX Microbial Detection System; BACTEC) mandated validating its use for culture of AF. For validation, 66 AF were cultured as follows: 1 drop of AF were inoculated onto sheep blood, MacConkey's, chocolate, colistin-nystatin agars and incubated aerobically, while 1 drop of AF was also inoculated on OxyPRAS Plus plated media (Oxyrase Inc; Brucella, Phenylethyl alcohol agar, Bacteroides bile esculin, and Laked kanamycin-vancomycin agar plates) and incubated anaerobically. Remaining AF was inoculated into BacTALERT® Microbial Detection System (BacTALERT; bioMerieux) Standard Anaerobic (SN) and FAN Aerobic (FA) blood culture bottles, and BACTEC Lytic Anaerobic/F non-resin media and BACTEC Plus Aerobic bottle. Cytospun Gram stains (GS; n=104) were performed on AF as well. Based on results of the validation, an additional 39 AF were also evaluated in a similar manner by inoculating 1 set of DAP and BACTEC blood culture bottles. Collective data, i.e. total number of organisms, were used as the 'gold standard'. For the validation, the sensitivity based on organisms detected for the DAP, BacTALERT and BACTEC was 86.5%, 72.7% and 82%, respectively. Comparing all data for only DAP and the BACTEC (n=105), the overall sensitivity was 83% and 82.9%, respectively. The cytospun GS was only 50% sensitive when compared to culture. GSs of all culture-negative specimens showed no organisms yet 93.6% demonstrated neutrophils; all culture-positive specimens showed few to many neutrophils. Based on organism groups, DAP missed 46% enterics while the BACTEC missed 53% anaerobes. Although both methods have equivalent sensitivities for AF cultures (83% vs. 82.9%), time-to-detection was generally faster for blood culture bottles while time-to-reporting with appropriate susceptibilities results was faster for DAP. Together, these results indicate that BACTEC bottles plus anaerobic primary plating media would be optimal for AF cultures and BACTEC appeared more sensitive than BacTALERT.

Author Disclosure Block:

B.A. Forbes: None. **K.L. Godwin:** None. **M.E. Wallace:** None. **C.D. Doern:** None. **J.L. Forbes:** None.

Poster Board Number:

SUNDAY-290

Publishing Title:

Comparison Of Body Fluid Cultures Inoculated In Blood Culture Bottles Versus Routine Media And Broth

Author Block:

A. Azar, N. D. Deschamp, R. C. Galloway, J. Liebl, K. L. Muldrew, M. A. Mahowald, **L. M. Stempak**; Univ. of Mississippi Med. Ctr., Jackson, MS

Abstract Body:

Background: When culturing body fluid from normally sterile sites, fluid is inoculated on routine media, and additional fluid may be added to a blood culture bottle. This study assessed the accuracy of blood culture bottles for recovery of clinically significant bacterial organisms from body fluids compared to the traditional methods. **Methods:** Retrospective data from the previous three years (7/13/2012 to 6/30/2015) was reviewed for body fluid specimens in which a blood culture bottle was submitted in addition to the standard fluid for routine culture. Blood bottles (Bactec PLUS Aerobic/F, Standard Anaerobic/F and/or PEDS Plus/F) were incubated on the automated instrument (BACTEC 9000, Becton Dickinson) for 5 days or until growth was detected. For cultures using routine media, blood, chocolate, and MacConkey agars and thioglycollate broth were inoculated, incubated at 37°C in 5% CO_2 and examined daily for 3 days. Results were critically compared between the two methods for the presence of true pathogens and likely contaminants (e.g. coagulase-negative *Staphylocccus*, *Bacillus* spp., coryneform bacteria).**Results:** During the study period, 486 specimens were examined by both methods and results were compared and categorized as follows: 1. Complete concordance: 440 (90.5%). 2. Additional significant pathogens isolated solely from blood culture bottles: 30 (6.2%); 5 were likely contaminants (1%). 3. Additional pathogens isolated by the routine method: 10 (2.1%); 2 likely contaminants (0.4%). 4. No correlation (different organisms identified by each method): 6 (1.2%). On further analysis, true bacterial pathogens and contaminants were found by each method.**Conclusions:** Blood culture bottles allowed for isolation of additional significant pathogens in about 6% of cases, with only occasional contaminants. This increased sensitivity can be attributed to the larger volume of specimen inoculated into the bottle and longer incubations time. Rarely, pathogens were only isolated in routine culture on solid media. This illustrates the utility of using both detection methods for the identification of bacterial pathogens in body fluid cultures, and demonstrates that blood culture bottles are as good as, if not better than traditional methods.

Author Disclosure Block:

A. Azar: None. **N.D. Deschamp:** None. **R.C. Galloway:** None. **J. Liebl:** None. **K.L. Muldrew:** None. **M.A. Mahowald:** None. **L.M. Stempak:** None.

Poster Board Number:

SUNDAY-291

Publishing Title:

Etiological Diagnosis of Bacterial Meningitis Using Filmarray Bcid Panel in Csf Samples: 6 Month Experience at Centro Hospitalario Pereira Rossell - Hospital Pediátrico, Montevideo, Uruguay

Author Block:

C. Gutierrez, M. I. Mota, A. Varela, F. Morosini, J. P. Gesuele, J. Prego, G. Algorta; Centro Hosp.ario Pereira Rossell, Montevideo, Uruguay

Abstract Body:

Bacterial meningitis has important sequelae burden and high mortality which have decreased thanks to early onset of antibiotic therapy; this however hampers the recovery of the responsible agent in cultures.In July 2015 the Film Array Blood Culture Identification (BCID) panel was introduced in our laboratory for the diagnosis of bacteremia. Here we report the off-label use of this panel in cerebrospinal fluid (CSF) samples.Biochemical and cytological findings in CSF samples of patients with clinical diagnosis of meningitis were observed from July to December 2015. To be analyzed with BCID panel the CSF had to comply with most of the following criteria: concentration of glucose <40 mg/dl, proteins >100 mg/dl, lactate >4,2 mmol/l, leukocyte count (WBC)> 1,000/µl, >75% neutrophils.6 of 232 CSF samples met the above criteria and were processed following the manufacturer's instructions for blood culture samples and analyzed with de BCID panel.**Table 1. Biochemical and cytological findings in CSF samples and FilmArray results.**

Sample	Proteins/Glucose (mg/dl)	Lactate (mmol/l)	WBC/µl (neutrophils %)	BCID panel
S1	235/37	13,36	14,400 (90%)	*S. pneumoniae*
S2	126/71	3,76	21,500 (90%)	*S. agalactiae*
S3	875/1	15,4	9,200 (90%)	*S. pneumoniae*
S4	0,96/Non detectable	8,94	1,400 (90%)	*Enterococcus* sp.
S5	320/7	11,77	3,800 (90%)	*N. meningitides*
S6	443/11	11,51	6,300 (90%)	*H. influenzae*

FilmArray detected 2 *Streptococcus pneumoniae*, 1 *Streptococcus agalactiae*, 1 *Enterococcus* sp., 1 *Neisseria meningitidis* and 1 *Haemophilus influenzae*.Gram stain examination showed neutrophils in all smears and gram positive cocci in S1 and S3 and no bacteria in S2, S4, S5 and S6.Latex agglutination test was positive only for *N.meningitidis* Y/W (S5).*E. faecalis* (S4) was

the only agent recovered from CSF cultures.For patients corresponding to S5 and S6, *N. meningitidis* W and *H. influenzae* (nontypeable) were recovered from blood culture.Unfortunately there was no more CSF sample left for further testing of the ones not confirmed by culture.Our results suggest that BCID panel may be used with CSF samples, providing rapid and accurate results.

Author Disclosure Block:

C. Gutierrez: None. **M.I. Mota:** None. **A. Varela:** None. **F. Morosini:** None. **J.P. Gesuele:** None. **J. Prego:** None. **G. Algorta:** None.

Poster Board Number:

SUNDAY-292

Publishing Title:

Use Of The Biofire Meningitis/Encephalitis Panel For The Detection Of Viruses In Csf

Author Block:

D. Sayre, L. Glaser, K. Alby; Hosp. of the Univ. of Pennsylvania, Philadelphia, PA

Abstract Body:

Introduction: Infectious encephalitis and meningitis are potentially life-threatening conditions caused by a host of viral, bacterial, and fungal pathogens. The clinical presentation of these conditions is often not dependent on the offending organism, leaving clinicians to utilize a broad spectrum of empiric therapies until more definitive laboratory data becomes available. The goal of this study was to establish the effectiveness and reliability of a recently approved multiplex panel for viral targets in our patient population.**Methods:** We retrospectively analyzed 32 patient samples as well as 18 patient samples known to be positive for either HSV, VZV, or enterovirus. The findings of the Biofire FilmArray ME panel were compared with conventional PCR assays and chart review.**Results:** The ME assay detected all targets previously found to be positive by standard PCR in samples obtained from outside laboratories (5 for HSV-2, 8 for VZV, 4 for enterovirus). However, additional targets were detected in three of the VZV samples (CMV, HSV-2, and *H. influenzae*), two (CMV and *H. influenzae*) of the three had sufficient residual sample for repeat testing and were negative upon repeat for the additional target. Of the 32 samples obtained from our hospital, 30 (94%) tested as negative for all viral targets. One sample (3%) tested positive for HHV-6, and one (3%) tested positive for enterovirus; no prior testing for these pathogens was previously performed but subsequent testing confirmed the observed results. Two non-viral targets were also detected. One *Cryptococcus*, confirmed by culture, and one *H. influenzae*, negative by Gram stain, culture, and chart review. Repeat testing of this sample was also negative.**Conclusions:** Overall, the ME panel demonstrated excellent sensitivity to detect multiple viral targets in CSF in a timely manner. However, a small number of the tests performed produced likely false positive bacterial results, a significant error given the severity of the disease and the aggressiveness of its therapy. This less than perfect specificity and analysis of the cost of test implementation suggest that this assay may be best utilized with some restrictions on testing and reporting by the laboratory.

Author Disclosure Block:

D. Sayre: None. **L. Glaser:** None. **K. Alby:** None.

Poster Board Number:

SUNDAY-293

Publishing Title:

Identification of Bacterial and Viral Pathogens in Previously Identified Negative Cerebrospinal Fluid Samples Using a Comprehensive Multiplex Pcr System

Author Block:

E. Lo[1], T. Lunt[2], E. Slechta[2], J. Daly[3], K. Hanson[2], A. Hemmert[1]; [1]Biofire Diagnostics, LLC, Salt Lake CIty, UT, [2]ARUP, Salt Lake CIty, UT, [3]Primary Children's Hosp., Salt Lake CIty, UT

Abstract Body:

Background: Central nervous system (CNS) infections, such as meningitis and/or encephalitis, are potentially life-threatening and require immediate and appropriate treatment. For suspected infections, cerebrospinal fluid (CSF) may be cultured for bacteria and fungi or tested by polymerase chain reaction (PCR) for viruses. The range of possible tests and test modalities makes pathogen etiology determination in CNS infection difficult and time-consuming. To aid in the identification of common causes of CNS infections, BioFire Diagnostics has developed the FilmArray™ (FA) Meningitis/Encephalitis (ME) panel recently cleared by the U.S. Food and Drug Administration (FDA) that simultaneously tests for fifteen bacteria, viruses, and fungi that may cause meningitis and/or encephalitis. Using a single-use disposable reagent pouch and, 200 µL of CSF the test is run and a result returned in about one hour.**Methods:** This study was performed to determine if a causative pathogen could be identified in previously characterized negative CSF samples. One-hundred (100) frozen, archived, de-identified patient CSF samples, previously identified to be pathogen negative by culture or specific PCR tests at Primary Children's Hospital or ARUP, were tested using the FilmArray ME panel. Any positive pathogen identification was confirmed using either a commercially available qPCR test or 16s sequencing.**Results:** For the majority of the 100 samples, no pathogen was identified. In eight of the samples, however, a virus or bacteria was discovered. Enterovirus was detected in five samples (5/100), human herpesvirus 6 in two (2/100), and *Haemophilus influenzae* in one (1/100).**Conclusions:** These data demonstrate that comprehensive molecular testing of CSF for patients suspected of meningitis or encephalitis will likely result in a higher rate of pathogen detection than selective testing for suspected etiologies. The FilmArray ME panel can provide valuable qualitative information to help clinicians make informed decisions through rapid and comprehensive identification of infectious etiologies in CNS disease.

Author Disclosure Block:

E. Lo: None. **T. Lunt:** None. **E. Slechta:** None. **J. Daly:** None. **K. Hanson:** None. **A. Hemmert:** None.

Poster Board Number:

SUNDAY-294

Publishing Title:

Multi-site Assessment of an Image Analysis Device for Urine Cultures

Author Block:

J. H. Glasson[1], M. Summerford[1], D. Olden[2], L. Riley[3], S. Young[4]; [1]LBT Innovations Ltd, Adelaide, Australia, [2]Hlth.scope Pathology, Clayton, Melbourne, Australia, [3]SydPath, ST Vincents Hosp., Darlinghurst, Sydney, Australia, [4]TriCore Reference Lab., Albuquerque, NM

Abstract Body:

The reporting of growth from agar plates has traditionally relied on experienced technologists manually handling and reading those plates for the presence, quantity and characteristics of the colonies. More recently, a number of image capture devices have become available and reporting from digital images is being increasingly adopted. The Automated Plate Assessment System (APAS®, LBT Innovations, Adelaide, South Australia) is able to automatically assesses plate images for the presence, quantity and characteristics of the colonies. With a decision algorithm, it stratifies cases by the need for further action such as manual review or identification and antibiotic susceptibility testing. This multi-site study was designed to establish the diagnostic performance of this device in the screening of urine cultures. A combined total of 9977 urines submitted for routine culture to one diagnostic laboratory in the USA (TriCore Reference Laboratories) and two laboratories in Australia (Healthscope Pathology, Victoria and SydPath, NSW) were assessed. A 1µL sample of each urine was inoculated onto TSA sheep blood agar and MacConkey with CV agar (Remel, Lenexa, USA) and incubated in air at 35^{o}C for 18h. The plates were independently read by three microbiologists and the consensus results were compared with those produced by APAS®. APAS® detected colonies on blood agar with a sensitivity of 99.0% and specificity of 84.5% while on MacConkey agar, the detection sensitivity was 99.5 % and specificity 98.8%. Colony counts from the device showed agreement in 8417/9977 (84.4%) cases. Of the discrepant counts, 1267/1560 (81.2%) were higher than the consensus results. APAS® separated plates requiring further action with a sensitivity of 97.3%. In this multi-site assessment, APAS® demonstrated a comparable performance in the detection, enumeration and colonial classification of isolates when compared with conventional plate reading. The device detected the most commonly encountered organisms found in uncomplicated urinary tract infections and segregated the reports and plates for more efficient handling.

Author Disclosure Block:

J.H. Glasson: D. Employee; Self; LBT Innovations Ltd. **M. Summerford:** D. Employee; Self; LBT Innovations Ltd. **D. Olden:** H. Research Contractor; Self; LBT Innovations Ltd. **L. Riley:** H. Research Contractor; Self; LBT Innovations Ltd. **S. Young:** H. Research Contractor; Self; LBT Innovations.com.

Poster Board Number:

SUNDAY-295

Publishing Title:

Enhanced Urine Culture Protocol Improves Detection of Clinically Relevant Microorganisms in Symptomatic Women

Author Block:

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Abstract Body:

Background: Bacteria that are not routinely cultivated using standard urine culture protocols have been identified by new techniques in the urine of patients with and without urinary symptoms. These findings raise a concern about the accuracy of current urine culture practices. We compared results from standard urine culture to an improved urine culture protocol for detection of clinically relevant microorganisms in women with urinary symptoms. **Methods:** After institutional review board approval, we prospectively enrolled 150 urogynecologic patients and dichotomized the group based on their UTI perception, using a Y/N response to "Do you feel you have a UTI?" In addition to a standard urine culture of catheterized urine specimens, bacterial growth was assessed using 3 versions of an expanded quantitative urine culture (EQUC) protocol, which uses different media, an expansion of environmental culturing conditions, and 3 volumes of urine plated (1µL, 10µL and 100µL) instead of the 1µL used in standard culture protocols. Bacterial growth detected with EQUC was identified using MALDI-TOF MS. Microbiota diversity was assessed using the average number of unique species per urine specimen. Women were treated clinically based on results from standard urine culture alone. **Results:** The 100µL EQUC protocol detected significantly more uropathogens (174, including 95 unique species) compared to standard culture (60, including 11 unique species). The standard culture missed 67% (122/182) of the uropathogens detected using the EQUC protocols. In the YES cohort, standard culture missed 50% (55/110) of the uropathogens. Standard culture detected most *Escherichia coli* (84% - 44/50), but detected only a minority of all other uropathogens (8% - 8/100). The optimal EQUC version [100µL urine plated onto Blood (5% CO_2), CNA (5% CO_2), and MAC (O_2) with 48 hours of incubation] detected more uropathogens 84% (152/182) than standard culture 33% (60/182). **Conclusions:** Improved methods of uropathogen detection may aid clinicians in selecting treatment for women reporting UTI symptoms. The optimal form of EQUC markedly improves uropathogen detection and supports the need for an immediate reassessment of standard urine culture practices.

Author Disclosure Block:

P.C. Schreckenberger: None. **T.K. Price:** None. **E.E. Hilt:** None. **T. Dune:** None. **C. Brincat:** None. **L. Brubaker:** None. **E.R. Mueller:** None. **K. Thomas-White:** None. **S. Kliethermes:** None. **A.J. Wolfe:** None.

Poster Board Number:

SUNDAY-296

Publishing Title:

A Comparison of Three Commercially Available Urine Culture Collection Systems on the Stability and Overgrowth of Common Urinary Pathogens

Author Block:

S. Volhejn, **S. E. Dale**; ACM Global Central Lab., Rochester, NY

Abstract Body:

Background: Boric acid has been used for >40 years as a preservative for urine culture specimens. Many manufacturers produce urine preservative tubes, however, few comparative stability and bacterial overgrowth studies exist for common uropathogens using different devices. Given the composition differences of urine preservative tubes and potential for clinical impacts when performing quantitative urine cultures with urine collected in these devices, we compared the stability and overgrowth of common uropathogens using the Andwin Scientific Boritex®, Sarstedt Urine Monovette® and BD Vacutainer® Culture Plus C&S Urine Preservation Systems. **Methods:** Eight urinary pathogens from the American Type Culture Collection were prepared in sterile urine samples. Serial dilutions of each organism were inoculated in duplicate into the Boritex, Monovette or Vacutainer tubes. One tube of each dilution was stored either ambient (20°C -25°C) or refrigerated (2°C - 8°C). At time zero (15 minutes), 24, 48, 72 and 96 hours each dilution was plated in triplicate. Following incubation, colony forming units (CFUs) were counted and averaged to determine stability and bacterial overgrowth. **Results:** Extended stability was achieved for up to 4 days when urine was stored at 2°C to 8°C for all organisms tested in the Boritex and Vacutainer tubes. All organisms, with the exception of *S. saprophyticus*, was stable for 4 days using the Monovette. At 20°C to 25°C, the Boritex and Vacutainer tubes showed stability for 4 days for all organisms, except *P. aeruginosa* (2 days). The Monovette exhibited stability from 1 to 4 days when stored at 20°C to 25°C. The Boritex and Vacutainers were susceptible to bacterial overgrowth (> 0.5 log CFU/ml) at 20°C to 25°C with *E. coli, K. pneumoniae, P. mirabilis, S. saprophyticus* and *E. faecalis*. The Monovette contained the highest concentration of boric acid and inhibited the overgrowth of all microorganisms with the greatest efficacy. **Conclusions:** Extended stability of common uropathogens can be achieved with Boritex and Vacutainer tubes stored at 2°C to 8°C and 20°C to 25°C for up to 96 hours, however, bacterial overgrowth is observed at 20°C to 25°C. The Monovette tube showed the least stability, but the high boric acid level in these transport tubes is likely responsible for an overall reduction in bacterial overgrowth.

Author Disclosure Block:

S. Volhejn: None. **S.E. Dale:** None.

Poster Board Number:

SUNDAY-297

Publishing Title:

Comparing Results from the Iris Iq® 200 Sprint™ Analyses with Routine Urine Analyzing on an Automated Bd Kiestra System, to Validate the Prediction of the Culture Negative Urine Samples

Author Block:

L. Leerbeck, J. Iversen, A. Lund, J. Knudsen; Depart of Clin Microbiol, Hvidovre Hosp., Hvidovre, Denmark

Abstract Body:

Background: In our fully automated department of clinical microbiology, we constantly have focus on reducing the response time on the urine analyses to provide results faster and thereby improve clinical decisions. We have performed a validation test on The Iris Diagnostics iQ© 200 SPRINT™ Automated Urine Microscopy Analyzer (iQ200 SPRINT) from Beckman Coulter, and compared the results with the routine urine analysis on the BD Kiestra™ Total Lab Automation system.**Methods:** We processed 528 clinical samples both through the iQ200 SPRINT and inoculated and cultured on the BD Kiestra system. The routine urine culture analyses considered the 10(3) colony forming units/mL as significant for *Escherichia coli* and *Staphylococcus saprophyticus*, and 10(4) for other typical uro-pathogens. The data for bacteria, white blood cells and small particles counted in samples by the iQ200 SPRINT and after culture were compared. We aimed to be able to predict the culture negative samples. **Results:** Using all three of the following thresholds for iQ200 SPRINT to report negative samples: White blood cells <40/µL, bacteria <5/µL, and small particles <3500/µL, 169 sample were identified. In 101 (60%) samples no growth were seen, in 64 (38%) insignificant growth were seen, and in 2.4%, 4 samples (10(4) yeasts, 10(4) hemolytic streptococci, 10(5) *Proteus mirabilis, and* 10(4) *Proteus vulgaris)* were missed. The negative predictive value was 98%. Screening with iQ200 SPRINT could have avoided 169 of 528 (32%) cultures performed. In these 32% of cases, the clinician could have had the negative result on the day of sampling, and the laboratory did not have to perform the cultivation. We found the iQ200 SPRINT to be a fast and user friendly instrument. **Conclusions:** The validation in our laboratory revealed acceptable results with a negative predictive value of 98%. In our fully automated laboratory, is the possibility for saving space in incubators and need for photo-reading of plates highly warranted. We conclude that the iQ200 SPRINT is a valuable urine analyzer that could make us reduce response time for negative samples and save resources as fewer samples needs cultivation.

Author Disclosure Block:

L. Leerbeck: None. **J. Iversen:** None. **A. Lund:** None. **J. Knudsen:** None.

Poster Board Number:

SUNDAY-298

Publishing Title:

Optoelectronic Detection of Bacterial Growth in Clinical Urine Samples

Author Block:

U. Aurbach[1], M. Eiden[2], S. Lim[2], P. Rhodes[2], **H. Wisplinghoff**[3]; [1]Wisplinghoff Lab., Cologne, Germany, [2]Specific Technologies, Mountain View, CA, [3]Inst. for Med. Microbiol., Univ. of Cologne, Cologne, Germany

Abstract Body:

Background: Optoelectronic detection and identification of microorganisms based on an inexpensive, printed, disposable colorimetric sensor array (CSA) responsive to the volatiles emitted into the headspace of culture bottles during growth has been shown to have the potential to shorten time to result in blood cultures. Similarly, using sensors placed in the lids of blood agar plates, it has been possible to identify presence and species on blood agar plates.**Methods:** This study was conducted to evaluate the potential use of optoelectronic detection more broadly in clinical microbiology. Urine samples from were tested with conventional cultural methods using TSA-blood-, CPS-, and CN-agar, all with and without CSA. Urine samples included were either sterile (with and without inhibitory substances) or were inoculated with one or more of the following bacterial pathogens at concentrations of 10^2-10^6 cfu/ml: E. coli and other Enterobacteriaceae, P. aeruginosa and other non-fermenting gram-negatives, Enterococcus spp., Staphylococcus spp., Streptococcus spp. All samples were run in duplicate.**Results:** A total of 200 samples were prospectively included. Time to positivity (detection time, all positive specimens) ranged from 4.6 to 11.6 hours, sensitivity of species identification (monomicrobial cultures) at 1, 2 and 3 hours after initial detection of growth showed a high correlation to MALDI-TOF identification but varied between different pathogen /media combinations pathogens and could be improved by adaption of algorithms to the respective media.**Conclusions:** CSA-based optoelectronic detection seems to be a promising addition to the routine clinical microbiology laboratory with the potential to significantly shorten the time to positivity and / or species identification in urine and potentially other clinical specimens.

Author Disclosure Block:

U. Aurbach: None. **M. Eiden:** D. Employee; Self; Specific Technologies. **S. Lim:** D. Employee; Self; Specific Technologies. **P. Rhodes:** D. Employee; Self; Specific Technologies. **H. Wisplinghoff:** I. Research Relationship; Self; Beckman Coulter, BioMerieux, Bruker Daltonics, Cepheid, Hologic, iSense, r-biopharm, Siemens, Specific Technologies. L. Speaker's Bureau; Self; Beckman Coulter, Bruker Daltonics.

Poster Board Number:

SUNDAY-299

Publishing Title:

High-Resolution Seven Snp-Based Test Allows Rapid Identification of Clonotypes and Prediction of Antimicrobial Susceptibility of Extraintestinal *Escherichia coli* Directly from Clinical Specimens

Author Block:

V. Tchesnokova[1], H. Avagyan[1], M. Muradova[1], S. Chattopadhyay[1], D. Chan[1], E. Rechkina[1], F. Fang[1], J. R. Johnson[2], E. V. Sokurenko[1]; [1]Univ. of Washington, Seattle, WA, [2]Univ. of Minnesota, Minneapolis, MN

Abstract Body:

Background: *Escherichia coli* is a highly clonal pathogen with strains belonging to a limited number of genetically related groups, often exhibiting characteristic antimicrobial resistance profiles.**Methods:** We developed a rapid method of clonotyping of extra-intestinal *E. coli* based on detection of the presence/absence of seven single-nucleotide polymorphisms (SNPs) within two genes (*fumC* and *fimH*). A reference set of 2,559 *E. coli* isolates, primarily of urinary origin, was used to evaluate the resolving power of the 7 SNP-based typing method.**Results:** A total of 54 unique SNP combinations ('septatypes') were identified in the reference strains, with clonal-group resolution power on par with traditional multi-locus sequence typing. In 72% of isolates the septatype identity predicted the sequence type identity with at least 90% (mean 97%) accuracy. Most septatypes exhibited highly distinctive antimicrobial susceptibility profiles. The 7 SNP-based test can be performed with high specificity and sensitivity using single or multiplex PCR and qPCR. In the latter format, *E. coli* presence and septatype identity was determined directly in urine specimens with bacterial loads as low as 10^2 cfu/mL within 45 minutes and, at clinically significant bacterial loads, with 100% sensitivity and specificity.**Conclusions:** The 7 SNP-based typing of *E. coli* can be used for both epidemiological studies and clinical diagnostics that could greatly improve the empirical selection of antimicrobial therapy.

Author Disclosure Block:

V. Tchesnokova: None. **H. Avagyan:** None. **M. Muradova:** None. **S. Chattopadhyay:** None. **D. Chan:** None. **E. Rechkina:** None. **F. Fang:** None. **J.R. Johnson:** None. **E.V. Sokurenko:** None.

Poster Board Number:

SUNDAY-300

Publishing Title:

A New Technique to Study Symptomatic Patients with Negative Urine or Semen Cultures

Author Block:

M. E. Ordonez Smith; Microbiol. Inst. of Colombia, Bogota, Colombia

Abstract Body:

A new technique is described in order to recover bacteria in urine or semen samples. Several urinary or prostatic symptomatic patients have negative urine or semen cultures; therefore we sought to look at a novel approach to address this diagnostic gap. All the urines (317) or semen (32) samples were processed immediately: 1. As the CLSI gold standard technique. 2. At the same time, they were done with the new technique, which consists in incubating the urine or semen samples at 37^0C for 24 hours. Each sample was smeared after 5 to 7 hours and after 24 hours, onto blood agar, and CLED agar in order to compare colony counts. This new technique gives results between 24 to 48 hours earlier than the standard method. The samples were studied from 208 symptomatic urines or semen from prostatitis (94 cases) or urinary infections (114 cases) and 109 from asymptomatic patients. Symptomatic patients had >10.000 CFU/mL after 5 to 7 hours of incubation a 40.8%, after 24 hours at 37^0C a 87.0% positive urine or semen cultures by the new method. Conversely, there were 19.5% positive cultures by the gold standard method ($p<0.05$, Table 1). This new technique not just can help to reduce costs in days of hospitalization, diagnostic tests, but it improves patients relieve in terms of urinary or prostatic symptoms. Here we show that bacteria grow better in the same urine or semen habitat (37^0C) rather than in artificial media.Keywords New technique, Urinary tract Infections, ProstatitisTABLE 1. Symptomatic patient’s samples

Colony counts of the bacteria isolated CFU/mL	Initial colony counts (cases) the standard technique	Samples after 5-7 hours in the incubator at 37^0C	Samples after 24 hours in the incubator at 37^0C
0 (no growth)	80	57	14
100-10.000	113	85	17
>10.100- 99.000	16	41	12
>100.000	31	57	197
Total cases	240	240	240

Author Disclosure Block:

M.E. Ordonez Smith: None.

Poster Board Number:

SUNDAY-301

Publishing Title:

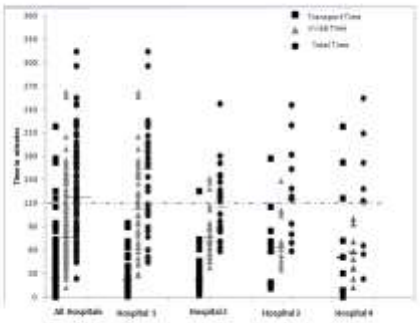
The Csf Gram Stain: Turn-Around Time Experience At One Health Care System

Author Block:

I. Agarwal, S. Das, R. B. Thomson, Jr; NorthShore Univ. Hlth.System, Evanston, IL

Abstract Body:

Bacterial meningitis is associated with high morbidity and mortality. Expedited specimen transport, rapid laboratory reporting of initial microscopic results can be crucial for initiation of appropriate therapy. In this study, we determined turn-around time (TAT) of CSF Gram stains from Emergency Department patients (ED) over a one year time period in a 4 hospital system to see if they met the proposed guidelines.Retrospective data collection of CSF Gram stain TATs from ED was done by extracting collection, laboratory receiving and reporting times from the hospital (EPIC) and laboratory (SCC) information systems. The time from sample collection to laboratory receipt was recorded as “transport time.” The time from sample receipt to result reporting was labeled “in laboratory time.” “Transport time” plus “in laboratory time” was labeled “total TAT.”Data for a total of 78 CSF specimens was collected. The overall TAT and TAT for each hospital are presented in the figure.If a 2 hour total time including a 1 hour transport and 1 hour in laboratory time is a proposed guideline recommendation, 44% specimens met the 2 hour total TAT, while 76% and 28% had a 1 hour transport time and in-lab time, respectively. When data from all 4 hospitals were combined, 2 out of 4 hospitals, met the recommended TAT for 50% of the total specimens processed. Among these, one laboratory, met the recommended in laboratory TAT for 67% of specimens and the other met the recommended total TAT for 54% of specimens.Guidelines suggesting a 2 hr TAT for CSF Gram stain reporting are not consistently achieved in our 4 hospital system. Our data show that unacceptable TATs are caused by extended transport and in-lab testing times. Plans for improvement will duplicate processes that allow some hospitals to meet acceptable TATs in hospitals that do not meet guidelines.



Scatterplot of TAT from All Hospitals and the Individual Hospitals. Horizontal dashed line represents recommended TAT of 2 hours. Median value is shown for each data set as a horizontal solid line.

Author Disclosure Block:

I. Agarwal: None. **S. Das:** None. **R.B. Thomson:** None.

Poster Board Number:

SUNDAY-302

Publishing Title:

Investigation of Bacterial and Viral Etiology in Community Acquired Central Nervous System Infections with Molecular Methods

Author Block:

H. Kahraman[1], **U. Onal**[1], A. Tünger[1], S. Alpçavuş[2], Z. Gülay[2], &. Şenol[3], H. Gazi[3], M. Avcı[4], B. Örmen[5], N. Türker[5], &. Köse[6], S. Ulusoy[1], M. Tasbakan[1], O. R. Sipahi[1], T. Yamazhan[1], H. Pullukçu[1]; [1]Ege Univ. Faculty of Med., İzmir, Turkey, [2]Dokuz Eylül Univ. Faculty of Med., İzmir, Turkey, [3]Celal Bayar Univ. Faculty of Med., Manisa, Turkey, [4]İzmir Teaching and Resaarch Hosp., İzmir, Turkey, [5]Ataturk Teaching and Resaarch Hosp., İzmir, Turkey, [6]Tepecik Teaching and Resaarch Hosp., İzmir, Turkey

Abstract Body:

Background: In this multicenter prospective cohort study it was aimed to evaluate the bacterial and viral etiology of community acquired infections of the central nervous system by standart bacteriological culture method and multiplex polymerase chain reaction method. **Methods:** A total 74 patients (aged 18-90) were hospitalized with central nervous system infections were enrolled into the prospective study between April 2012 and February 2014. Demographic information of patients were collected. Cerebrospinal fluid (CSF) samples of patients were examined for bacterial and viral pathogens by standart bacteriological culture methods, bacterial multiplex PCR (Seeplex meningitis-B ACE Detection (Streptococcus pneumoniae, Neisseria meningitidis, Haemophilus influenzae, Listeria monocytogenes, Group B streptococcus) and viral multiplex PCR (Seeplex meningitis-V1 ACE Detection kits herpes simplex virus-1 (HSV1), herpes simplex virus-2 (HSV2), varisella zoster virus (VZV), cytomegalovirus (CMV), Ebstain Barr virus (EBV) ve human herpes virus 6 (HHV6)) (Seeplex meningitis-V2 ACE Detection kits (enteroviruses)). **Results:** A total of 74 patients (24 female, 50 male, aged 43,9±17,7) fulfilled the study inclusion criteria. CSF cultures were positive in 10 patients (9 S. pneumoniae, 1 H. influenzae). Pathogens were shown by multiplex PCR in 43 patients. One type of bacteria were detected in 16 patients (13 S. pneumoniae, 2 N. meningitidis, 1 H. influenzae) and single type of virus were isolated in 14 patients (8 enterovirus, 2 HSV1, 1 HSV2, 2 VZV and 1 EBV). Besides, it is noteworthy that multipl pathogens detected such as bacteria- virus combination in 9 patients, viral-viral detections in 3 patients and two different bacterias in 1 patient in our study. **Conclusions:** These data suggest that multiplex PCR methods may increase the isolating of pathogens in central nervous system infections. Further studies are needed for the clinical significance of mixed detections.

Author Disclosure Block:

H. Kahraman: None. **U. Onal:** None. **A. Tünger:** None. **S. Alpçavuş:** None. **Z. Gülay:** None. **&. Şenol:** None. **H. Gazi:** None. **M. Avcı:** None. **B. Örmen:** None. **N. Türker:** None. **&. Köse:** None. **S. Ulusoy:** None. **M. Tasbakan:** None. **O.R. Sipahi:** None. **T. Yamazhan:** None. **H. Pullukçu:** None.

Poster Board Number:

SUNDAY-303

Publishing Title:

Draft Genome Sequence of *Klebsiella pneumoniae* ST-14 Harboring *bla*$_{\text{CTX-M-15}}$, *bla*$_{\text{DHA-1}}$, *bla*$_{\text{TEM-1B}}$, *bla*$_{\text{NDM-1}}$, *bla*$_{\text{SHV-28}}$, & *bla*$_{\text{OXA-1}}$ Isolated from a Patient in Lebanon

Author Block:

S. Tokajian[1], J. A. Eisen[2], G. Jospin[2], G. Matar[3], G. F. Araj[3], D. A. Coil[2]; [1]Lebanese American Univ., Byblos Campus, Lebanon, [2]Univ. of California Davis, Davis, CA, [3]American Univ. of Beirut, Beirut, Lebanon

Abstract Body:

Extended-spectrum β-lactamase (ESBL)-producing *Klebsiella pneumoniae* constitutes one of the most common multidrug-resistant (MDR) groups of gram-negative bacteria involved in nosocomial infections worldwide. Emergence and rapid dissemination of ESBL-producing bacteria has led to the increased utilization of carbapenems, consequently leading to the occurrence of isolates with resistance genes that code for carbapenemases (1).In this study we report on the molecular characterization through whole genome sequencing of a highly drug resistant *K. pneumoniae* KGM-IMP216 isolated from a urine sample collected from a patient in Lebanon. A Nextera XT kit (Illumina, San Diego, CA, USA) was used to simultaneously fragment and adapter tag the library. The library was sequenced using the MiSeq v3 600-cycle kit (Illumina, San Diego, CA, USA) to perform 300-bp paired-end sequencing. Quality trimming and error correction of the reads resulted in 4,192,299 high-quality reads. The final draft genome sequence consisted of 77 contigs that contained 5,731,500 bases with 57% G+C content. The sequence type of *K. pneumoniae* KGM-IMP216 was ST-14. Automated annotation was performed using the RAST annotation server (2), which revealed the presence of several virulence determinants including adhesins. ResFinder additionally, revealed that the isolate harbored *bla*$_{\text{CTX-M-15}}$, *bla*$_{\text{DHA-1}}$, *bla*$_{\text{TEM-1B}}$, *bla*$_{\text{NDM-1}}$, *bla*$_{\text{SHV-28}}$, and *bla*$_{\text{OXA-1}}$ genes (3). The concomitant presence of other resistance determinants, including resistance to macrolide (*msrE* and *mphE*), phenicol, sulfonamide (*sul1* and *sul2*), tetracycline (*tetA*), trimethoprim (*drfA14*) and aminoglycoside (*aac(6')lb-cr)*, was another important finding.To the best of our knowledge we report for the first time the sequencing of *K. pneumoniae* harboring *bla*$_{\text{CTX-M-15}}$, *bla*$_{\text{DHA-1}}$, *bla*$_{\text{TEM-1B}}$, *bla*$_{\text{NDM-1}}$, *bla*$_{\text{SHV-28}}$, and *bla*$_{\text{OXA-1}}$ with the sequence type ST-14. The potential role of *K. pneumonia*e as a reservoir for ESBL and carbapenemases genes is along with the presence of key factors that favor the spread of antimicrobial resistance, a clear cause of concern.

Author Disclosure Block:

S. Tokajian: None. **J.A. Eisen:** None. **G. Jospin:** None. **G. Matar:** None. **G.F. Araj:** None. **D.A. Coil:** None.

Poster Board Number:

SUNDAY-304

Publishing Title:

Molecular Diversification in Epidemic Carbepenem-Resistant *K. pneumoniae* (Crkp) Clonal Group 258 (Cg258)

Author Block:

L. Chen[1], H. Xiong[2], E. G. Pamer[2], J. D. Pitout[3], M. D. Adams[4], B. N. Kreiswirth[1]; [1]PHRI-Rutgers Univ, Newark, NJ, [2]MSKCC, New York, NY, [3]Univ of Calgary, Calgary, AB, Canada, [4]JCVI, La Jolla, CA

Abstract Body:

Background: Our previous study showed that Kp ST258 is a hybrid strain distinguished into two major clades with different capsule polysaccharide (*cps*) regions. Clade I strains evolved from clade II by *cps* replacement. Our recent molecular surveillance demonstrated the emergence of novel *cps* groups in ST258. Here we use whole genome sequencing (WGS) to understand the molecular evolution in CG258.**Methods:** 39 CRKp CG258 isolates (21 ST258, 14 ST11, 2 ST437 and 2 ST340) from US, China, India, Argentina, Colombia, Brazil, and Turkey were selected for WGS using Pacific Biosciences RSII and Illumina NextSeq. The sequences were compared to other genomes from our in-house database and in the public domain. 9 CG258 isolates with different *cps* were examined in a murine pneumonia model for virulence and clearance by neutrophils and monocytes.**Results:** Among the 39 isolates, 26 KPC-2, 6 KPC-3, 3 NDM-1, 1 OXA-163 and 1 OXA-48 were identified. Core SNPs analysis clustered the 39 genomes with other CG258 from the public database, but separated CG258 from the genomes of other CGs. However, deeper analysis among the 39 CG258 genomes revealed high genetic diversity, including (1) extensive *cps* variations: 21 different *cps* types (7 in ST258, 10 in ST11, 2 in ST437 and 2 in ST340), and two truncated *cps* due to an IS insertion; (2) putative donor STs of novel *cps*: ST258 *cps-3* carries the same *cps* as one ST111, while ST258 *cps-4* harbors the same *cps* as an ST16 in our database; (3) large non-contiguous, non-*cps* chromosomal recombination regions in different ST258 strains; (4) unique genetic regions: ST258 (*cps-1* to *-7*) have unique ICE and prophage sequences in comparison to the genomes of ST11, -340 and -437; and (5) different CR genes and plasmids: ST258 are associated with KPC, while ST11 also harbor other CR genes, on different plasmids. Novel *cps* groups in ST258 mainly carry $bla_{\text{KPC-2}}$ which is located on a novel plasmid. Upon inoculation into mouse lungs, CG258 isolates differ in terms of in vivo expansion and clearance by neutrophils.**Conclusions:** Cps replacement is largely driving the molecular evolution in CG258, however, additional chromosomal recombination and plasmid acquisition also contribute to reconstructing CG258, which may create strains of novel phenotypes.

Author Disclosure Block:

L. Chen: None. **H. Xiong:** None. **E.G. Pamer:** None. **J.D. Pitout:** None. **M.D. Adams:** None. **B.N. Kreiswirth:** None.

Poster Board Number:

SUNDAY-305

Publishing Title:

The Genome of ST307, an Emergent *Klebsiella pneumoniae* Clone Producing KPC and CTX-M-15

Author Block:

L. Villa[1], C. Feudi[1], D. Fortini[1], M. Iacono[2], J. Pires[3], C. Mammina[4], C. Bonura[4], A. Endimiani[5], **A. Carattoli**[1]; [1]Istituto Superiore Sanità, Roma, Italy, [2]Roche Diagnostic S.P.A., Monza, Italy, [3]Inst. of Infectious Diseases, BERN, Switzerland, [4]Univ. of Palermo, Palermo, Italy, [5]Univ. of Bern-Inst. of Infectious Diseases, Bern, Italy

Abstract Body:

Background: *Klebsiella pneumoniae* producing KPC(KP-KPC) of the ST258 and related variants (Clonal Group CG258) spread worldwide. Here, we report the complete genome sequencing of the emerging KP-KPC clone ST307. This clone differently by CG258 produces the ESBL CTX-M-15.**Methods:** 94 KPC-KP isolates from a surveillance study conducted in 2014 in Neonatal Intensive Care Units of Palermo, Italy, were analyzed for ESBL, carbapenemase, PMQR, and plasmid content and typed by MLST. ST307 genome was compared against ST258 reference genomes NJST258 and KPNIH1.**Results:** Multifocal dissemination of KPC-3-producing KP clones was observed, the predominant KPC-3-CG258 clone was identified in 38/94 (40%) patients, but in 27/94 (28%) the ST307 was found. ST307 also carried *aac(6')-Ib-cr* and *qnrB* genes. The complete sequence of the ST307 strain KP-48, isolated from urine, revealed that the assembled genome size was 5,687,551 bp in length. 6,080 predicted coding sequences (CDSs), 76 tRNAs, and 25 rRNAs were identified in the genome (GenBank Acc. No. LKAB00000000). Three extrachromosomal elements were identified: the two IncFIIK plasmids pKpQIL and pKN3 carrying $bla_{\text{KPC-3}}$ and $bla_{\text{CTX-M-15}}$ genes, respectively and a novel phage. The pKN3 plasmid also contained two novel virulence clusters, encoding the enzymes for glycogen synthesis and a complete urea transport system. A second phage (HK97-like) was identified, integrated in the chromosome. The wzi-173 capsular antigen allele for this strain was exacerbated from the genome sequence, defining the KN2 capsular type. Interestingly, the ST307 genome also carried a second capsular cluster, whose best match was found against *Enterobacter aerogenes* genomes. Putative VirB Type IV secretion and iron uptake proteins were identified in regions of major discordance with the ST258 genome.**Conclusions:** The ST307 clone has different resistance and virulence features than CG258. The characteristics identified in silico -the urea transport system, KN2 capsular type, and the Enterobacter-like additional capsular cluster- may favor its expansion as a persistent colonizer of the urinary tract.

Author Disclosure Block:

L. Villa: None. **C. Feudi:** None. **D. Fortini:** None. **M. Iacono:** None. **J. Pires:** None. **C. Mammina:** None. **C. Bonura:** None. **A. Endimiani:** None. **A. Carattoli:** None.

Poster Board Number:

SUNDAY-306

Publishing Title:

Genomic and Functional Characterization of IncX3 Plasmids Harbouring *bla*$_{SHV-12}$ in *Escherichia coli* from Human and Animal Origin

Author Block:

A. Liakopoulos, A. Kant, F. Harders, M. Brouwer, D. Ceccarelli, H. Smith, D. Mevius; CVI of Wageningen Univ. and Res. Ctr., Lelystad, Netherlands

Abstract Body:

Background: Only a few IncX3 plasmids have been characterised, limiting our insight into the biology of this plasmid subtype. The aim of this study was to elucidate the biology of IncX3 plasmids encoding the *bla*$_{SHV-12}$ gene from diverse origins by sequencing and functional analysis. **Methods:** Nine epidemiologically unrelated isolates exhibiting extended-spectrum cephalosporin-resistant phenotype were included in the study. Species identification was performed using MALDI-TOF MS, while the genetic relatedness was assessed by MLST. The presence of ESBL/AmpC genes was determined by microarray analysis, PCR amplification and sequencing. Transformation experiments were performed to assess plasmid location of the ESBL/AmpC genes.Plasmids were characterized by PCR-based replicon typing, S1-PFGE, mating and stability assays. Quantification of the plasmid copy numbers and the fitness cost are currently under way. Plasmid DNA was extracted from transformants and sequenced using 150 bp paired-end libraries on an Illumina MiSeq sequencer. High quality filtered reads were assembled *de novo* and annotated.**Results:** All isolates were identified as *E. coli* belonging to five different STs and were recovered from human infections (ST69, n=1), food-producing animals (ST117, n=5; ST315, n=1) and retail meat (ST115, n=1; ST410 n=1). The *bla*$_{SHV-12}$ gene was located on non-typeable plasmids based on standard PBRT analysis (approximately 45 Kb), with stability ranging between 99 and 100% over 200 generations and transfer frequencies between $2.3*10^{-6}$ and $7.0*10^{-4}$ transconjugants per donor cell.Plasmid sequences varied in size between 43,506 and 48,414 bp, while 65 to 73 coding sequences were predicted per plasmid. All plasmids were found to belong to the IncX3 subgroup and co-carried *qnrS*1 (n=8) or *bla*$_{TEM-1}$ (n=1). Plasmid backbone shared high similarity to plasmid pIncX-SHV (JN247852), including sequences encoding replication, partitioning, conjugation/type IV secretion system, transcriptional activator and putative DNA transfer proteins.**Conclusion:** Our data underscore the potential of spreading and maintenance of these plasmids encoding for decreased susceptibility to first-line antimicrobial agents and limiting the effective treatment options for human infections.

Author Disclosure Block:

A. Liakopoulos: None. **A. Kant:** None. **F. Harders:** None. **M. Brouwer:** None. **D. Ceccarelli:** None. **H. Smith:** None. **D. Mevius:** None.

Poster Board Number:

SUNDAY-307

Publishing Title:

Longitudinal Kpc-Producing *k. Pneumoniae* (Kp) Bloodstream Isolates (Bsi) Exhibit Differences In Colistin (Col) Susceptibility, Virulence, And Chromosomal And Plasmid Genes

Author Block:

M. Nguyen[1], S. Cheng[1], L. Chen[2], R. Shields[1], B. Kreiswirth[2], C. Clancy[1]; [1]Univ. of Pittsburgh, Pittsburgh, PA, [2]PHRI-Rutgers, Newark, NJ

Abstract Body:

Background: Molecular mechanisms of virulence among KPC-Kp are poorly understood. We recovered COL-susceptible (S) and -resistant (R) ST258 KPC-Kp BSIs from a patient (pt) who failed COL treatment (BSI Number 1 and Number 2, respectively). We hypothesized that genomes of the BSIs would be closely related, but virulence would be attenuated in BSI Number 2. **Methods:** We performed whole genome sequencing using Illumina MiSeq, and tested BSIs for virulence in a mouse model of intra-abdominal infection (IAI). **Results:** Genomes differ by 25 single nucleotide polymorphisms (SNPs) and 16 insertion-deletions (15 intergenic, 1 frameshift). Core genes containing non-synonymous SNPs include pmrB and ramR. PmrB encodes the sensor in a two component system that regulates lipopolysaccharide modifications; pmrB mutations have been implicated in COL R. RamR is a transcriptional repressor that controls expression of multidrug efflux genes and regulates virulence in Gram negative bacteria. In our large repository, 3 KPC-Kp from unique patients collected years apart have core genomes that are virtually identical to each of BSI Number 1 and Number 2 (0-2 SNPs). Therefore, BSI Number 2 is likely a circulating hospital strain that was acquired independently, rather than a strain that evolved from BSI Number 1 during treatment. BSI Number 2 is ~100 kb larger than strain Number 1 due to plasmid differences. BSIs Number 1 and Number 2 contain 250 kb and 350 kb that are unique, respectively. BSI Number 2 carries pKLN-IT, an $IncFII_k$-FIB-like plasmid that contains Fec-like iron (III) uptake and glutathione ABC transport systems. At high inocula, BSI Number 1 caused significantly greater mortality than BSI Number 2 during IAI. However, at lower inocula, BSI Number 2 persisted for significantly longer periods and at higher burdens within IA abcesses. **Conclusions:** A COL-R ST258 KPC-Kp BSI was less able to kill mice than a closely-related COL-S BSI, but caused more severe and persistent disease during sublethal IAI. KPC-Kp chromosomal genes (such as pmrB and ramR, which regulate multiple targets) and plasmid genes (such as those involved in iron uptake) may make unique contributions to virulence, at different stages of infection. Individual pts can be infected with multiple KPC-Kp strains, with different resistance and virulence profiles.

Author Disclosure Block:

M. Nguyen: None. **S. Cheng:** None. **L. Chen:** None. **R. Shields:** None. **B. Kreiswirth:** None. **C. Clancy:** None.

Poster Board Number:

SUNDAY-308

Publishing Title:

Comparative Genomic of Clonal Complex (Cc) 11 of Carbapenem Resistant *K. pneumoniae* (Cr-Kp) Clinical Isolates from Brazil

Author Block:

J. K. Palmeiro[1], A. L. Grazziotin[2], R. F. de Souza[3], N. M. Vidal[2], M. A. Schörner[4], T. M. Venancio[2], L. M. Dalla-Costa[1]; [1]HC-UFPR; FPP/IPPPP, Curitiba, Brazil, [2]Lab. de Química e Função de Proteínas e Peptídeos, CBB/UENF, Campos de Goytacazes, Brazil, [3]LEEP/ICB/USP, São Paulo, Brazil, [4]HU-UFSC, Florianópolis, Brazil

Abstract Body:

Background: Over the past decade CR-Kp has been recognized in health-care settings as a cause of difficult-to-treat infections associated with high mortality. Here we report a comparative genomic analysis of nosocomial Kp isolates representing different times and resistance profiles in Southern Brazil. **Methods:** A total of 51 Kp clinical isolates recovered during 2005-2012 were studied. AST was performed using agar dilution. PCR was used to detect resistance genes. PFGE was carried out to discriminate strains. Based on isolated timing, resistance profiles and genetic features, six Kp isolates were sequenced on a Illumina HiSeq 2000 platform using the RAPID method. Genomes were assembled using Velvet and SSPACE and annotated using RAST, Prokka, BLAST, ARDB and CGE servers. **Results:** In 2010, KPC-Kp (KpA2 and KpA3) caused an outbreak at our university's hospital. KpD8/KpC9 and KpB10/KpC2 were isolated before and after the outbreak, respectively. All isolates showed multidrug resistance (MDR) profiles and belonged to CC11: four ST437 (PFGE profile A and G) and two ST11 (PFGE profile L and Q). Resistance genes *bla*$_{KPC-2}$, *bla*$_{CTX-M2}$, *bla*$_{CTX-M3}$, *bla*$_{CTX-M15}$, *bla*$_{TEM-1B}$, *bla*$_{OXA-1}$, *bla*$_{OXA-2}$, aac(3')-IId, aac(6')-Ib-cr, aph(3')-Ia, ant(2")-Ia, ant(4')-Ia, aadA and *qnrB1* were variedly distributed among the isolates and presumably contributed to the diverse MDR profiles. The *bla*$_{KPC-2}$ genes were found to be located within Tn4401b and to be associated with plasmids of similar Inc groups. Such groups were markedly different from the ones found in our non-KPC isolates. KpC9 was unique because it did not have *bla*$_{KPC-2}$ but was resistant to carbapenem, thus suggesting the involvement of other resistance genes. **Conclusions:** Our isolates belong to CC11 and have *bla*$_{KPC-2}$ associated to Tn4401b. We have found the emergency of multi-resistant Kp in our samples was parallel to radical shifts in plasmid composition, thus suggesting that horizontal gene transfer was key to the evolution of the 2010 outbreak.

Author Disclosure Block:

J.K. Palmeiro: None. **A.L. Grazziotin:** None. **R.F. de Souza:** None. **N.M. Vidal:** None. **M.A. Schörner:** None. **T.M. Venancio:** None. **L.M. Dalla-Costa:** None.

Poster Board Number:

SUNDAY-309

Publishing Title:

Single-molecule Sequencing of Pooled Conjugable Plasmids from Clinical Multidrug Resistance *Klebsiella pneumoniae* Revealed Transposons and Resistant Genes Horizontal Transfer Profile

Author Block:

Y. Jiang, Y. Wang, X. Hua, Z. Ruan, Y. Feng, P. Shen, Y. Yu; Sir Run Run Shaw Hosp., Coll. of Med., Zhejiang Univ., Hangzhou, China

Abstract Body:

Background: Plasmids play an important role in horizontal gene transfer (HGT), particularly in the dissemination of antibiotic resistance. To investigate the transposons and resistant genes HGT profile in plasmids, we designed a single-molecule real time (SMRT) sequencing assay of pooled conjugable plasmids from clinical multidrug resistance (MDR) *Klebsiella pneumoniae*.**Methods:** 53 nonduplicated MDR *K. pneumoniae* strains were obtained from affiliated hospital of Zhejiang University in China in 2009. All of these strains were confirmed harboring at least one comjugable plasmid. Plasmid conjugation was carried out by filter mating assay. The antimicrobial susceptibility were determined by K-B method. An S1 nuclease assay was performed in transconjugant strains to estimate the size of large plasmids in the presence of genomic DNA using PFGE. All conjugable plasmids from transconjugant strains were abstracted and pooled, and then they were sequenced by SMRT technique using PacBio platform.**Results:** The 53 clinical *K. pneumoniae* strains showed MDR phenotype against β-lactams, aminoglycosides, fluoroquinolones, and so on. Most of these resistance profiles could be transferred to transconjugants by plasmid conjugation. The 53 conjugable plasmids were 34kb to 355kb in size as estimate. With complete SMRT sequencing and assembling, 389 assembled fragments which have been designated as "unitigs" were acquired. All of unitigs were annotated by gene prediction and BLAST tools. 84 of 389 unitigs were deemed as plasmid fragments based on their gene annotation. There were 85 resistant genes (30 non-redundant genes) found in 84 plasmid fragments using the ResFinder tool, such as aminoglycoside resistant gene *aph(3')-Ia*, *rmtB*; β-lactamase gene bla_{SHV}, bla_{TEM}, bla_{CTX-M}, bla_{KPC}; fluoroquinolone resistant gene *qnrB4*, *qnrS1*; tetracycline resistant gene *tet*, and so on. Furthermore, 259 genes related with transposons or IS elements (96 non-redundant) were found using IS-Finder, including Tn3, Tn501, IS1, IS4, IS26, IS501, IS903 and so on. Most of HGT elements were related with resistant genes.**Conclusions:** We concluded that transposons and resistant genes were diversity in clinical MDR *K. pneumoniae* strains.

Author Disclosure Block:

Y. Jiang: None. **Y. Wang:** None. **X. Hua:** None. **Z. Ruan:** None. **Y. Feng:** None. **P. Shen:** None. **Y. Yu:** None.

Poster Board Number:

SUNDAY-310

Publishing Title:

Global Analysis of Insertion Sequence (IS) Elements

Author Block:

M. D. Adams[1], B. Bishop[2], M. S. Wright[1]; [1]J. Craig Venter Inst., La Jolla, CA, [2]J. Craig Venter Inst., Rockville, MD

Abstract Body:

Background: Insertion sequence (IS) elements are active in many bacterial species and can have significant contributions to genome diversity. IS elements participate in the acquisition and spread of antimicrobial resistance genes in multidrug-resistant *Acinetobacter baumannii* and *Klebsiella pneumoniae* and have led to the sharp increase in carbapenem resistance in both species. Few studies have addressed genome-wide patterns of IS distribution using the wealth of genomic data that now exists for several pathogens. Comprehensive analysis of the IS element locations is important for understanding their evolutionary impact, but defining their insertion sites in draft genomes is challenging because typical sequencing and assembly methods do not resolve individual IS copies. **Methods:** We developed and applied a computational pipeline (ISseeker) to identify and annotate the termini of IS elements at contig edges in draft genome assemblies and applied the method to analysis of IS*Aba* and IS*Kpn* elements in all publicly available *A. baumannii* (>900) and *K. pneumoniae* (>750) genome sequences and evaluated the locations in each genome in the context of the core phylogeny for each species.**Results:** IS elements and insertion locations were more abundant and diverse in *A. baumannii* genomes than in *K. pneumoniae* genomes. In *K. pneumoniae*, IS elements associated with the bla_{KPC} gene are shared by most ST258 genomes, whereas in *A. baumannii*, the abundant IS elements are different in distinct phylogenetic groups. The location of IS elements relative to coding regions varies by the element, with some more likely to disrupt genes and others predominantly in intergenic regions. Multiple independent insertion events were found in several genomic regions, suggesting that those regions may play important roles in antimicrobial resistance or host adaptation.**Conclusions:** IS elements have played a larger role in shaping *A. baumannii* genomes than *K. pneumoniae* genomes, based on currently available sequence data.

Author Disclosure Block:

M.D. Adams: None. **B. Bishop:** None. **M.S. Wright:** None.

Poster Board Number:

SUNDAY-311

Publishing Title:

Novel Pathogenic *Acinetobacter* Species Isolated at a Large Tertiary Care Facility

Author Block:

J. Stam[1], R. Clifford[1], E. Snesrud[1], L. Appalla[1], F. Onmus-Leone[1], P. Mc Gann[1], A. Ong[1], R. Maybank[1], Y. Kwak[1], P. Waterman[2], M. Hinkle[1], E. Lesho[1]; [1]Walter Reed Army Inst. of Res., Silver Spring, MD, [2]Armed Forces Hlth.Surveillance Ctr., Silver Spring, MD

Abstract Body:

Background: *Acinetobacter baumannii* is a serious threat to public health because of its ability to rapidly acquire drug resistance. To examine the epidemiology of multidrug resistant *A. baumannii* in a large tertiary care hospital, we performed whole genome sequencing on 1024 isolates collected from 2005 through 2011. We found that 55 isolates first identified as *A. baumannii* are members of distinct, but closely related species. Several may define novel pathogenic *Acinetobacter* species. **Methods:** All isolates identified as *A. baumannii* were provided by the hospital microbiology laboratory. Strains were reanalyzed on automated identification / antibiotic susceptibility testing platforms. Whole genomic sequencing was performed on the Illumina MiSeq or NextSeq platforms. Draft genome sequences were assembled and analyzed using a bioinformatics pipeline developed by MRSN.**Results:** The non-baumannii isolates were associated with bloodstream, sterile tissue, urinary tract, respiratory and wound infections, in addition to surveillance cultures. Seven isolates were multidrug resistant.Whole genome clustering based on core genome SNPs indicated that 55 isolates are not *A. baumannii*. The BLAST-based average nucleotide identity (ANIb) of these isolates to the *A. baumannii* reference strain ACICU ranged from 86.8% to 91.4%.The non-baumannii strains fall into three main branches. One strain, MRSN11738, is quite diverged from all others, showing only 75.9% ANIb to ACICU. Twenty-five isolates are more similar to *A. baumannii.* Three of these form one cluster, while the rest make up a cluster that includes *Acinetobacter genomosp*. 13TU. The remaining 29 strains are more similar to *A. calcoaceticus*, with ANIbs to reference strain PHEA-2 ranging from 96.1% and 96.7%. Isolates in this branch of the phylogenetic tree show more genomic diversity than those that are more similar to *A. baumannii*. These strains fall into at least 10 subgroups.**Conclusions:** Using whole genome sequencing we identified 55 healthcare associated *Acinetobacter* that are not *A. baumannii*. Isolates from several of these putative novel pathogenic species exhibit multidrug resistant phenotypes. Further characterization of these strains is ongoing.

Author Disclosure Block:

J. Stam: None. **R. Clifford:** None. **E. Snesrud:** None. **L. Appalla:** None. **F. Onmus-Leone:** None. **P. Mc Gann:** None. **A. Ong:** None. **R. Maybank:** None. **Y. Kwak:** None. **P. Waterman:** None. **M. Hinkle:** None. **E. Lesho:** None.

Poster Board Number:

SUNDAY-312

Publishing Title:

Rapid Identification New Delhi Metallo-β-Lactamase-5 (Ndm-5) in US Hospital Populations Using Prospective Genomic Surveillance

Author Block:

X. Zhao, N. Li, N. D. Pecora, L. Bry; Brigham and Women's Hosp., Harvard Med. Sch., Boston, MA

Abstract Body:

Background: The rapid spread of NDM-5 producing *Enterobacteriaceae* has raised serious concerns for healthcare systems. Within our hospital, multiple instances of NDM-5 have been identified in patients with international exposures. We present findings from our prospective genomic surveillance program that rapidly identified two *bla*$_{\text{NDM-5}}$-carrying *Escherichia coli* (*E.coli)* isolates using whole genome sequencing (WGS). We present the use of actionable data from genomic findings to support ongoing surveillance and support of patient care.**Method:**27 CRE isolates were collected from BWH during 2015. Species were identified by Vitek and phenotypic resistance determined by MIC and disk diffusion. WGS was performed on the Illumina MiSeq and generated paired-end reads of 250 bp with 90X average coverage. A locally developed clinical pipeline handled the assembly and analysis of resistance genes, mobile elements, and strain types, and further integrated epidemiologic data to identify local and international characteristics of strains.**Results:**Of the 27 sequenced isolates, 2 *E.coli* strains (8761 and 1465) carried *bla*$_{\text{NDM-5}}$. Epidemiologic analyses identified that 8761 was isolated from a patient who had recent hospitalizations in China and 1465 was from USA patient with hospitalizations in India. In addition to beta-lactam antibiotics, these strains were highly resistant to other drug classes. Multiple plasmids were identified in both isolates, including Inc group F, A/C2, and IncX3 plasmid which have played crucial roles in the dissemination of the *bla*$_{\text{NDM.}}$**Conclusion:** Our genomic surveillance program identified *bla*$_{\text{NDM-5}}$ positive *E.coli* isolated from two patients from different countries indicating spread of NDM carrying *E.coli* across international borders. Therefore, international cooperation between healthcare institutes will be required for robust surveillance and control of these drug resistant pathogens. With international travel, urban medical centers need to remain vigilant for the pathogens and forms of drug resistance that may spread from other locations. Clinical use of bacterial WGS provides a valuable tool to track and rapidly identify underlying causes of resistance and virulence, further supporting clinical actions taken to reduce the transmission and spread of these organisms.

Author Disclosure Block:

X. Zhao: None. **N. Li:** None. **N.D. Pecora:** None. **L. Bry:** None.

Poster Board Number:

SUNDAY-313

Publishing Title:

The Use of Comparative Genomics to Characterize the Diversity of *Acinetobacter baumannii* in a Health Care Institution

Author Block:

L. Wallace[1], T. H. Hazen[1], S. C. Daugherty[1], S. Nagaraj[1], J. Johnson[2], A. D. Harris[2], D. A. Rasko[1]; [1]Univ. of Maryland Inst. for Genome Sci., Baltimore, MD, [2]Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract Body:

Despite the increasing prevalence of the nosocomial pathogen *Acinetobacter baumannii*, little is known about the genomic components which contribute to clinical presentation of this important pathogen. Whole genome comparisons of *A. baumannii* have focused on specific genomic regions associated with phenotypes in a limited number of genomes, often associated with antimicrobial resistance. In this work we describe the results of a whole genome comparative analysis of 254 surveillance isolates of *Acinetobacter* species isolates, 203 of which were identified as *A. baumannii*. These isolates were obtained as part of an infection control active surveillance program at the University of Maryland Medical Center. The collection of surveillance isolates includes both carbapenem sensitive and resistant isolates obtained from either sputum or perirectal swabs. Based on the whole genome phylogeny, the *A. baumannii* isolates belong to two major phylogenomic lineages. Results from multi-locus sequence typing indicated that one of the major phylogenetic groups of *A. baumannii* was comprised solely of strains from the international clonal lineage 2. The genomic content of the *A. baumannii* isolates was examined using Large-Scale BLAST Score Ratio analysis to identify genes that are associated with carbapenem sensitive and resistant isolates, as well as genes associated with the source of isolation. This analysis revealed a number of genes that were exclusive or present at a greater frequency among these groups, This study is the most comprehensive genomic comparison of *Acinetobacter* isolates to date and provides important information that will contribute to our understanding of the success of *A. baumannii* as a human pathogen.

Author Disclosure Block:

L. Wallace: None. **T.H. Hazen:** None. **S.C. Daugherty:** None. **S. Nagaraj:** None. **J. Johnson:** None. **A.D. Harris:** None. **D.A. Rasko:** None.

Poster Board Number:

SUNDAY-314

Publishing Title:

Exploration Of An *acinetobacter Johnsonii* clinical Strain Genome Revealed The Presence Of Extensive Horizontal Gene Transfer (Hgt)

Author Block:

S. Montaña[1], **K. Chiem**[2], S. T. J. Schramm[2], G. M. Traglia[1], G. Parmeciano Di Noto[1], M. Almuzara[3], C. Barberis[3], M. E. Tolmasky[2], C. Vay[3], C. Quiroga[1], A. Iriarte[4], M. S. Ramírez[2]; [1]IMPaM, UBA-CONICET, Buenos Aires, Argentina, [2]CSUF, Fullerton, CA, [3]FfyB, UBA, Buenos Aires, Argentina, [4]IIBCE, Montevideo, Uruguay

Abstract Body:

Background: In recent years, *Acinetobacter* spp. such as *A. johnsonii* has been considered an emergent pathogen that can cause human infections. While most *A. johnsonii* isolates are susceptible, strains harboring a variety of β-lactamases have recently been described. Here, we present research on the genome of an Aj2199 clinical strain that was co-producing PER-2 and OXA-58. **Methods:** A draft sequence for *A. johnsonii* Aj2199 was obtained using Illumina MiSeq. SPAdes assembler was used for *de novo* assembly and RAST server to predict open reading frames (ORF). OrthoMCL method was used to identify homologous genes. Different softwares (ARG-ANNOT, ISfinder, PHAST, etc) were used to explore the genome. PCR reactions and conjugation assays were also carried out.**Results:** Assembled contigs totaled 3,803,969 base-pairs with an N50 contig size of 70,198 and has a G+C content of 41.4 %. A total of 3737 possible ORFs were predicted.Genomic comparison identified 1204 gene families in *A. johnsonii* strains, while 240 were exclusively found in Aj2199. The 240 gene families were comprised of 254 genes, of which, more than 164 of these were annotated as hypothetical proteins and 24 were mobile elements. Moreover, 46 complete insertion sequences (IS) and truncated IS as well as the presence of three intact prophages were found. The analysis of $bla_{\text{PER-2}}$ genetic context revealed the presence of the previously described context from the *Enterobacteriacea* family suggesting genetic exchange.**Conclusions:** Genomic analysis of Aj2199 showed evidence of extensive HGT events indicated by the presence of a wide variety of different mobile elements and resistance determinants in Aj2199. The data obtained from our genomic analysis strongly suggested the promiscuity of *A. johnsonii* to easily acquire exogenous DNA from other species. The detection of antimicrobial resistance determinants and mobile elements expose the potential for *A. johnsonii* to evolve into a multiresistant bacterium and will explain the further spread of these genes into other species.

Author Disclosure Block:

S. Montaña: None. **K. Chiem:** None. **S.T.J. Schramm:** None. **G.M. Traglia:** None. **G. Parmeciano Di Noto:** None. **M. Almuzara:** None. **C. Barberis:** None. **M.E. Tolmasky:** None. **C. Vay:** None. **C. Quiroga:** None. **A. Iriarte:** None. **M.S. Ramírez:** None.

Poster Board Number:

SUNDAY-315

Publishing Title:

Genome Sequencing Reveals Global Spread of Stable Chromosomal CTX-M-15 in *Escherichia coli* ST648

Author Block:

N. Stoesser[1], H. Phan[1], D. Eyre[1], A. Sheppard[1], P. Turner[2], R. Sebra[3], A. Kasarskis[3], C. Moore[1], D. Dance[4], P. Newton[4], R. Phetsouvanh[4], T. Peto[1], S. Walker[1], D. Crook[1]; [1]Univ. of Oxford, Headington, United Kingdom, [2]Cambodia-Oxford Med. Res. Unit, Siem Reap, Cambodia, [3]Icahn Inst. for Genomics, New York, NY, [4]Lao-Oxford-Mahosot Hosp.-Wellcome Trust Res. Unit, Vientiane, Lao People's Democratic Republic

Abstract Body:

Background:A subset of clinical clones of *E. coli* (e.g. ST131, ST73) cause a disproportionate burden of global *E. coli*-attributable disease. The association of these clones with broad-spectrum antimicrobial resistance genes is a major clinical problem.Recently, ST648 has emerged as another common lineage associated with the ESBL gene $bla_{\text{CTX-M}}$, in particular $bla_{\text{CTX-M-15}}$. We sought to identify the genetic basis for this association using whole genome sequencing.**Methods:**We identified 27 novel, non-duplicate, ST648 sequences from our Modernising Medical Microbiology (MMM) databank of >1500 *E. coli* sequences (Illumina; clinical/carriage isolates, UK/South-East Asia; 2004-2012), and 90 from data in the sequencing read archive (identified from Enterobase).Reads from both sources were mapped and *de novo* assembled, and resistance genes identified with an in-house resistance gene database/gene-finding tool. Phylogenies were created using ClonalFrameML; a time-scaled analysis was performed in BEAST on a subset of sequences for which isolation dates were available.We also performed long-read PacBio sequencing on 1 $bla_{\text{CTX-M-15}}$-positive isolate (la_12008-2) in order to confirm the chromosomal/plasmid structures and genetic context of $bla_{\text{CTX-M-15}}$.**Results:**66/117 (56%) isolates were $bla_{\text{CTX-M-15}}$-positive. Chromosomal integration of $bla_{\text{CTX-M-15}}$ was confirmed in 27/66 (41%) cases in several contexts: interrupting a fructose transporter (n=5) or *lacY* (n=1), and downstream of *nanM* (n=2) or *asd-dprA* genes (n=19). The latter context was confirmed in la_12008-2, with a 5.12Mb chromosome containing $bla_{\text{CTX-M-15}}$ in a resistance gene cassette. 18 other cases with this chromosomal context were internationally distributed (UK, Germany, Laos, Cambodia, Thailand) and clustered on the phylogeny, suggestive of stable inheritance and dispersal since 2002.**Conclusions:**Chromosomal acquisition and stable inheritance of $bla_{\text{CTX-M-15}}$ represents a significant genetic mechanism for its dissemination in the major clinical lineage *E. coli* ST648.

Author Disclosure Block:

N. Stoesser: None. **H. Phan:** None. **D. Eyre:** None. **A. Sheppard:** None. **P. Turner:** None. **R. Sebra:** None. **A. Kasarskis:** None. **C. Moore:** None. **D. Dance:** None. **P. Newton:** None. **R. Phetsouvanh:** None. **T. Peto:** None. **S. Walker:** None. **D. Crook:** None.

Poster Board Number:

SUNDAY-316

Publishing Title:

Genomic and Epidemiological Determinants of *Enterobacter cloacae* Transmission

Author Block:

S. E. Whitefield[1], L. L. Washer[2], C. A. Scipone[2], C. R. Dombecki[2], D. W. Newton[2], E. S. Snitkin[1]; [1]Univ. of Michigan Med. Sch., Ann Arbor, MI, [2]Univ. of Michigan Hlth.System, Ann Arbor, MI

Abstract Body:

Background: The emergence of healthcare associated carbapenem resistant *Enterobacteriaceae* (CRE) infections is concerning because carbapenems are a last line treatment for resistant gram-negative infections. Prior CRE work has focused on *Klebsiella pneumoniae*, due to widespread dissemination of *K. pneumoniae* carbapenemase (KPC)+ strains. Less is known about the genetic epidemiology of other CRE, such as *Enterobacter cloacae.* In 2015, 8 *E. cloacae* isolates were identified from clinical samples of patients who underwent a procedure in our hospital that utilized duodenoscopes with elevator channels. This type of device has been linked to previous CRE outbreaks, raising concerns that contaminated devices mediated transmission. **Methods:** We sought to determine if duodenoscopes at our institution were a vehicle for CRE transmission, through a combined epidemiological investigation and whole genome sequencing of isolates from 77 *E. cloacae* infections in our hospital since 2012. We compared genetic distances and gene content between isolates to rule out transmissions and infer factors that contribute to dissemination of *E. cloacae* strains.**Results:** Comparison of genetic distances between the 5 available isolates from patients undergoing endoscopy, definitively ruled out duodenoscope-mediated transmission among 3 patients. Of the remaining isolates with no duodenoscope exposure, 55 showed no evidence of transmission, and 17 were found in 6 putative transmission clusters. The genetically homogeneous transmission clusters are in stark contrast with the diverse population of *E. cloacae* circulating in our hospital, which spans the full diversity of all ~300 *E. cloacae* genomes in Genbank. Inspection of the largest cluster in the context of previously sequenced strains revealed that all five members carry a KPC gene, and a close genetic relationship with isolates from a recent *E. cloacae* CRE outbreak in Minnesota.**Conclusions:** Despite most CRE *E. cloacae* at our hospital not encoding carbapenemases, we find the majority of transmissions involve KPC+ strains. The observation that the most prevalent strain also recently caused an outbreak suggests an emerging lineage of CRE *E. cloacae* that is stably associated with a KPC plasmid and transmissible in hospital settings.

Author Disclosure Block:

S.E. Whitefield: None. **L.L. Washer:** None. **C.A. Scipone:** None. **C.R. Dombecki:** None. **D.W. Newton:** None. **E.S. Snitkin:** None.

Poster Board Number:

SUNDAY-317

Publishing Title:

Prediction of Resistance Phenotypes from Microbial Genomes

Author Block:

M. FELDGARDEN, A. Badretdin, V. Brover, M. DiCuccio, D. Haft, A.Prasad, M. Shumway, W. Klimke; NCBI/NLM/NIH/DHHS, Bethesda, MD

Abstract Body:

Antimicrobial resistance (‘AMR’) is a major public health problem, especially now that multi- and totally drug-resistant clinical isolates have been observed. In the U.S., the National Strategy on Combating Antibiotic Resistant Bacteria has called for a better understanding of how bacterial genotype is related to antimicrobial resistance phenotype.To facilitate linking phenotype to genotype, NCBI has built on the existing BioSample database that provides descriptions of biological source material including isolate metadata for microbial pathogens. BioSamples are linked to sequence data providing direct links between published sequenced data, isolate metadata, and AMR phenotypes. The antibiotic resistance phenotypes are stored as an antibiogram table linked to the BioSample record. Phenotype fields include antibiotic compound, measurement data, typing method and platform, and antimicrobial susceptibility phenotypes using a standardized template with controlled vocabularies. Standardized fields streamline submissions as well as provide a structured format for researchers to comprehensively search across all records to find those relevant to their research. To date, NCBI has received 789 antibiograms.To identify AMR genes, NCBI is collaborating with external expert groups curating AMR genes to produce a high-quality reference database with up-to-date nomenclature; the database contains 4868 resistance gene proteins belonging to 1130 gene families. To identify resistance proteins in annotated genomes, we use a dual strategy of protein BLAST and hidden Markov models. To test the accuracy of this method, we assessed the correlation between our predicted AMR genotypes and resistance phenotypes in 248 clinical isolates. We found 94.3% of predicted genotypes were consistent with resistance phenotypes. The false positive and negative resistance genes resulted from limitations of currently accepted sequencing and assembly technology and software.The NCBI reference database of AMR sequences has been made available (see link at http://www.ncbi.nlm.nih.gov/pathogens). The linking of detailed antimicrobial susceptibility data to well-annotated bacterial genomes and the curated AMR gene reference set in publicly accessible databases at NCBI will be critical in the fight against antimicrobial resistance.

Author Disclosure Block:

M. Feldgarden: None.

Poster Board Number:

SUNDAY-318

Publishing Title:

Identification of Pathogenicity-associated Loci in *Klebsiella pneumoniae* clinical Isolates

Author Block:

R. M. Martin, K. Rao, W. Wu, L. Zhao, D. Manthei, L. Roberts, J. Cao, P. N. Malani, M. A. Bachman; The Univ. of Michigan, Ann Arbor, MI

Abstract Body:

Klebsiella pneumoniae is a leading cause of healthcare-associated infections (HAI) in the U.S. *K. pneumoniae* commonly colonizes hospitalized patients, and a subset of colonized patients goes on to develop extra-intestinal infections such as urinary tract infections, septicemia, and pneumonia. Though several virulence genes have been identified to date, the bacterial factors that determine whether an isolate causes disease or remains a colonizer are poorly understood. We hypothesize that horizontally-acquired genes promote extra-intestinal infection in colonized patients. To identify host factors and bacterial genes associated with *K. pneumoniae* infection, we performed a case-control study comparing infected patients meeting standard case definitions (n=39) and asymptomatic colonized patients (n=78), matched by age, sex, and sample collection date. Construction of a logistic regression model identified patient factors significantly associated with and predictive of infection (Area Under the Curve [AUC] of Receiver Operating Characteristic curve = 0.882). We then constructed a *K. pneumoniae* pan-genome, collapsed orthologous genes into "gene bins" and used read mapping from whole genome sequencing to compare the frequency of genes in each "gene bin" among colonizing and disease-causing strains. Using this Pathogenicity-Associated Locus Sequencing (PAL-Seq) method, we identified 5 gene bins associated with infection after adjustment for patient factors (P = 0.014-0.03). Incorporation of three of these gene bins improved the predictive power of our logistic regression model (Table; AUC = 0.951; P <.001). Our results suggest that genes in the accessory genome of *K. pneumoniae* are associated with HAIs and could provide a basis for novel predictive diagnostic approaches to identify colonized patients at risk of subsequent *K. pneumoniae* infection.

Table. Findings from final model of invasive *Klebsiella pneumoniae* infection

Variable	**OR (95% Confidence Interval)**	***P***
White race	0.03 (<0.01-0.75)	.032
Minimum serum glucose (mg/dL)	1.08 (1.01-1.16)	.026
BMI (kg/m^2)	0.69 (0.50-0.95)	.022
Fluid/electrolyte disorder	22.9 (1.6-329)	.021
Gene Bin: Metalloid resistance	157 (3.34-7350)	.010

Gene Bin: Putative deoxygluconate dehydrogenase	17.8 (2.2-143)	.007
Gene Bin: Hypothetical protein	16.9 (1.59-179)	.019

Author Disclosure Block:

R.M. Martin: None. **K. Rao:** None. **W. Wu:** None. **L. Zhao:** None. **D. Manthei:** None. **L. Roberts:** None. **J. Cao:** None. **P.N. Malani:** None. **M.A. Bachman:** None.

Poster Board Number:

SUNDAY-319

Publishing Title:

A New Automated Gram Smear Reading and Image Acquisition System for the Clinical Microbiology Laboratory

Author Block:

M. Grimaldi, **M. Oggioni**, A. Bielli, C. Lacchini, G. Lombardi, C. Vismara, G. Gesu; ASST Grande Ospedale Metropolitano Niguarda, Milano, Italy

Abstract Body:

Background: The microbiology laboratory has been automated with the implementation of different systems. Microscopy involves reading many Gram smear (GM) that are time demanding and tiresome to the eyes. The Metafer (MF) (MetaSystems) is a fully automated slide scanning and image recording platform that handles different sample types. High quality images are generated by MF with the future possibility to transfer to WASPLab^{TM} (WL)(Copan), next to same sample culture images (CI). The objective of this study was to evaluate the MF automated GM digital images acquisition in order to facilitate GM reading and results interpretation.**Methods:** WASP^{TM} (WA), prepared GM smears (N=427), 327 were sputa, broncoaspirates, bile, pus, exudate, surgical biopsies, throat, lesions, vaginal, uretrhal, wounds and abscess swabs, etc. and 100 blood cultures were used for this study. GMs were read in duplicate, one with the MF and the other with a manual microscope. Results were compared and discrepancies recorded. MF first scans the GM at 10x then reads at 63x, under oil, 9x4 field's set points on the smear and uses a scanning algorithm to recognize the presence of the sample on the slide. All samples were WA seeded on agars plates as per SOP. Gram (G) results were compared to bacteria culture.**Results:** In the 427 WA prepared smear, 327 different samples had concordant G results with both manual and automated microscopy readings. Concordant G and culture results were found in 269 samples, only 58 had discordant G and culture results due to presence of non-pathogenic bacterial flora. Both G readings and culture results of the 100 blood culture had 100% concordance. The smear pattern recognition resulted in better performance, with overall process improvement. The MF smear image acquisitions at 10x allows the assessment of smear quality, presence of cells identification and counting, while the images captured at 63x, permits a digital magnification up to 150x for better bacteria identification.**Conclusions:** The MF GM scanning and images acquisition was simple to use and reliable for reading GM prepared with the WA automation. The ability of MF to capture images and the future implementation to transfer to WL system, placing on the same screen (GM and CI), provides working time optimization, increases results reporting capabilities.

Author Disclosure Block:

M. Grimaldi: None. **M. Oggioni:** None. **A. Bielli:** None. **C. Lacchini:** None. **G. Lombardi:** None. **C. Vismara:** None. **G. Gesu:** None.

Poster Board Number:

SUNDAY-320

Publishing Title:

First Evaluationof the Prelud, a New Inoculation Automated System

Author Block:

p. grohs[1], L. Armand-Lefevre[2]; [1]Hosp. Européen Georges Pompidou, PARIS, France, [2]Hosp. Claude Bernard Bichat, PARIS, France

Abstract Body:

Background: The Prelud is a new inoculation automated system (I2a, Montpellier, France) designed to plate liquid specimens and swabs. It has a plating control system (PCS), a camera that detects the presence of the droplet and streaks on the medium. We evaluated here, (i) the performance of inoculation with the Prelud compared to manual method (ii) the PCS and (iii) the absence of cross contamination. **Methods:** Bacterial counts and isolated species of 277 urinary specimens, with bacteria on direct smear examination, were compared using the Prelud and the manual method inoculation. The PCS was assessed on 113 (207 media) urinary specimens. The number of different morphological and clinical relevant colonies was compared on 43 genital and 26 multi-drug resistant (MDR) bacteria positive rectal screening samples (E-swab). Quality of streaking was compared on all specimens. Microbiological cross-contamination was also evaluated. **Results:** A total of 346 specimens were processed. Among the 277 urinary samples tested, 216 (78%) showed a positive culture. Bacterial count were identical using the two methods in 142/216 (66%) samples and differed with less than 1 Log_{10} CFU/ml in 70/216. In 203/216 (94%) urinary samples, the same bacterial species were isolated with the two methods. Discrepancies concerned mostly samples (10/13) with less than 10 colonies ($<10^3$CFU/ml) on manual media *vs* no colony on Prelud media. The PCS triggered 68 alerts on 207 urinary media plated. Using the camera, 50/68 alerts were easily invalided. For 18/68 the droplet and/or the streaks could not be seen and a new medium was plated manually, most of them (13/18) concerning the Drigalski medium. No difference was observed comparing the MDR screening rectal samples. Few discrepancies (7/46 with +/- 1 colony) were observed for genital specimens, without any difference in clinical relevant colonies. No cross contamination was observed. Globally, the quality of streaking was higher with the Prelud. **Conclusions:** The Prelud is an innovative system that will improve the traceability of the inoculation step and the streaking quality of specimen. It has an important tool, the CPS, avoiding the risk of inoculation failed. Additional studies are merited to test the full potential of this instrument during the workflow of a laboratory, and to test the antibiotics susceptibility module.

Author Disclosure Block:

P. grohs: None. **L. Armand-Lefevre:** None.

Poster Board Number:

SUNDAY-321

Publishing Title:

Improvements in Time to Result Associated with the Bd Kiestra Inoqula

Author Block:

M. Maley, S. Neville, H. Ziochos, M. Chater, E. Treadwell, G. Parisi; Sydney South West Pathology Service, Sydney, Australia

Abstract Body:

Background: Processes in bacteriology laboratories have remained unchanged for years. Recently instruments have been introduced that automate many of the repetitive, labour intensive tasks often accompanied by claims for improved performance and efficiency. The SSWPS Microbiology lab is a large diagnostic lab operating 7 am to midnight 7 days per week. The laboratory introduced the BD Kiestra InoqulA in January 2014 to help cope with a steadily increasing workload unmatched by staff resources. This study aims to describe the impact on time to result for a range of culture based and non-culture-based tests.**Method:**Time from specimen collection to final result (TTR) was extracted from the LIS for deep wound and urine culture and for group B Streptococcus (GBS) and MRSA culture-based screening tests. Similar data was obtained for the non-culture-based tests urine pneumococcal antigen (UrPnAg) and urine and deep wound microscopy. The median TTR as well as the proportion of tests completed within a target time were determined for each month and an interrupted time series analysis was performed for the 12 months pre- and post-InoqulA implementation.**Results:**There was a reduction in median TTR and an increase in tests completed within target time for all culture based tests post-InoqulA. The effect was greatest for deep wound cultures with a reduction in median TTR from 68h 33m to 49h 13m (19h 19m reduction, $p<0.001$) and increase in proportion completed within 72hrs (58 to 84%). For MRSA screen culture there was an 8h 56m reduction in median TTR, and an increase from 35 to 61% completed within 60hrs ($p<0.001$). There was a lesser improvement for GBS screen (reduction in median TTR of 4h 27m, $p=0.33$; 42 to 55% by 48hrs) and urine culture (median TTR reduction 1h 42m, $p=0.06$, 40 to 45% completion by 24hrs). Reduction in median TTR and increased completion within target time was also noted for tests not performed by the InoqulA (UrPnAg - 2h 21m reduction, $p<0.001$; deep wound swab microscopy - 11h 20m reduction, $p<0.001$; urine micro - 45 to 60% within 6hrs, $p=0.03$).**Conclusion:**The InoqulA improved TTR for a range of culture based and non-culture based tests most likely because of a reduction in delay from specimen receipt to incubation, and from the reassignment of staff from specimen processing to specimen receipt and data entry and prioritisation of rapid diagnostic tests.

Author Disclosure Block:

M. Maley: None. **S. Neville:** None. **H. Ziochos:** None. **M. Chater:** None. **E. Treadwell:** None. **G. Parisi:** None.

Poster Board Number:

SUNDAY-322

Publishing Title:

The Telegram of the 21st Century: The Digital Gram Stain

Author Block:

R. M. Martinez, B. C. Shoemaker, J. A. Riley, D. M. Wolk; Geisinger Hlth.Systems, Danville, PA

Abstract Body:

Background: Although the Gram stain is an essential clinical tool, many laboratories struggle to maintain competency for technologists, especially those who read stains infrequently. The need for a second review is common when performing Gram stains as they are often subject to variability due to inconsistent staining techniques, antibiotic pressure, as well as artifacts. Telemicroscopy utilizes technology to improve diagnostic accuracy, by providing expert consultation for technologists who are uncertain of their results.**Materials and Methods:** Geisinger Medical Laboratories is an eight hospital integrated health service organization, serving >2.6 million residents throughout 44 counties in Pennsylvania. Geisinger Medical Center serves as the full service reference laboratory for 4 limited- service laboratories (Gram stain reading, no culture work-up) and 3 partial-service laboratories (Gram stain reading, limited culture work-up). The TeleGram© program consists of infrastructure and policies to share both still and live images [Olympus BX40, BX41 microscope, Nikon cellSense software (version 1.7.1)] with the reference laboratory via Microsoft Lync/Skype [Logitech 920 camera (version 2013)]. From April 2014 through January 2016, the TeleGram© result, which is a consensus finding, was manually recorded and followed by culture review to determine patient impact. Results were imported into excel (ver. 4.3) for data analysis.**Results:** There were 65 unique consults placed by 3 remote sites (1 limited- and 2 partial-service laboratories). A change in initial interpretation was observed in 38.5% (25/65) of consults, of which, 92% (23/25) of changes positively impacted the patient, compared to culture. Overall, 84.6% (55/65) of TeleGram© results correlated with the culture result.**Conclusions:** Telemicroscopy offers an easy to use and relatively inexpensive solution to provide formal and informal second opinions to various sections of the laboratory. Gram stain consultations from remote hospital sites can improve patient care in an integrated hospital system.

Author Disclosure Block:

R.M. Martinez: J. Scientific Advisor (Review Panel or Advisory Committee); Self; BioFire. **B.C. Shoemaker:** None. **J.A. Riley:** None. **D.M. Wolk:** I. Research Relationship; Self; Cepheid, Accelerate, Luminex, GenMark, BioRad, Roche. J. Scientific Advisor (Review Panel or Advisory Committee); Self; Cepehid, Accelerate, Waters.

Poster Board Number:

SUNDAY-323

Publishing Title:

Two Stepwise Molecular Approaches of Separating *Staphylococcus aureus* from Coagulase Negative Staphylococci Isolated from Clinical Samples in Ibadan South-West, Nigeria

Author Block:

C. O. Ezeamagu[1], O. E. Fagade[2], S. I. Smith[3], A. A. Ogunjobi[4]; [1]Babcock Univ., Ogun State, Nigeria, [2]Babcock Univ., Ibadan, Nigeria, [3]Nigerian Inst. For Med. Res., Yaba, Lagos, Lagos, Nigeria, [4]Univ. of Ibadan, Ibadan, Nigeria

Abstract Body:

Background: *Staphylococcus aureus* is an important cause of nosocomial and community associated infections as well as notorious for its drug resistance potential. There are reports of inconsistencies in the presumptive diagnosis of *S. aureus* using commercial and conventional coagulase test methods. This study reports a simple, rapid, feasible, accurate and cost-effective molecular technique based on PCR-RFLP that separate and identify to species level *S. aureus* from other Coagulase Negative Staphylococci.**Methods:**Five hundred clinical samples were collected and investigated at the Microbiology Laboratory of University College Hospital, Ibadan. Specimens were streaked on mannitol salt agar and incubated for 18-24 hr. Colonies showing yellow coloration or whitish color were picked and sub-cultured on tryptone soya, blood and nutrient agars, incubated at 37^{o}C for between 18-24 hrs. Suspected staphylococcal colonies were tested for catalase, coagulase and hemolysis on blood agar. DNA was extracted using the boiling method, while *Staphylococcus* spp were identified by PCR-RFLP supplemented with specific primers. PCR products were electrophoresed on 1.5% agarose gel, and bands visualized with UV illuminator.**Results:** Fifty clinical isolates of Staphylococci were recovered from the specimens. 6 (12%) and 44 (88%) of these gave positive and negative results respectively for conventional tube coagulase test. The PCR-RFLP supplemented with species-specific primers classified these isolates into three distinct species; *S. epidermidis* (86%), *S. aureus* (12%) and *S. xylosus* (2%). However, this current technique classified the isolates into two RFLP patterns representing *S. aureus* and other Coagulase Negative Staphylococci respectively which correlated with coagulase test results.**Conclusions:Conclusion:** Since coagulase test could be sometimes misleading and inconclusive, this method could be substituted for routine laboratory program.

Author Disclosure Block:

C.O. Ezeamagu: None. **O.E. Fagade:** None. **S.I. Smith:** None. **A.A. Ogunjobi:** None.

Poster Board Number:

SUNDAY-324

Publishing Title:

Development of a Phage Protein-Mediated *Staphylococcus aureus* Detection Tool

Author Block:

H. Matsui[1], J. Uchiyama[2], M. Tsuda[1], S. Matsuzaki[3], T. Nakae[1], H. Hanaki[1]; [1]Kitasato Univ., Tokyo, Japan, [2]Azabu Univ., Kanagawa, Japan, [3]Kochi Univ., Kochi, Japan

Abstract Body:

Background: Bacteriophages infect the host cells by binding on the specific cell surface receptor via the adsorption protein located at the tip of the phage tail. Therefore, the proteins have a potential utility for the development of a specific host cell detection tool. *Staphylococcus aureus* is a terrestrial bacterium that often infects children, elders and immunocompromised patients in hospitals. Thus, rapid and simple detection of this pathogen is urgent. We report here a novel method that detects *S. aureus* cells using an adsorption protein ORF16 of phage S24-1.**Methods:** The PCR-amplified *orf16* gene was cloned into an expression vector pCold II, and the recombinant ORF16 was expressed in *E.coli*. Purified rORF16 was either immobilized nitrocellurose membrane and conjugated with gold colloid particles. A chromato-strip was developed based on the principal of sandwich-type lateral flow test. The strains to be tested were pretreated with a lytic enzyme, e.g. lysostaphin. The detection limit was assessed using two strains each of MRSA and MSSA. The cross-reactivity was determined with 24 and 4 strains of bacteria and fungus, respectively. Reliability was tested using a total 50 *S. aureus* strains from clinical sources.**Results:** The size of purified rORF16 appeared as expected (~75 kDa) on SDS-PAGE. A whole operation including receptor extraction and chromatography took only 20 min. The detection limit of both MRSA and MSSA appeared ~10^6 CFU/test without noticeable variation among strains. All the strains subjected to the cross reactivity test were negative with an only exception that *Staphylococcus saprophyticus* showed false-positive reaction. All 50 clinical isolates of *S. aureus* showed positive results without exception.**Conclusions:** We developed a simple and rapid *S. aureus* detection tool using the phage tail adsorption protein. The method could be extended to the development of detection tools for other bacterial species .

Author Disclosure Block:

H. Matsui: None. **J. Uchiyama:** None. **M. Tsuda:** None. **S. Matsuzaki:** None. **T. Nakae:** None. **H. Hanaki:** None.

Poster Board Number:

SUNDAY-325

Publishing Title:

Clinical Evaluation of a New Chromogenic Medium for the Isolation of *Staphylococcus aureus* in Various Samples Including Cystic Fibrosis Patients

Author Block:

V. Tafani, J. Safrani-Lahyani, S. Trouillet-Assant, M. Chiganne, F. Vincent, **F. Laurent**; Hospices Civiles de Lyon - French Natl. Reference Ctr. for Staphylococci, Lyon, France

Abstract Body:

About 20% of the population is colonized with *S. aureus* in their nose, skin or other body sites and 60% of the population can be transient carrier. Colonisation has been identified as a risk factor for *S. aureus* infection. Therefore a sensitive and rapid identification of *S.aureus* carriage could be useful to prevent the risk of healthcare-associated infection.A clinical performance evaluation was conducted for Cystic Fibrosis (CF, n=133) patients using respiratory samples, or for non-CF patients (n=271) using nasal, Ears-Nose-Throat, respiratory, genital, pus, skin (Burn Unit patients), stool specimens.Respiratory samples from CF patients were tested on chromID™ S. aureus ELITE (SAIDE) medium, chromID™ S. aureus (SAID), BBL™ CHROMagar™ Staph aureus) (BBL) and Columbia blood agar + CNA (CNA). All media were incubated at 36°C until 72 hours and 3 readings were performed after 24 h, 48 h and 72 h. Samples from non-CF patients were tested on SAIDE, SAID and CAN media and read after 20 hours.Implementation of chromID™ S. aureus ELITE in routine globally improves the sensitivity of S. aureus detection at 24h comparatively to all other media (conventional or chromogenic) whatever the samples and the patients. Of note, for CF patients, chromID™ S. aureus ELITE shows a better sensitivity at each reading time than all other media with a very high specificity.

	Cystic Fibrosis Patients N = 133			Non Cystic Fibrosis Patients N = 271
	Performance at 24H	Performance at 48H	Performance at 72H	Performance at 24H
SAIDE	Se = **87.7% (64/73)** Sp = **100% (60/60)**	Se = **97.3% (71/73)** Sp = **100% (60/60)**	Se = **98.6% (72/73)** Sp = **100% (60/60)**	Se = **89.8% (53/59)** Sp = **97.6% (207/212)**
SAID	Se = **65.8% (48/73)**	Se = **90.4% (66/73)**	Se = **95.9% (70/73)**	Se = **83.1% (49/59)** Sp = **96.7% (205/212)**

	Sp = **96.7% (58/60)**	Sp = **93.3% (56/60)**	Sp = **91.7% (55/60)**	
BBL™	Se = **82.2% (60/73)** Sp = **100% (60/60)**	Se = **90.4% (66/73)** Sp = **96.7% (58/60)**	Se = **93.2% (68/73)** Sp = **96.7% (58/60)**	Not tested
CNA*	Se = **84.9% (62/73)** Sp = **100% (60/60)**	Se = **90.4% (66/73)** Sp = **100% (60/60)**	Se = **91.8% (67/73)** Sp = **100% (60/60)**	Se = **88.1% (52/59)** Sp = **100% (212/212)**

** Data for CNA agar are obtained after confirmation by mass spectrometry, explaining the 100% of specificity.*

Author Disclosure Block:

V. Tafani: None. **J. Safrani-Lahyani:** None. **S. Trouillet-Assant:** None. **M. Chiganne:** None. **F. Vincent:** None. **F. Laurent:** E. Grant Investigator; Self; Biomerieux.

Poster Board Number:

SUNDAY-326

Publishing Title:

The Investigation of *Streptococcus agalactiae* Colonization in Last Trimester Pregnants by Using Standard Culture and Molecular Methods

Author Block:

N. Kuru[1], O. Kuru[2], A. Tuten[1], **N. Gonullu**[1]; [1]Cerrahpaşa Faculty of Med. Istanbul Univ., Istanbul, Turkey, [2]Hacettepe Faculty of Med. Hacettepe Univ., Ankara, Turkey

Abstract Body:

Background: Group B streptococcus (GBS) is a leading cause of sepsis, menengitis, and death among newborns. GBS colonization in pregnant women is the most important risk factor. The most common method to diagnose the presence of GBS in pregnant women is the culture. Unfortunately, although the standard culture is a high sensitive and specific method, it takes long time. A rapid test that could accurately detect GBS carriage at the time of labour may be ideal for screening programme. Our prospective study was designed to detect GBS colonization in pregnant women at third trimester using real-time PCR and culture methods.**Methods:**Vaginal swab specimens from one hundred women between 35-37 weeks of gestation who were attending to antenatal outpatient unit of Obstetrics and Gynecology Department of Cerrahpasa Medical Faculty were taken for screening of GBS. Selective Todd-Hewitt broth medium was selected as culture media. DNA test was based on real-time PCR assay targeting cfb gene region.**Results:**In our study, rates of GBS colonization was %5 and %7 by culture and PCR methods, respectively. Sensitivity and specificity for real-time PCR were 100% and 97.9%, respectively using culture as the gold standard. When we compared GBS colonization with age groups, education levels, number of previous pregnancies, smoking habits, history of antibiotic use, and contraceptive method, there were no significant differences.**Conclusions:**Our results indicated that the PCR technique proved to be as sensitive as the culture method. We also demonstrate that the PCR is a very useful screening method and may provide a diagnostic tool for GBS detection potentially allowing a more effective intrapartum antibiotic prophylaxis and lower infant morbidity and mortality.

Author Disclosure Block:

N. Kuru: None. **O. Kuru:** None. **A. Tuten:** None. **N. Gonullu:** None.

Poster Board Number:

SUNDAY-327

Publishing Title:

Development Of Immunoassays For *burkholderia Pseudomallei* Typical And Atypical lipopolysaccharide Strain Typing

Author Block:

T. Nualnoi[1], M. H. Norris[2], A. Tuanyok[2], P. J. Brett[3], M. N. Burtnick[3], P. S. Keim[4], E. W. Settles[4], C. J. Allender[4], D. P. AuCoin[1]; [1]Univ. of Nevada Sch. of Med., Reno, NV, [2]Univ. of Florida, Gainesville, FL, [3]Univ. of South Alabama, Mobile, AL, [4]Northern Arizona Univ., Flagstaff, AZ

Abstract Body:

Burkholderia pseudomallei is a Gram-negative bacillus and the causative agent of melioidosis, a severe infection prominent in Southeast Asia and northern Australia. Lipopolysaccharide (LPS) is one of the most important virulence factors used by *B. pseudomallei*. To date, four different *B. pseudomallei* LPS structures (typical LPS, atypical LPS types B and B2, and rough LPS) have been identified. It has been demonstrated *in vitro* that different LPS types may impact disease severity. However, the association between LPS types and clinical manifestations is not understood, in part because there is no effective method for distinguishing between the LPS types. This led us to develop antigen capture immunoassays for identifying LPS types of *B. pseudomallei*. To generate monoclonal antibodies (mAbs) specific to atypical LPS, mice were immunized with purified LPS type B or B2. Two mAbs, 3A2 and 5B4, were isolated from mice immunized with B2 LPS, but none were isolated from B LPS-immunized mice. Immunoblot analysis and surface plasmon resonance were exploited for mAb characterization and selection. Both 3A2 and 5B4 are cross-reactive to B LPS; however, 3A2 is preferable because of a higher affinity. The assays were further developed using capsular polysaccharide (CPS)-specific mAb 4C4 for bacterial capture, and horseradish peroxidase (HRP)-labeled 4C7 and HRP-labeled 3A2 (specific to typical and atypical LPS, respectively) for detection. The evaluations performed with 197 strains of *Burkholderia* and non-*Burkholderia* species showed that the assays are selectively reactive to pathogenic *B. pseudomallei* and *B. mallei* strains and have an accuracy of 98.5% (0 false positives and 3 false negatives) for LPS typing. The results suggest that the assays are effective and applicable for *B. pseudomallei* LPS typing and use of the assay would provide helpful information for clinicians involved in melioidosis research.

Author Disclosure Block:

T. Nualnoi: None. **M.H. Norris:** None. **A. Tuanyok:** None. **P.J. Brett:** None. **M.N. Burtnick:** None. **P.S. Keim:** None. **E.W. Settles:** None. **C.J. Allender:** None. **D.P. AuCoin:** None.

Poster Board Number:

SUNDAY-328

Publishing Title:

Improved Performance and Time-to-results for *Acinetobacter baumannii* / *haemolyticus* Isolates with the Updated Beckman Coulter Gram-negative Id Product

Author Block:

D. Nothaft, J. Bobolis, D. Carpenter, O. Madriaga, L. Smoot, T. Wong, **C. Beck**; Beckman Coulter Microbiol., West Sacramento, CA

Abstract Body:

Background: *Acinetobacter baumannii* has a remarkable ability to acquire antibiotic resistance, and pandrug-resistant infections have been documented globally. A predominant source of nosocomial infections, the organism survives extended periods of time in hospital environments. Thus, early and accurate identification (ID) and therapy are paramount for patient health and outbreak prevention. In this study, we report on new panel completion criteria for MicroScan's Gram-negative ID product (NID) which enhance ID performance of *Acinetobacter baumannii* / *haemolyticus* (ABH). The new criteria complete the majority of ABH isolates at 16 h with a high degree of accuracy. **Methods:** The updated MicroScan NID product was evaluated using 323 ABH isolates. Panels were inoculated and incubated following manufacturer's instructions for use. The time-to-results, measured from start of panel incubation to obtaining final results, and the percentage of correct and incorrect results were used to assess performance.**Results:** Tested on the WalkAway instrument, the majority of ABH isolates [66.9% (216/323)] completed at 16 h and all identified correctly. The remaining 107 isolates completed at 42 h and 98.1% (105/107) identified correctly. The two incorrect results were identified as *A. lwoffii*. Overall, 99.4% (321/323) of ABH isolates identified correctly, and only 0.9% (3/321) required additional tests to confirm a low-probability correct ID.A subset (n=257) was tested manually and on the autoSCAN-4 (AS-4) instrument with similar results. With the AS-4, 87.9% (226/257) completed at 16 h and all identified correctly. Manually, 94.6% (243/257) completed at 16 h and all identified correctly. Overall at 42 h, 99.6% (256/257) were identified correctly on both methods, with one isolate generating an incorrect result of *A. lwoffii*.**Conclusions:** The updated Beckman Coulter Gram-negative ID product with new panel completion criteria and updated organism database provides timely, accurate identification results for *Acinetobacter baumannii* / *haemolyticus* isolates.Beckman Coulter, the stylized logo and the Beckman Coulter product and service names mentioned herein are trademarks or registered trademarks of Beckman Coulter, Inc. in the United States and other countries.

Author Disclosure Block:

D. Nothaft: None. **J. Bobolis:** None. **D. Carpenter:** None. **O. Madriaga:** None. **L. Smoot:** None. **T. Wong:** None. **C. Beck:** None.

Poster Board Number:

SUNDAY-329

Publishing Title:

Identifying *Burkholderia cepacia* Complex and *Pseudomonas aeruginosa* Isolates from Cystic Fibrosis Patients with the Updated Microscan Dried Gram-negative Id Product

Author Block:

C. Beck, L. Smoot, J. Bobolis, T. Wong, **D. Nothaft**; Beckman Coulter Microbiol., West Sacramento, CA

Abstract Body:

Background: MicroScan recently updated the database for the Dried Gram-negative Identification product (NID). Included in the update were the addition of 39 taxa (new to NID product), nomenclature updates, and enhanced identification of challenging species including those species isolated from cystic fibrosis (CF) patients. *Burkholderia cepacia* complex and *Pseudomonas aeruginosa* are increasingly recovered from CF patients with more advanced disease, and these pose a challenge for commercial systems due to heterogeneity within the *B. cepacia* complex and the higher prevalence of mucoid strains. Accurate identification is critical to patient care.**Materials:** To identify these isolates and expand proper patient care and management, 153 known domestic CF isolates including 32 *B. cepacia* complex and 121 *P. aeruginosa* were tested. Reference identification testing was performed following the manufacturers' instructions for use and sequencing of 16S rDNA was included for discrepant isolates. NID panels were inoculated and incubated following manufacturer's instructions for use. The percentage of correct & incorrect results were used to assess product performance.**Results:** A correct identification was obtained for 98.7% (151/153) of the isolates. An incorrect identification was obtained for 1.3% (2/153) of the isolates. The mis-identifications were within the *Pseudomonas* genus; one identified as a *P. fluorescens/putida* and the other as *P. stutzeri*.**Conclusions:** MicroScan's updated NID database accurately identifies these challenging isolates from CF patients.Beckman Coulter, the stylized logo and the Beckman Coulter product and service names mentioned herein are trademarks or registered trademarks of Beckman Coulter, Inc. in the United States and other countries.

Author Disclosure Block:

C. Beck: None. **L. Smoot:** None. **J. Bobolis:** None. **T. Wong:** None. **D. Nothaft:** None.

Poster Board Number:

SUNDAY-330

Publishing Title:

Multicenter Evaluation Supports Accuracy of the Beckman Coulter Gram-negative Identification Product with Improved Database for Clinically Significant Bacteria

Author Block:

J. A. Hindler[1], P. C. Schreckenberger[2], J. Tjhio[2], L. Mann[3], C. Beck[4], D. Nothaft[4], D. Carpenter[4], O. Madriaga[4], L. Smoot[4], T. Wong[4], **J. Bobolis**[4]; [1]UCLA David Geffen Sch. of Med., Los Angeles, CA, [2]Loyola Univ. Med. Ctr., Maywood, IL, [3]Individual Contributer, Sacramento, CA, [4]Beckman Coulter Microbiol., West Sacramento, CA

Abstract Body:

Background: The MicroScan Gram-negative Identification (NID) organism database was revised and includes 39 new Gram-negative organisms - 22 fermentative (F) taxa and 17 non-fermentative (NF) taxa - along with updated nomenclature. A multicenter study was performed to evaluate the accuracy of the updated NID product. **Methods:** The MicroScan NID product was evaluated at two sites with 609 fresh clinical isolates comprised of 55 F and NF Gram-negative taxa. NID panels were processed in a WalkAway instrument, and the identifications were generated using the updated NID organism database. Percent correct and incorrect results were used to assess accuracy of the updated NID panel. Sequencing of 16S rDNA was included for discrepant isolates. Thirteen isolates were identified to only genus-level using the reference methods available.**Results:** A correct identification was obtained for 98.6% (414/420) of the F Gram-negatives and for 94.7% (179/189) of the NF Gram-negatives. Furthermore, the clinically significant species *A. baumannii / haemolyticus*, *E. coli*, *P. aeruginosa,* and *P. mirabilis* were correct at 100%, 97.4%, 97.8%, and 96.4% respectively. Overall, a correct species-level identification was obtained for 97.4% (593/609) of all isolates, and only 3.1% (19/593) required additional tests to confirm a low-probability correct identification. Incorrect species-level identifications were obtained for 2.5% (15/609) of the isolates, which includes the 13 isolates that had only genus-level reference identifications. A very rare biotype was obtained for 0.2% (1/609) - a single *E. aerogenes* isolate.**Conclusions:** The results of the evaluation with fresh clinical isolates show that the MicroScan Dried Gram-negative ID panel with an updated database provides accurate identification results for clinically important Gram-negative bacteria.Beckman Coulter, the stylized logo and the Beckman Coulter product and service names mentioned herein are trademarks or registered trademarks of Beckman Coulter, Inc. in the United States and other countries.

Author Disclosure Block:

J.A. Hindler: None. **P.C. Schreckenberger:** None. **J. Tjhio:** None. **L. Mann:** None. **C. Beck:** None. **D. Nothaft:** None. **D. Carpenter:** None. **O. Madriaga:** None. **L. Smoot:** None. **T. Wong:** None. **J. Bobolis:** None.

Poster Board Number:

SUNDAY-331

Publishing Title:

Impact of Organism Identification Method on Central Line Infection Designation

Author Block:

E. J. Gomez[1], K. Alby[2], A. L. Roberts[3], N. A. Ledeboer[4], C. D. Doern[5], **K. V. Sullivan**[1]; [1]The Children's Hosp. of Philadelphia, Philadelphia, PA, [2]The Hosp. of the Univ. of Pennsylvania, Philadelphia, PA, [3]Thomas Jefferson Univ. and Hosp., Philadelphia, PA, [4]Med. Coll. of Wisconsin, Milwaukee, WI, [5]Virginia Commonwealth Univ., Richmond, VA

Abstract Body:

Background: According to the National Healthcare Safety Network (NHSN), the assignment of central line-associated bloodstream infection (CLABSI) status requires isolation of the same commensal organism on 2 or more blood cultures (BC). The accuracy and reliability of the organism identification (ID) method in use may impact on CLABSI status and reported incidence rates. **Methods:** The Theradoc system was used to identify all CLABSI cases involving commensal organisms and meeting NHSN criteria at The Children's Hospital of Philadelphia from June 1, 2013-June 30, 2015. Routinely, first isolates undergo ID. ID of subsequently recovered isolates is referred for 72 hours. For the study, first and subsequent isolates were ID'd using Vitek 2 (V2); BD Phoenix (BDP); Micro Scan Auto Scan-4 (MSAS); Bruker Biotyper (BB); and Vitek MS (VMS). All streptococci underwent optochin susceptibility and bile solubility testing. **Results:** 13 CLABSIs involved coagulase-negative staphylococci. In 11, there was 100% concordance across all 5 ID methods in all isolates tested. In one case, 4 methods reported *S. epidermidis* in 2/2 isolates tested but BDP reported *S. schleiferi* for both isolates. In the other case, 4 methods reported *S. hominis* in 2/2 isolates tested but MSAS reported *S. epidermidis* for both. 8 cases involved viridans group streptococci. Isolates from 2 positive blood cultures were tested for each case. BB and VMS reported all isolates as *S. mitis* or *S. oralis.* BDP reported 2 cases with differing IDs (*S. acidominimus* and *S. salivarius*; *S. oralis* and *S. constellatus*). A third case yielded no ID on both isolates. V2 also reported 2 cases with differing IDs (*S. sanguinis* and *S. mitis/oralis*; *S. mitis/oralis* and *G. adiacens*). MSAS reported discordant IDs in 6/8 cases, 5 with erroneous IDs of *S. pneumoniae.* **Conclusion:** For BSI involving coagulase-negative staphylococci, the ID method did not impact on assignment of CLABSI status. For cases involving viridans group streptococci, automated biochemical methods yielded cases with differing IDs on the first and subsequent isolates recovered. Use of these methods may lead to lower reported CLABSI rates.

Author Disclosure Block:

E.J. Gomez: None. **K. Alby:** None. **A.L. Roberts:** None. **N.A. Ledeboer:** None. **C.D. Doern:** None. **K.V. Sullivan:** None.

Poster Board Number:

SUNDAY-332

Publishing Title:

Ceftolozane-Tazobactam (C/T) is Effective against Most Multidrug-Resistant (Mdr) *P. aeruginosa* (*pa*) Infections, but Resistance May Emerge on Therapy

Author Block:

G. Haidar, C. J. Clancy, R. K. Shields, Y. Doi, B. A. Potoski, M. H. Nguyen; Univ. of Pittsburgh, Pittsburgh, PA

Abstract Body:

Background:KPC-producing *Enterobacter* spp. are emerging. Gentamicin (GEN) is active against the majority of carbapenem-resistant *K. pneumoniae*. The activity of GEN and other AGs against KPC-producing *Enterobacter* is not known. **Methods:** We compared the *in vitro* susceptibility of KPC-producing (KPC+) and non-producing (KPC-) *Enterobacter* isolates to aminoglycosides (GEN, tobramycin TOB, amikacin AMK). We determined the presence of KPC, ESBL and AME genes by PCR.**Results:**21 KPC+ (12 *E. aerogenes* (EA) and 9 *E. cloacae* (EC)) and 44 KPC- isolates (12 EA and 32 EC) were compared. 71% (15) and 7% (3) of KPC+ and KPC- isolates were resistant to GEN, respectively ($p<0.0001$). Corresponding rates were 67% (14) and 9% (4) for TOB ($p<0.0001$), and 9% (2) and 0% for AMK ($p=0.10$). AMEs were more common among KPC+ (95%) than KPC- isolates (20%, $p<0.0001$), as were ESBLs (41% vs. 30%, $p<.0001$). Likewise, mean and median numbers of AMEs were higher for KPC+ (4 and 5) than KPC- isolates (0.4 and 0). The most common AME genes among KPC+ isolates were: *aac(6')-Ib* (62%), *aadA2* (62%), *aac(6')-II* (57%) and *aadA1* (57%). The presence of either *aac(6')-Ib* or *aac(6')-II* was associated with resistance to all 3 AGs. The presence of any one of *aac(3)-II* and *–IV*, *aadA1* and *aadA2* was associated with resistance to GEN and TOB but not AMK. The presence of *aph(3')-Ia* was associated with GEN resistance. GEN, TOB and AMK MICs were significantly increased as the number of β lactamases (either KPC or ESBL) increased (<0.001). There was a direct correlation between presence of KPC and presence of *aac(6')-Ib* and *aph(3')-Ia*, and between presence of ESBL and *aac(6')-II* and *aph(3')-Ib* (all $p<0.01$).**Conclusion:**A significant majority of KPC+ *Enterobacter* spp. were resistant to GEN and TOB. Associations between presence of AME, KPC and ESBL genes suggest that they may be acquired on the same plasmids. The high prevalence of KPC+ isolates with *aac(6')-Ib*, known to confer resistance to GEN, TOB and AMK, suggests that these AG are unlikely to have a significant role in the treatment of KPC+ *Enterobacter* infections.

Author Disclosure Block:

G. Haidar: None. **C.J. Clancy:** None. **R.K. Shields:** None. **Y. Doi:** E. Grant Investigator; Self; The Medicines Company Advisory Board: Meiji, Tetraphase. **B.A. Potoski:** None. **M.H. Nguyen:** None.

Poster Board Number:

SUNDAY-333

Publishing Title:

Risk Factors and Outcome of Levofloxacin-Resistant *Elizabethkingia meningoseptica* Bacteremia in Adult Patients in Taiwan

Author Block:

Y-T. Lin, Y-C. Huang, F-D. Wang; Taipei Veterans Gen. Hosp., Taipei, Taiwan

Abstract Body:

Background: *Elizabethkingia meningoseptica* is an emerging nosocomial pathogen and inherently resistant to many antimicrobial agents commonly used to treat gram-negative bacterial infections. *E. meningoseptica* bacteremia is associated with high mortality rates, and levofloxacin has been considered as a therapeutic agent based on in-vitro susceptibility. However, investigations into the risk factors and outcomes for levofloxacin-resistant *E. meningoseptica* bacteremia have never been reported. **Methods:** Adult patients with *E. meningoseptica* bacteremia were identified retrospectively in a medical center in Taiwan from January 2012 to June 2015. We compared clinical features and outcomes of patients with levofloxacin-resistant (MIC > 2 μg/mL) and levofloxacin-susceptible *E. meningoseptica* bacteremia (MIC ≤ 2 μg/mL). Risk factors for levofloxacin-resistant *E. meningoseptica* bacteremia and its mortality were analyzed by logistic regression. Antimicrobial susceptibilities were determined by E test and interpreted by the Clinical and Laboratory Standards Institute MIC breakpoints for other non-*Enterobacteriaceae* spp. **Results:** A total of 93 patients with *E. meningoseptica* bacteremia were identified and 51 patients had levofloxacin-resistant *E. meningoseptica* bacteremia. The 14-day mortality of levofloxacin-resistant *E. meningoseptica* bacteremia was significantly higher than that of levofloxacin-susceptible *E. meningoseptica* bacteremia (56.9 % vs 26.2 %; p=0.003). Levofloxacin-resistant *E. meningoseptica* bacteremia is associated with higher APACHE II score. Previous exposure to β-lactam/β-lactamase inhibitor within 30 days prior to bacteremia (OR, 2.08; 95% CI, 0.87-4.97; $p = 0.099$) and APACHE II score (OR, 1.08; 95% CI, 1.02-1.14; $p = 0.008$) were the independent risk factors for levofloxacin-resistant *E. meningoseptica* bacteremia. Regarding the risk factor for mortality, levofloxacin-resistant *E. meningoseptica* bacteremia (OR, 4.8; 95% CI, 1.77-12.96; $p = 0.002$) was found to be the independent risk factor for 14-day mortality. **Conclusion:** Levofloxacin-resistant *E. meningoseptica* bacteremia had a marked effect on patient mortality. Better surveillance and control measures are necessary to deal with invasive levofloxacin-resistant *E. meningoseptica* infections.

Author Disclosure Block:

Y. Lin: None. **Y. Huang:** None. **F. Wang:** None.

Poster Board Number:

SUNDAY-334

Publishing Title:

Clinical Characteristics and Outcomes in Patients with Hematogenous Vertebral Osteomyelitis and Infective Endocarditis Co-Infection

Author Block:

K-H. Park[1], Y-M. Lee[2], O-H. Cho[3], J. Woo[1], S. Park[4], Y. Chong[5], S-H. Kim[5], S-O. Lee[5], M. Lee[1], S-H. Choi[5], I-G. Bae[3], Y. Kim[5], J. Woo[5]; [1]Kyung Hee Univ. Sch. of Med., Seoul, Korea, Republic of, [2]Inje Univ. Coll. of Med., Seoul, Korea, Republic of, [3]Gyeongsang Natl. Univ. Hosp., Jinju, Korea, Republic of, [4]Dongguk Univ. Ilsan Hosp., Seoul, Korea, Republic of, [5]Asan Med. Ctr., Seoul, Korea, Republic of

Abstract Body:

Background: The relationship between hematogenous vertebral osteomyelitis (HVO) and infectious endocarditis is uncertain. This study investigates the incidence and risk factors of infective endocarditis in patients with HVO, and the outcome of HVO with and without associated with infective endocarditis.**Methods:** A retrospective review was conducted of all cases of HVO from January 2015 to December 2012, occurring in 5 tertiary-care hospitals in the Republic of Korea.**Results:** A total of 345 patients with microbiologically diagnosed HVO were included. The median age was 65 years, and 181 (52.5%) were male. The most common underlying condition was diabetes (29.6%), followed by liver cirrhosis (9.3%) and malignancy (9.0%). Overall, of the 345 patients with HVO, 17 (4.9%) patients had concomitant infective endocarditis. There was no infective endocarditis in 89 patients with non-bacteremic HVO. Among remaining 256 patients with bacteremic HVO, the rates of endocarditis were 27% (8/30) for *Streptococcus*/*Enterococcus* spp., 10% (7/73) for methicillin-resistant *Staphylococcus aureus*, 2% (2/93) for methicillin-susceptible *S. aureus*, and 0% (0/54) for aerobic gram-negative bacteria. The median durations of antibiotic therapy were 67 and 69 days for the non-endocarditis and endocarditis groups, respectively (P = .58). Clinical outcomes were similar between the groups, including in-hospital mortality (11.8% vs. 8.5%; P = 0.65), recurrence (9.7% vs 13.3%; P = .65), and length of hospital stay (median 53 vs. 56 days; P = 0.65).**Conclusions:** Overall incidence of infective endocarditis was low (4.9%) in patients with microbiologically diagnosed HVO. However, bacteremic HVO due to *streptococcus*/*enterococcus* spp. and MRSA were frequently associated with infective endocarditis. For such high-risk patients, echocardiography should be performed as routine and switch to oral antibiotic therapy should be only considered after excluding infective endocarditis.

Author Disclosure Block:

K. Park: None. **Y. Lee:** None. **O. Cho:** None. **J. Woo:** None. **S. Park:** None. **Y. Chong:** None. **S. Kim:** None. **S. Lee:** None. **M. Lee:** None. **S. Choi:** None. **I. Bae:** None. **Y. Kim:** None. **J. Woo:** None.

Poster Board Number:

SUNDAY-335

Publishing Title:

High-Level Daptomycin-Resistant (Dap-R) *Streptococcus mitis* Is Virulent in Experimental Endocarditis (Ee) and Enhances Survivability During Dap Treatment vs. Its Dap-Susceptible (Dap-S) Parental Strain

Author Block:

C. Garcia-de-la-Maria[1], J. M. Pericas[1], Y. Armero[1], A. Moreno[1], N. N. Mishra[2], M. J. Rybak[3], T. T. Tran[4], C. A. Arias[4], P. M. Sullam[5], Y. Q. Xiong[2], A. S. Bayer[2], J. M. Miro[1]; [1]Hosp. Clinic-IDIBAPS, Barcelona, Spain, [2]LA Biomed Res. Inst Torrance & Geffen Sch. of Med. at UCLA, Torrance, CA, [3]Anti-Infective Res. Lab. Wayne State, Detroit, MI, [4]UT Med. SCh., Houston, TX, [5]UCSF & VA Med. Ctr., San Francisco, CA

Abstract Body:

Background: *S. mitis* has a unique propensity to evolve rapid, durable and high-level DAP-R *in vitro* and *in vivo*. This study investigated the intrinsic fitness and DAP survivability of a DAP-R *S. mitis* strain *vs.* its DAP-S parental strain in an *in vivo* EE co-infection model.**Methods:** Strains: DAP-S 351 (DAP MIC = 0.5 ug/ml) was isolated from a clinical case of IE; its DAP-R variant (D_6-6; DAP MIC >256 ug/ml) was isolated from rabbit EE vegetations after 48 h. of DAP treatment (*AAC, 2013; 57:2319-25*). EE model: Aortic valve indwelling catheter EE model in rabbits. EE was induced by IV challenge of ~$2x10^6$ cfu/ml of a mixed DAP-S and DAP-R inoculum (1:1 ratio) 24 h post-catheterization. Studies: A) One group of animals were sacrificed 24 h. after the IV co-infection challenge; and, B) Another group was treated for 48 h. with IV DAP (6 mg/kg/d) + gentamicin (GEN, 1 mg/kg/8h). Animals were sacrificed after six drug half-lives. Target tissues were quantitatively cultured in parallel on plain and DAP-containing (8 ug/ml) plates to detect retention of the DAP-R phenotype.**Results:**

	Vegetations		**Kidney**		**Spleen**	
Study group	IR (%)	Median (IQR) log cfu/g tissue	IR (%)	Median (IQR) log cfu/g tissue	IR (%)	Median (IQR) log cfu/g tissue
Rabbits not treated with antibiotics and sacrificed at 24 h						
DAP-S strain	5/5 (100)	10.1 (9.4-10.2)[a]	5/5 (100)[b]	3.2 (2.7-4)	5/5 (100)[c]	5.3 (4.5-5.6)
DAP-R strain	4/5 (80)	6.6 (5.7-6.9)[a]	0/5 (0)[b]	0 (0-0)	0/5 (0)[c]	0 (0-0)

Rabbits treated with DAP+GEN and sacrificed after 48 h of treatment						
DAP-S strain	0/6 (0)	0 (0-0)	0/6 (0)	0 (0-0)	0/6 (0)	0 (0-0)
DAP-R strain	6/6 (100)	8.5 (6.3-9)	5/6 (83)	2.4 (2-2.5)	3/6 (50)	3.4 (2.7-3.5)
^{a}P = .009; ^{b}P = .008; ^{c}P = .008 IR, rate of animals with infected valve vegetations, kidney and spleen/total number of animals.						

In the absence of antibiotic therapy, both strains induced IE, although the DAP-S parental strain was significantly more fit in terms of target tissue seeding and proliferation. In contrast, after DAP + GEN treatment, only DAP-R strains survived in target tissues. All DAP-R variants from target tissues maintained high-level DAP-R after passage in the EE model.**Conclusions:** DAP-R variants can cause EE, with their survival amplified in the presence of DAP combination therapy. Further studies are needed to confirm that DAP + GEN therapy can effectively prevent the emergence of DAP-R variants *in vivo*.

Author Disclosure Block:

C. Garcia-de-la-Maria: None. **J.M. Pericas:** None. **Y. Armero:** None. **A. Moreno:** None. **N.N. Mishra:** None. **M.J. Rybak:** None. **T.T. Tran:** None. **C.A. Arias:** None. **P.M. Sullam:** None. **Y.Q. Xiong:** None. **A.S. Bayer:** None. **J.M. Miro:** None.

Poster Board Number:

SUNDAY-336

Publishing Title:

Unravelling the Characteristics of Three Common Clonal Complexes (Ccs) of Methicillin-Susceptible *Staphylococcus aureus* (Mssa) Causing Complicated Catheter-Related Bacteremia (Crb)

Author Block:

D. Pérez-Montarelo[1], E. Viedma[1], M. Fernández de Mera[1], N. Larrosa[2], N. Fernández-Hidalgo[2], B. Almirante[2], F. Chaves[1]; [1]Hosp. Univ. 12 Octubre, Madrid, Spain, [2]Hosp. Univ. Vall d'Hebron, Barcelona, Spain

Abstract Body:

Background: Previous studies have suggested that MSSA strains causing CRB belonging to particular CCs are more prone to develop endovascular complications. Our aim was to explore the characteristics of a selection of the three most common CCs MSSA isolates producing complicated CRB using in vitro and in vivo models.**Methods:** Six CRB MSSA (CC5, CC30, CC45) with different *agr* functionality were selected. The capacity to form biofilm, to adhere to collagen, the susceptibility to antimicrobial peptides produced by neutrophils (hNP1) and the virulence in the *Galleria Mellonella* model were investigated. Three independent experiments were included in each assay. Their genomes were sequenced by NGS.**Results:** Five of the six strains were capable to form biofilm. No differences were detected among different CCs. Dysfunctional *agr* strains showed a higher biofilm formation than those with a functional *agr* (p: 0.024). The percentage of collagen binding ranged between 3.2% and 23.8%. CC45 (19.1%) and CC30 (18.9%) strains showed a higher percentage of collagen binding than CC5 (4.4%), p: 0.045. This effect could be attributed to the presence of collagen adhesion gene *cna* in both CC45 and CC30 but not in CC5. No differences in collagen binding were detected between *agr* functional (16.8%) and dysfunctional strains (8.8%), p:0.17. The percentage of bacterial survival to hNP1 varied from 49.9% to 58.9%. All CCs showed similar survival percentages (54.4% CC45, 54.6% CC30 and 54.8% CC5) and no differences were detected between strains with different *agr* functionality. The studies in *G. Mellonella* model revealed a lower larvae mean survival time in CC45 and CC5 strains than in CC30 ($p < 0.001$). Those strains with a functional *agr* showed a lower larvae mean survival time compared to the ones with a dysfunctional *agr* ($p<0.001$). In addition, strains carrying *cna* gene resulted in lower larvae mean survival time than strains without this gene (p: 0.016).**Conclusions:** Our study suggested differences in the behaviour of MSSA strains causing complicated BRC according to their genetic background. Even so, the three CCs presented characteristics that favour the virulence of these strains.

Author Disclosure Block:

D. Pérez-Montarelo: None. **E. Viedma:** None. **M. Fernández de Mera:** None. **N. Larrosa:** None. **N. Fernández-Hidalgo:** None. **B. Almirante:** None. **F. Chaves:** None.

Poster Board Number:

SUNDAY-337

Publishing Title:

Epidemiology, Clinical Characteristics And Antimicrobial Susceptibility Profiles Of An Emerging Mdr Opportunistic Pathogen, Corynebacterium Striatum

Author Block:

A. R. McMullen, N. Anderson, M. Wallace, A. Shupe, C-A. Burnham; Washington Univ. Sch. of Med., St Louis, MO

Abstract Body:

Objective: Routine species level identification of clinically significant coryneform bacteria has revealed that *Corynebacterium striatum* is frequently isolated, especially in patients who are immunosuppressed or have an indwelling medical device. For many of these patients, long courses of antimicrobial therapy are required. Our objective was to characterize the epidemiology and antimicrobial susceptibility profiles of *C. striatum* infections.**Methods:** Between 2012 and 2015, at Barnes Jewish Hospital, a 1250 bed, tertiary care medical center, approximately 275 unique *C. striatum* isolates were recovered. Susceptibility testing was performed using gradient diffusion assays on Mueller Hinton Agar with blood. MIC values were interpreted according to CLSI guidelines (M45).**Results:** *C. striatum* was isolated from a variety of sources, including blood, LVADs, tissue and hardware/indwelling devices. The % susceptible, MIC range and MIC_{50} and MIC_{90} for antimicrobial agents tested are found in Table 1. All isolates tested were susceptible to vancomycin and linezolid. Most of the isolates were resistant to clindamycin, ciprofloxacin, and ceftriaxone. For 18 of the isolates, daptomycin susceptibility testing was performed and 16 (89%) of the isolates were susceptible. The two resistant isolates had a daptomycin MIC of >256 µg/ml.**Conclusion:** Many strains of *C. striatum* are multi-drug resistant, and antimicrobial susceptibility testing is important to guide appropriate therapy. Future studies to explore the activity of newer generation anti-Gram positive agents against this species are needed.**Table 1.** Antimicrobial susceptibly of *C. striatum* recovered from clinical specimens

Antibiotic	**% Susceptible**	**MIC range (µg/ml)**	**MIC_{50} (µg/ml)**	**MIC_{90} (µg/ml)**
Vancomycin (n = 225)	100.0	0.5 - 2.0	1.0	2.0
Linezolid (n = 50)	100.0	0.25 - 2.0	1.0	1.0
Daptomycin (n = 18)	88.9	0.064 - >256	0.25	0.25
Tetracycline (n = 224)	39.3	<0.5 - >256	>64	>256
Clindamycin (n = 183)	0.5	<0.5 - >256	>256	>256
Ciprofloxacin (n = 211)	0.0	>32	>32	>32
Ceftriaxone (n = 162)	0.0	32 - >256	>256	>256

Author Disclosure Block:

A.R. McMullen: None. **N. Anderson:** None. **M. Wallace:** None. **A. Shupe:** None. **C. Burnham:** None.

Poster Board Number:

SUNDAY-338

Publishing Title:

Clinical Outcomes and Risk Factors of Ampc Producing Enterobacteriaceae

Author Block:

R. Chavada[1], D. Tong[2], G. O'Kane[1], M. Maley[3]; [1]Pathology North, Gosford Hosp., Gosford,NSW, Australia, [2]Pharmacy Dept., Gosford Hosp., Gosford, NSW, Australia, [3]SSWPS, Livperpool Hosp., Liverpool, NSW, Australia

Abstract Body:

Introduction: Blood stream infections (BSI) caused by multidrug resistant Gram negative (MDRGN) enterobacteriaceae are rising throughout the world. While the focus has shifted to carbapenem resistant enterobacteriaceae (CRE) and extended spectrum beta-lactamase (ESBL) due to limited therapeutic antimicrobial options, higher mortality and increasing healthcare costs, there is little data on outcomes in BSI caused by AmpC producing enterobacteriaceae (AmpC-E). The aim of this study was to assess the clinical outcomes and risk factors for AmpC-E BSI.**Methods:**This was a retrospective matched case control study over a period of 3 years from 2 tertiary care centres. Cases (patients with BSI due to AmpC-E) were matched with controls (standard enterobacteriaceae [SE]) in ratio of 1:2. AmpC detection was done by both phenotypic methods using a boronic acid (inhibitor) and clavulanate (inducer) and genotypic method (multiplex PCR). Clinical information was gathered from electronic medical records. Statistical analysis was done using SPSS (IBM v23.0, California).**Results:**There were 28 cases of BSI with AmpC-E which were matched to 56 cases of BSI due to SE (by age, comorbidities and site of infection). Mean age and proportion of male patients was higher in patients with AmpC-E (71.3 vs. 68.6, p=0.16 and 68% vs 61 %, p=0.32). Similarly, AmpC-E group had longer hospital stay (12.5 vs. 9.5, p=0.14), slightly higher APACHE2 score (19.5 vs 18.2, p=0.188) and more patients with healthcare associated onset (46.4% vs 30.3%, p=0.147). Indwelling urinary catheters and being on immunosuppressants were also similar in the two groups. Rates of admission to ICU (39% vs. 17.8%, p=0.33), surgery in hospital (53.5% vs. 12.5%, p=0.001) and mortality at 30 days (21.4% vs 14.2%, p=0.408) were higher in the AmpC-E group. On multivariate logistic regression analysis AmpC-E bacteraemia was significantly higher surgical setting compared to bacteraemia with SE with an OR of 8.2 (IQR 2.7-25.1, p=0.001).**Conclusion:**Incidence of AmpC-E BSI is higher in setting of recent surgery due to likely increased LOS and prolonged prior duration of antibiotics administered. Mortality and other clinical outcomes did not differ in this study.

Author Disclosure Block:

R. Chavada: None. **D. Tong:** None. **G. O'Kane:** None. **M. Maley:** None.

Poster Board Number:

SUNDAY-339

Publishing Title:

Evaluation of Clinical Outcomes of *Pseudomonas* Infections Stratified By Carbapenem Minimum Inhibitory Concentration

Author Block:

L. L. Benitez, T. S. Patel, K. Rao, J. Nagel; Univ. of Michigan Hlth.System, Ann Arbor, MI

Abstract Body:

Background: The Clinical and Laboratory Standards Institute revised the carbapenem susceptibility breakpoint for *Pseudomonas spp.* from 4 mg/L to 2 mg/L. However, there are minimal data evaluating the impact of carbapenem MIC on clinical outcomes associated *Pseudomonas* infections.**Methods:** A single-center retrospective cohort study was conducted in adult patients with bloodstream or respiratory infections caused by Pseudomonas treated with meropenem or imipenem for greater than 48 hours. Patients infected with Pseudomonas with carbapenem MIC ≤ 2 mg/L were compared to those with carbapenem MIC ≥ 4 mg/L. All-cause 30-day mortality, hospital length of stay (LOS), and 30-day hospital readmission rates were compared between cohorts. Logistic regression was used to elucidate independent risk factors for mortality.**Results:** A total of 134 patients with bloodstream infections or respiratory infections caused by Pseudomonas were included; 82 patients had an isolate with MIC ≤ 2 mg/L. All-cause 30-day mortality (22.0% vs. 17.3%, p = 0.66) and 30-day hospital readmission (20.3% vs. 25.6%, p = 0.63) were similar between the carbapenem MIC ≤ 2 mg/L group and the carbapenem MIC ≥ 4 mg/L group, respectively. The only independent predictor for mortality was immunocompromised status (odds ratio [OR], 5.1; 95% confidence interval [95% CI], 1.2-22.2; p = 0.03). Hospital LOS was significantly longer in the carbapenem MIC ≥ 4 mg/L group (20.5 days (interquartile range [IQR], 11-49.3) vs. 16.5 days (IQR, 8-28.5), p = 0.03).**Conclusions:** Patients with bloodstream infections or respiratory infections caused by *Pseudomonas* have similar mortality rates and hospital readmission rates irrespective of carbapenem MIC. Patients infected with isolates with carbapenem MIC ≥ 4 mg/L had significantly longer hospital LOS.

Author Disclosure Block:

L.L. Benitez: None. **T.S. Patel:** None. **K. Rao:** None. **J. Nagel:** None.

Poster Board Number:

SUNDAY-340

Publishing Title:

The Mechanism of Resistance to Vancomycin in Slow-VISA Possibly Responsible for Recurrent MRSA Infection

Author Block:

Y. Katayama[1], Y. Aiba[2], M. Sekine[1], M. Miyazaki[3], T. Takata[4], M. Matsuo[1], T. Azechi[5], T. Hishinuma[1], H. Hanaki[6], K. Hiramatsu[7]; [1]Juntendo Univ. Faculty of Med., Tokyo, Japan, [2]Juntendo Univ. Graduate Sch. of Med., Tokyo, Japan, [3]Fukuoka Univ. Chikushi Hosp., Fukuoka, Japan, [4]Fukuoka Univ. Sch. of Med., Fukuoka, Japan, [5]Juntendo Univ. Hosp., Tokyo, Japan, [6]Kitasato Inst. for Life Sci., Kitasato Univ., Tokyo, Japan, [7]Graduate Sch. of Med., Juntendo Univ., Tokyo, Japan

Abstract Body:

Background: The Vancomycin(VAN)-intermediate *Staphylococcus aureus* (VISA) strains generally grow slowly, and form colonies on VAN-containing agar-plates after 48 hours of incubation in population analysis. However, we have found a curious group of VISA strains whose colonies appear only after 72 hours incubation. We designated them 'slow-VISA'(sVISA) (1). As compared to extant VISA strains, sVISA strains had 1) significantly prolonged doubling-times, 2) increasing resistance to VAN after prolonged incubation, 3) unstable phenotypic expression, 4) RNA polymerase subunit gene mutations are frequently detected. We investigated a characterization of sVISA in this study.**Materials and Methods:** 1)We established 26 (26/34[76.5%]) sVISA strains by selecting hVISA Mu3 with VAN 6mg/L. Whole genome sequence of the 26 sVISAs were determined and SNPs analysis were performed. 2) We detected sVISA phenotype in 214 clinical MRSA isolates obtained from blood cultures in 18 Japanese hospitals(2), using by E-test.**Results:** We found mutations in the gene(s) involved in various metabolic pathways such as purine/pyrimidine synthesis, PRPP (5P-D-ribosyl-1pyrophosphate) synthesis (*prs*), and in Embden-Meyerhof pathway. These mutations seemed to change the flow of metabolites in the cell and promote cell-wall peptidoglycan synthesis by concentrating energy and metabolites into the pathway. There were 24 sVISA-converted strains, in which the most frequent SNPs were on the transcription machinery, i.e, in *rpoB* or *rpoC* genes.**Conclusions:** The sVISA spontaneously returns to hVISA when the threat of VAN is lifted. Now, we perform metabolomic analysis among sVISA. Moreover, we detected 18 sVISA ($MIC_{VAN} > 4$ mg/L) in 214 clinical MRSA isolates. We attempt to analyze the relationship between the detection of sVISA and the clinical manifestation, medication history and prognosis of the patients.

Author Disclosure Block:

Y. Katayama: None. **Y. Aiba:** None. **M. Sekine:** None. **M. Miyazaki:** None. **T. Takata:** None. **M. Matsuo:** None. **T. Azechi:** None. **T. Hishinuma:** None. **H. Hanaki:** None. **K. Hiramatsu:** None.

Poster Board Number:

SUNDAY-341

Publishing Title:

Case Control Study of Pneumonia Patients with *Streptooccus anginosus* Group Identified from Sputum

Author Block:

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Abstract Body:

Background: Even though *Streptococcus anginosus* group (SAG) was previously thought to rarely cause pneumonia, they have emerged as a causative microorganism of pneumonia particularly in the elderly people, and it was reported recently that 3-4% of the cases of community-acquired pneumonia was caused by SAG. Hence, we have a policy to identify SAG as a routine culture altohgh identification of SAG from sputum is not routinely performed in most microbiology laboratories. The purpose of this study was to clarify which SAG was related to pnemonia, clinical characteristics of patients with SAG pneumonia, and radiologic features. **Methods:** We retrospectively investigated that clinical characteristics and radiological features of 27 patients with pneumonia (PWP) caused by SAG, and also compared between PWP and 37 patients without pneumonia (PWOP) detected SAG from sputum to research difference of them from September 2009 and November 2015. **Results:** In univariate analysis, PWP were statistically more likely to have bed-ridden status (p<0.01) and also had more comorbid conditions such as cerebrovascular diseases and dementia (p<0.01) than PWOP. Among PWP cases, nursing- and healthcare-associated pneumonia (NHCAP) was the most frequent cases (59.3%). *S. anginosus* was significantly higher cultured with PWP compared to PWOP (p=0.013). Polymicrobial pathogens that includes drug-resistant strains such as methicillin-resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa* other than SAG detected more frequently (p=0.014) in PWP, however, surprisingly 40% cases were recovered after administering antibiotics covered only SAG. The distribution of pneumonia was the most frequently observed in bilateral lower lobe on computed tomography (CT) scans, which suggest that SAG is related to aspiration pneumonia. **Conclusions:** The present study describes that SAG must be recognized as a very important pathogen causes pneumonia especially when *S. anginosus* was detected in elderly patients with bed-ridden status. Previous article also reported that *S. anginosus* was mostly detectd in patients with respiratory infection. Therefore, our study provides an encouraging signal that SAG causes acute pneumonia and physicians and laboratory technicians need to identify SAG actively from now on.

Author Disclosure Block:

J. Hirai: None. **M. Hagihara:** None. **T. Kinjo:** None. **Y. Yamagishi:** None. **J. Fujita:** None. **H. Mikamo:** None.

Poster Board Number:

SUNDAY-342

Publishing Title:

Prevalence and Risk Factors of *Helicobacter pylori* Infection in the Northwest Region of Cameroon

Author Block:

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Abstract Body:

Background: *Helicobacter pylori* remain a public health problem and a major cause of peptic ulcer disease and gastric, with a prevalence of about 70% and 14-30% in developing and developed countries respectively.**Methods:** This study was carried out in Tubah District Hospital in the Northwest region to assess the prevalence and risk factors associated to *H. pylori*. This study runs from March to August 2014 with ethical clearance from the institutional ethical board. In all 400 subjects between 16-73 years were recruited. An open ended questionnaire was administered to all participants to capture information on socio-demographic data, risk **factors and Knowledge. Blood samples were collected and tested for the presence of the parasite using the Pylori test strip (dipstick). All diagnosed cases were treated using triple therapy.Results:**The prevalence of *H. pylori* in the study population was 240(60%). There was a significant difference $p<0.05$ in the prevalence of *H. pylori* among the age group, gender, occupation, educational level, monthly income and number of occupant in a house. The highest prevalences 112(82.2%), 186(75.0%), 160(66.6%), 96(40%), 152(63.3%) and 166(69.2%) was seen in the age group >34 years, female gender, students, those who attained primary education, monthly income <50,000frs CFA and > 10 occupants in a house respectively. The higher proportion 160 (61%) had poor knowledge about the disease recorded the highest prevalence 126(78.8%; $p=0.02$). A multivariate analysis showed a positive correlation between sex, age, number of house occupant, Knowledge of the disease, monthly income and occupation and the prevalence of *H. pylori.* Although no significant difference between the different treatment options most subjects 224(56%) prefer traditional herbs to synthetic drugs.**Conclusions:** Data from this study showed that gender, age, number of house occupant, knowledge, monthly income and occupation are risk factors of *H. pylori.*

Author Disclosure Block:

L.E. Abongwa: None. **M.Y. Elvis:** None.

Poster Board Number:

SUNDAY-343

Publishing Title:

Comparison of the Prevalence of Group A *Streptococcus* and *Fusobacterium necrophorum* at a Pediatric Hospital

Author Block:

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Abstract Body:

Background: *F. necrophorum*, a causative agent of Lemierre's syndrome, has recently been identified to be an important pathogen of bacterial pharyngitis with higher prevalence than group A *Streptococcus* (GAS) in adolescents and young adults. *F. necrophorum* pharyngitis is clinically indistinguishable from GAS pharyngitis and is not recoverable in routine aerobic throat culture. This study aimed to determine the prevalence of *F. necrophorum* compared to GAS to guide testing recommendations for patients with pharyngitis symptoms.**Methods:** Patients presenting to the CHLA emergency department who had a beta-streptococcus screen test ordered were enrolled prospectively in the study. For GAS, rapid antigen direct test (RADT) and culture were performed. *F. necrophorum* was detected by culture with selective and differential anaerobic medium and PCR. Medical chart abstraction was performed on all patients.**Results:** The study consisted of 196 patients, ages 1-20 years (mean: 7.8), most of whom had no significant comorbidities (95%). 11 (5.6%) were positive for GAS by RADT. Culture identified an additional 23 (13.4%) GAS and 4 group C and G streptococci. 8 (4.1%) patients had positive *F. necrophorum* by culture and PCR, ages 5-20 years (mean: 12 y). One patient was both GAS and *F. necrophorum* positive. All 8 (100%) *F. necrophorum* positive patients reported sore throat, 7 (87.5%) with fever, 7 (87.5%) with absence of cough, 1 (12.5%) with lymphadenopathy, and 4 (50%) with exudate. Comparatively, 28 (74%) of the 38 BS positive reported fever, 76% with no cough, 11% with lymphadenopathy, 32% with exudate, and 82% with sore throat. One *F. necrophorum* and 18 GAS culture positive patients were prescribed antibiotics at the time of visit.**Conclusions:** This study confirms the role of *F. necrophrorum* as a significant pathogen of pharyngitis. The prevalence of *F. necrophrorum* pharyngitis in our institution was lower than previously reported, which may be explained by the high number of patients < 10 y (67%) in our study. The vast majority of patients positive for *F. necrophrorum* did present with true signs and symptoms of pharyngitis that is indistinguishable from GAS.

Author Disclosure Block:

T.T. Van: None. **L.M. Cox:** None. **M.E. Cox:** None. **J. Dien Bard:** None.

Poster Board Number:

SUNDAY-344

Publishing Title:

***Candida spp*. Airway Colonization: A Potential Risk Factor for *Acinetobacter baumannii* Ventilator-Associated Pneumonia**

Author Block:

X. Tan[1], S. Zhu[1], D. Yan[1], W. Chen[2], R. Chen[1], J. Zou[3], J. Yan[3], X. Zhang[4], D. Farmakiotis[5], E. Mylonakis[5]; [1]Overseas Med. Ctr., Nanfang Hosp., Southern Med. Univ., Guangzhou, China, [2]The People's Hosp. of Qingyuan, Jinan Univ. Hosp., Qingyuan, China, [3]Nanfang Hosp., Southern Med. Univ., Guangzhou, China, [4]Southern Med. Univ., Guangzhou, China, [5]Div. of Infectious Diseases, Rhode Island Hosp., Warren Alpert Med. Sch. of Brown Univ., Providence, RI

Abstract Body:

This retrospective study was conducted to identify potential risk factors for *Acinetobacter baumannii (A. baumannii)* ventilator-associated pneumonia (VAP) and evaluate the association between *Candida* spp. airway colonization and *A. baumannii* VAP. Intensive care unit (ICU) patients who were on mechanical ventilation (MV) for at least 48 hours were divided into the following groups: patients with and without *Candida* spp. airway colonization; colonized patients receiving antifungal treatment or not; patients with *A. baumannii* VAP and those without VAP. Logistic regression analysis and propensity score matching were used to identify factors independently associated with *A. baumannii* VAP. Among 618 eligible patients, 264 (43%) had *Candida* spp. airway colonization and 114 (18%) developed *A. baumannii* VAP. Along with MV for ≥7 days (adjusted odds ratio [aOR] 8.9, 95% confidence intervals [95%CI] 4.9-15.8) and presence of a central venous catheter (aOR 3.2, 95%CI 1.1-9), *Candida* spp. airway colonization (aOR 2.6, 95%CI 1.6-4.3) was identified as an independent risk factor for *A. baumannii* VAP. Patients with *Candida* spp. airway colonization were more likely to develop *A. baumannii* VAP than non-colonized patients (23% *vs*. 15%, P=0.01 and 34% vs. 15%, P<0.001 in propensity score-matched subgroups). Administration of antifungal agents was not associated with *A. baumannii* VAP (29% vs. 21%, P=0.153), but with higher in-hospital mortality (53% *vs.* 39%, P=0.037). *Candida* spp. airway colonization (43%) and *A. baumannii* VAP (18%) were common in ICU patients who were on mechanical ventilation for at least 48 hours. *Candida* spp. airway colonization was an independent risk factor for subsequent *A. baumannii* VAP.

Author Disclosure Block:

X. Tan: None. **S. Zhu:** None. **D. Yan:** None. **W. Chen:** None. **R. Chen:** None. **J. Zou:** None. **J. Yan:** None. **X. Zhang:** None. **D. Farmakiotis:** None. **E. Mylonakis:** None.

Poster Board Number:

SUNDAY-345

Publishing Title:

Procalcitonin as a Predictor of Bacteremia in Early Postoperative Period in Adult Cardiosurgical Patients

Author Block:

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Abstract Body:

Background: Bacteremia is a serious post-op complication. We study the value of procalcitonin (PCT) as a predictor of bacteremia in adult patients underwent cardiac surgery. **Methods:** The retrospective 6-years study included 10158 pairs of simultaneously taken blood samples for cultivation and PCT testing from 4113 post-op patients with suspected bacteremia. Blood cultures incubation was performed using BacT/Alert 3D (bioMerieux, France). Identification of positive cultures was done by VITEK 2 compact (bioMerieux, France). PCT blood plasma concentration was determined by Vidas (bioMerieux, France). Data are presented as means and standard error of the mean (M±m) and were compared by Student`s t-test, p-values of <0.05 were considered statistically significant.**Results:** Bacteremia developed in average on day 6 of complicated post-op period (min - on day 2, max - on day 124). The whole rate of bacteremia was 20.7% (2105/10158): 972 cases (46.2%) were caused by Gram-positive cocci, 702 (33.3%) - by Gram-negative bacteria, 338 (16.1%) - by yeasts; in 93 cases (4.4%) mixed cultures were detected. Analysis included only monocultures (n=2012). Mean PCT levels were significantly higher in bacteremia cases (14,35±0.91 ng/mL) compared with negative blood cultures (7.35±0.26 ng/mL), p=0. The highest PCT levels were found in Gram-negative bacteremia (26.03±2.13 ng/mL) without significant differences between Enterobacteriaceae (30.56±4.05 ng/mL) and non-fermenters (22.79±2.21 ng/mL), p=0.07. Statistically significant differences by PCT levels between groups of bacteremia caused by Gram-negative bacteria (26.03±2.13 ng/mL) and Gram-positive cocci (7.24±0.88 ng/mL, p=0), *Candida spp.* (9.02±1.84 ng/mL, p=0) and contamination cases (9.92±2.79 ng/mL, p = 0) were found. The mean PCT levels in bacteremia caused by CNS (5.94±0.87 ng/mL), *S. aureus* (4.04±0.9 ng/mL), Enterococci (15.72±3.52 ng/mL), *Candida spp.* (9.02±1.84 ng/mL), contamination (9.92±2.79 ng/mL) and negative blood culture cases (7.35±0.26 ng/mL) were not statistically differ between each other.**Conclusions:** High PCT blood plasma levels during early post-op period in cardiosurgical patients may predict the Gram-negative bacteremia. Together with clinical data it may contribute to rational choice of empirical antibiotic therapy.

Author Disclosure Block:

D.A. Popov: None. **S.T. Ovseenko:** None. **T.Y. Vostrikova:** None.

Poster Board Number:

SUNDAY-346

Publishing Title:

Evaluation of Vancomycin Utilization in a Pediatric Hospital Before and After 2011 Idsa Recommendations

Author Block:

S. Tremblay[1], P. Bedard[1], C. Sallaleh[2], Y. Zilber[2]; [1]CHU Ste-Justine, Montreal, QC, Canada, [2]Univ. de Montreal, Montreal, QC, Canada

Abstract Body:

Pediatric references suggest starting doses of Vancomycin between 40 and 60 mg/kg/day divided every 6 hours. In 2011, IDSA indicated that targeting trough concentrations of 15-20 mg/L should be considered in children with serious infections. At CHU Sainte-Justine(CHUSJ) , since these recommandations, the initial dosage of vancomycin is 15mg/kg/dose every 6 hours. The dosage is then adjusted to target trough concentrations of 10-20mg/L. The aim of this study was to compare use, monitoring, response and toxicity of vancomycin at CHUSJ before and after the publication of IDSA recommendations.Hospitalized children at CHUSJ between January 2006 and November 2015 with ages between 3 months and 18 years who were treated with systemic vancomycin for at least 24 hours with at least one measured serum level were included in this study. Patients were divided in two groups; group A received vancomycin between January 2006 and December 2009 and group B received this antibiotic between January 2012 and November 2015. 80 patients were chosen with Excel software, 40 patients for each time frame. Patients hospitalized in oncology or neonatal units and patients < 3 months were excluded. For each patient demographic, clinical, microbiologic and pharmacokinetic datas were collected. Descriptive statistics were performed for the studied population. Both groups were comparable. The mean daily dose of vancomycin received was higher in group B(55 mg/kg/day vs 46,6 in group A). Vancomycin was given every 6 hours in 85% of group A patients while every 6 hours in 60% and every 4 hours in 20% of group B patients. 25% of patients reached a through value of 10 mg/L in group A and 72,5% in group B. There was no differences for through value > 15 mg/L. There was no significant differences for creatinine, neutropenia, eosinophilia, Red Man syndrom and other side effects. An elevation of blood serum creatinine >25% was observed in 71.4% of patients with through >15 mg/L.The utilization of higher vancomycin dosages allowed to attain through levels >10 mg/L in a higher proportion of patients. There was no significant differences in side effects in our 2 groups except for a trend toward an elevation of blood creatinine with higher through levels. Our group was small and further studies are needed.

Author Disclosure Block:

S. Tremblay: None. **P. Bedard:** None. **C. Sallaleh:** None. **Y. Zilber:** None.

Poster Board Number:

SUNDAY-347

Publishing Title:

Salivary Shedding of Epstein-Barr, Cytomegalovirus and HHV-8 DNA Correlate with Long-Term Plasma HIV RNA Detectability in HIV-Positive Men Who Have Sex with Men (MSM)

Author Block:

R. Scaggiante[1], S. Andreis[1], M. Basso[1], C. Del Vecchio[1], C. Mengoli[1], L. Sarmati[2], M. Cruciani[3], G. Palù[1], **S. G. Parisi**[1]; [1]Padova Univ, Padova, Italy, [2]Roma Univ, Roma, Italy, [3]ULSS 20, Verona, Italy

Abstract Body:

Background: To evaluate Cytomegalovirus (CMV), Epstein-Barr virus (EBV) and Human Herpesvirus-8 (HHV-8) DNA salivary shedding in MSM with 48 months (mo) of a persistent detectable or undetectable plasma HIV viremia (pHIV).**Methods:** Adult MSM, no oral lesions, positive CMV, EBV and HHV-8 serology provided an unstimulated saliva sample (SlSp). Viral DNA was quantified by a in-house real-time PCR. pHIV was analyzed for 24 mo prior to the first SlSp (T0) and for a 24-mo period between T0 and T1 (second SlSp); MSM were categorized as with pHIV suppressed (SS) or not suppressed (NS).**Results:** 121 MSM included (median age 44 yrs, mean CD4+ cells/mm^3 at T0 580, mean nadir value 345 cells/mm^3); HHV-8 was tested in 73 MSM. EBV and CMV shedding at T0 and T1 are reported in figure 1. No CMV and EBV shedding was more frequent in SS MSM at T0 (26.7 % vs 10 %, p=0.02); at T1 57.9% of SS were still negative but 100% of NS became positive (p=0.04). Isolated EBV detection was similar at T0 in the SS and NS group: but more NS turned EBV and CMV pos at T1 (14.3% vs 2.3 %, p=0.04). Combined CMV and EBV shedding was lost in 90% of SS at T1 versus 30% of NS MSM (p=0.01), this figure increased in NS (18% to 28%). Mean pHIV (copies/ml) was significantly higher in MSM with CMV and EBV shedding (47994) both respect those with isolated EBV (13585) and with no shedding (8425) (p=0.03). At T0 HHV8 was detected in 29 MSM, (39.7%) (17 still positive at T1); at T1 all HHV-8+/CMV+ MSM were NS respect to 36% of HHV8+/CMV- pts (p=0.01).**Conclusions:** : A persistent pHIV detectability influenced CMV, EBV and HHV-8 salivary burden both in the short and in the long period in immunocompetent MSM.

Figure 1. Different patterns of EBV DNA and CMV DNA salivary shedding in MSM with pHIV suppressed and with pHIV not suppressed at T0 and T1.

MSM with pHIV suppressed (SS, 71 pts)

T0	T1			
	EBV neg CMV neg 17 (24%)	EBV pos CMV neg 51 (71.8%)	EBV neg CMV pos 1 (1.4%)	EBV pos CMV pos 2 (2.8%)
EBV neg - CMV neg 19 pts (26.7%)	11 pts (57.9%)	8 pts (42.1%)	0 pts	0 pt
EBV pos – CMV neg 44 pts (62%)	5 pts (11.3%)	38 pts (86.4%)	0 pts	1 pt (2.3%)
EBV neg – CMV pos 0 pt	-	-	-	-
EBV pos - CMV pos 8 pts (11.3%)	1 pt (12.5%)	5 pts (62.5%)	1 pt (12.5%)	1 pt (12.5%)

MSM with pHIV not suppressed (NS, 50 pts)

T0	T1			
	EBV neg CMV neg 5 (10%)	EBV pos CMV neg 29 (58%)	EBV neg CMV pos 2 (4%)	EBV pos CMV pos 14 (28%)
EBV neg - CMV neg 5 pts (10%)	0 pts	3 pts (60%)	1 pt (20%)	1 pt (20%)
EBV pos – CMV neg 35 pts (70%)	5 pts (14.3%)	24 pts (68.6%)	1 pt (2.8%)	5 pts (14.3%)
EBV neg – CMV pos 1 pt (2%)	0 pts	0 pts	0 pts	1 pt (100%)
EBV pos - CMV pos 9 pts (18%)	0 pts	2 pts (22.2%)	0 pts	7 pt (77.8%)

Author Disclosure Block:

R. Scaggiante: None. **S. Andreis:** None. **M. Basso:** None. **C. Del Vecchio:** None. **C. Mengoli:** None. **L. Sarmati:** None. **M. Cruciani:** None. **G. Palù:** None. **S.G. Parisi:** None.

Poster Board Number:

SUNDAY-348

Publishing Title:

Prevalence and Genotype Distribution of High Risk *Human papillomavirus* and Cervical Cytology Abnormalities at Selected Obstetrics and Gynecology Clinics in Addis Ababa, Ethiopia

Author Block:

K. ALI[1], K. TULU[2], I. MOHAMMED[2], M. DIFABACHEW[1], D. SOLOMON[3], T. HAILE[4], R-J. TEN HOVE[1], T. HAILU[5], Z. LAKEW[6]; [1]INTERNatl. CLINICAL LABORATORIES, ADDIS ABABA, Ethiopia, [2]ADDIS ABABA Univ., ADDIS ABABA, Ethiopia, [3]ST. PAUL's MILLINIUM Med. Coll., ADDIS ABABA, Ethiopia, [4]SinamokshEthio Women's Hlth.Special Clinic, ADDIS ABABA, Ethiopia, [5]ARMAUER HANSEN RESEARCH Inst. (AHRI), ADDIS ABABA, Ethiopia, [6]HEMEN MATERNAL AND CHILDREN Hosp., ADDIS ABABA, Ethiopia

Abstract Body:

Cervical cancer is a preventable disease affecting an estimated 530,000 women each year and leading to nearly 275,000 deaths. Human papillomavirus (HPV) has been recognized as an important cause of cervical cancer and it is implicated in 99.7% of cervical squamous cell cancer cases in the world. In Ethiopia, every year 7095 women diagnosed with cervical cancer and 4732 die from the disease. Very low screening practice and inadequate screening coverage in Ethiopia makes cervical cancer as one of the major public health concern.This study was aimed to assess the prevalence and genotype distribution of High Risk Human Papilloma Virus (HR HPV) and Cervical Cytology abnormalities at selected Obstetrics and Gynecology clinics in Addis Ababa, EthiopiaInstitutional based cross sectional study design was used from 15th June to 10th October 2015. Cervical samples were collected from the os of the cervix using Abbott cervi-cyt collection material for HR HPV DNA and cyto-brush for Pap smear screening. A total of 366 participants were enrolled based on the set inclusion criteria. HR HPV DNA was analyzed using Abbott Real Time PCR and cervical cytology screening using conventional Pap smear techniques. Data entry was performed using Epi-data ver 3.1 and data analysis was performed by using STATA ver 11.0.The overall HR HPV prevalence was 13.7% (50/366), with 76% (38/50) of Other HR HPV genotypes. Abnormal cytology was observed in 13.1% (48/366) with 81.3%, 12.5%, and 6.3%, are LSIL, ASCUS and HSIL respectively.In this study, Non-16/18 genotypes contributed the largest proportion of the overall HR HPV. The highest frequency of HR HPV positives was women without cervical cytology abnormality. The HR HPV with Pap smear co-screening in women whose age is >30 shall be in place. Further evaluation between the two screening methods against a perfect reference method shall be performed.

Author Disclosure Block:

K. Ali: None. **K. Tulu:** None. **I. Mohammed:** None. **M. Difabachew:** None. **D. Solomon:** None. **T. Haile:** None. **R. Ten hove:** None. **T. Hailu:** None. **Z. Lakew:** None.

Poster Board Number:

SUNDAY-349

Publishing Title:

Whole Genome Analyses Reveal Multiple Viral Pathogens in Respiratory Illness Specimens from United States Army Basic Trainees

Author Block:

J. Hang, T. J. Vento, E. A. Norby, R. G. Jarman, R. A. Kuschner, P. B. Keiser, L. N. Binn; Walter Reed Army Inst. of Res., Silver Spring, MD

Abstract Body:

Background: Human adenoviruses (AdV), in particular types 4 and 7, frequently cause acute respiratory disease (ARD) during basic military training. AdV4 and AdV7 vaccines have been proven safe and effective and have substantially reduced the ARD risk. It is important to identify other respiratory pathogens and assess their potential impact on military readiness. We investigated respiratory specimens collected from a group of basic trainees to identify their viral etiologies. **Methods:** In 2002, during a period when the adenovirus vaccines were not available, throat swabs were taken from recruits with respiratory disease at Fort Jackson, South Carolina. The swabs were inoculated into A549 cells and observed for viral cytopathic effects (cpe) and tested in neutralization assays with antisera for AdV types 3, 4, 7. Nucleic acids purified from viral culture supernatants were sequenced using Illumina MiSeq and Nextera methods. The viral genome sequences were assembled using Roche GS Data Analysis Software and applied to whole genome comparative analysis using Geneious R8.**Results:** The study enrolled 184 military recruits with febrile ARD, afebrile ARD and pneumonia. Viral culture and neutralization assays identified 86 AdV-4 isolates (46.7%) and 23 other AdVs or picorna-like cpe viruses (12.5%). Sequencing 103 cpe-positive samples confirmed these results and determined the genotypes: 78 AdV4, four AdV3, one AdV2, and 18 Coxsackie virus A-21 (CAV 21) and one enterovirus, D68. Two samples were positive for both AdV4 and CAV 21. The analyses suggest that each of these virus types originated from a single strain. The identified genotypes are phylogenetically close to but distinct from those found during other years or in other military/non-military sites.**Conclusions:** These results confirmed AdV4 is the predominant respiratory pathogen in military recruits. The genome sequence analysis shows the temporal and demographic variability of adenoviruses. CAV 21 has been a significant respiratory pathogen and needs to be evaluated for its current significance in military recruits. Next-generation sequencing enables accurate pathogen identification and provides information for improved disease surveillance and prevention.

Author Disclosure Block:

J. Hang: None. **T.J. Vento:** None. **E.A. Norby:** None. **R.G. Jarman:** None. **R.A. Kuschner:** None. **P.B. Keiser:** None. **L.N. Binn:** None.

Poster Board Number:

SUNDAY-350

Publishing Title:

Clinical Scoring System to Predict Life-Threatening Respiratory Syncytial Virus (Rsv) Infections in Adults

Author Block:

S. Park, Y. Jang, T. Kim, S. Hong, J. Jung, Y. Chong, S-O. Lee, S-H. Choi, Y. Kim, J. Woo, S-H. Kim; Asan Med. Ctr., Seoul, Korea, Republic of

Abstract Body:

Background: RSV is a significant cause of considerable morbidity and mortality in elderly persons as well as in young children. However, there are limited data on clinical prediction models for life-threatening RSV infection in adults, which could guide the appropriate use of antiviral agents.**Methods:** We conducted a retrospective cohort study in a tertiary referral hospital during a 2-year period. All adult patients with RSV infection confirmed by PCR on nasopharyngeal swab who visited emergency department were enrolled. Primary outcome was life-threatening RSV infection (admission to intensive care unit or need to ventilator care or in-hospital death).**Results:** A total of 227 patients were analyzed. Median age was 65 (IQR 57-74). Of these 227 patients, 216 (95%) had underlying chronic medical condition; 48 (21%) had chronic pulmonary diseases, 69 (30%) solid tumors, and 22 (10%) hematologic diseases. Life-threatening RSV infection occurred in 34 (15%) patients including 17 (8%) fatal infections. By logistic regression, lower respiratory infection (OR 4.8, 95% CI 2.0 to 11.6, *P*=0.001), fever ≥38ºC (OR 2.4, 95% CI 1.1 to 5.3, *P*=0.05), and chronic lung disease (OR 2.8, 95% CI 1.3 to 6.1, *P*=0.03) was found to be independent predictors for life-threatening RSV infection. We developed simple clinical scoring using these variables (lower respiratory tract infection= score 2, fever=score 1, chronic lung disease=score 1) to predict life-threatening RSV infection. A score of ≥2 differential life-threatening RSV from non life-threatening RSV with 82.4 sensitivity (95% CI, 65.5 - 93.2) and 51.3 specificity (95% CI, 44.0 - 58.5).**Conclusions:** Using clinical scoring based on lower respiratory infection, fever, and chronic lung disease appears to be useful for outcome prediction and risk stratification to select patients who may need early antiviral therapy.

Author Disclosure Block:

S. Park: None. **Y. Jang:** None. **T. Kim:** None. **S. Hong:** None. **J. Jung:** None. **Y. Chong:** None. **S. Lee:** None. **S. Choi:** None. **Y. Kim:** None. **J. Woo:** None. **S. Kim:** None.

Poster Board Number:

SUNDAY-351

Publishing Title:

Molecular Characterization of Circulating Strains of Influenza B Between 2014-2015 in Egypt

Author Block:

E. A. Ayoub[1], M. A. Aziz[1], N. Nabil[1], S. El Refaay[2], M. Genedy[2], A. Kandil[2], G. Defang[1]; [1]US Naval Med. Res. Unit 3, Cairo, Egypt, [2]Ministry of Hlth.and Population, Cairo, Egypt, Cairo, Egypt

Abstract Body:

Infection with influenza can result in a wide a range of clinical presentations and in some instances result in fatal disease. Monitoring genetic changes of influenza viruses is crucial for vaccine strain selection, detection of drug resistance and determination of virulence markers. Herein we analyzed the HA and NA genes of influenza B viruses circulating among influenza-like illness (ILI) patients in Egypt during winter of 2014/15.Patients were considered to have an ILI if they met the WHO criteria of fever ≥38°C and cough. Oropharyngeal swabs in viral transport media collected from seven sites within Egypt were diagnosed by real-time PCR to determine influenza subtypes. Positive influenza B samples were inoculated on MDCK cells. The supernatant was tested by PCR to confirm influenza B, and representative isolates were chosen for Sanger's sequencing.Sequence analysis of the HA gene of 40 isolates showed that all viruses were within the Influenza B/Yamagata lineage with 28 isolates clustered within clade 3 and 12 within clade 2. The two clades were differentiated by substitutions at HA1 positions 48, 108, 150, 165, 181 and 229; viruses in clade 3 encode R48, P108, I150, Y165, T181 and D229. There was no specific geographical region or time interval associated with the distribution of both clades. Clade 2 sequences had more than 99% nucleotide (nt) similarity to B/Massachuetts/02/2012, the vaccine strain for 2014/15 season in northern hemisphere. Clade 3 sequences demonstrated 97% nt similarity to the same strain and showed more than 99% nt similarity to B/Phuket/3073/2013, the vaccine strain for 2014/15 for the southern hemisphere and the 2015/16 northern hemisphere candidate vaccine, however clade 2 viruses showed 97% nt similarity to the same strain. The NA sequences showed the same pattern of the HA phylogenetic tree in which the sequences clustered within clade 2 and 3 viruses. Mutations; R152K, G109E, D198N, and G402S previously reported to be associated with resistance to NA inhibitors were not detected.Phylogenetic analysis of influenza B viruses from Egypt during 2014/15 showed no Victoria lineage viruses. Our data supports the WHO recommendation of replacing B/Massachuetts/02/2012 with B/Phuket/3073/2013 in the influenza vaccine composition for 2015/16 season.

Author Disclosure Block:

E.A. Ayoub: None. **M.A. Aziz:** None. **N. Nabil:** None. **S. El Refaay:** None. **M. Genedy:** None. **A. Kandil:** None. **G. Defang:** None.

Poster Board Number:

SUNDAY-352

Publishing Title:

Severe Fever with Thrombocytopenia Syndrome Associated with Hemophagocytosis Lymphohistiocytosis

Author Block:

H. Oh[1], M. Kim[1], J-O. Lee[1], H. Kim[2], E. Kim[1], P. Choe[2], W. Park[2], J. Bang[2], K. Park[2], S. Park[2], H. Kim[1], N. Kim[2], M-d. Oh[2], K-H. Song[1]; [1]Seoul Natl. Univ. Bundang Hosp., Seongnam, Korea, Republic of, [2]Seoul Natl. Univ. Coll. of Med., Seoul, Korea, Republic of

Abstract Body:

Background: Severe fever with thrombocytopenia syndrome (SFTS) is new emerging zoonosis. Hemophagocytic lymphohistiocytosis (HLH) is life-threatening syndrome caused by hyper-inflammation. Here we report the case of SFTS associated HLH.**Case report:** A 62-year-old man was admitted to local hospital with generalized tonic clonic seizure-like movement. He suffered from 8 days of fever and chill. Brain magnetic resonance imaging and electroencephalography were normal. First seizure spontaneously terminated and his mentality was alert during post-ictal period. His peak body temperature was 39.4℃. Initial laboratory finding revealed leukopenia ($1,900\times10^6$/L) and thrombocytopenia ($107,000\times10^6$/L). Since his mentality became confused gradually and fever persisted, he was transferred to referral hospital. Leukopenia was stationary ($2,400\times10^6$/L), but thrombocytopenia got worsened ($49,000\times10^6$/L). He had hypertriglyceridemia (627 mg/dL) and hyperferritinemia (832 ng/mL). Cerebrospinal fluid analysis showed mild elevation of protein without pleocytosis. Bone marrow examination and trans-jugular liver biopsy were performed and showed hemophagocytic histiocytes. Serological and molecular tests for possible infectious causes were negative except SFTS virus polymerase chain reaction (PCR). The PCR tests were performed by not only serum but also bone marrow and liver biopsy specimens. All of them indicated positive. Definite diagnosis was SFTS associated HLH. During 2 weeks of systemic steroid, intravenous immunoglobulin and conservative management, he was fully recovered from HLH.**Discussion:** The present case met five of eight criteria of HLH. SFTS virus PCR results were positive in all clinical specimens. We could make definite diagnosis of SFTS associated HLH. HLH can be rapidly progressing and life-threatening without adequate treatment, active investigation of possible infectious causes of HLH is crucial. We confirmed that SFTS could be one of causes of HLH. Thus, clinicians need to be aware of it, especially in endemic area.

Author Disclosure Block:

H. Oh: None. **M. Kim:** None. **J. Lee:** None. **H. Kim:** None. **E. Kim:** None. **P. Choe:** None. **W. Park:** None. **J. Bang:** None. **K. Park:** None. **S. Park:** None. **H. Kim:** None. **N. Kim:** None. **M. Oh:** None. **K. Song:** None.

Poster Board Number:

SUNDAY-353

Publishing Title:

Norovirus Infection of Children in Egypt Between 2005 and 2009 Showed Distinct Molecular Clustering Based on Time of Year and Location

Author Block:

R. Nada, I. Nakhla, S. Lizewski, I. Raafat, M. Kamel, M. Mostafa, A. Hamad, H. Mohammady; US. Naval Med. Res. Unit-3, Cairo, Egypt

Abstract Body:

Background: Noroviruses (NoVs) are a worldwide leading cause of acute viral gastroenteritis across all ages and is a major cause of acute gastroenteritis outbreaks in industrialized countries. This study investigated the prevalence and genotype distribution of noroviruses in pediatric patients presenting with diarrhea from three different regions in Egypt. It also studied the phylogeny of the predominating norovirus genotypes.**Methods:** Archived stool samples collected from 930 diarrheal patients enrolled in hospital-based, pediatric diarrhea surveillance studies between 2005 and 2009 were tested for the presence of norovirus using real-time PCR (Q-PCR) based on amplification of ORF1-ORF2 junction. Circulating norovirus genotypes for both GI and GII were identified using DNA sequencing of regions C and D of ORF2.**Results:** Noroviruses were detected in 177 (19%) patients, of which, 105 (11%) had norovirus as the sole pathogen. GII was more common (n=150; 16.1%) than GI (n=34; 3.6%). Seven patients were co-infected with noroviruses GI and GII. Both genogroups had a very high association ($p<0.0001$) with vomiting. Seasonality of norovirus infections was consistent during the five years of the study. Norovirus infections peaked in the warm months with 91% of norovirus GI and 68% of norovirus GII infections occurring between May and October. Sequence analysis of GI revealed equal distribution of GI.2 and GI.3 (15%) followed by GI.6 (12%). The other G1 genotypes were detected in smaller frequencies ranging from 6 to 9%. . GII.4 was the most abundant genotype (42%) identified in norovirus GII cases followed by GII.3 (27%) and GII.1 (7%). Other norovirus GII genotypeswere identified in lower frequencies ranging from 5% to 1%. Phylogenetic analysis of GII.3 and GII.4 norovirus strains showed clear association between time period and geographical location with norovirus clusters identified which might indicate the complexity of noroviruses epidemiology and the high infectivity of these viruses that might appear in the form of mini-outbreaks.**Conclusions:** Results of this study provide valuable information regarding the epidemiology of noroviruses infections and confirm that these viruses are responsible for a significant portion of acute gastroenteritis in outpatient children in Egypt.

Author Disclosure Block:

R. Nada: None. **I. Nakhla:** None. **S. Lizewski:** None. **I. Raafat:** None. **M. Kamel:** None. **M. Mostafa:** None. **A. Hamad:** None. **H. Mohammady:** None.

Poster Board Number:

SUNDAY-354

Publishing Title:

Measles a Considerable Public Health Problem in Oromia Regional State of Ethiopia

Author Block:

M. Getahun, E. Assefa, Y. HaileMariyam, A. Asha, B. Beyene; Ethiopian Publ. Hlth.Inst., Addis Ababa, Ethiopia

Abstract Body:

Background: Measles is a highly contagious acute febrile rash viral infection causing large outbreaks and complications like brain damage, blindness, and deafness. Despite the availability of safe and cost effective vaccine, measles remained endemic with persistent periodic outbreaks in the Horn of Africa, being one of the five major causes of childhood illness in Ethiopia. The aim of this study was to characterize laboratory confirmed measles cases from the case based surveillance data in the highly measles affected Oromia Regional State of Ethiopia, 2008-2015. **Methods:** A suspected measles case was defined as any person presenting with fever, maculopapular rash and at least one of the three, cough, coryza or conjunctivitis or a patient in whom a clinician suspects measles. A blood sample was collected from suspected cases and information about the patient was captured by case reporting format (CRF). Samples were transported to the National Measles Laboratory where tested for Measles IgM by ELISA technique. Case based surveillance data were entered and analyzed using Epi-Info 3.5.4 software.**Results:** From 2008-2015, a total of 13,303 samples were tested for measles IgM among 15,004 cases notified with blood and CRF from Oromia Regional State. Of the tested samples, 5336 (40.1%) had confirmed measles (IgM positive) with 7608 negative and 359 equivocal result. Patients with age group 1-4 years were the most affected followed by ≥15 years.. The highest number of laboratory confirmed measles cases were detected in 2015 contributed for 20% of all years. Confirmed cases were found distributed throughout all zones of the regional state. A seasonal variation noted with the highest occurrence in the hot-dry season of the year, peak in February (figure 1).**Conclusions:** Measles infection was found increasing from year to year, mostly affecting children 1-4 years. Seasonal variation was seen reaching its peak in hot-dry season. It is highly recommended to improve routine immunization, and conduct a wide age group campaign. In addition to genotyping the circulating strain of measles, evaluating the knowledge, attitudes and practice of the parents and health care professionals about measles infection and vaccination is important.

Author Disclosure Block:

M. Getahun: None. **E. Assefa:** None. **Y. HaileMariyam:** None. **A. Asha:** None. **B. Beyene:** None.

Poster Board Number:

SUNDAY-355

Publishing Title:

An Under-Recognized Arbovirus in Nova Scotia, Canada: Seroprevalence of Jamestown Canyon Virus

Author Block:

G. Patriquin[1], E. Schleihauf[2], B. Johnston[3], K. Dimitrova[4], A. Mask[5], M. Traykova-Andonova[4], D. Haldane[3], M. Drebot[4], T. Cole[6], R. Lindsay[4], T. Hatchette[3]; [1]Dalhousie Univ., Halifax, NS, Canada, [2]Publ. Hlth.Agency of Canada, Halifax, NS, Canada, [3]Nova Scotia Hlth.Authority, Halifax, NS, Canada, [4]Publ. Hlth.Agency of Canada, Winnipeg, MB, Canada, [5]Publ. Hlth.Agency of Canada, Ottawa, ON, Canada, [6]Nova Scotia Dept. of Hlth.and Wellness, Halifax, NS, Canada

Abstract Body:

Background: Jamestown Canyon Virus (JCV) is a mosquito-borne arbovirus in the California serogroup (CSG) within the Bunyaviridae family. The white-tailed deer is thought to be its primary reservoir host. The virus has been described in North America, as infections ranging from asymptomatic to meningoencephalitis. Our preliminary serological data demonstrated exposure to JCV among white-tailed deer and humans. Prior data were limited to two district health authorities (DHAs) in Nova Scotia (NS).**Objective:** To determine and compare the JCV seroprevalence of each DHA in NS.**Methods:** Randomly-selected anonymized residual sera from specimens submitted for diagnostic testing in 2012 in each DHA were screened for JCV antibodies, and then confirmed using plaque reduction neutralization assay (PRNT). A PRNT titre ≥1:20 was considered positive. Additional PRNT endpoint titrations were required in some cases to discriminate between JCV and other CSG viruses. Seroprevalence estimates and 95% confidence intervals (CIs) were calculated using the Clopper-Pearson Exact method. Design weights accounted for regional oversampling in the provincial estimate (SAS v9.4; SAS Institute, Inc., Cary, NC, USA). Population estimates for 2014 were based on Statistics Canada census data.**Results:** A total of 251 samples across 9 NS DHAs were tested, with an overall seroprevalence of JCV of 21.2% (95% CI 16.1-27.0). Seroprevalence based on DHA ranged from 12.9% to 48.2%. The areas of lowest seroprevalence were in DHAs containing NS's two largest urban centres, Halifax and Sydney. DHA 1, located in southern NS had a significantly higher seroprevalence of 48.2% (CI 35.1-61.3), than all other DHAs combined (20.5% (CI 14.8-26.2)), with a p-value <0.05.**Conclusions:** The seroprevalence of JCV in NS is high and varies based on DHA. There have been no known clinical cases of JCV infection in the province, suggesting the possibility of an under-recognized zoonotic disease in NS.

Author Disclosure Block:

G. Patriquin: None. **E. Schleihauf:** None. **B. Johnston:** None. **K. Dimitrova:** None. **A. Mask:** None. **M. Traykova-Andonova:** None. **D. Haldane:** None. **M. Drebot:** None. **T. Cole:** None. **R. Lindsay:** None. **T. Hatchette:** None.

Poster Board Number:

SUNDAY-356

Publishing Title:

Adenovirus 8 (D Strain) Associated Conjunctivitis Outbreak in a Pediatric Nursery Ward of a Tertiary Care Hospital

Author Block:

G. Satpathy, A. K. Mishra, R. Tandon; All India Inst. of Med. Sci., New Delhi, India

Abstract Body:

Objectives: The objective was to determine the microbial aetiology in a cluster of conjunctivitis cases occurring in a pediatric nursery of our hospital using classical microbiological and molecular methods and to do the molecular phylogenetic study of the causative viruses.**Methods:** Seven babies (9-66 days old) in the paediatric nursery ward developed conjunctivitis along with one of their mothers simultaneously. Conjunctival specimens were subjected to bacterial culture, direct immunofluorescence assay for *C trachomatis* antigen detection, viral culture and PCR assay for detection of conjunctivitis causing viruses (target genes: VP 1 region of EV 70 and Coxscakiae virus A 24 and 302 bp region of Adeno Virus hexon protein coding gene). Nested PCR assay was done to amplify 1004bp and 956bp internal region of Adeno Virus hexon gene, nucleotide sequencing was done and a phylogenetic tree was drawn using MEGA 4 software along with 30 different Adeno virus hexon gene sequences from gene bank and nucleotide sequences of 9 Adeno virus isolates from our laboratory from sporadic conjunctivitis cases.**Results:** Three of the babies and one of their mother were positive in diagnostic Adeno Virus PCR assay. Adeno viruses could be isolated in *Hep* 2 cells from 3 of these babies. All of them were negative for *C trachomatis* antigen detection and bacteria could be isolated from all eyes (*S epidermidis*: 6, *S aureus*: 1., *E coli:* 1). The isolated Adenoviruses demonstrated 98% sequence homology with Adeno virus 8 in the hexon gene sequences, In phylogenetic analysis they clustered with Strain D of Adeno Virus.**Conclusion:** Adeno Vorus 8 (Strain D) was concluded as the causative agent of conjunctivitis in the paediatric nursery.; this was also the causative virus in the sporadic conjunctivitis cases occurring in Delhi. Use of molecular diagnostics along with nucleotide sequencing could identify the causative virus within a short time.

Author Disclosure Block:

G. Satpathy: None. **A.K. Mishra:** None. **R. Tandon:** None.

Poster Board Number:

SUNDAY-357

Publishing Title:

Norovirus Seroprevalence in the United States, 1999-2004

Author Block:

A. E. Kirby, J. Barton, M. Sizemore, E. Anderson, C. L. Moe; Emory Univ., Atlanta, GA

Abstract Body:

Norovirus is the most common cause of foodborne gastroenteritis, however, estimates of the full burden of infection are limited. Very few cases seek medical care and testing, and epidemiologic studies focus on specific populations or severe cases. Thus, the burden of endemic norovirus causing mild or moderate disease is not known. To estimate the total burden of norovirus infection in the US, we determined the seroprevalence of antibodies to norovirus in a nationally representative sample. Banked serum specimens from adults aged 16-49 collected during the 1999-2000 and 2003-2004 cycles of the National Health and Nutrition Examination Survey were tested against a panel of norovirus virus-like particles (VLPs): GI.1 Norwalk, GI.4, GII.3, GII.4 US95/96, GII.4 Farmington Hills, GII.4 New Orleans, and GIV.1. Serum samples were diluted 1:50 for ELISA. Samples with an optical density greater than 1.0 were considered seropositive. Most samples (87.6%) were seropositive for at least one VLP, and 18.2% were seropositive for all seven VLPs. The VLPs with the highest seroprevalence were GII.4 US95/96 and GII.4 Farmington Hills, at 71.4% and 70.6%, respectively. GII.4 New Orleans, which had not yet emerged at the time of sample collection, had a seroprevalence of 65.9%. GI.1 Norwalk and GII.3 had similarly high seroprevalences, at 68.8% and 69.6%, respectively. The VLP with the lowest seroprevalence was GI.4 (44.7%). Half of all samples (49.6%) were seropositive for GIV.1. Very high titers (OD>2.5)-consistent with recent infection-were much less frequent, ranging from 4.9% to 30.9%. There were no significant findings when the results were stratified by subject age. Although this study found slightly lower seroprevalence to norovirus than previous reports, the majority of subjects had evidence of norovirus infection. This is the first nationally representative serosurvey for norovirus in the US and one of the few studies to assess prevalence of GIV.1 infection.

Author Disclosure Block:

A.E. Kirby: C. Consultant; Self; Takeda Vaccines. **J. Barton:** None. **M. Sizemore:** None. **E. Anderson:** None. **C.L. Moe:** E. Grant Investigator; Self; Takeda Vaccines, GE.

Poster Board Number:

SUNDAY-358

Publishing Title:

Norovirus and Rotavirus Coinfection in Mexican Children During 2014-2015

Author Block:

R. Infante-Ramirez[1], D. Perez-Lugo[1], J. Lopez-Franco[1], C. Delgado-Gardea[1], M. De La O-Contreras[1], G. Erosa-Dela Vega[1], M. Gonzalez-Horta[1], B. Sanchez-Ramirez[1], A. Torres Reyes[1], E. Guevara-Macias[2], F. Zavala-Diaz de la Serna[1], C. Romo-Saenz[3], J. Contreras Cordero[3]; [1]Univ. Autonoma de Chihuahua, CHIHUAHUA, Mexico, [2]Hosp. Infantil de Especialidades, CHIHUAHUA, Mexico, [3]Univ. Autonoma de Nuevo Leon, MONTERREY NL, Mexico

Abstract Body:

Severe acute diarrhea is an intestinal inflammatory type alteration caused by various infectious agents, bacteria, parasites fungi and virus; In Mexico to viral agents it is recognized as the leading cause of childhood gastroenteritis , followed by bacteria and parasites. Globally, in infants under five, Rotavirus is the most important cause of mobility and mortality. Within *Caliciviridae* family, Norovirus is the most common cause of outbreaks on non-bacterial gastroenteritis in all age patients, while in Mexico we had reported only a 3% of cases caused by this viral agent, both due to the easy dispersion by oral-fecal route. The purpose of this study was detected confection Rotavirus-Norovirus in children with acute diarrhea during 2014-2015.**Methods:** A total of 116 stool samples of children under 5, with acute diarrhea were studied. Samples were collected in Pediatric Hospital in Chihuahua, Mexico, during 2014-2015. Viral RNA was extracted by Trizol® method. Molecular detection of Rotavirus and Norovirus by two steps RT-qPCR was done. Rotavirus cDNA was synthesized for generating a fragment of 876pb of the VP4 protein amplified with primers PA1/CON2. To detect Norovirus we used specific primers JV13 and JV12, amplifying a 326 bp fragment of gen RdRp. Amplicons was analyzed by Software Kodak 1D®in 1%agarose gel. The results reveal coinfection Norovirus-Rotavirus in the children samples. The presence of the Norovirus was 18.96% (22) of the samples analyzed. Gene four of Rotavirus (VP8) was detected in 38 samples. **Conclusions:** During 2014-2015 a significant increase in norovirus strains in children with acute diarrhea, three times higher than what we had reported in previous periods was observed. Moreover Rotavirus also showed increase, so it is necessary to continue with the epidemiological surveillance.

Author Disclosure Block:

R. Infante-Ramirez: None. **D. Perez-Lugo:** None. **J. Lopez-Franco:** None. **C. Delgado-Gardea:** None. **M. De La O-Contreras:** None. **G. Erosa-Dela Vega:** None. **M. Gonzalez-Horta:** None. **B. Sanchez-Ramirez:** None. **A. Torres Reyes:** None. **E. Guevara-Macias:**

None. **F. Zavala-Diaz de la Serna:** None. **C. Romo-Saenz:** None. **J. Contreras Cordero:** None.

Poster Board Number:

SUNDAY-359

Publishing Title:

Recurrent Respiratory Tract Infections in Children - A Prospective Cohort Study

Author Block:

L. Toivonen[1], S. Karppinen[1], L. Schuez-Havupalo[1], T. Teros-Jaakkola[1], J. Vuononvirta[2], Q. He[2], M. Waris[1], V. Peltola[1]; [1]Univ. of Turku, Turku, Finland, [2]Natl. Inst. for Hlth.and Welfare, Turku, Finland

Abstract Body:

Background: The disease burden of recurrent respiratory tract infections is unclear. We identified young children with recurrent respiratory infections in order to characterize the clinical manifestations and short-term consequences. **Methods:** In this prospective cohort study, 1089 children were followed from birth to two years of age for respiratory infections by a daily symptom diary. Background information was gathered by questionnaires. In a subgroup of 714 children, nasal swab samples taken during respiratory infections were analyzed for viruses, and nasopharyngeal swabs collected at two months of age were cultured for bacteria. The 10% of children with the highest number of annual respiratory illness days were defined to have recurrent respiratory infections.**Results:** A total of 99,587 respiratory illness days, 12,118 episodes of acute respiratory infections, and 5981 physician visits for acute respiratory infection were documented. The median number of annual respiratory illness days was 44 per child, and the 90th percentile was 98 days. Children above the 90th percentile (n = 109) had a median of 113 days with respiratory symptoms, 9.6 acute respiratory infection episodes, 6.0 physician visits, 1.8 episodes of acute otitis media, and 2.5 antibiotic treatments per child per year. Of these children, 60% were diagnosed with ≥3 episodes of acute otitis media, 33% with a wheezing illness, 73% received ≥3 antibiotic treatments, and 14% were hospitalized for an acute respiratory infection. Tympanostomy tubes were inserted to 35% and adenoidectomy was performed for 13% of these children. Older siblings, early nasopharyngeal colonization with Streptococcus pneumoniae, and an acute respiratory infection before three months of age were associated with recurrent respiratory tract infections ($P<0.0001$, $P=0.005$, and $P<0.0001$, respectively).**Conclusions:** Recurrent respiratory tract infections cause a substantial disease burden. Children with recurrent respiratory infections frequently use healthcare services and antibiotics.

Author Disclosure Block:

L. Toivonen: None. **S. Karppinen:** None. **L. Schuez-Havupalo:** None. **T. Teros-Jaakkola:** None. **J. Vuononvirta:** None. **Q. He:** None. **M. Waris:** None. **V. Peltola:** None.

Poster Board Number:

SUNDAY-360

Publishing Title:

Diagnostic Usefulness of CMV-Specific T Cell Immunity in Predicting CMV Infection after Kidney Transplantation

Author Block:

T. Kim, S. Park, M-C. Kim, Y. Jang, H-J. Lee, S-M. Kim, J. Jung, S. Shin, Y-H. Kim, H. Sung, Y. Chong, S-O. Lee, S-H. Choi, Y. Kim, J. Woo, D. Han, S-H. Kim; Asan Med. Ctr., Seoul, Korea, Republic of

Abstract Body:

Background: Cytomegalovirus (CMV) is one of the most important opportunistic infections in transplant recipients. Currently pretransplant serostatus for CMV IgG is the only laboratory test for stratifying the risk of CMV infection after solid organ transplant, while T cell-mediated immunity is important for controlling CMV infection. We thus evaluated the usefulness of the pretransplant CMV-specific ELISPOT assay in kidney transplant (KT) recipients for predicting CMV infections after transplant. **Methods:** All adult recipients admitted to the KT institute without donor CMV-seropositive and recipient seronegative (D+/R-) between March 2014 and March 2015 were enrolled. CMV pp65 and IE1-specific ELISPOT assays were performed before KT. The primary outcome was the incidence of CMV infection at 6 months after KT. CMV infection was defined in the presence of CMV antigenemia, CMV syndrome, or tissue-invasive CMV disease. The cut-off values for pp65 and IE1 were selected as the point on ROC curves that maximized the sum of the sensitivity and specificity. **Results:** A total of 199 transplant recipients involving 150 (75%) living-donor KT, 32 (16%) deceased-donor KT, 8 (4%) kidney-pancreas transplants, and 9 (5%) pancreas transplants were enrolled. CMV infections occurred in 73 (37%) patients. Of 199 patients, 65 (33%) had negative pp65-specific ELISPOT results (<10 spots/2.0×10^5 cells) and 134 (67%) had positive results. There was no significant difference in CMV infections between those with negative results and with positive results (43% [28/65] vs. 34% [45/134]) ($P = 0.19$). In addition, there was no difference in CMV infection between those with negative IE1-specific ELISPOT results (< 7 spots/2.0×10^5 cells, 38% [58/152]) and with positive results (32% [15/47]) ($P = 0.44$). The sensitivity, specificity, positive predictive value, and negative predictive value of the pp65-specific ELISPOT for predicting CMV infection were 38% (95% confidence interval [CI], 27%-50%), 71% (95% CI, 62%-78%), 43% (95% CI, 31%-56%), and 66% (95% CI, 58%-74%), respectively. **Conclusions:** Pretransplant CMV-specific ELISPOT results did not predict the development of subsequent CMV infections after transplant in KT recipients with moderate risk.

Author Disclosure Block:

T. Kim: None. **S. Park:** None. **M. Kim:** None. **Y. Jang:** None. **H. Lee:** None. **S. Kim:** None. **J. Jung:** None. **S. Shin:** None. **Y. Kim:** None. **H. Sung:** None. **Y. Chong:** None. **S. Lee:** None. **S. Choi:** None. **Y. Kim:** None. **J. Woo:** None. **D. Han:** None. **S. Kim:** None.

Poster Board Number:

SUNDAY-361

Publishing Title:

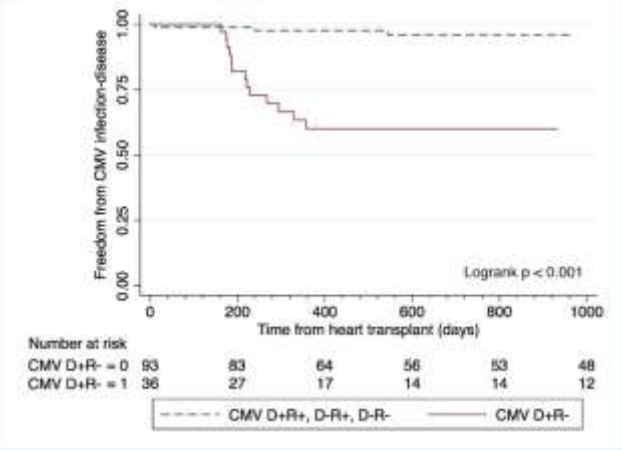
Post-prophylaxis Risk of Cmv Infection in Heart Transplant (Ht)

Author Block:

J. B. Doyon, A. Galar, J. M. Stempel, M. M. Givertz, F. M. Marty, S. Koo; Brigham and Women's Hosp., Boston, MA

Abstract Body:

Background: Although CMV viremia and disease are known complications of HT, the optimal prophylactic strategy is not well defined. **Methods:** We performed a cohort analysis of all HT recipients at Brigham and Women's Hospital from 1/08- 12/14 to identify risk factors for CMV infection, using Cox proportional hazards models.**Results:** The median age of our 129-patient cohort was 51 years (IQR 42, 62); 89 (69%) were male. Most common indications for HT were nonischemic (45, 35%) or ischemic (35, 27%) cardiomyopathy, or congenital heart disease (17, 13%). Median duration of CMV prophylaxis, mostly valganciclovir, was 144 days (IQR 110, 181). CMV viremia developed in 17 patients, 6 of whom also had CMV disease. CMV donor+/recipient- (D+R-) status was the only factor associated with CMV infection (HR 10.0, 95% CI 3.3, 30.9). CMV events predominantly occurred in CMV D+R- patients in the 6-month interval following discontinuation of CMV prophylaxis (Figure). There was no association between CMV infection and episodes of treated acute rejection, modeled as a time-varying covariate, humoral rejection, or the use of additional basiliximab induction in patients with renal dysfunction at the time of HT. CMV infection was associated with an increased risk of subsequent cardiac allograft vasculopathy (HR 8.8, 95% CI 2.6, 29.2), which developed in 4 patients who had CMV infection. **Conclusions:** The risk of CMV infection was higher in CMV D+R- patients with CMV events typically occurring soon after discontinuation of CMV prophylaxis through the first post-HT year. Longer CMV prophylaxis duration and increased surveillance after stopping CMV prophylaxis may be indicated to reduce the rate of CMV infections and the subsequent development of cardiac allograft vasculopathy.



Author Disclosure Block:

J.B. Doyon: None. **A. Galar:** None. **J.M. Stempel:** None. **M.M. Givertz:** None. **F.M. Marty:** None. **S. Koo:** None.

Poster Board Number:

SUNDAY-362

Publishing Title:

Slipping Through the Cracks: Late Onset BK Viremia

Author Block:

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Abstract Body:

Introduction: Most studies indicate that viremia caused by BK virus (BKV), the step preceding BKV associated nephropathy (BKVN) in kidney transplant recipients (KTRs), occurs within one year of transplantation. In a retrospective analysis, we have found that a significant percentage of patients develop viremia after one year. Due to varying screening protocol compliance, KTRs remain at risk for BKV and subsequent allograft injury. Due to a lack of universal screening protocol and systemic screening, KTRs remain profoundly at risk for being undiagnosed with BKV and subsequent allograft injury. **Methods:** At a single center study, we screened for BK viremia in KTRs transplanted between 2003-2013, and found 50 patients with viremia. We divided patients into two groups: patients who had BK viremia diagnosed less than one year after transplant and patients who tested positive after one year. They were deemed "*early*" and "*late*" BK virus patients accordingly. We then compared the "early" and "late" BK cohorts for immunosuppression, ESRD diagnosis, patient characteristics, and graft outcomes.**Results**: 30% of our cohort presented with BK viremia after year one of transplant**.** Other than recipient age and creatinine at baseline, we found few diagnostic clues to differentiate between the onset of BK viremia between the "early" and "late" BKV patient cohorts.**Conclusions:** Screening for BKV after one year of transplant could prevent BKVN. A more formalized and prolonged screening strategy is indicated for BK virus in KTRs.

Table 1: Patient Characteristics	Early n=35	Late n=15	*P*-Value
Days to BK Viremia Diagnosis	142.9 ± 14.8	977.7 ± 141.2	0.0001
Male *n*	24	11	1.00
Recipient Age (yrs)	56.2 ± 1.7	50.0 ± 3.2	0.05
Donor Age (yrs)	47.9 ± 3.0	43.0 ± 3.6	0.15
Donor-Recip. Gender Mismatch *n*	20	4	0.07
Live Organ Donation *n*	17	8	1.00
HLA Mismatches	4.0 ± 0.3	4. 5± 0.4	0.37
CMV Status Donor-Recip Mismatch *n*	7	7	0.09
Delayed Graft Function *n*	8	5	0.49
Diabetes *n*	11	1	0.08

Table 2: Immunosuppression & Kidney Function	Early n=35	Late n=15	*P*-Value
Mycophenolate Mofetil *n*	28	14	0.41
Prednisone *n*	9	7	0.19
Mycophenolate Mofetil *n*	28	14	0.41
Creatinine at Baseline mg/dL	1.1 ± 0.3	1.9 ± 0.2	0.001
Creatinine at Diagnosis mg/dL	1.6 ± 0.1	2.2 ± 0.3	0.7
Rise in Creatinine from Baseline to Diagnosis mg/dL	0.80 ± 0.2	0.3 ± 0.1	0.007
BK nephropathy *n*	9	3	1.0

Author Disclosure Block:

J. Gandhi: None. **D. DeWolfe:** None. **F. Cardarelli:** None. **M. Pavlakis:** None. **C.S. Tan:** None.

Poster Board Number:

SUNDAY-363

Publishing Title:

Immunogenicity of Standard vs. High Dose of Inactivated Influenza Vaccine in Solid Organ Transplant Recipients: A Randomized Controlled Trial

Author Block:

M. Mombelli, N. Rettby, M. Perreau, M. Pascual, G. Pantaleo, O. Manuel; CHUV, Lausanne, Switzerland

Abstract Body:

Background: Although high-dose (HD) influenza vaccine improves protection against influenza in the elderly, the effect among solid organ transplant (SOT) recipients is unknown. We assessed the immunogenicity and safety of HD vs. standard dose (SD) influenza vaccination in SOT recipients.**Methods:** SOT recipients were prospectively randomized to receive 15 µg (SD) or 30 µg (HD) of the 2014/15 influenza vaccine. Immunogenicity was evaluated using a hemagglutination inhibition assay (HIA). Vaccine response was defined as a 4-fold increase of the HIA titer for at least one of the 3 viral strains. Geometric mean titers (GMT) and seroprotection rate (SP) (HIA ≥40) were also analyzed. Follow up period was 6 months.**Results:** 39 patients (30 kidney and 9 liver transplant recipients) received the SD and 40 (33 kidney and 7 liver) the HD influenza vaccination. Baseline characteristics were similar between groups. Rate of systemic adverse events (AEs) to vaccination was similar between groups (23.1% SD and 22.5% HD) and no serious vaccine-related AEs were observed. 28% of patients in the SD group compared to 42.5% in the HD group (p=0.18) responded to vaccination at 2 weeks, with a significant difference observed for the response to influenza B (5.1% vs 22.5%; p=0.026). GMTs at 2 weeks were 97.8 and 138.5 (p=0.26) for H1N1, 147.5 and 198.9 (p=0.17) for H3N2, and 82.1 and 118.0 (p=0.04) for influenza B in SD vs. HD, respectively. Most patients were seroprotected at 6 months (79% for H1N1, 90% for H3N2, and 91% for B) without differences between groups. In univariate analysis, HD vaccine (OR 1.88; p=0.19) was positively associated whereas vaccination during the first 2 years post transplant (OR 0.33; p=0.03) was negatively associated with vaccine response. No biopsy proven rejections after vaccination were observed. Only one patient (in the HD group) developed laboratory-confirmed influenza.**Conclusions:** HD influenza vaccine was safe and well tolerated and may increase antibody responses in SOT recipients. If confirmed in a larger population, HD influenza vaccination may become an appropriate strategy to reduce the burden of influenza after SOT.

Author Disclosure Block:

M. Mombelli: None. **N. Rettby:** None. **M. Perreau:** None. **M. Pascual:** None. **G. Pantaleo:** None. **O. Manuel:** None.

Poster Board Number:

SUNDAY-364

Publishing Title:

Infectious Complications after Transplantation from Donors with Circulatory Determination of Death - A Case-Control Study

Author Block:

V. Fehr, C. Benden, P. Dutkowski, I. Inci, R. Lenherr, T. Mueller, B. Müllhaupt, C. Oberkofler, O. de Rougemont, **N. Mueller**; Univ. Hosp. Zurich, Zurich, Switzerland

Abstract Body:

Background: Since re-introduction of donation after circulatory determination of death (DCD) in 2011, a total of 47 Maastricht III donors were recruited at our institution. To date little is known on the incidence of infectious disease (ID) after DCD transplantation. ID events were compared between patients with organs from DCD donors and DBD donors (Donation after Brain determination of Death). **Methods:** A case-control design matched a case DCD patient with two control DBD patients transplanted with the same organ immediately before and after the case patient. The goal of a 1:4 case-control matching could not be achieved for all patients, due to overlapping patients. ID events were systematically collected during the first year after transplant. Follow-up observation time was one year. Organs allocated to other centers were not considered. **Results:** 32 cases were matched to 89 controls (kidney (N=20), liver (N=8) lung (N=4). Incidence of proven bacterial infections (PI) (HR 2.0, 95% CI 1.3-3.2, p=0.003) was higher in recipients of a DCD organ. This difference was driven by the kidney group (2.4, 1.4-4.2, p=0.003). For lung and liver, the limited case numbers only allowed to observe a trend toward significance. Dominant pathogens were *E. coli* (27.9% of PI) for kidney recipients and *Enterococcus* spp. for liver (36.3% of all PI) as well as lung (15.3% of all PI) transplant recipients. Main sites of infection were the urinary tract in kidney (80.2%), bacteremia and liver infections (each 24%) in liver and respiratory tract infections (48.7%) in lung recipients. No difference was observed for neither viral nor fungal infections. One-year graft function did not differ between cases and controls. **Conclusions:** Causes for the higher incidence of bacterial ID events remain to be determined, but did not affect graft function or mortality at one year. Potential causes could be the higher rate of delayed graft function after DCD kidney transplantation, or the warm ischemia time of the donor predisposing for bacterial colonization of the graft.

Author Disclosure Block:

V. Fehr: None. **C. Benden:** None. **P. Dutkowski:** None. **I. Inci:** None. **R. Lenherr:** None. **T. Mueller:** None. **B. Müllhaupt:** None. **C. Oberkofler:** None. **O. de Rougemont:** None. **N. Mueller:** None.

Poster Board Number:

SUNDAY-365

Publishing Title:

Candiduria in Renal Transplant Recipients: Incidence, Treatment and Outcome

Author Block:

B. Denis, D. Chopin, P. Piron, M. Resche-Rigon, M-N. Peraldi, S. Bretagne, M. Gits-Muselli, I. Abboud, J-M. Molina; AP-HP, Hôpital Saint Louis, Paris, France

Abstract Body:

Background: Few studies have addressed the epidemiology and management of candiduria in renal transplant recipients (RTR); antifungal therapy is often prescribed, although the effect of treatment is uncertain. **Methods:** A single-center retrospective study between 2010-2014 in France was undertaken to assess the incidence, treatment and outcome of candiduria in RTR, with a comparison between treated and untreated patients.Candiduria was defined as a urine culture with ≥ 1000 CFU/mL of *Candida* species and clearance of candiduria as a negative urine culture within a month following candiduria diagnosis. Patients with an undisputable antifungal therapy indication at candiduria diagnosis (ie *Candida* species contamination of preservation fluid, candidemia at candiduria diagnosis and known digestive perforation during surgery) were excluded. Adequacy of treatment was evaluated using the 2009 IDSA guidelines. Candiduria clearance, severe complications and death rates were estimated by Kaplan-Meier methods and the effect of treatment by Cox models.**Results:** During the study period, 52/1223 patients (4.3%) had ≥ 1 episode of candiduria, 42 (81%) were female, 18 (35%) had diabetes, 120 candiduria episodes were recorded, leading to an incidence of 0.023 /person-year. Candiduria was asymptomatic in 51 (98%) patients. *Candida glabrata* was the most frequent pathogen identified (48% of episodes). Antifungal therapy was initiated in 14 episodes (12%), in compliance with guidelines in all cases. Candiduria clearance did not differ significantly between treated and untreated patients (HR, 0.6; 95% CI, 0.3-1.1; p=0.10). Three patients (6%) developed severe complications, in the first 2 weeks after transplantation, and 8 (15%) died. Antifungal treatment had no impact on the likelihood of severe complications or death (HR, 1.1; 95% CI, 0.3-4.8; p = 0.89).**Conclusions:** Candiduria was rare among RTR and usually asymptomatic. Treatment of asymptomatic candiduria did not impact clearance rate nor the rate of severe complications or death. However, candiduria management in the immediate post-transplant period should be extremely cautious and antifungal treatment might be beneficial during this period.

Author Disclosure Block:

B. Denis: None. **D. Chopin:** None. **P. Piron:** None. **M. Resche-Rigon:** None. **M. Peraldi:** None. **S. Bretagne:** L. Speaker's Bureau; Self; Gilead. N. Other; Self; Educational program : Astellas, Congress symposium : Gilead and Bio-Rad, travel grants : Pfizer. **M. Gits-Muselli:**

None. **I. Abboud:** None. **J. Molina:** J. Scientific Advisor (Review Panel or Advisory Committee); Self; Gilead, BMS, ViiV, Janssen. N. Other; Self; Research grants : Gilead.

Poster Board Number:

SUNDAY-366

Publishing Title:

Bacteremias Following Autologous Stem Cell Transplantation for Hematologic Malignancies and Solid Tumors: Risk Factors and Outcomes

Author Block:

A. Kothari, S. P. Susanibaradaniya, M. Mohan, M. E. Saylors, K. T. Lusardi, J. R. Crescencio, M. J. Burgess, J. R. Bariola; UAMS, Little Rock, AR

Abstract Body:

Background: Autologous peripheral blood stem cell transplantation (APBSCT) is a commonly used treatment for multiple myeloma. Febrile neutropenia (FN) following APBSCT is seen in 45-83% of cases. Bacteremia occurs in 7-50% of all patients undergoing APBSCT. The Myeloma Institute at University of Arkansas for Medical Sciences conducts over 400 APBSCT procedures inpatient and outpatient each year. This retrospective cohort study characterizes the risk factors and outcomes associated with bacteremia following APBSCT. **Methods:** Retrospective chart review of patients who underwent APBSCT for hematologic or solid tumor malignancy from May 2014 to March 2015. Patients who had at least 1 positive blood culture within 60 days post-APBSCT were included in the bacteremia cohort. Others formed the non-bacteremia cohort. Cultures determined as contaminants were excluded. **Results:** During the study period, 363 APBSCTs were completed. Bacteremias were observed in 46/363 (13%) transplants. A total of 62 distinct isolates were identified in 54 episodes of bacteremia. 63% were caused by Gram positive organisms. *E. faecium* was the most common Gram positive isolate (14/39, 36 %), and the most frequent isolate overall (14/62, 23%). Vancomycin resistance was present in 93% of *E. faecium* isolates. Incidence of *Pseudomonas* spp. and *S. aureus* was 3% each (n=2), yeasts were seen in 7% (n=4) of cases. Median time between APBSCT and onset of bacteremia was 4.5 days (mean 4.3$\pm$6.6 days, 95% CI 2.3-6.2) and median time to culture clearance was 3 days (mean 3.4$\pm$1.7 days, 95% CI 2.9-3.9). Duration of neutropenia was higher in the bacteremia cohort (median days 10.0 vs 6.0, $p<0.001$). Elevated creatinine prior to transplant was a significant risk factor for bacteremia ($p=0.002$), while use of melphalan in the conditioning regimen was protective ($p<0.0001$). Bacteremia was associated with a significantly decreased survival at 3 (OR 0.15; CI 0.06, 0.38) and 6 months (OR 0.24; CI 0.11, 0.51) post-transplant. **Conclusions:** We found a high incidence of *E. faecium*, and a low incidence of MRSA and *Pseudomonas* spp. bacteremias following APBSCT in our patient population. Bacteremia was associated with decreased survival. Knowledge about local epidemiological data is important to determine empiric antibiotics for FN.

Author Disclosure Block:

A. Kothari: None. **S.P. Susanibaradaniya:** None. **M. Mohan:** None. **M.E. Saylors:** None. **K.T. Lusardi:** K. Shareholder (excluding diversitied mutual funds); Self; USD 5000 stock in Gilead. **J.R. Crescencio:** None. **M.J. Burgess:** None. **J.R. Bariola:** None.

Poster Board Number:

SUNDAY-367

Publishing Title:

An Assessment Of *legionella* pcr Using Bronchoalveolar Samples In Hematopoietic Cell Transplantation

Author Block:

S. Sivagnanam[1], K. Kurosawa[2], C. C. S. Yeung[1], D. SenGupta[2], D. Hoogestraat[2], S. M. Butler-Wu[2], **S. A. Pergam**[1]; [1]Fred Hutchinson Cancer Res. Ctr., Seattle, WA, [2]Univ. of Washington Med. Ctr., Seattle, WA

Abstract Body:

Background: Legionellosis is associated with significant morbidity and mortality in hematopoietic cell transplant (HCT) recipients. These patients are at risk for non-*pneumophila Legionella* infections which are missed by the commonly used urinary antigen test. Furthermore, although *Legionella* culture is the gold standard diagnostic test, it is infrequently performed, time consuming and may delay treatment. We therefore assessed the utility of a real-time PCR for *Legionella* using stored bronchoalveolar lavage (BAL) samples from HCT recipients.**Methods:** We retrieved stored BAL specimens from HCT recipients who had a positive *Legionella* culture and/or Gimenez stain suggestive of *Legionella* on histopathology over a 15 year period (2000-2015) from the Seattle Cancer Care Alliance. A PCR-based assay for *Legionella* was validated using these and other stored BALs. We retrospectively reviewed medical records to determine additional microbiological/clinical diagnoses.**Results:** There were 19 *Legionella*-positive BAL specimens from 15 HCT recipients (culture positive, n=9; "possible *Legionella*" on histopathology, n=10; positive by both, n=1). The median time for finalized culture result was 12 (IQR 10, 13) days. PCR correctly identified all culture positive samples (*L. micdadei*, n=4; *L. pneumophila*, n=3; *L. wadsworthii*, n=1; *L. tusconensis*, n=1). No *Legionella* were detected from BALs known to be *Legionella* culture negative; we noted no cross-reactivity against other common and rare respiratory pathogens. All samples positive by Gimenez stain alone tested negative by PCR. Four of these had other pathogens (*Aspergillus* [n=2], respiratory syncytial virus [1] and influenza A [1] pneumonia); the etiology for the pulmonary findings for the others (n=3) were unknown.**Conclusions:** A real-time PCR for *Legionella* appeared to be as sensitive and specific as culture in detecting multiple *Legionella* species from BALs in HCT recipients. As *Legionella*-related clinical and radiological features are indistinguishable from other major post-transplant infections, the addition of a *Legionella*-specific PCR to standard BAL panels could help to more rapidly identify clinically important non-*pneumophila Legionella* species in these high-risk patients.

Author Disclosure Block:

S. Sivagnanam: None. **K. Kurosawa:** None. **C.C.S. Yeung:** H. Research Contractor; Self; Gilead. **D. SenGupta:** None. **D. Hoogestraat:** None. **S.M. Butler-Wu:** None. **S.A. Pergam:** None.

Poster Board Number:

SUNDAY-368

Publishing Title:

Oral Serum Derived Bovine Immunoglobulin for Treatment of Infectious Diarrhea Due to Norovirus & Cryptosporidiosis in Solid Organ Transplant Patients

Author Block:

M. GELFAND, K. Cleveland, M. Talwar; UTHSC, MEMPHIS, TN

Abstract Body:

Background: Diarrhea (D) is commonly seen in organ transplant patients. Current options to treat many causes of infectious D are limited. Oral immunotherapy with various immunoglobulin (Ig) preparations such as intravenous Ig and hyperimmune bovine colostrum has been previously attempted. Due to exposure of bovine herds to *Cryptosporidium* (C) as well as animal strains of norovirus (N) with possible development of antibodies against these organisms, use of oral serum derived bovine Ig (OSDBI), a medical food, might confer some efficacy in treatment of infectious D caused by N and C. **Methods** Two organ transplant recipients were evaluated for prolonged watery nonbloody D with weight loss. Neither experienced fever, abdominal pain, or nausea. Patient A had undergone a deceased donor renal transplant 6 years earlier and developed weight loss and D due to N which persisted despite symptomatic therapy and reduction of antirejection medications. After 4 months of attempts to manage D, a 6 week course of OSDBI (5 grams orally BID) was begun. Patient B presented with acute D due to C 6 weeks after following a combined kidney/pancreas transplant. D and continued weight loss persisted over the next 4 months despite reduction in immunosuppressive medications, symptomatic treatment, and 3 separate 14-day courses of nitazoxanide (1000 mg orally BID). 14 days of OSDBI (5 grams orally BID) was given. **Results** Patient A experienced resolution of D and weight gain within 7 days of beginning treatment with OSDBI. Following treatment, stool N PCR was negative. Patient B had resolution of D within 3 days of beginning OSDBI treatment and stool testing for (C) with antigen assay, PCR, and modified acid-fast smear was negative. **Conclusions** While no specific data on the antibody activity of OSDBI against C or N have been published, such activity is likely to be present in the preparation derived from large bovine herds. OSDBI, generally safe and modestly priced, may offer a therapeutic option for treatment of infectious D. *In vitro* and controlled clinical studies of OSDBI for specific antimicrobial activity will be essential in supporting further use in gastrointestinal infections.

Author Disclosure Block:

M. Gelfand: L. Speaker's Bureau; Self; Entera Health. **K. Cleveland:** None. **M. Talwar:** None.

Poster Board Number:

SUNDAY-369

Publishing Title:

A Case-Series Of Multi-Drug Resistant (Mdr) Fusariosis At A Large Cancer Center

Author Block:

N. Piccicacco[1], **Y. Pasikhova**[2], A. Baluch[2]; [1]Morton Plant Hosp., Clearwater, FL, [2]Moffitt Cancer Ctr., Tampa, FL

Abstract Body:

Background:*Fusarium* species display *in vitro* resistance to most antifungals with a resultant high mortality rate in immunocompromised patients. Treatment is generally empiric and without susceptibility data. Since optimal therapy and duration is currently unknown, we report 4 patients with failure to empiric fusariosis therapy to observe clinical outcomes and treatment regimens.**Methods:**Retrospective case-series of fusariosis, with antifungal susceptibility data, at an NCI designated cancer center.**Results:**We identified 4 patients with culture proven MDR *Fusarium* infections. Patients were predominately female with a median age of 44 years and BMI of 20.9 kg/m^2. 50% were HSCT recipients and the other 50% AML patients. Only 1 patient had severe neutropenia at the time of diagnosis. All received anti-mold prophylaxis prior to diagnosis. Liposomal amphotericin B (LAmB) was the most utilized antifungal, with a median dose of 6.5 mg/kg/day. Combination therapy (LAmB + voriconazole) was used in 1 patient. All patients had susceptibility testing sent secondary to clinical failure. Treatment duration was not well defined, but ranged from 8 to 18 weeks. Cases are outlined in *Table 1*.**Conclusion:**Routine mold prophylaxis in high-risk patients needs to be continuously monitored as MDR mold infections are emerging and routine susceptibility data is often not obtained.***Table 1.*** **Fusariosis Cases**

	Case Number 1	**Case Number 2**	**Case Number 3**	**Case Number 4**
Diagnosis	Disseminated cutaneous infection	Fungemia	Abdominal abscesses	Osteomyelitis
Antifungal MICs (units)	Ampho B ≥ 8 Itra ≥ 16 Vori = 4 Posa = 2	Ampho B = 2 Itra ≥ 16 Vori ≥ 16 Posa ≥ 16	Ampho B = 2 Itra ≥ 16 Vori = 8 Posa ≥ 16	Ampho B = 2 Itra ≥ 16 Vori ≥ 16 Posa ≥ 16
Treatment	LAmB 5mg/kg/day (increased to 10mg/kg/day) +	LAmB 10mg/kg/day; changed to Amphotericin B Deoxycholate 1.5mg/kg/day	LAmB 3mg/kg/day; changed to Voriconazole 200mg BID	Itraconazole 200mg BID; changed to Voriconazole 200mg BID;

	Voriconazole 200mg BID			changed to LAmB 3mg/kg/day
Duration of Treatment	8 weeks	At least 8 weeks + secondary prophylaxis	At least 12 weeks of PO voriconazole	18 weeks of total therapy
All-cause Mortality (days from diagnosis)	53	17	--	--

Author Disclosure Block:

N. Piccicacco: None. **Y. Pasikhova:** None. **A. Baluch:** None.

Poster Board Number:

SUNDAY-370

Publishing Title:

Comparison of Two Matrix-assisted Laser Desorption/Ionization Time-of-flight Mass Spectrometry (Maldi-Tof Ms) Systems Using a Formic Acid Extraction Method for the Identification of Common and Uncommon Yeast Isolates

Author Block:

H-S. Lee, J. Shin, M-J. Choi, E. Won, S-H. Kim, S-P. Suh, D-W. Ryang; Chonnam Natl. Univ. Med. Sch., Gwangju, Korea, Republic of

Abstract Body:

Background: The introduction of MALDI-TOF MS provides rapid and accurate identification (ID) of clinical isolates of fungal species. While direct on-plate formic acid extraction (FA) is simpler, Bruker Biotyper MS recommends an in-tube formic acid plus acetonitrile (FA/ACN) extraction for the ID of yeasts. We evaluated the Biotyper using the FA method for the ID of a wide spectrum of clinically relevant yeast species, in comparison with VITEK MS.**Methods:** A total of 298 clinical yeast isolates, including 158 isolates of four common *Candida* species (*C. albicans, C. tropicalis, C. glabrata,* and *C. parapsilosis*) and 140 isolates of 38 uncommon yeast species, were analyzed. In both VITEK MS and Biotyper (cutoff value of 1.7) systems, a simple FA smear was used for protein extraction, but additional FA/ACN methods were used for Biotyper. The ID results were compared to those of sequence-based ID.**Results:** For all 298 yeast isolates, 88.6% and 89.3% were correctly identified by VITEK MS and Biotyper, respectively. Both systems correctly identified 100% of 158 isolates of four common *Candida* species. However, the rates of correct ID for the 140 uncommon isolates were 75.7% and 77.1% by VITEK MS and Biotyper, respectively. For uncommon yeast isolates, Biotyper showed a higher rate of mis-ID (12.1% vs. 2.9%, $P = 0.005$) but a lower rate of no ID (0.7% vs. 11.5%, $P < 0.0001$) than VITEK MS. Especially, Biotyper often yielded incorrect ID of *Cryptococcus neoformans* [58.3% vs. 0% (Biotyper vs. VITEK), $P = 0.005$], but VITEK yielded incomplete ID of *Candida orthopsilosis* [0% vs. 100% (Biotyper vs. VITEK), $P < 0.0001$]. Using Biotyper for the ID of uncommon yeast species, either FA or FA/ACN extraction presented comparable rates of correct ID [77.1% vs. 82.9% (FA vs. FA/ACN)], incomplete ID (10.0% vs. 5.0%), mis-ID (12.1% vs. 10.7%), and no ID (0.7% vs. 1.4%).**Conclusions:** Simple FA extraction by Biotyper shows a comparable ID performance with VITEK MS for most clinical yeast isolates, except for *C. neoformans*, and both systems more accurately identify four common *Candida* species (100%) than uncommon yeast species (76-77%).

Author Disclosure Block:

H. Lee: None. **J. Shin:** None. **M. Choi:** None. **E. Won:** None. **S. Kim:** None. **S. Suh:** None. **D. Ryang:** None.

Poster Board Number:

SUNDAY-371

Publishing Title:

Evaluating Maldi-Tof in Derm-Fungal Infections in Clinical Microbiology

Author Block:

J. G. Thomas[1], M. Zaccagnini[2], **J. Kuzyck**[3], K. Kasarda[1]; [1]Allegheny Halth Network, Pittsburgh, PA, [2]Gannon Univ., Erie, PA, [3]Allegheny Hlth.Network, Pittsburgh, PA

Abstract Body:

Background: The integration of new methods including PCR and mass spectrometry (MS) has changed strategies/work flow in clinical microbiology, dramatically. Here, we focus on potential benefits of Matrix Assisted Laser Desorption Ionization -Time of Flight (MALDI-TOF) specifically in derm/fungal detection to facilitate rapid reporting/cost reduction, and a potential referee in selection of an MS instrument. **Methods:** The Allegheny Health Network (AHN) Epidemiology Report was used to record dermatologic fungal isolates from skin, hair and nails for 6 years (January 2009 - April 2015), provided by the 6 hospital consortium to the Core Microbiology Laboratory at AGH, representing a total of 2000 acute care beds, 77outpatient care clinics and 4 private dermatologic Groups. Fungal isolates were identified by standard culture techniques including 3 non and selective/differential media with DTM and Tap Water Media. Lab Core was used as external resource • Fu • Two fungal libraries [FDA/cleared and RUO (research use only)] were provided by each of two MS ID Systems (BD/Bruker and BMX/Vitek MS) to compare/contrast detection strengths/weaknesses with our library **Results:** Over the 6-year study, culture-positive patients yielded 1487 results, 287 patients with multiple isolates, highlighting 67 identifiable fungal/yeast isolates. • Top five species were *Trichophyton* (599), *Candida* (89*), Alternaria* (87), *Fusarium* (72) and *Penicillium* (68). • MALDI-TOF/BMX FDA cleared library matched with 14/67 species, MALDI-TOF/BD cleared with 10/67. Of top 5 species, neither cleared library matched 4, only correlating with Candida species. Of the Candida species, MALDI-TOF/BMX matched 9/11, BD 8/11. **Conclusions:** Our culture data revealed a rich and diverse derm/fungal population, some with multiple species from multiple sites . MS ID Systems with cleared FDA libraries matched culture positive isolates infrequently, primarily yeast, offering a potential differential in MS selection.

Author Disclosure Block:

J.G. Thomas: None. **M. Zaccagnini:** None. **J. Kuzyck:** None. **K. Kasarda:** None.

Poster Board Number:

SUNDAY-372

Publishing Title:

Evaluation of Rapid Microbial Identification and Susceptibility Testing from Positive Blood Cultures Using Maldi-Tof Ms After Minimal Incubation on Solid Media

Author Block:

S. L. Mitchell, K. Alby; The Univ. of Pennsylvania, The Perelman Sch. of Med., Philadelphia, PA

Abstract Body:

Bloodstream infections (BSI) are a leading cause of patient morbidity and mortality. Rapid identification of organisms from BSI is critical for initiating targeted antimicrobial therapy. Additionally, rapid identification can have positive impacts in other patient outcomes, such as decreased length of stay, reduced antibiotic usage and lower costs. Currently, the turn-around-time (TAT) from a positive blood culture to identification is approximately two days, with antimicrobial susceptibility testing (AMT) requiring an additional day. Although many methods exist for the rapid identification of organisms from positive blood cultures, they do not provide detailed or definitive susceptibility information. We therefore assessed the utility of MALDI-TOF to identify organisms from a positive blood culture bottle after only four hours of growth and whether this limited growth could yield accurate AMT results compared to overnight cultures. For identification, of 119 isolates tested, 100 (84%) were in agreement to the species level with the standard blood culture workup. Sixteen (13.4%) did not generate an identification and two (1.7%) resulted in misidentifications. Specifically, 49/57 (85.9%) Gram-negatives and 51/57 (89.5%) Gram-positives were properly identified. 1/2 (50%) anaerobic organisms and 0/4 (0%) yeast were correctly identified to the species level. Twenty-four isolates (12 Gram-positive and 12 Gram- negative) were blindly selected for AMT using Vitek 2. Twenty-one (87.5%) gave the same susceptibility profile compared to those grown overnight. Specifically, 11/12 (91.6%) Gram-negatives and 10/12 (83.3%) Gram-positives gave comparable AMT profiles. Three organism profiles were inconsistent (12.5%). Of those, one Gram-negative and one Gram-positive resulted in minor errors (8.3%) and one Gram-positive resulted in a very major error (4.2%). In conclusion, MALDI-TOF was able to accurately identify the majority of organisms after only four hours of growth resulting in identification TAT of approximately five hours and an AMT TAT of approximately one day. This study identifies a method using common commercial instruments that can provide a way to improve upon identification and detailed susceptibility information.

Author Disclosure Block:

S.L. Mitchell: None. **K. Alby:** None.

Poster Board Number:

SUNDAY-373

Publishing Title:

Evaluation of Rgm Medium for Detection and Identification of Nontuberculous Mycobacteria from Patients with Cystic Fibrosis

Author Block:

R. Plongla[1], C. L. Preece[2], J. D. Perry[2], P. H. Gilligan[1]; [1]UNC Sch. of Med., Chapel Hill, NC, [2]Northumbria Univ., Newcastle upon Tyne, United Kingdom

Abstract Body:

Overgrowth of cultures with bacteria or fungi is a challenge for isolation of nontuberculous mycobacteria (NTM) from patients with cystic fribrosis (CF). RGM medium (RGM) is a novel selective agar containing OADC supplement and 4-antimicrobial mixture, and is designed to improve detection of NTM. We evaluated the identification of NTM on this medium using MALDI-TOF MS (bioMérieux) and recovery of NTM on RGM from CF respiratory specimens.41 archived isolates (11 *M. abscessus* subsp. *abscessus*: MABS; 11 *M. abscessus* subs. *massiliense*: MABM; 5 *M. chelonae*: MCHEL; 6 *M. fortuitum* complex: MFORTG; 3 *M. immunogenum*: MIMM and 5 *M. mucogenicum*: MMUC) were grown on RGM and *Burkholderia cepacia* selective agar (BCSA). After 72-96h incubation at 30^{O}C, archived isolates were identified by MALDI-TOF MS using bM Vitek MS research use-only system (RUO) with SARAMIS v4.12 database. 116 consecutive respiratory samples (66 expectorated sputa, 44 deep pharyngeal swabs, 6 bronchoaveolar lavage fluids) from 115 CF patients were inoculated directly on RGM and BCSA and were observed after 4, 7, 10 and 14 days of incubation at 30^{O}C in air. MALDI-TOF MS or sequencing of 16s rRNA gene was used to identify the organisms.All 41 archived isolates grew on RGM but 1 MABS, 4 MFORTG and 4 MMUC did not grow on BCSA. All NTM on both media could be correctly identified by MALDI-TOF MS with $\geq$75.0% confidence value, except 1 MIMM on BCSA. Of 116 samples, 19 and 7 mycobacteria were isolated on 17 (14.7%) and 7 (6.0%) of RGM and BCSA, whereas other bacteria and fungi grew on 10 (8.6%) RGM and 38 (32.8%) BCSA (Table). 34 samples had concomitant AFB cultures (AFBC). 5 (14.7%) and 3 (8.8%) samples had 6 and 3 mycobacteria on RGM and AFBC. 10 (29.4%) LJ media were overgrown and discarded.RGM has a higher recovery rate of NTM and is more selective than BCSA. MALDI-TOF MS can be used to identify mycobacteria on RGM. There is a potential to obviate the AFBC, however studies to compare RGM with AFBC and the recovery of *M. avium-intracellurare* complex on RGM are needed.

Table: Isolates recovered on RGM, BCSA and AFBC from respiratory specimens of patients with CF

Organisms	RGM (n=116)	BCSA (n=116)	RGM (n=34)	AFBC(n=34)

M. abscessus complex	9	6	3	3
M. chelonae	9	0	2	0
M. immunogenum	0	1	0	0
M. avium-intracellulare complex	1	0	1	0
Glucose-nonfermenters	9	20	2	10 contaminated LJ
Yeasts	1	7	1	
Molds	0	11	0	

Author Disclosure Block:

R. Plongla: N. Other; Self; Rongpong Plongla received travel award from bioMerieux for presenting an abstract at ASM General Meeting 2015, New Orleans. **C.L. Preece:** None. **J.D. Perry:** I. Research Relationship; Self; John D. Perry has received funding for Research or Consultancy from diagnostics companies including bioMérieux, Becton Dickinson and Lab M (bioMérieux have a potential commercial interest in RGM media. **P.H. Gilligan:** None.

Poster Board Number:

SUNDAY-374

Publishing Title:

Identification of *Burkholderia* and Uncommon Glucose Nonfermenters Isolated from Cystic Fibrosis Patients Using Maldi-Tof Ms

Author Block:

R. Plongla[1], T. Panagea[2], M. C. Jones[3], P. H. Gilligan[1]; [1]UNC Sch. of Med., Chapel Hill, NC, [2]Sismanogleio-A. Fleming Gen. Hosp., Athens, Greece, [3]UNC Hlth.Care, Chapel Hill, NC

Abstract Body:

With MALDI-TOF MS, Gram-negative glucose nonfermeters (GNGNF) can be identified quickly however the database is incomplete. We evaluated the performance of an updated database of bioMérieux (bM) Vitek MS *in vitro* diagnostic (IVD) system for the identification of GNGNF other than *Pseudomonas, Achromobacter, Chryseobacterium, B. gladioli* and *B. multivorans*.MALDI-TOF MS (bM, Durham, NC) was performed on 155 archived CF isolates after 24h, 48h and 72h incubation on 5% sheep blood agar (SBA), MacConkey agar (MAC) and *Burkholderia cepacia* selective agar (BCSA). Spectra were analyzed using Vitek MS research use-only system (RUO) with SARAMIS v4.12 database, bM IVD system with the current v2.0, the new v3.0 and draft v4.0 Knowledge Base (KB) database. The results were compared with identification by the *Burkholderia cepacia* Research Laboratory and Repository at the University of Michigan or 16s rRNA gene sequencing.The satisfactory results were achieved at 48.6%, 33.1%, 81.1% and 88.0% on SBA using RUO, v2.0, v3.0 and v4.0. There were the same trends on MAC (23.6% v 26.6% v 51.5% v 63.4%) and BCSA (41.1% v 28.7% v 74.7% v 84.0%). Of 1298 spectra analyzed, the incorrect identifications were 0.5%, 42.3%, 2.2% and 1.4% using RUO, v2.0, v3.0 and v4.0, which occurred on the uncommon members of *B. cepacia* complex. On v4.0, *B. cepacia*, *B. cenocepacia*, *B. vietnamiensis* and *B. contaminans* had 63.6%, 95.2%, 95.8% and 94.4% satisfactory results on SBA (Table). Identifications of *Pandoraea*, *Cupriavidus* and *Bordetella* on v4.0 were similar to v3.0 (88.9%, 66.7%, 57.1%). *Herbaspirillum* was added to the v4.0 database and had satisfactory results of 88.9%, 100% and 100% on SBA, MAC and BCSA.There is a continuing improvement to identify the uncommon GNGNF isolates especially on *B. cenocepacia* and *B. contaminans*. SBA and BCSA are preferred over MAC. The incorrect identification rate was fewer on v4.0. Improving identification of *B. cepacia*, *Cupriavidus* and *Bordetella* is still challenging.

Table: Performance of RUO SARAMIS, Vitek MS IVD v2.0, v3.0, v4.0 KB databases on selected organisms

	Satisfactory results (%)			
Organism (n)	SARAMIS	v2.0	v3.0	V4.0
B.cepacia (11)	0.0	87.9	27.3	63.6

*B.cenocepacia** (42)	44.4	0.0	87.3	95.2
*B.contaminans** (12)	0.0	0.0	91.7	94.4
*Pandoraea** (24)	68.5	0.0	88.9	88.9
Ralstonia (14)	76.2	85.7	83.3	85.7
*Herbaspirillum** (3)	55.6	0	0	88.9
Cupriavidus (6)	61.1	33.3	66.7	66.7
*Not on the current v2.0 KB database				

Author Disclosure Block:

R. Plongla: N. Other; Self; Rongpong Plongla received travel award from bioMerieux for presenting an abstract at ASM General Meeting 2015, New Orleans. **T. Panagea:** None. **M.C. Jones:** None. **P.H. Gilligan:** None.

Poster Board Number:

SUNDAY-375

Publishing Title:

Maldi-Tof Ms with Protein Extraction Using Naoh-Hcl Neutralization is Simple, and Cost-Effective Urine Preparation for Direct Identification of Uropathogens

Author Block:

J. Kim; Coll. of Med., Intl. St. Mary`s Hosp., Catholic Kwandong Univ., Incheon, Korea, Republic of

Abstract Body:

Background: The direct identification of bacteria in urine samples by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) can dramatically increase the usefulness of this method, significantly reducing the time for microorganism identification. The aim of this study was to develop a rapid, simple, cost-effective and reliable protocol using very common reagents, NaOH-HCl to identify bacteria directly from urine samples on MALDI-TOF MS Bruker Biotyper (Bruker Daltonics, Leipzig, Germany) and to establish a protocol combining the conventional screening tests.**Methods:** In May and July 2015, 122 urine samples showing positivity on Gram stain were selected. After first centrifugation at 2,000 x g, the pellet was assigned for gram positive cocci (GPC) positive urine samples and the supernatant for gram negative bacteria (GNB) positive urine samples. The 0.01M NaOH-0.01M HCl neutralization method was used for the bacteria protein extraction. We evaluated the analytical performance and compared the conventional method (MicroScan, Beckman-Coulter, USA) and MALDI-TOF MS with Gram stain and screening device, Sysmex UF 1000i (UF 1000i ; bioMe´rieux, Marcy l'E´toile, France) for identification of the urinary tract pathogens. **Results:** An 84.3% (70/83) concordant rate was obtained in urine samples showing single morphotype on gram stain with bacterial counts of > 1 X 10^5 CFU/ml. Among them, *Escherichia coli* showed 90.9% (55/61) concordant rate. The MALDI-TOF MS combined with single morphotype on gram stain and UF 1000i counts ≥1 x 10^6 bacteria/mL identified this microorganism in 73.6% (67/91). In this study, the detection limit of this method was 10^7 CFU/ml both to sterile DW and sterile urine specimens.**Conclusions:** MALDI-TOF MS with protein extraction using NaOH-HCl neutralization allows a rapid, simple, cost-effective and accurate bacterial identification directly from infected urine samples especially when major uropathogens such as *E. coli* are involved. MALDI-TOF MS combined gram positive results and UF 1000i count ≥ 10^6 bacteria/mL may improve the bacterial identification efficiency in routine microbiology laboratory.

Author Disclosure Block:

J. Kim: None.

Poster Board Number:

SUNDAY-376

Publishing Title:

More Species, Less Time to Result - How Maldi-Tof Ms Changes Medical Microbiology

Author Block:

A. B. Pranada[1], M. Kostrzewa[2], M. Bienia[1], F. Pranada[1]; [1]MVZ Dr. Eberhard & Partner Dortmund (ÜBAG), Dortmund, Germany, [2]Bruker Daltonik GmbH, Bremen, Germany

Abstract Body:

Background: Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) has been introduced for microorganism identification in many clinical diagnostics laboratories. High discriminatory power and speed of this technology allow more accurate and rapid results.We evaluated the impact of MALDI-TOF MS on our own clinical microbiology practice in a major independent laboratory in Germany by reviewing data obtained over a period of six years. A huge dataset for identification by MALDI-TOF MS of bacteria, filamentous fungi and mycobacteria as well as direct identification from positive blood cultures and detection of resistance markers is presented.**Materials and Methods:** A MALDI Biotyper system (Bruker Daltonik GmbH, Germany) was established in our laboratory in December 2009 as supplement to the existing conventional methods. Identification and specimen data from the LIS and the MALDI statistical system before (2009) and after (2010-2015) introduction of the new method were analyzed for changes in rapidity and reporting. **Results:** For the whole time period n=938,123 data records for reported identification results were analyzed (n=123,900 in 2009 and n=814,223 for 2010-2015). MALDI-TOF MS was performed for n=152,917 analytes. Average log(score) was 2,134 with 92.8 % successful identifications (76.7 % at high confidence level, 16.1 % at low confidence level). Log(score) values improved over time with 64.0 % of identifications at high confidence level in 2010 and 80.0% in 2015.Regarding the discriminatory power, during 2009 a total of 285 different species from 112 genera were finally reported to the clinicians. This continuously increased to 581 different species (+104 %) from 174 genera (+55 %) in 2015.For specimens with anaerobe bacteria the median time to final report decreased by 1 day after introduction of MALDI-TOF MS. **Conclusion and Outlook:** Introduction of MALDI-TOF MS into our medical microbiology laboratory has decreased the time to the final report and significantly increased the variety of microorganisms identified. In the future this may lead to a better understanding in infectious diseases and causing agents. A comprehensive dataset for MALDI-TOF MS in routine could be presented.

Author Disclosure Block:

A.B. Pranada: None. **M. Kostrzewa:** D. Employee; Self; Bruker Daltonik GmbH. **M. Bienia:** None. **F. Pranada:** None.

Poster Board Number:

SUNDAY-377

Publishing Title:

Optimal Turnaround Time for Direct Identification of Microorganisms by Mass Spectrometry in Blood Culture

Author Block:

A. Randazzo, J-S. Goffinet; Cliniques Sud Luxembourg, Arlon, Belgium

Abstract Body:

Background: During the past few years, several studies describing direct identification of bacteria from blood culture using mass spectrometry have been published. These methods cannot, however, be easily integrated into a laboratory workflow because of the high hands-on time they require. In this paper, we propose a new method of identification with a short hands-on time and a turnaround time smaller than 15 min once the bottle became positive and the moment we have identified the pathogen. **Materials and methods:** Blood culture bottles from adult patients were collected at the Laboratory of Clinical Biology of the Cliniques du Sud Luxembourg in Arlon (Belgium) and incubated on a BacT/Alert 3D system (Biomérieux®, Marcy-l'Etoile, France). Positive blood bottles were homogenized and 600 µL of blood were transferred to an Eppendorf tube where 600 µL of lysis buffer were added. The mix was homogenized during 5 s by vortexing. A centrifugation step of 4 min at *10,500 g* was performed and the supernatant was discarded. The pellet was then washed with the wash buffer from Vitek® MS Blood Culture Kit and a sample of the pellet was loaded in quadruplicate into wells of a Vitek® MS-DS plate. Each well was covered with a saturated matrix solution of Vitek® MS-CHCA and a matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry analysis was performed. Species were identified using the software Myla 3.2.0-2 (Biomérieux®, Marcy-l'Etoile, France). **Results:** We analyzed 266 positive blood culture bottles. A total of 279 microorganisms grew in 261 cultures, while the remaining five bottles stayed sterile after 48 h of incubation in subculture. Our method reaches a probability of detection of 77,8 % (203/261) with a positive predictive value of 99,51% (202/203). We obtained 95,4 % of correct identification for the Gram-negative bacilli (145/152) and 50,9% for the Gram-positive cocci (59/116). The 5 negative cultures showed also a negative result for the rapid identification and thus were considered as false positive. **Conclusion:** We developed a new method for the identification of microorganisms using mass spectrometry, directly performed from a positive blood culture. This one has short hands-on and turnaround times and can easily take place in the workflow of a laboratory, with comparable results in performance to other known methods.

Author Disclosure Block:

A. Randazzo: None. **J. Goffinet:** None.

Poster Board Number:

SUNDAY-378

Publishing Title:

Identification of *Streptococcus pneumoniae* by Maldi-Tof Ms: Comparison of Molecular Weight Typing and the New Matching System in Bruker Database 4613 and 5627

Author Block:

E. Thidholm[1], S. Lehmann[2], O. Altun[1], **V. Ozenci**[1]; [1]Karolinska, Stockholm, Sweden, [2]Bruker, Stockholm, Sweden

Abstract Body:

Background: Identification of *S. pneumonia* with Bruker microflex mass spectrometry Bruker database 4613 have been showed to be partially successful. Analysis of the protein masses in the range 2-20 kDa showed that the alpha-hemolytic streptococci have very similar mass spectra. The aim for this study was to develop and validate a method based on specific peaks present in *S.pneumoniae* or in other alpha-hemolytic streptococci's spectra and to compare this approach with Bruker database 4613 and newly developed 5627 database. **Material/methods:** Strains of *S. pneumonia* and other alpha-hemolytic streptococci isolated from positive blood culture bottles were diagnosed by optochin susceptibility. Mass spectra from ten *S. pneumoniae* and ten other alpha-hemolytic streptococci was overlaid and analyzed to distinguish peaks, specific for *S. pneumonia* or the other alpha-hemolytic streptococci using Bruker database 4613 . The developed spectra, containing two peaks specific for the other alpha-hemolytic streptococci were defined. The two peaks were then matched to clinical isolates of 47 *S. pneumoniae* and 32 other alpha-hemolytic streptococci. **Results:** Discrimination by matching against two peaks, specific for the other alpha-hemolytic streptococci resulted in 31/32 (97%) and 45/47 (96%) correct identification result for other alpha-hemolytic streptococci and *S. pneumoniae* respectively using Bruker database 4613 . One of the two *S. pneumoniae* strains with discrepant result had both peaks while the other one had only one of the two peaks. The other alpha-hemolytic streptococcus with discrepant result showed no match on the two specific peaks. Identification of the isolates by Bruker database 5627 was successful in 30/32 (94%) and 47/47 (100%) of other alpha-hemolytic streptococci and S. pneumoniae respectively considering the four highest score values. **Conclusion:** The result showed that analysis of differences in peaks might lead to improved identification of S. pneumoniae with Bruker database 4613. In addition newly developed Bruker database 5627 is reliable in identification of S. pneumoniae.

Author Disclosure Block:

E. Thidholm: None. **S. Lehmann:** None. **O. Altun:** None. **V. Ozenci:** None.

Poster Board Number:

SUNDAY-379

Publishing Title:

Identification of Microorganisms Using Short-term Culture Followed by Maldi-Tof Ms

Author Block:

M. Almuhayawi, P. Lüthje, **V. Ozenci**; Karolinska, Stockholm, Sweden

Abstract Body:

Background: Polymicrobial bloodstream infections are related to high morbidity and mortality. Identification of microorganisms from polymicrobial infections by conventional microbiological methods is time-consuming and delays effective treatment of the patients. In this study, we evaluated the possibility to identify species from polymicrobial infections by MALDI-TOF MS after short-term incubation under selective conditions. **Methods:** Clinical polymicrobial blood culture (BC) bottles were analyzed after culture positivity; simulated polymicrobial samples were prepared by mixing two positive clinical monomicrobial BC bottles. Subcultures were inoculated on appropriate agar plates with antibiotic discs, i.e. linezolid and aztreonam for selection of Gram-negative and Gram-positive species, respectively. After an incubation of 6 hours, MALDI-TOF MS analysis was performed from growth around the selective discs. Results were compared with results from standard methods. **Results:** A total of 73 polymicrobial cultures were analyzed, including clinical microbiological samples (n=19) and simulated samples (n=54). In 47/73 cultures (64%), two of two bacterial species could be identified by the present method within 6 hours after BC positivity. In another 23/73 cultures (32%), at least one species was correctly identified. Only in three samples, no identification was obtained by this method. The most commonly detected pathogens, *E. coli* and *Staphylococcus spp.* could be identified in 35/38 (92%) and 42/46 (91%) of positive samples. In general, Gram-negative species were identified more often (62/65, 95%) than Gram-positive species (58/77, 75%; P<0.001). *Candida* spp. could not be identified by MALDI-TOF MS after short-term incubation. When BC medium was investigated directly, two of two bacterial species could be identified in 2/73 (3%) and one species in 41/73 (56%), mostly a Gram-positive species. **Conclusion:** In this study, we demonstrate that that short-term culture followed by MADLI-TOF MS can help to identify bacterial pathogens from polymicrobial species without the need of overnight incubation.

Author Disclosure Block:

M. Almuhayawi: None. **P. Lüthje:** None. **V. Ozenci:** None.

Poster Board Number:

SUNDAY-380

Publishing Title:

Evaluation of the 2415 *m/z* Peak in the MALDI-TOF Mass Spectra of *Sphylococcus aureus* to Infer Methicillin Resistance

Author Block:

D. D. Rhoads, H. Wang, S. M. Harrington, G. W. Procop, S. S. Richter; Cleveland Clinic, Cleveland, OH

Abstract Body:

Background: Methicillin resistant *S. aureus* (MRSA) isolates are *S. aureus* that express the *mecA* gene. Typically, *mecA* is a component of an SCC*mec* mobile genetic element. Some SCC*mec* types also contain the *psm-mec* gene, and MALDI-TOF mass spectrometry (MS) can detect the PSM-mec peptide at 2415 *m/z*. This MS peak has been used to infer *mecA* carriage and methicillin resistance in *S. aureus* (doi: 10.1016/j.ijmm.2014.07.005). This previous study also demonstrated that strains lacking a delta-toxin peak at 3007 and 3037 *m/z* (which implies *agr* dysfunction) never had a detectable 2415 *m/z* peak. In the current investigation, we evaluate the validity of using MS to infer *mecA* carriage in *S. aureus* clinical isolates. **Methods:** 283 consecutive blood cultures from unique patients, which grew only *S. aureus* were considered. These primary specimens were cultured March through November of 2015. Results obtained during routine clinical testing using the Verigene Gram-Positive Blood Culture Test (Nanosphere; Northbrook, IL) and VitekMS (bioMerieux; Durham, NC) were reviewed retrospectively to determine the presence or absence of three variables for each isolate: the *mecA* gene, a PSM-mec peak (2415 ± 4 *m/z*), and a delta-toxin peak (3007 ± 4 or 3037 ± 4 *m/z*). **Results:** The results are summarized in the Table. A delta-toxin peak was not prominent in 9% (25/283) of the isolates, including 2 isolates that had both *mecA* and a 2415 *m/z* peak.Table. Correlation of 2415 *m/z* and *mecA* in *S. aureus* isolates

S. aureus isolates	2415 *m/z* (no. isolates)	
	Detected	Not detected
mecA positive	53	84
mecA negative	4	142

Conclusion: In this study, a 2415 *m/z* peak detected by routine MS had low sensitivity (39%; 53/137) but high specificity (97%; 142/146) for predicting *mecA* carriage in *S. aureus* clinical isolates. Excluding isolates that lack a delta-toxin peak did not significantly improve the predictive value of the test, so it may be unnecessary or suboptimal to screen for delta-toxin before evaluating the presence or absence of the 2415 *m/z* peak. The positive predictive value

(93%; 53/57) of the 2415 *m/z* peak is high enough to consider reporting *S. aureus* isolates as MRSA using MS data alone.

Author Disclosure Block:

D.D. Rhoads: None. **H. Wang:** None. **S.M. Harrington:** None. **G.W. Procop:** None. **S.S. Richter:** I. Research Relationship; Self; bioMerieux, BD Diagnostics, OpGen, Nanosphere, Pocared.

Poster Board Number:

SUNDAY-381

Publishing Title:

Matrix-assisted Laser Desorption Ionization-time of Flight Mass Spectrometry-based Identification of *Staphylococcus aureus* and Methicillin Resistance Through Detection of a Single Peak

Author Block:

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Abstract Body:

Background: Laboratory diagnosis of Methicillin-Resistant *Staphylococcus aureus* (MRSA) by culture is a two-step process which includes identification of a colony followed by phenotypic or genotypic susceptibility testing. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) is increasingly used to rapidly identify *S. aureus* colonies. In this study, we evaluated the accuracy of MALDI-TOF for simultaneous detection of methicillin resistance through detection of a single SCC*mec*-associated peak.**Methods:** 235 clinical isolates of *S. aureus*, including 80 MSSA and 155 MRSA, identified by the Bruker Biotyper (Bruker Daltonics) and tested for methicillin sensitivity with mecA PCR and broth microdilution (MicroScan Walkaway, Beckman Coulter), were evaluated. The MALDI-TOF spectra was reanalyzed with the MALDI Biotyper MRSA Prototype software (Bruker Daltonics) for the presence of a peak with an identity of 2413 m/z . Multilocus sequence typing (MLST) and staphylococcal cassette chromosome mec (SCC*mec*) typing were performed by sequencing and PCR, respectively.**Results:** None of 80 MSSA isolates had the 2413 m/z peak. The 2413 m/z peak was present in 40 out of 155 MRSA isolates. The assay had a sensitivity and specificity of 25.8% and 100%, respectively. Strain typing of 50 MRSA isolates did not show a clear association between MLST type and presence of the 2413 m/z peak (ST1, 0/1; ST5, 14/19; ST8, 0/23; ST30 0/1; ST36, 1/1; ST87 0/1; ST80, 0/1; ST105, 1/1; ST254, 0/1; ST2247, 0/1;). However, MLST types that are frequently associated with SCC*mec* type II more commonly had the 2413 m/z peak (ST5, 14/19; ST36, 1/1; ST105 1/1). SCC*mec* typing is being conducted to confirm a relationship between SCC*mec* type II and MALDI detection of the 2413 m/z peak.**Conclusions:** MALDI-TOF MS can simultaneously identify *S. aureus* and methicillin resistance with a high positive predictive value. Although the sensitivity of 2413 m/z peak is low at our institution, it may be high in locales where SCC*mec* type II is prevalent.

Author Disclosure Block:

I. Budvytiene: None. **H.W. Moon:** None. **G. Shi:** None. **N. Banaei:** None.

Poster Board Number:

SUNDAY-382

Publishing Title:

Rapid Identification of Oxa-48 Producing *Klebsiella pneumoniae* Using Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (Maldi-Tof Ms)

Author Block:

B. Rodriguez-Sanchez, C. Sanchez-Carrillo, E. Cercenado, M. Marin, A. Ruiz, E. Bouza; Inst. de Investigación Sanitaria Gregorio Marañon/ Hosp. GU Gregorio Marañon, Madrid, Spain

Abstract Body:

Background: The presence of OXA-48 Carbapenem-resistant *Enterobacteriaceae* (CRE) has increased dramatically in the last years. Their rapid and accurate detection is currently one of the main challenges of the microbiology laboratory. At present, the standard for detection is based on the amplification of the responsible plasmid. Alternatively, the detection by MALDI-TOF MS, that identifies the hydrolysis products of different carbapenems, is reliable but cumbersome. In this study, a simpler, direct MALDI-TOF MS protocol was applied in order to find distinctive protein profiles in resistant and sensitive *Klebsiella pneumoniae*, correlating with the reference method.**Methods:** Overall, 183 *K. pneumoniae* isolates from clinical samples were included in this study: 79 carbapenem-sensitive and 104 hosting the OXA-48 carbapenemase. For comparison reasons, 7 VIM- and 3 KPC-expressing *K. pneumoniae* were also analysed. The reference method consisted of a real-time PCR kit (RealCycler OXVIKP, Progenie, Spain). All the isolates were directly identified by MALDI-TOF MS (Bruker Daltonics, Germany) and their protein spectra were further analysed using Flex Analysis 3.3.80 software (Bruker Daltonics).**Results:** The peak analysis showed that the protein profile of the two groups of Klebsiella isolates could be differentiated: two distinctive peaks of 3073 and 3707 m/z were present in spectra from OXA-48 *K. pneumoniae*, concordant with the real-time PCR results, and these peaks were consistently absent in the control group of Carbapenem-sensitive *K. pneumoniae* isolates. Besides, a 5377 m/z peak was also characteristic of OXA-48 *K. pneumoniae* isolates, although its intensity was more variable. The combination of these three peaks was not present in other *K. pneumoniae* isolates containing VIM or KPC carbapenemases.**Conclusions:** Although our results are preliminary, peak analysis of the CRE isolates identified by MALDI-TOF MS could provide a rapid and reliable detection of OXA-48 carbapenemases. The turnover time for this test is 10 min and does not require additional reagents to those used for the identification of the microorganism.

Author Disclosure Block:

B. Rodriguez-Sanchez: None. **C. Sanchez-Carrillo:** None. **E. Cercenado:** None. **M. Marin:** None. **A. Ruiz:** None. **E. Bouza:** None.

Poster Board Number:

SUNDAY-383

Publishing Title:

An Accelerated Workflow for Mbt-Astra

Author Block:

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Abstract Body:

Background: Antimicrobial resistance is an increasing health care problem. Since rapid identification of species has been accelerated by use MALDI-TOF MS, a gap arises until information about the resistance status is available. Recently, a semiquantitative MALDI-TOF approach, MBT-ASTRA, has been described facilitating resistance detection in two to five hours (Lange et al., 2015). The published protocol requires significant time-consuming hands-on work. Here, we describe a simplified workflow for higher throughput. **Methods:** Fresh overnight cultures of 40 *E.coli* were analyzed by Etest and the MALDI-TOF based assay. Growth of bacteria in BHI without CTX and in the presence of 20 g/ml CTX after 2 h incubation was determined using semi-quantitative MS. After growth, cell suspensions of the 80 setups were transferred to a 96-well 0.2 µm GHP filter plate (Pall Corp.) and centrifuged (3.000 x g, 5 min). Cells remaining on the filter were washed with 100 µl H_2O and 50 µl ethanol (75%). Lysis was performed directly on the filter by adding 10 µl of formic acid (70 %) and 10 µl ACN supplemented with internal standard. Lysates were recovered in a fresh microtiter plate by centrifugation. 1 µl of lysate was spotted on a MALDI target and overlaid with HCCA matrix. MS profile spectra were acquired on a microflex benchtop mass spectrometer (Bruker Daltonik GmbH). Relative protein amounts in each well were calculated using the internal standard. Ratio of the protein amounts (BHI plus CTX setup/BHI only setup) was calculated (relative growth, RG).**Results:** Etest analysis revealed 20 CTX-resistant and twenty CTX-susceptible *E.coli* strains. MBT-ASTRA were in 100% concordance with the Etest results. Employing MBT-ASTRA shortened the time until resistance information was available by several hours. The use of filter plates facilitates the parallel processing of up to 48 samples on one plate at the same time. This significantly reduced hands-on work and accelerated the workflow.**Conclusion:** Results of the MBT-ASTRA workflow were in concordance to Etest results. Time to result was about about 3.5 hours for 40 samples starting from a fresh overnight culture. The use of filter plates for sample processing significantly reduced hands-on work and accelerated sample handling.

Author Disclosure Block:

K. Sparbier: D. Employee; Self; Bruker Daltonik GmbH. **B. Wegemann:** D. Employee; Self; Bruker Daltonik GmbH. **M. Kostrzewa:** D. Employee; Self; Bruker Daltonik GmbH.

Poster Board Number:

SUNDAY-384

Publishing Title:

Fluconazole and Amphotericin B: Working Together is Better Against Cryptococcus Gattii

Author Block:

N. d. Ribeiro, J. R. A. Santos, D. A. Santos; Univ.e Federal de Minas Gerais - UFMG, belo horizonte, Brazil

Abstract Body:

Background: Amphotericin B and fluconazole are the most used antifungals for the treatment of cryptococcosis caused by *Cryptococcus gattii*, a main pathogen of cryptococcosis in healthy patients. However, there is a controversy regarding the combination of amphotericin B and fluconazole for fungal infections, due potential antagonism. Here, we evaluated the interaction in vivo between fluconazole and amphotericin B against *C. gattii.* **Methods:** Six mice per group were inoculated by the intratracheal route with 1×10^6 cells of L27/01*C. gattii* strain. Fluconazole (15 or 150 mg/kg/day) and amphotericin B (0.5 mg/kg/day) alone or in combination were administered intraperitoneally (i.p.) from one day post-infection. Animals were monitored daily for the survival curve or mice were euthanized 14 or 80 days post-treatment for CFU/ g lungs or brain quantification.**Results:** All the treatments significantly prolonged the median survival of mice infected. Treatment with 150 mg/kg/day fluconazole alone or in combination with amphotericin B and 15 mg/kg/day fluconazole in combination with amphotericin B improved the median survival of mice treated with antifungals in monotherapy. Animals infected and treated with fluconazole 150 alone or in combination showed significant reduction of Log_{10} CFU/g in the lungs, but no significant reduction of fungal burden in lungs occurred in other groups. The fungal burden analysis in the brain of mice infected and treated with fluconazole 150 and amphotericin B post 80 days proved to be sterile in comparison to mice treated with fluconazole 150 alone.**Conclusions:** This study provided new insights into the fluconazole and amphotericin B combination and reinforced a success of interactions between these antifungals.

Author Disclosure Block:

N.D. Ribeiro: None. **J.R.A. Santos:** None. **D.A. Santos:** None.

Poster Board Number:

SUNDAY-385

Publishing Title:

Two Voriconazole-Resistant Strains of *Aspergillus fumigatus* with *CYP51A* TR46/Y121F/T289A Genotype in China

Author Block:

W. Liu, Q. Wang, H. Zhang, Z. Wan, R. Li; Peking Univ. First Hosp., Beijing, China

Abstract Body:

Azole-resistant strains of Aspergillus fumigatus harboring TR46/Y121F/T289A mutations in CYP51A gene have been increasingly found in Europe and Asia. Here, we report 2 voriconazole(VRC)-resistant A.fumigatus strains with TR46/Y121F/T289A in CYP51A gene from an invasive aspergilosis (IA) patient in China. Three sequential strains were isolated from the sputum and BALF of a IA patient. Antifungal susceptibility testing was assayed by using the CLSI microbroth dilution method M38-A2 procedure. And CYP51A gene sequencing and microsatellite genotyping were also performed for the 3 isolates. Consequently, despite of the first isolate being susceptible, the other 2 isolates presented high-level resistance to VRC (MIC ≥8μg/ml). Sequencing analysis for the CYP51A gene showed that the TR46/Y121F/T289A mutations occurred in CYP51A gene. Microsatellite genotyping indicated that 2 resistant strains in this report showed different genotype to that being susceptible, as well as to those strains of A. fumigatus with VRC-resistance reported previously in Europe and Asia. This description for the VRC-resistant A. fumigatus with TR46/Y121F/T289A alternations in CYP51A gene indicated an urgent need for antifungal susceptibility surveillance of pathogenic Aspergillus spp. in China.

Author Disclosure Block:

W. Liu: None. **Q. Wang:** None. **H. Zhang:** None. **Z. Wan:** None. **R. Li:** None.

Poster Board Number:

SUNDAY-386

Publishing Title:

Re-Purposing of the Kinase Inhibitor Sorafenib as a Growth Inhibitor of the Fungal Pathogen Histoplasma Capsulatum

Author Block:

C. Berkes, J. Franco, M. Lawson, D. Trinh; Merrimack Coll., North Andover, MA

Abstract Body:

Background: Histoplasmosis is a respiratory disease caused by the dimorphic fungal pathogen *Histoplasma capsulatum*, which primarily affects immunocompromised individuals. New drugs to treat histoplasmosis are needed, however, efforts to identify new mechanistic classes of anti-fungal compounds have lagged, due in part to the high cost of drug development. In recent years, there has been renewed interest in the repurposing of mammalian kinase inhibitors in order to target fungal, protozoan, and even prokaryotic pathogens. MAP kinases constitute a highly conserved family of proteins present in all eukaryotes. In yeasts, the high osmolarity glycerol (Hog) MAP kinase, a direct homolog of human p38 MAP kinase, is essential for coordination of stress response upon exposure to osmotic shock, reactive oxygen and nitrogen species, temperature extremes, and other forms of cellular stress. Given that *Histoplasma capsulatum* is an intracellular pathogen that colonizes alveolar macrophages, it must be able to utilize robust stress response mechanisms in order to survive and replicate within the hostile environment of the macrophage phagosome. **Methods:** In preliminary studies, we have found that two multi-kinase inhibitors - Sorafenib and SC-1 - inhibit growth of *Histoplasma* in culture and within the context of a macrophage infection model. Here, we expand our search for kinase inhibitors that inhibit *Histoplasma* growth by screening the GlaxoSmithKline PKIS (Protein Kinase Inhibitor Set) using disc diffusion and liquid dilution assays**Results:** Liquid dilution assays show that Sorafenib and a closely related structural derivative, SC-1, inhibit growth of the G217B and G186AR strains of *Histoplasma capsulatum*, with MICs in the high nanomolar/low micromolar range. Screening of the full GSK kinase inhibitor set is currently underway.**Conclusions:** Our work has shown that re-purposing of kinase inhibitors may be an effective avenue of anti-*Histoplasma* drug identification. Future research will be aimed at building the structure-activity relationship, utilizing biochemical approaches to confirm drug targets, and investigating the effectiveness of these compounds on other dimorphic fungal pathogen species.

Author Disclosure Block:

C. Berkes: None. **J. Franco:** None. **M. Lawson:** None. **D. Trinh:** None.

Poster Board Number:

SUNDAY-387

Publishing Title:

The Inhibition of Yeast-to-Hypha Conversion Induced by Amphotericin B in *Candida albicans* Is Dependent on Its Antifungal Effect

Author Block:

J. Argüelles; Univ. de Murcia, Murcia, Spain

Abstract Body:

The morphological transition from yeast to hypha is considered a factor of virulence in the opportunistic pathogenic fungus *Candida albicans* (Gow *et al*., 2002). It has been proposed that sub-lethal concentrations of Amphotericin B (AMB) induce a complete suppression of the capacity to issue germ-tubes, which is the first step of dimorphic conversion in *C. albicans.* Here, we have investigated the *in vitro* correlation between the fungicidal effect of AMB and its hypothetical inhibitory action on hypha formation in the *C. albicans* wild-type strain CEY.1 (CAI.4-*URA*$^{+}$). For germ-tube induction, fresh cultures were supplemented with sterile human serum (10%) and simultaneously transferred from 28 to 37ºC. AMB concentrations below or around the MIC_{90} (0.12 mg/L) added to exponential CEY.1 cells caused a weak reduction in the percentage of human serum-induced germ-tube formation at 37ºC compared with an untreated control (Guirao-Abad *et al.*, 2015). However, the dimorphic transition was drastically suppressed after addition of potentially lethal doses of AMB, which also brought about a severe degree of cell killing. These actions on cell growth and hypha formation are reversible, conditioned to the presence of the antifungal in the culture medium. In contrast, an identical experimental approach carried out with the fungistatic compound 5-fluorocytosine had no significant effect on the level of the germ-tube formation. Together, these results strongly point to a close correlation between the fungicidal action of AMB and its ability to impair morphogenetic conversion in *C. albicans*. This relationship might be relevant for therapeutical purposes, particularly in the light of the ability of *C. albicans* to develop biofilms as a main pathogenesis mechanism.

Author Disclosure Block:

J. Argüelles: None.

Poster Board Number:

SUNDAY-388

Publishing Title:

***In Vitro* Nail Penetration of Nitric Oxide Releasing Formulations for the Topical Treatment of Onychomycosis**

Author Block:

K. McHale, M. Martin, B. Johnston, Y. Zhang, R. Doxey, S. Hollenbach, N. Stasko; Novan Therapeutics, Durham, NC

Abstract Body:

Background: Onychomycosis is a common fungal infection of the nail caused primarily by dermatophytes. Topical treatments are favored over orally administered agents due to their side-effect profile and reduced risk of drug-drug interactions. However, efficacy rates for current topical therapies are hampered due to low penetration of drug across the nail plate. Nitric oxide-based topical therapies are promising due to the ability of the gaseous species to readily diffuse across the nail. Previous experiments have demonstrated the potent fungicidal effect of Novan's nitric oxide-releasing drugs in vitro. The aim of this study was to evaluate the ability of nitric oxide, released from several unique drug product formulations (gel, cream, lacquer), to penetrate the human nail and effectively kill the fungal infection. **Methods:** *In vitro* human nail penetration was evaluated (MedPharm, Guilford, UK) utilizing the ChubTur® infected human nail assay. *Tricophyton rubrum (T. rubrum)* was inoculated to the underside of human nails and allowed to establish infection for 14 days. Following the establishment of infection, the nails were mounted into modified Franz cells and topical treatments were applied to the top of the nail plate. The penetration of several unique nitric oxide-releasing formulations was evaluated by assessing the viability of *T. rubrum* after exposure via bioluminescence quantitation of ATP. **Results:** All nitric oxide-releasing topical formulations demonstrated effective fungal killing in 24 hrs, after a single treatment application. **Conclusions:** The mean reductions of viable fungal hyphae observed with various nitric oxide-releasing formulations ranged from 82% - 99 with several candidate formulations performing as well or better than 10% efinaconazole in this assay (83% mean reduction, Jublia®). The minimal difference in fungicidal effects between the various nitric oxide formulations suggests that penetration of nitric oxide gas through the nail plate was sufficient to kill the fungal infection after a single treatment application. Taken together these data demonstrate that nitric oxide-releasing treatments, with rapid penetration of the nail plate and eradication of fungal infection, are comparable to efinaconazole and represents promising novel, topical therapies for the treatment of onychomycosis.

Author Disclosure Block:

K. McHale: D. Employee; Self; Novan Therapeutics. K. Shareholder (excluding diversitied mutual funds); Self; Novan Therapeutics. **M. Martin:** D. Employee; Self; Novan Therapeutics.

B. Johnston: D. Employee; Self; Novan Therapeutics. K. Shareholder (excluding diversitied mutual funds); Self; Novan Therapeutics. **Y. Zhang:** D. Employee; Self; Novan Therapeutics. K. Shareholder (excluding diversitied mutual funds); Self; Novan Therapeutics. **R. Doxey:** D. Employee; Self; Novan Therapeutics. K. Shareholder (excluding diversitied mutual funds); Self; Novan Therapeutics. **S. Hollenbach:** D. Employee; Self; Novan Therapeutics. K. Shareholder (excluding diversitied mutual funds); Self; Novan Therapeutics. **N. Stasko:** A. Board Member; Self; Novan Therapeutics. D. Employee; Self; Novan Therapeutics. K. Shareholder (excluding diversitied mutual funds); Self; Novan Therapeutics.

Poster Board Number:

SUNDAY-389

Publishing Title:

Evaluation of Fungicidal Potential of *Nicotiana tabacum l.* and *Ocimum sanctum l.* Against *Aspergillus flavus* and *Aspergillus niger*

Author Block:

Z. Naeem, A. Maimoona, K. Jabeen, B. Khan; Lahore Coll. for Women Univ., Lahore, Lahore, Pakistan

Abstract Body:

The present study was undertaken to evaluate the antifungal activity of a locally found and ethno-botanically important plants *Ocimum sanctum* Linn. and *Nicotiana tabacum* Linn. The reason for selecting those plants is that both are rich in secondary plant metabolites. The first step was the extraction of powdered plant material in methanol of both plants in order to get their crude extracts. Extraction was carried out by means of maceration technique followed by fractionation (liquid-liquid partitioning). Qualitative phytochemical tests were carried out to confirm the absence or presence of flavanoids, glycosides, phenolics, saponins, steroids, tannins and tri-terpenes. For carrying out antifungal activity two test fungal strains belonging to genus Aspergillus were selected i.e *A. niger* and *A. flavus*. In *O. sanctum*, n-hexane fraction is most effective (100%) at conc. of 0.3% against *A. niger*. On contrary to *O. sanctum* in which maximum inhibition was given by *A. niger*, in *N. tabacum* maximum control for fungal growth has been recorded in butanol fraction at 96% at the concentration of 0.1% against *A. flavus*. Hence these plants can be used for manufacturing drugs to preserve our cereals and to treat fungal infections as both possess significant antifungal activity.

Author Disclosure Block:

Z. Naeem: None. **A. Maimoona:** None. **K. Jabeen:** None. **B. Khan:** None.

Poster Board Number:

SUNDAY-391

Publishing Title:

HXP124, a Plant Defensin, Represents a Novel Antifungal Therapeutic

Author Block:

N. L. van der Weerden[1], B. Hayes[2], M. Baker[1], K. Harris[2], M. Bleackley[2], **M. Anderson**[2]; [1]Hexima Limited, Melbourne, Australia, [2]La Trobe Univ., Melbourne, Australia

Abstract Body:

Background: Plant defensins with potent antifungal activity have the potential to be developed as effective and robust treatments for fungal infections in humans. The cysteine-stabilised structure of plant defensin's makes them extremely stable and able to withstand extremes of pH and temperature. Here we investigate the antifungal properties of a novel plant defensin, HXP124, against a range of clinically important fungal pathogens.**Methods:** HXP124 was recombinantly expressed using the Pichia pastoris yeast expression system and purified using a scalable two-step purification method. The activity of HXP124 against a range of fungal and bacterial pathogens was tested using the microbroth dilution method and the ability of HXP124 to kill human U937 cells was examined by monitoring the uptake of propidium iodide using FACS.**Results:** HXP124 has potent antifungal activity against a range of human fungal pathogens including Candida spp, Cryptococcus spp, dermatophytes and non-dermatophytic moulds. HXP124 is highly selective for fungal cells having no effect on mammalian cells, including red blood cells, at concentrations more than 50 times the MIC for fungus. HXP124 is also fungicidal, killing fungal cells within 1 h of exposure after the induction of reactive oxygen species and disruption of fungal membranes. Plant defensins contain four disulphide bonds which stabilise the three-dimensional structure. As such, they are stable at extremes of pH (<2) and temperature (>95°C). HXP124 can be produced recombinantly in yeast with very high yields. Its small size and positive charge makes it easy to purify and highly pure protein can be achieved with a simple two-step purification process.**Conclusions:** HXP124 is a potent antifungal molecule and an excellent candidate for the development of a novel antifungal therapeutic useful in a number of clinical settings.

Author Disclosure Block:

N.L. van der Weerden: A. Board Member; Self; Hexima Limited. D. Employee; Self; Hexima Limited. K. Shareholder (excluding diversitied mutual funds); Self; Hexima Limited. **B. Hayes:** I. Research Relationship; Self; Hexima Limited. **M. Baker:** D. Employee; Self; Hexima Limited. **K. Harris:** I. Research Relationship; Self; Hexima Limited. **M. Bleackley:** I. Research Relationship; Self; Hexima Limited. **M. Anderson:** A. Board Member; Self; Hexima Limited. D. Employee; Self; Hexima Limited. K. Shareholder (excluding diversitied mutual funds); Self; Hexima Limited.

Poster Board Number:

SUNDAY-392

Publishing Title:

Use of the Non-Essential Yeast Deletion Collection to Define the Antifungal Mechanisms of Plant Defensins

Author Block:

K. Parisi[1], M. Bleackley[1], N. van der Weerden[2], M. Anderson[1]; [1]La Trobe Univ., Melbourne, Australia, [2]Hexima Limited, Melbourne, Australia

Abstract Body:

Background: Plant defensins are potent antifungal peptides that represent a novel class of antifungal drug with excellent potential for clinical application. Plant defensins share a similar three-dimensional structure but vary significantly in sequence. This variation in sequence is thought to account for the different mechanisms of action observed for plant defensins. We have identified several novel antifungal plant defensins with high sequence variability and potentially novel mechanisms of action.**Methods:** To define the mechanism of action of each defensin we employed a *Saccharomyces cerevisiae* gene deletion library that contains 5000 unique knockout strains. Each knockout strain is identifiable by an individual barcode incorporated into the gene during deletion. A pool of this library was treated in triplicate with eight different defensins alongside an untreated control. Following defensin treatment, genomic DNA was isolated from the treated yeast. The barcoded region was amplified and sequenced using next-generation sequencing to determine the abundance of each strain. From this analysis, we determined the fitness of 5000 yeast deletion strains in the presence of a specific defensin.**Results:** We identified several strains that were sensitive or resistant to all eight defensins indicating that elements of the activity of plant defensins are conserved. These strains may represent general stress response genes. However, the clustering revealed elements that are unique to each defensin and suggests at least three different mechanisms of action. Two of the defensins we tested appear to have a conserved mechanism of action which correlates with their degree of sequence identity.**Conclusions:** Understanding how plant defensins exert their antifungal activity will assist in selection of defensins with different antifungal mechanisms which can be used in therapeutics to create broad spectrum resistance to fungi.

Author Disclosure Block:

K. Parisi: I. Research Relationship; Self; Hexima Limited. **M. Bleackley:** I. Research Relationship; Self; Hexima Limited. **N. van der Weerden:** A. Board Member; Self; Hexima Limited. D. Employee; Self; Hexima Limited. K. Shareholder (excluding diversitied mutual funds); Self; Hexima Limited. **M. Anderson:** A. Board Member; Self; Hexima Limited. I. Research Relationship; Self; Hexima Limited. K. Shareholder (excluding diversitied mutual funds); Self; Hexima Limited.

Poster Board Number:

SUNDAY-393

Publishing Title:

Effects of Aqueous and Ethanolic Extracts of *Heinsia crinita* on the Pathogenic Fungi of *Carica papaya*

Author Block:

E. Enemo, D. Anthony; Univ. of Uyo, Uyo, Nigeria

Abstract Body:

Background: The need for edible medicinal sources of antifungal agents has risen in recent times owing to the increasing resistance of pathogenic fungi to synthetic antifungal agents. Hence, this research.**Methods:** Pawpaw fruits were bought from three different markets in Uyo metropolise(Itam, Use and Akpana Ndem markets). Sterilised surgical blade was used to cut out 3mm by 3mm from the diseased area of the pawpaw fruits. This was inoculated on a Potato Dextrose Agar(PDA) media and incubated at room temperature for 5 days. Observed mixed growths were subcultured to get pure cultures. For the exact identification of the isolates further molecular methods will be employed(which is beyond the scope of this work). The fungi isolates were re-inoculated into healthy matured pawpaw fruits to test their pathogenicity. All the isolates were able to produce spoilage with EKP41 being the most pathogenic, and EKP12 being the least pathogenic. Fungi spores were obtained by growing the isolates in peptone broth. The effects of the extracts were tested on the spores using both Agar Disc Diffusion and Agar Well Diffusion methods at two levels (125mg/ml) and (50mg/ml).**Results:** Of the seven fungal samples, only EKP41 exhibited slight sensitivity to the extracts.**Conclusions:** The result confirms *Heinsia crinita's* antifungal property. Hence, the consumption of *Heinsia crinita* is recommended for curing fungal infections of pawpaw origin, especially of the EKP41 source.

Author Disclosure Block:

E. Enemo: None. **D. Anthony:** None.

Poster Board Number:

SUNDAY-394

Publishing Title:

***In Vitro* Anti-Dermatophyte Activities of Some Nigerian Medicinal Plants**

Author Block:

C. C. Ekwealor, C. A. Oyeka, V. N. Anakwenze, C. M. Ogbukagu; NNAMDI AZIKIWE Univ., AWKA, Nigeria

Abstract Body:

Background: Medicinal plants are plants whose parts are used in the treatment of different diseases. They are becoming popular because of their inability to cause side effects. This study is to determine the anti-dermatophyte activities of some Nigerian medicinal plants against dermatophytes recovered from rice farmers in Anambra State, Nigeria.**Methods:** Samples collected from 201 rice farmers with lesions suggestive of cutaneous mycoses were identified as *Microsporum audouinii*, *Microsporum ferruginieum*, *Trichophyton megninii*, *Trichophyton tonsurans* and *Trichophyton rubrum*. Dried leaves of *Lawsonia inermis*, *Cassia alata* and *Mitracarpus villosus* were extracted by soxhlet method using methanol as solvent. Anti-dermatophyte activities of the methanol and aqueous extracts of the leaves were studied using disc diffusion method at varying concentrations (10mg, 20mg, 40mg, 80mg). Discs impregnated with 2% DMSO and 2mg ketoconazole were used as negative and positive controls respectively. Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentrations (MFC) of the extracts were determined using broth dilution method.**Results:** Growth of the dermatophytes were inhibited by methanol extracts of the plants at various concentrations. The inhibition zones increased with increase in concentration of the extracts. At 10mg/disc, methanol extracts of *L. inermis* inhibited all isolates with an inhibition zone ranging from 7.8mm-16mm diameter while *C. alata* inhibited only *M. audouinii* with an inhibition zone of 8.2mm in diameter. *Mitracarpus villosus* extract showed no inhibition at this concentration. However, the highest diameter zone of inhibition (16.8mm) was observed with *L. inermis* against *T. rubrum* at 80mg/disc. All dermatophytes were susceptible to aqueous extracts of all the plants with *L. inermis* having diameter zone of inhibition of 9.0mm-14.5mm, *C. alata* 6.4mm-8.2mm and *M. villosus* 6.0mm-7.8mm. MIC was observed at ≥25mg/ml for all the plants. *L. inermis* and *C. alata* recorded an MFC of ≥50mg/ml and ≥100mg/ml respectively against the dermatophytes while no fungicidal action was recorded with *M. villosus***Conclusions:** Methanol and aqueous extracts of the plants inhibited the dermatophytes and so could be a good source for the production of plant based antifungal drugs for treatment and control of tinea infections

Author Disclosure Block:

C.C. Ekwealor: None. **C.A. Oyeka:** None. **V.N. Anakwenze:** None. **C.M. Ogbukagu:** None.

Poster Board Number:

SUNDAY-395

Publishing Title:

Candiduria and Bacteriuria in Children Attending Primary School in Amassoma Community, Niger Delta Region of Nigeria

Author Block:

O. A. Olorode, **O. A. Olorode**; Niger Delta Univ., Faculty of Pharmacy., Yenagoa Ba, Nigeria

Abstract Body:

Candiduria and Bacteriuria infections can be severe and it is associated with considerable morbidity if left untreated. Can these infections co-infect in primary school children? This study was conducted between January and October, 2015 to determine the frequency of bacteriuria and candiduria of apparently healthy children of different age groups and sex attending primary schools in Amassoma, Nigeria. Mid-Stream Urine (MSU) was examined in 142 children using selective media. The isolated organisms were characterised and identified on the basis of their cultural morphology, Gram stain and biochemical properties. Antibiotic susceptibility of isolated organisms was evaluated by standard procedure. Stastitical analysis showed no significant difference in the effectiveness of susceptible antimicrobial agents. The findings showed asymptomatic Bacteriuria and Candiduria in 91 (64.1%) and 97 (68.3%) samples respectively; the subjects who had staphylococcal co-infection with Candidiasis and other infections were 75 cases (52.8%), with female preponderance over males. Microorganisms isolated include *Staphylococcus aureus* (20.8%), *Candida glabrata* (14%), *Staphylococcus epidemidis* (13.3%), *Candida albicans* (6.8%); *Candida tropicalis* (5%), *Bacillus species* (4.3%), *Candida parapsilosis* (3.2%), *Enterococcus species* (2.2%), Klebsiella species (1.4%) other *Staphylococcus* species were (9.7%). *Staphylococcus,Bacillus and Enterococcus* species isolated were highly susceptible to Levofloxacin (97.4%), Gentamycin (93.2%), Ciprofloxacin (93.1%), Streptomycin (92.6%), moderately susceptible to Rifampicin (71.2%); least susceptible to Erythromycin (58.9%) but resistant to Chloramphenicol (85.4%). All *Candida* isolates were most susceptible to Fluconazole (87.9%) followed by Nystatin (71%), moderately susceptible to Ketoconazole (68.1%) and highly resistant to itraconazole (8.1%). Observation showed that the most infected subjects were females between ages of 5 and 12yrs.

Author Disclosure Block:

O.A. Olorode: None. **O.A. Olorode:** None.

Poster Board Number:

SUNDAY-396

Publishing Title:

Characterization of *Myo*-inositol Transport in *Pneumocystis* Species; A Potential New Drug Target

Author Block:

M. T. Cushion[1], M. S. Collins[1], K. Lynch[1], T. Sesterhenn[1], M. J. Linke[2]; [1]Univ. of Cincinnati Coll. of Med., Cincinnati, OH, [2]Cincinnati VA Med. Ctr., Cincinnati, OH

Abstract Body:

Background: Fungi in the genus *Pneumocystis* cause an oftentimes lethal pneumonia in humans and other mammals with compromised immune status. The niche of these fungi include patients with underlying chronic diseases such as COPD or HIV and those receiving anti-inflammatory or immunosuppressive agents. PCP is not responsive to standard antifungal therapy with few treatment alternatives besides trimethoprim-sulfamethoxazole. We recently reported *Pneumocystis* spp. were auxotrophic for *myo*-inositol, a polyol that is essential for viability, and instead uses transporters to satisfy this requirement. There is but a single such transporter (ITR1) in *P. jirovecii*, the species infecting humans, providing a potential new drug target as humans can both synthesize and transport *myo*-inositol. In the present study we characterized *myo*-inositol transport in *Pneumocystis*.**Methods:** *P. carinii* obtained from the lungs of immunosuppressed rats was used in uptake studies with *myo*-[2-^{3}H] inositol to determine the K_m, substrate specificities, and critical determinants of substrate recognition. Uptake was measure by liquid scintillation in the presence or absence of 50-100X monosaccharides and *myo*-inositol stereoisomers; with varying concentrations of proton or sodium transport inhibitors; acidic and basic pH, and *myo*-inositol biosynthesis inhibitors. Yeast *itr1/itr2* mutants were used to further evaluate function of the ITR1 from *P. carinii, P. murina,* and *P.jirovecii*. Statistical significance was measured by ANOVA with Dunnett's multiple comparison test.**Results:** The K_m was in the range of other high affinity *myo*-inositol transporters in yeast. No other hexose or pentose sugar inhibited uptake of the radioactive label besides *myo*-inositol. A similar high affinity was observed with the stereoisomers and inositol derivatives. Transport was proton but not sodium dependent. As expected, inhibitors of *myo*-inositol synthesis (e.g. lithium chloride) had no effects on uptake or viability. The ITR1 genes of the *Pneumocystis* species could complement uptake and growth of the double yeast mutants.**Conclusions:** *Pneumocystis* spp. rely on high affinity *myo*-inositol transport which offers a potential new drug target.

Author Disclosure Block:

M.T. Cushion: None. **M.S. Collins:** None. **K. Lynch:** None. **T. Sesterhenn:** None. **M.J. Linke:** None.

Poster Board Number:

SUNDAY-397

Publishing Title:

Type-Iii Secretion Proteins as a Broadly Protective Subunit Vaccine against *Salmonella enterica* Serovars

Author Block:

V. VISHWAKARMA, F. J. MARTINE-BASSERA, P. KUMAR, O. ARIZMENDI, M. M. PRESSNALL, W. D. PICKING, W. PICKING; Univ. OF KANSAS, LAWRENCE, KS

Abstract Body:

Salmonella enterica is an important foodborne pathogen. Infection of livestock is common and a threat to the food animal industry. Non-typhoidal *S. enterica* is the leading cause of hospitalization and death resulting from contaminated food in the US. Currently, no broadly protective vaccine is available against the many *S. enterica* serovars. We have genetically fused SPI-1 and SPI-2 tip and first translocator proteins, SipB/SipC and SseB/SseC, respectively, to produce recombinant fusion proteins S1F and S2F, respectively, for immunization of mice. S1F and S2F, alone or together, were administered intramuscularly with monophosphoryl lipid-A (MPL) and Alhydrogel as adjuvant system. Serum and bone marrow were isolated post-immunization to assess immunogenicity; cecum samples were sectioned and stained to determine cecal inflammation. Both fusions elicited a high serum IgG response alone and together. Antibody secreting cells (ASC) isolated from bone marrow of immunized mice showed moderate to high frequencies of IgG ASCs against the proteins of S1F, high frequencies to proteins in S2F, while the highest frequencies were detected in the S1S2 combination vaccinated group. Similarly, unique cytokine secretion patterns were detected in S1S2 vaccinated mice. When mice were challenged with *S.* Typhimurium or *S.* Enteritidis, the S1S2 vaccine formulation elicited the highest protection against death as well as prevention of cecal inflammation. These results demonstrate the proof of concept in a small animal model that the S1S2 subunit vaccine can provide broad coverage to protect against all *S. enterica* serovars which may be transformative to the livestock industry and improve human health.

Author Disclosure Block:

V. Vishwakarma: None. **F.J. Martine-bassera:** None. **P. Kumar:** None. **O. Arizmendi:** None. **M.M. Pressnall:** None. **W.D. Picking:** None. **W. Picking:** None.

Poster Board Number:

SUNDAY-398

Publishing Title:

Association of Phenotyic and Genotypic Fluoroquinolone Susceptibility Among Non-Typhoidal Salmonella Isolates From Jeddah, Ksa

Author Block:

A. Ali, R. A. Lahzah; King Abdulaziz Univ., Jeddah, Saudi Arabia

Abstract Body:

Non-typhoid Salmonella (NTS) infect 250 to 3200 per 100,000 individuals from all over the world. NTS infection is relatively high in Jeddah (ranges between 44-132) as compared to other cities of Saudi Arabia due to high influx of people from across the globe for pilgrimage. Fluoroquinolone (FQ) is the drug of choice for the treatment of NTS infections. However, miss-use of FQs has led to increase in FQ resistance. Genotypic FQ resistance has been associated with mutations in *gyrA* and *parC* genes. This study primarily explored the phenotypic FQ susceptibility among clinical NTS isolates from Jeddah, Saudi Arabia. Secondly, study also explored any correlation between phenotypic and genotypic FQ resistance.48 NTS isolates were collected from a public sector hospital in Jeddah during 2014. FQ susceptibility was determined using Clinical and Laboratory Standards Institute methodology. Presence of mutations for FQs resistance was detected in *gyrA* and *parC* genes by PCR- based gene-sequencing method.Phenotypic antibiogram revealed 38% (18/48) resistance for FQ among NTS isolates. Genotypic resistance revealed mutations in *gyrA* and *parC* genes among 39% (7/18) of FQ resistant isolates. 43% (3/7) of FQ resistant isolates showed mutations at two codons 83 (S83F, S83Y) and 87, (D87G, D87Y, D87W) of *gyrA* gene. Two resistant isolates showed triple mutations i.e. at codons 83 and 87 of *gyrA* and codon 80 (S80I and S80W*)* of *pyrC* gene. While one of each resistant isolate revealed mutation at codon 87 of *gyrA* and 57 (S57T) of *pyrC* gene. 55% (6/11) intermediate susceptible isolates for FQ revealed mutations in *gyrA* and *pyrC* genes. Of these 83% had single mutation at codon 83 of *gyrA* gene whereas, 17% (1/6) revealed double mutation at codon 83 of *gyrA* and 57 of *pyrC* gene. None of the FQ susceptible isolates showed any mutations in *gyrA* or *parC* genes.Occurrence of mutations at only four codons in *gyrA* and *parC* genes among FQ resistant isolates may assist in development of rapid molecular method for FQ resistance detection. Presence of mutations among more than fifty percent of intermediate susceptible FQ NTS isolates could also serve as a predictor for pre-resistant isolates. However, absence of mutation in about sixty percent of resistant isolates stress further investigation to explore an alternate resistance mechanism for FQ among NTS.

Author Disclosure Block:

A. Ali: None. **R.A. Lahzah:** None.

Poster Board Number:

SUNDAY-399

Publishing Title:

High Prevalence and Characterization of CTX-M-15-Producing *Salmonella enterica* Serotype Virchow in Chicken in Korea

Author Block:

S-K. Lim, D. Moon, S-R. Kim, G-C. Jang, H-S. Lee; Animal and Plant Quarantine Agency, Anyang, Korea, Republic of

Abstract Body:

Background: *Salmonella enterica* serotype Virchow (*S.* Virchow) carrying CTX-M-15 type extended-spectrum beta-lactamase (ESBL) has rapidly emerged in humans in Korea recently. Third generation cephalosporins are widely used in both human and veterinary medicine to treat important bacterial infections. The aim of this study was to investigate prevalence and characterization of ceftiofur resistant *S.* Virchow isolated from food animals and carcasses in Korea. **Methods**: *S.* Virchow strains were isolated from animal faecal and animal carcass samples in slaughterhouse during 2011-2014. Serotyping by agglutination, antimicrobial susceptibility testing by broth dilution method, identification of ESBL genes and plasmid replicon types by polymerase chain reaction and sequencing, and conjugation by filter mating of the ceftiofur resistant *S.* Virchow were performed. **Results:** From 2011 to 2014, a total of 103 ceftiofur resistant *S.* Virchow were isolated from chicken (n=42), pigs (n=3), cattle (n=1), and chicken carcasses (n=57). The percentage of ceftiofur resistant *S.* Virchow isolates dramatically increased from 0.7% (2/268) in 2011 to 20.3% (74/364) in 2014. The ceftiofur resistant *S.* Virchow isolates were recovered from 59 chicken farms, 3 pig farms and 22 slaughterhouses. All ceftiofur resistant *S.* Virchow carried the $bla_{\text{CTX-M-15}}$ gene. Among them, 18 isolates simultaneously co-carried $bla_{\text{CTX-M-15}}$ and $bla_{\text{CMY-2}}$ genes. The $bla_{\text{CTX-M-15}}$ gene was located on transferrable IncI (n=17) and IncHI1&HI2 (n=18) plasmids. Identical PFGE patterns were observed in isolates from same or different farms and slaughterhouses. **Conclusions:** These results suggested that $bla_{\text{CTX-M-15}}$ gene could be disseminate via plasmid transfer and clonal spread in chicken production system.

Author Disclosure Block:

S. Lim: None. **D. Moon:** None. **S. Kim:** None. **G. Jang:** None. **H. Lee:** None.

Poster Board Number:

SUNDAY-400

Publishing Title:

Detection of Plasmid-Encoded Fosfomycin Resistance Gene *Fosa3* in *Salmonella* Isolates from Food-Producing Animals

Author Block:

H-X. Jiang, W-H. Zhang, X-X. Zhang, Z-L. Zeng; South China Agricultural Univ., Guangzhou, China

Abstract Body:

The purpose of this study was to investigate the occurrence of plasmid-mediated fosfomycin-resistant genes in *Salmonella* isolates from food-producing animals and retail pork.A total of 285 non-duplicate *Salmonella* isolates obtained from retail pork (n=126), pigs (n=90) and chickens (n=69) in China during 2014-2015 were screened for the presence of plasmid-mediated fosfomycin-resistant genes (*fosA3*, *fosA and fosC2*) by PCR and sequencing. The positive isolates were further tested for the presence of other associated resistance genes (ESBLs and PMQR genes). Plasmids were characterized by conjugation, replicon type and S1-PFGE. The genetic relatedness was determined by PFGE and MLST. The genetic context was determined by PCR mapping and sequencing. In total, eight (2.8%) isolates showed resistance to fosfomycin and 5 (1.8 %) were positive for *fosA3*, no *fosC2* or *fosA* gene was detected. All of the five *fosA3*-positive isolates were recovered from chickens of two different serotypes (3were *S.Typhimurium*, 2 were *S. Indiana*). CTX-M type ESBLs were found in all five fosA3-carrying isolates (two CTX-M-27, two CTX-M-14 and one CTX-M-65), PMQR genes (*aac-(6')-Ib-cr* and /or *oqxAB*) was identified in two isolates. PFGE results showed that these *fosA3*-carrying isolates belonged to two different clonal groups. MLST analysis showed all of the five fosA3-positive isolates belonged to ST17. All fosA3 genes were located on IncN /IncA/C ~150kb plasmids with co-transfer $bla_{\text{CTX-M}}$ genes in 3 isolates. Genetic-environment analysis showed the fosA3 genes were flanked by IS26 with a genetic structure (IS26-fosA3-orf1-orf2- IS26).Our results indicated that both clonal spread of resistant strains and horizontal transmission of the plasmids contributed to the dissemination of *fosA3*-positive *Salmonella* isolates, the coexistence of $bla_{\text{CTX-M}}$ gene is also a public health concern. Close monitoring of spread tread of the *fosA3* gene is necessary.

Author Disclosure Block:

H. Jiang: None. **W. Zhang:** None. **X. Zhang:** None. **Z. Zeng:** None.

Poster Board Number:

SUNDAY-401

Publishing Title:

Antimicrobial Susceptibility and Molecular Characterization of *Shigella* Clusters Associated with Men Who Have Sex with Men (MSM)

Author Block:

A. Bicknese[1], D. Campbell[2], A. Bowen[2], J. Grass[2], J. Hurd[2], J. C. Norton[3], A. McCullough[1], J. Chen[1], Z. Rigney[3], J. Concepcion-Acevedo[2], D. Wagner[1], J. Folster[2]; [1]IHRC, Atlanta, GA, [2]CDC, Atlanta, GA, [3]ORISE, Oak Ridge, TN

Abstract Body:

Shigella causes an estimated 500,000 illnesses annually in the United States, and transmission is usually fecal-oral. Shigellosis is a public health problem in the MSM-community. The National Antimicrobial Resistance Monitoring System (NARMS) at CDC tests every 20th Shigella isolated in the United States for antimicrobial resistance and tested 84 isolates from 8 MSM-associated clusters (2013-2015), identified by epidemiological and laboratory investigations.*Shigella* were speciated at State Public Health laboratories. Broth microdilution (ThermoScientific Sensititre) was performed to determine minimum inhibitory concentrations of 14 antimicrobial agents. Pulsed-field-gel-electrophoresis (PFGE) and whole genome sequencing (WGS) analysis were also performed. Seven clusters were caused by *S. sonnei* and one by *S. flexneri.* Fifteen PFGE Xbal patterns were found among the 84 isolates tested from these clusters. Resistance for up to 12 antimicrobial agents was observed, and resistance varied between clusters and even within PFGE patterns. All eight clusters had multidrug resistance (MDR, resistance in 3 or more CLSI antimicrobial classes) isolates. One cluster had 67% MDR isolates, another had 86% MDR isolates and the remaining six had 100% MDR isolates. The top three resistance patterns among MSM-associated clusters included resistance or non-susceptibility to at least one clinically important antimicrobial (ampicillin, trimethoprim/sulfamethoxazole, ciprofloxacin, or azithromycin); preliminary WGS (Illumina MiSeq) analysis using ResFinder (cge.cbs.dtu.dk) identified beta lactamase genes (primarily *bla*$_{\text{TEM}}$), trimethoprim/sulfamethoxazole resistance genes (*dfrA*1 and *sul1/2*), mutations in *gyrA*, and a macrolide resistance gene (*mphA*). Plasmids IncFIB and IncFII were identified by PlasmidFinder (cge.cbs.dtu.dk). Further studies are necessary to define risk factors for acquisition of MDR *Shigella* infections, determine how antimicrobial resistance affects transmission and success of treatment with antimicrobials, and tailor prevention strategies for at-risk groups including MSM communities.

Author Disclosure Block:

A. Bicknese: None. **D. Campbell:** None. **A. Bowen:** None. **J. Grass:** None. **J. Hurd:** None. **J.C. Norton:** None. **A. McCullough:** None. **J. Chen:** None. **Z. Rigney:** None. **J. Concepcion-Acevedo:** None. **D. Wagner:** None. **J. Folster:** None.

Poster Board Number:

SUNDAY-402

Publishing Title:

***In Vitro* Activity of Pexiganan (Px) and 8 Comparator Antimicrobials against 230 Isolates Including 93 *Pasteurella spp* and 47 Anaerobic Bacteria Recovered from Animal Bite Wounds**

Author Block:

E. J. C. Goldstein[1], K. L. Tyrrell[2], D. M. Citron[2]; [1]R.M. Alden Res. Lab, Santa Monica, CA, [2]R.M. Alden Res. Lab, Culver City, CA

Abstract Body:

Background: Annually, &gt4 million Americans will be bitten by a dog and an additional ½ million will be bitten by a cat. While 15-20% will become infected, ~80% will use topical forms of self-administered therapy prior to seeking medical attention. Pexiganan, a 22-amino acid synthetic cationic analogue of peptide magainin II, acts by selectively damaging bacterial cell membranes. PX is in Ph3 clinical development as a topical cream (0.8%) for treatment of mild infections of diabetic foot ulcer and has potential for other skin and skin structure infections. The aim of this study was to evaluate the in vitro of typical aerobic and anaerobic bite wound isolates. **Methods:** Some of the strains were recovered during the past 3 years, although many of the unusual species were older. About 10% were from European and Canadian patients. Anaerobic isolates were tested by the agar dilution method, for all drugs except PX, which was tested using Brucella broth supplemented with vitamin K and hemin, with and without 5% lysed horse blood (CLSI M11-A8). Agar is not suitable for testing PX due to interference by the calcium concentration in agar. Aerobic organisms were tested by broth microdilution for penicillin (PCN), amoxicillin-clavulanate (A-C), piperacillin-tazobactam (P-T), meropenem (MR), clindamycin (CM), doxycycline (DX), ceftriaxone (CX), and moxifloxacin (MX). **Results:** *Pasteurella* spp. MIC_{90}s ranged from 8-32 µg/ml. The no. of isolates and MIC 90%s were as follows: *P. multocida* (31), 32 µg/ml; *P septica*, (22), *P canis (15) and P dagmatis (15)* all 16 µg/ml; and *P stomatis* (10), 8µg/m; *Bergeyella zoohelcum* (11), 16 µg/ml; *Moraxella* spp. (16), 16 µg/ml; *Neisseria zoodagmatis* (14), 2 µg/ml; *Eikenella corrodens* (32), 8 µg/ml. For anaerobes, the MIC 90%s were as follows: *Bacteroides pyogenes*, (15), 4 µg/ml; *Prevotella heparinolytica.* (12) 8 µg/ml; *Fusobacterium canifelinum* (10), 16 µg/ml; *F russii* (10), 4 µg/ml. None of the higher PX MICs was related to resistance in any of the other antimicrobial agents. **Conclusions:** PX showed a high level of activity against this diverse group of animal bite isolates. The concentration of PX in the cream is 8,000 µg/ml, more than 60 times the highest MIC obtained. PX shows great potential for early topical therapy of animal bite wounds.

Author Disclosure Block:

E.J.C. Goldstein: H. Research Contractor; Self; ipexium. **K.L. Tyrrell:** None. **D.M. Citron:** None.

Poster Board Number:

SUNDAY-403

Publishing Title:

Microbiological Safety of Tulathromycin on Human Gut Flora in Chemostats

Author Block:

H. Hao, S. Zhou, Z. Liu, Y. Wang, Z. Yuan; Huazhong Agricultural Univ., Wuhan, China

Abstract Body:

To evaluate microbiological safety of tulathromycin on human gut flora, low to high concentration of tulathromycin (0, 0.1, 1 and 10μg/mL) was administrated into Chemostat models. Short chain fatty acids (SCFAs), population and resistance of four dominant human interstinal bacterial was monitored daily. Colonization barrier of each community was determined by 3 successive daily challeges of *Salmonella typhimurium*. The resistance phenotype, genotype, transferability, virulence genes and pathogenicity of *Enterococus.faecalis* were determined before and after treatment with tulathromycin. Results showed that 10μg/mL tulathromycin would significantly decrease the population but increase the resistance rate of *Enterococci* and *Bacteroides fragilis*. The 100 μg/mL tulathromycin significantly decreased population of *Escherichia coli*, *Bifidobacterium* and *B.fragilis* and SCFAs, but largely increased resistant rate of *Enterococci* and selected multidrug resistant *E.faecalis*. The selected resistant *E. faecalis* always carried *ermB* gene located in transposons Tn154 and had ability of horizontal gene transfer (HGT). The *ermB* gene in selected resistant *E. faecalis* had a positive correlation with virulence gene of *esp* and *cylA*, but a negative relationship with *gelE* virulent gene. The *E. faecalis* harboring *esp* and *cylA* had higher pathogenicity. Conclusively, microbiological acceptable daily intake (mADI) of tulathromycin was calculated as 4.58μg/kg bw/day. The higher concentration of tulathromycin would disturb colonization resistance of human intestinal flora and increase antimicrobial resistance development, horizontal gene transfer and virulence of pathogens. The antimicrobial resistant pathogens with high pathogenicity may pose risk to human health.

Author Disclosure Block:

H. Hao: None. **S. Zhou:** None. **Z. Liu:** None. **Y. Wang:** None. **Z. Yuan:** None.

Poster Board Number:

SUNDAY-405

Publishing Title:

***Dfra14* Trimethoprim Resistance Gene Associated to a Small Plasmid Is Present in Chilean *Shigella sonnei* Strains**

Author Block:

A. MIRANDA, B. ÁVILA, P. DÍAZ, L. RIVAS, K. BRAVO, J. ASTUDILLO, C. BUENO, M. ULLOA, G. HERMOSILLA, F. DEL CANTO, J. SALAZAR, **C. S. TORO**; Programa de Microbiología y Micología, ICBM - Facultad de Med., Univ. de Chile, Santiago, Chile

Abstract Body:

Background: The most common mechanism of trimethoprim (TMP)-resistance is the acquisition of dihydrofolate reductase enzyme insensitive to this drug. Previous molecular characterization of TMP-gene resistance in Chilean isolates of *S. sonnei* looking for *dfrA1* and *dfrA8*, showed solely the presence of *dfrA8* (formerly *dhfr*IIIc). However, these genetic markers were absent in *S. sonnei* strains further isolated during a nationwide outbreak in 2009.**Methods:** To identify the TMP resistance gene in these strains, a genomic DNA library from a TMP-resistant (TMP^R) *S. sonnei* representative strain for the outbreak was used to clone, select and identify a TMP-resistance marker.**Results:** The TMP^R clone was sequenced by primer walking, identifying the presence of the *dfrA14* in the *sul2-strA'-dfrA14-'strA-strB* gene arrangement, harbored in a native 6,779-bp plasmid. The same plasmid was isolated by transforming with a ~4.0 MDa plasmid from several TMP^R *S. sonnei* strains into *E. coli*. This plasmid, named pABC-3, was homologous to pCERC-1, but different due to the absence of an 11-bp repetitive unit, and present only in *dfrA14*-positive strains. The distribution of *dfrA1*, *dfrA8* and *dfrA14* TMP-resistance genes, was characterized in 126 TMP^R *S. sonnei* strains. Only one strain (0.8%) out of the 126 strains, isolated before the outbreak was negative for the three TMP resistance genes, and just 4% displayed two resistance genes. Most of the strains (95%) carried only one of the three TMP resistance gene assessed; all strains obtained during the outbreak harbored only *dfrA14*, whereas, *dfrA8* was the most abundant gene marker before outbreak and after the outbreak *dfrA1* seems have appeared in circulating strains. According PFGE, *dfrA14*-positive strains were clustered in a genetically related group including some *dfrA1*- and *dfrA8*-positive strains; meanwhile other genetic group included most of *dfrA8*-positive strains. This distribution also correlated with the isolation period, showing a dynamics of trimethoprim genetic markers prevalent in Chilean *S. sonnei* strains.**Conclusions:** To our knowledge, *dfrA14* gene associated to a small non-conjugative plasmid was detected for the first time in *Shigella*.

Author Disclosure Block:

A. Miranda: None. **B. Ávila:** None. **P. Díaz:** None. **L. Rivas:** None. **K. Bravo:** None. **J. Astudillo:** None. **C. Bueno:** None. **M. Ulloa:** None. **G. Hermosilla:** None. **F. Del canto:** None. **J. Salazar:** None. **C.S. Toro:** None.

Poster Board Number:

SUNDAY-406

Publishing Title:

Identification and Characterization of *Salmonella enterica* Serotype Newport Isolates with Decreased Susceptibility to Ciprofloxacin in the United States

Author Block:

D. Campbell[1], A. Bicknese[2], A. McCullough[2], B. Karp[1], J. Folster[1]; [1]CDC, Atlanta, GA, [2]IHRC, Atlanta, GA

Abstract Body:

Nontyphoidal *Salmonella* (NTS) causes an estimated 1.2 million illnesses, 23,000 hospitalizations, and 450 deaths each year in the United States. Resistance to fluoroquinolones, which are used to treat severe NTS infections in adults, has historically been associated with chromosomal mutations in *gyrA* and *parC* (quinolone resistance determining regions; QRDRs), but plasmid-mediated quinolone resistance (PMQR) genes have recently emerged. Since 1996, the National Antimicrobial Resistance Monitoring System at the Centers for Disease Control and Prevention (NARMS-CDC) has conducted broth microdilution susceptibility testing on *Salmonella* to determine minimum inhibitory concentrations (MIC) for up to 15 drugs. Decreased susceptibility to the fluoroquinolone ciprofloxacin (DSC), defined as MIC $\geq$ 0.12 mg/L, increased from 0.4% in 1996 to 4.3% in 2014 in NTS surveillance isolates. Approximately 20% of NTS surveillance isolates with DSC were nalidixic acid susceptible, suggesting they may have PMQRs; Newport was the second most common serotype among these isolates. To investigate and characterize DSC among *Salmonella enterica* serotype Newport, we examined 38 isolates with DSC including 37 from patients (routine surveillance and outbreak) and one isolate from food (outbreak) in the United States from 1996 through 2014. Mechanisms of resistance and resistance plasmids were identified by PCR and sequencing analysis. Pulsed-field gel electrophoresis (PFGE) analysis was used to determine genetic relationships among these isolates.Twenty-seven Newport isolates (71%) contained a PMQR gene, and all had the *qnrB* gene. No additional PMQRs genes were identified. Seven isolates (18%) contained a QRDR, and these were the S83Y, S83F, or D87G *gyrA* mutations. Four isolates (11%) had no QRDR or PMQR resistance determinants detected. Several resistance plasmids were identified among PMQR-positive isolates including ColE, IncP, IncI1, and IncA/C. Also, eight PFGE patterns were found among 26 isolates that contained a PMQR gene, suggesting that multiple plasmid transmission and not clonal expansion is involved.Additional studies are needed to determine the sources of infection and to investigate DSC mechanisms among additional NTS serotypes.

Author Disclosure Block:

D. Campbell: None. **A. Bicknese:** None. **A. McCullough:** None. **B. Karp:** None. **J. Folster:** None.

Poster Board Number:

SUNDAY-407

Publishing Title:

Tenofovir Alafenamide (Taf) Has Wide Efficacious Range for Treatment of Hiv-1 Infection: Pharmacokinetic-Pharmacodynamic (Pk-Pd) Relationships from a Phase 3 Study

Author Block:

L. S. Ting, J. Zack, M. Yan, L. Zhong, M. S. Rhee; Gilead Sci. Inc., Foster City, CA

Abstract Body:

Background: Tenofovir alafenamide (TAF) is a novel prodrug of tenofovir (TFV) that has been coformulated with emtricitabine (F) into a fixed dose combination tablet (F/TAF) as a stand-alone N(t)RTI backbone. We conducted a randomized, double blind, active controlled study in virologically suppressed HIV-1 infected patients receiving F/TDF-containing regimens to evaluate the efficacy and safety of switching from F/TDF to F/TAF vs continuing F/TDF while remaining on the same third agent. Study drugs were taken without regard to food. **Methods:** TAF area under the curve over dosing interval (AUC_{tau}) and maximum concentration (C_{max}) were estimated from sparse PK data collected across 6 study visits using an established population PK model. The efficacy endpoint for PK-PD analysis was the proportion of subjects with HIV-1 RNA <50 copies/mL at Week 48 as defined by the FDA snapshot algorithm. The percentage of virologic success at Week 48 was summarized by exposure subgroups (i.e. quartiles of TAF AUC and C_{max}).**Results:** 663 patients were randomized (1:1) and treated. Through Wk 48, high virologic success (HIV-1 RNA <50 copies/mL) was maintained in both treatment groups: F/TAF 94.3% (312/333) vs F/TDF (307/330) 93.0% (difference +1.3%, 95% CI: -2.5% to +5.1%), demonstrating noninferiority of F/TAF to F/TDF. TAF PK exposures were available for 292 F/TAF treated subjects. Mean (% coefficient of variation) TAF AUC_{tau} was 137.2 (48.1), with a wide range of 30.3 to 466.7 ng*h/mL. The rates of virologic success were uniformly high across quartiles of TAF exposures (Table). There were no apparent trends with virologic success and TAF exposure.**Conclusions:** These data demonstrate that TAF (10 mg with boosted regimen or 25 mg with unboosted regimen) was efficacious across wide ranges of exposures in virologically suppressed HIV-1 infected patients.**Table. Percentage of Virologic Success by FDA snapshot algorithm at Week 48 Across Quartiles of TAF Exposure**

Quartile	TAF AUCtau Quartile Range (ng*h/mL)	N	Virologic Success (HIV-1 RNA < 50 copies/mL)
1	30.3 to 87.6	73	93.2%
2	87.6 to 129.5	73	95.9%
3	129.8 to 173.1	73	97.3%

4	173.8 to 466.7	73	95.9%

Author Disclosure Block:

L.S. Ting: D. Employee; Self; Gilead Sciences Inc.. K. Shareholder (excluding diversitied mutual funds); Self; Gilead Sciences Inc. **J. Zack:** D. Employee; Self; Gilead Sciences Inc.. K. Shareholder (excluding diversitied mutual funds); Self; Gilead Sciences Inc. **M. Yan:** D. Employee; Self; Gilead Sciences Inc.. K. Shareholder (excluding diversitied mutual funds); Self; Gilead Sciences Inc. **L. Zhong:** D. Employee; Self; Gilead Sciences Inc.. K. Shareholder (excluding diversitied mutual funds); Self; Gilead Sciences Inc. **M.S. Rhee:** D. Employee; Self; Gilead Sciences Inc.. K. Shareholder (excluding diversitied mutual funds); Self; Gilead Sciences Inc..

Poster Board Number:

SUNDAY-408

Publishing Title:

Combination Antiretroviral (cARV) Drug Loaded Nanoparticles (NPs) Are Efficacious in Humanized (Hu-BLT) Mice

Author Block:

S. Mandal[1], G. Kang[2], Y. Yuan[2], W. Lu[2], P. K. Prathipali[1], Q. Li[2], **C. J. Destache**[1]; [1]Creighton Univ., Omaha, NE, [2]Univ. of Nebraska-Lincoln, Lincoln, NE

Abstract Body:

Background: The use of elvitegravir (EVG) + tenofovir alafenamide fumurate (TAF) + emtricitabine (FTC) along with cobicistat is a new formulation that has recent been approved for oral therapy. We are the first to report the use of EVG+TAF+FTC NP formulation for treatment of HIV-1 in humanized BLT mice.**Methods:** cARV-loaded nanoparticles (NPs) were formulated by oil-in-water (o-w) and water-in-oil-in-water (w-o-w) emulsion methodology. Poly(lactic-co-glycolic acid) (PLGA), a biodegradable/biocompatible polymer was used to formulate the TAF+EVG drug-loaded NPs using our standard (o-w) methodology. FTC loaded NPs were formulated using w-o-w emulsion method. Hu-BLT mice (n=12) with functional human immune reconstitution were randomly divided into treatment (Rx; n=6) and control (Ctr; n=6). Hu-BLT mice were intravaginally infected with transmitted/founder viruses (WITO.c/2474 and SUMA.c/2821) at 2.5 x 10^5 $TCID_{50}$ each. Two weeks later, baseline plasma was drawn for viral load (pVL). Rx group mice received 500 mg/kg (200 mg/kg each of TAF+EVG; 100 mg/kg FTC) cARV NPs by SubQ injection in 2 mL D5W starting 3 weeks after HIV-1 challenge. Four doses of cARV NPs were administered over 6 weeks. Ctr mice received 2 mL D5W. All mice had blood drawn for pVL using qRT-PCR every other week for 10 weeks. On the opposite week of pVL, blood was drawn for TAF+EVG+FTC trough drug levels and analyzed by LC-MS/MS.**Results:** TAF+EVG entrapment efficiency averaged 40 and 45% for each drug in the combination NPs and FTC entrapment averaged 62%. Baseline pVL averaged 1.2 x 10^4 $\pm$ 5.3 x 10^4 copies/mL for Rx mice and 1.7 x 10^5 $\pm$ 8.3 x 10^4 copies/mL for Ctr mice. At the end of the four doses, all Rx mice had non-detectable pVL (< 800 copies/mL) compared to Ctr mice 1.7 x 10^6 $\pm$ 7.9 x 10^5 copies/mL, ($p < 0.01$). Non-detectable viral load continued for an additional 3 weeks after the last cARV NP dose without re-dosing. All trough plasma drug levels were > 400 ng/mL for all ARV drugs.**Conclusions:** cARV NPs given SubQ demonstrate sustained release efficacy in this animal model of HIV-1.

Author Disclosure Block:

S. Mandal: None. **G. Kang:** None. **Y. Yuan:** None. **W. Lu:** None. **P.K. Prathipali:** None. **Q. Li:** None. **C.J. Destache:** None.

Poster Board Number:

SUNDAY-409

Publishing Title:

Enhanced Exposure of Tenofovir-diphosphate (Tfv-Dp) in Peripheral Blood Mononuclear Cells (Pbmc) by Tenofovir Alafenamide (Taf) Compared with Tenofovir Disoproxil Fumarate (Tdf)

Author Block:

L. S. Ting, J. Zack, M. Yan, L. Zhong, J. Ling, L. Miles, M. S. Rhee; Gilead Sci. Inc., Foster City, CA

Abstract Body:

Background: TAF is a novel prodrug of tenofovir (TFV) that has been coformulated with emtricitabine (F) into a fixed dose combination tablet (F/TAF) as an N(t)RTI backbone. We conducted a randomized, double blind, active controlled study in virologically suppressed HIV-1 infected patients receiving F/TDF-containing regimens to evaluate the efficacy and safety of switching from F/TDF to F/TAF vs continuing F/TDF while remaining on the same third agent. Study drugs were taken without regard to food. **Methods:** Patients were randomized 1:1 to switch to F/TAF or continue F/TDF while remaining on the same third agent. Randomization was stratified by the third agent [boosted protease inhibitor (F/TAF 200/10 mg) vs unboosted agent (F/TAF 200/25 mg)]. A trough blood sample was collected after 4 weeks of treatment for determination of intracellular TFV-DP in PBMCs. TFV-DP concentrations in PBMCs between F/TAF and F/TDF groups were compared and 90% confidence intervals (CI) for the ratio of the geometric least-squares means (GMR) were calculated.**Results:** 663 patients were randomized (1:1) and treated. Through Wk 48, high virologic success (HIV-1 RNA <50 c/mL) was maintained in both treatment groups: F/TAF 94.3% (312/333) vs F/TDF 93.0% (307/330). Intracellular TFV-DP concentration was measured for 304 subjects receiving F/TAF and 265 subjects receiving F/TDF. TFV-DP concentrations in the F/TAF group were consistently higher than those of the F/TDF group regardless of the third agent. The mean (% coefficient of variation) TFV-DP concentration in PBMC was 192.37 (114.1) and 44.26 (139.0) pg/million cells in the F/TAF and F/TDF group, respectively. The GMR (90% CI) was 416.1% (362.4% to 477.8%), indicating TFV-DP concentrations were >4-fold higher in the TAF group compared with the TDF group, with a range of 1.7 to 9.5-fold higher in the TAF group among various third agents.**Conclusions:** TAF provided enhanced delivery of TFV into PBMCs, resulting in > 4-fold higher intracellular levels of TFV-DP when compared to TDF. These results are consistent with the high efficacy of F/TAF seen in the study and historic data on TFV-DP exposure in PBMC with TAF 25 mg as a single agent and that of TAF 10 mg administered as the single tablet regimen elvitegravir/cobicistat/F/TAF.

Author Disclosure Block:

L.S. Ting: D. Employee; Self; Gilead Sciences Inc.. K. Shareholder (excluding diversitied mutual funds); Self; Gilead Sciences Inc. **J. Zack:** D. Employee; Self; Gilead Sciences Inc.. K. Shareholder (excluding diversitied mutual funds); Self; Gilead Sciences Inc. **M. Yan:** D. Employee; Self; Gilead Sciences Inc.. K. Shareholder (excluding diversitied mutual funds); Self; Gilead Sciences Inc. **L. Zhong:** D. Employee; Self; Gilead Sciences Inc.. K. Shareholder (excluding diversitied mutual funds); Self; Gilead Sciences Inc. **J. Ling:** D. Employee; Self; Gilead Sciences Inc.. K. Shareholder (excluding diversitied mutual funds); Self; Gilead Sciences Inc. **L. Miles:** D. Employee; Self; Gilead Sciences Inc.. K. Shareholder (excluding diversitied mutual funds); Self; Gilead Sciences Inc. **M.S. Rhee:** D. Employee; Self; Gilead Sciences Inc.. K. Shareholder (excluding diversitied mutual funds); Self; Gilead Sciences Inc..

Poster Board Number:

SUNDAY-410

Publishing Title:

Pharmacokinetic-Pharmacodynamic (Pk-Pd) of Emtricitabine/Tenofovir Alafenamide (F/Taf) Demonstrated Wide Exposure Range Associated with Clinical Safety

Author Block:

L. S. Ting, J. Zack, M. Yan, L. Zhong, M. S. Rhee; Gilead Sci. Inc., Foster City, CA

Abstract Body:

Background: Tenofovir alafenamide (TAF) is a novel prodrug of tenofovir (TFV) that has been coformulated with emtricitabine (F) into a fixed dose combination tablet (F/TAF) as an N(t)RTI backbone. We conducted a randomized, double blind, active controlled study in virologically suppressed HIV-1 infected patients receiving F/TDF-containing regimens to evaluate the efficacy and safety of switching from F/TDF to F/TAF vs continuing F/TDF while remaining on the same third agent. Study drugs were taken without regard to food. **Methods:** Patients were randomized 1:1 to switch to F/TAF or continue F/TDF while remaining on the same third agent. The TAF and TFV PK area under the curve over dosing interval (AUC_{tau}) and maximum concentration (C_{max}) were estimated via population PK analysis. The safety endpoints for PK-PD analysis were selected gastrointestinal (GI) adverse event (diarrhea, nausea, vomiting, abdominal pain, change in hip and spine bone mineral density (BMD), and change in selected lipid parameters. Subjects were grouped into quartile subgroups based on TAF and TFV exposures for evaluation of exposure-safety trends.**Results:** 663 patients were randomized and treated (F/TAF 333 vs F/TDF 330). Drug related serious adverse events were rare (0 vs 0.3%). Drug discontinuation due to adverse events (AEs) was low (2.1% vs 0.9%). In F/TAF-treated patients, TAF and TFV PK exposures were available for 292 and 328 subjects, respectively. No trends in GI AEs were observed across wide range of TAF exposures (Table). Similarly, no trends with TFV exposures were noted. The changes in BMD (hip and spine) and fasting lipids at Week 48 were comparable across TAF and TFV exposure quartiles, with no trends noted.**Conclusions:** These data demonstrate that TAF is well tolerated with no trends of safety signal across wide ranges of TAF exposures in virologically suppressed HIV-1 infected patients.**Table.Percentage of Subjects with Selected Adverse Events or Change from baseline at Week 48 by TAF AUC_{tau} Quartile Subgroup (N=73)**

Quartile range of TAF AUC_{tau} (ng*h/mL)	**1Q (30.3 to 87.6)**	**2Q (87.6 to 129.5)**	**3Q (129.8 to 173.1)**	**4Q (173.8 to 466.7)**
Diarrhea	13.7%	8.2%	9.6%	4.1%
Nausea	6.8%	2.7%	6.8%	1.4%

Vomiting	4.1%	1.4%	4.1%	4.1%
GI and abdominal pain	6.8%	1.4%	0%	6.8%
Hip BMD % change, mean (SD)	1.14 % (2.323)	1.38% (3.055)	1.31% (2.283)	0.81% (3.196)
Spine BMD % change, mean (SD)	1.28 % (3.398)	1.56% (2.994)	2.00% (3.113)	1.34% (3.319)
Change in total cholesterol, mean (SD)	11.9 mg/dL (38.35)	13.7 mg/dL (34.43)	12.6 mg/dL (27.89)	12.0 mg/dL (29.28)

Author Disclosure Block:

L.S. Ting: D. Employee; Self; Gilead Sciences Inc.. K. Shareholder (excluding diversitied mutual funds); Self; Gilead Sciences Inc. **J. Zack:** D. Employee; Self; Gilead Sciences Inc.. K. Shareholder (excluding diversitied mutual funds); Self; Gilead Sciences Inc. **M. Yan:** D. Employee; Self; Gilead Sciences Inc.. K. Shareholder (excluding diversitied mutual funds); Self; Gilead Sciences Inc. **L. Zhong:** D. Employee; Self; Gilead Sciences Inc.. K. Shareholder (excluding diversitied mutual funds); Self; Gilead Sciences Inc. **M.S. Rhee:** D. Employee; Self; Gilead Sciences Inc.. K. Shareholder (excluding diversitied mutual funds); Self; Gilead Sciences Inc..

Poster Board Number:

SUNDAY-411

Publishing Title:

Switch from Tdf Regimens to E/C/F/Taf Is Associated with Improved Bone Mineral Density, Decreased Serum Pth and Decreased Bone Turnover Biomarkers

Author Block:

E. T. Overton[1], P. Shalit[2], G. Crofoot[3], P. Benson[4], D. Murphy[5], Y-P. Liu[6], D. SenGupta[6], S. McCallister[6]; [1]The Univ. of Alabama at Birmingham Sch. of Med., Birmingham, AL, [2]Peter Shalit and Associates, Seattle, WA, [3]Gordon Crofoot MD, PA, Houston, TX, [4]Be Well Med. Ctr., Berkley, MI, [5]Clinique Med.e l'Actuel, Montreal, QC, Canada, [6]Gilead Sci., Inc., Foster City, CA

Abstract Body:

Background: HIV infection increases the risk of low bone mineral density (BMD) and fragility fracture; tenofovir disoproxil fumarate (TDF) is associated with decreased BMD. Tenofovir alafenamide (TAF) is a novel tenofovir (TFV) prodrug that reduces TFV plasma levels by 90% with less impact on BMD. We present an analysis of changes in BMD, parathyroid hormone (PTH), and serum bone turnover markers through 48 weeks in subjects switching to elvitegravir/cobicistat/emtricitabine/TAF (E/C/F/TAF) from TDF-containing regimens in Study 109. **Methods:** 1436 virologically suppressed adults on 1 of 4 TDF-containing regimens for ≥96 weeks were randomized (2:1) to E/C/F/TAF or to continue their prior regimen. Pre-specified secondary endpoints included percentage change (ANOVA) in hip and spine BMD, assessed by DEXA. Changes in serum bone biomarkers (PTH; bone procollagen type 1 N-terminal propeptide, P1NP; C-type collagen sequence, CTx) were assessed by Wilcoxon rank sum test.**Results:** Subjects switching to E/C/F/TAF had increased hip and spine BMD at 48 weeks compared to decreases in those remaining on TDF (p<0.001; Table). Median PTH decreased following switch to E/C/F/TAF, compared with increases in the TDF group. Bone turnover biomarkers decreased significantly in the E/C/F/TAF switch group. The changes in BMD and bone turnover results were consistent when stratified by pre-switch regimen, except serum CTx, for which there was no difference between the switch and the Atripla groups.**Conclusion:** Switching to E/C/F/TAF significantly improves spine and hip BMD, decreases PTH and decreases serum bone turnover biomarkers at 48 weeks. Long-term follow-up is needed to demonstrate changes in osteoporosis and fragility fracture risk.

% Changes from Baseline at Week 48			
-	**E/C/F/TAF**	**FTC/TDF + 3rd agent**	**p**
N (number of treated subjects)	959	477	
Mean (SD) **Hip BMD**	1.47 (2.71)	-0.34 (2.83)	<0.001
Mean (SD) **Spine BMD**	1.56 (3.84)	-0.44 (4.14)	<0.001

Median (Q1, Q3) **PTH**	-3.4 (-24.6, 21.7)	7.7 (-12.0, 40.0)	<0.001
Median (Q1, Q3) **P1NP**	-29.65 (-43.37, -12.97)	2.89 (-14.23, 23.34)	<0.001
Median (Q1, Q3) **CTx**	-3.1 (-16.7, 14.5)	2.9 (-10.0, 20.8)	<0.001

Author Disclosure Block:

E.T. Overton: H. Research Contractor; Self; ETO has served as the principal investigator for industry trials supported by Bavarian Nordic, ViiV and AbbVie. **P. Shalit:** C. Consultant; Self; Gilead, BMS, Merck, Janssen. I. Research Relationship; Self; Gilead, Janssen, Glaxo Smith Kline. L. Speaker's Bureau; Self; Gilead, Janssen, BMS, Merck. **G. Crofoot:** F. Investigator; Self; Jansen, Viiv, Glaxo, Pfizer, Merck, Gilead. J. Scientific Advisor (Review Panel or Advisory Committee); Self; Viiv, Gilead. **P. Benson:** None. **D. Murphy:** C. Consultant; Self; Janssen, Gilead, Abbvie, BMS, Merck, Viiv. F. Investigator; Self; Janssen, Gilead. I. Research Relationship; Self; Janssen, Gilead. J. Scientific Advisor (Review Panel or Advisory Committee); Self; Janssen, Gilead, Abbvie, BMS, Merck, Viiv. K. Shareholder (excluding diversitied mutual funds); Self; Gilead. L. Speaker's Bureau; Self; Gilead. **Y. Liu:** D. Employee; Self; Gilead Sciences, Inc. **D. SenGupta:** D. Employee; Self; Gilead Sciences, Inc. **S. McCallister:** D. Employee; Self; Gilead Sciences, Inc..

Poster Board Number:

SUNDAY-412

Publishing Title:

Three-day Per Week Atripla® in Patients with Sustained Viral Suppression

Author Block:

E. Martínez[1], J. Rojas[1], J. L. Blanco[1], S. Sanchez[1], A. Marcos[1], M. Lonca[1], B. Torres[1], A. Gonzalez-Cordon[1], A. Romero[2], J. M. Gatell[1]; [1]Hosp. Clínic and Univ. of Barcelona, Barcelona, Spain, [2]Dept. of Epidemiology and Evaluation, IMIM (Hosp. del Mar Med. Res. Inst.), Barcelona, Spain

Abstract Body:

Background: Antiretroviral drugs contained in single tablet Atripla® could allow for longer than once-daily (OD) dosing. We hypothesized that simplifying Atripla® OD to 3-day per week would be able to maintain viral suppression and less toxic. **Methods:** HIV+ adults on Atripla® OD, with HIV-1 RNA <37 copies/mL ≥2 years, CD4 >350/mm^3 , and no prior virological failure or resistance mutations to study drugs were randomized to maintain their OD regimen or to reduce it to 3 days (Mondays, Wednesdays, and Fridays) a week (3W) (ClinicalTrials.gov: NCT01778413). Plasma HIV-1 RNA was measured at baseline, 12, and 24 weeks in both arms, and also at 1, 2, 4, 6, and 8 weeks in the 3W. Pittsburg Sleep Quality Index (PSQI), body mass index (BMI), bone mineral density (BMD), CD4 and CD8 cells, ultrasensitive HIV-1 RNA (1 copy/mL), plasma 25OH vitamin D and efavirenz levels, estimated flomerular filtration rate (CPK-EPI), fasting blood lipids and urinary proteins were measured at baseline and 24 weeks. **Results:** Sixty-one patients included: 89% men, HIV-1 RNA <1 copy/mL 72%, median age 48 years, BMI 24 kg/m^2, CD4 563/mm^3, CD4/CD8 1.1, PSQI score 4, lumbar T-score -1.3, femur T-score -1.3, plasma 25OH vitamin D 18 ng/mL, plasma efavirenz 2.1 mg/L, CPK-EPI 101 mL/min, total chol 194 mg/dL, HDL chol 47 mg/dL, triglycerides 99 mg/dL, and urine protein/creatinine 75 mg/g, albumin/creatinine 4 mg/g, and beta-2-microglobulin 194 μg/g. All patients completed the study. Out of 333 plasma samples for HIV-1 RNA measurement during the study, none had ≥37 copies/mL. At 24 weeks, total chol and femur T-score significantly increased, while PSQI, plasma efavirenz, albumin/creatinine and beta-2-microglobulin in urine significantly decreased in the 3W arm relative to OD arm (table). **Conclusions:** Three-day per week Atripla® in patients with sustained viral suppression is a feasible option that should be further confirmed in larger clinical trials.

Median (IQR) changes from baseline to 24 weeks			
	Atripla OD (n=31)	Atripla 3W (n=30)	P-value for the difference between arms

Total colesterol (mg/dL)	-5 (-14 to +4)	+4 (0 to +16)	0.019
HDL cholesterol (mg/dL)	-3 (-4 to +1)	-4 (-6 to +3)	0.636
CD4 cells /mm^3	-15 (-73 to +50)	+5 (-52 to +1169	0.132
Ultrasensitive plasma HIV-1 RNA (copies/mL)	0.0 (0.0 to 0.0)	0.0 (0.0 to 0.0)	0.601
Efavirenz plama levels (mg/dL) (12h after dose, OD arm; 60 hours after dose, 3W arm)	+0.2 (0 to +0.4)	-1.3 (-1.8 to -0.8)	<0.005
25OH Vitamin D (ng/mL)	+0.1 (-7.7 to +3.7)	0 (-6.3 to +5.7)	0.630
Pittsburg Sleep Quality Index (PSQI) score	-0.5 (-1.3 to 0)	-1 (-2 to -1)	0.038
Lumbar T-score	0.0 (-0.1 to 0)	0 (-0.2 to +0.1)	0.815
Femur T-score	0.0 (-0.2 to +0.1)	+0.1 (0 to +0.1)	0.010
Urine protein/creatinine (mg/g)	-6 (-17 to +4)	-16 (-33 to -3)	0.144
Urine albumin/creatinine (mg/g)	0 (-1 to +1)	-1 (-3 to 0)	0.047
Urine beta-2-microglobulin (microg/g)	+312 (-45 to 1300)	-158 (-474 to -61)	0.003

Author Disclosure Block:

E. Martínez: E. Grant Investigator; Self; MSD. J. Scientific Advisor (Review Panel or Advisory Committee); Self; MSD, Janssen. **J. Rojas:** None. **J.L. Blanco:** E. Grant Investigator; Self; BMS, MSD. J. Scientific Advisor (Review Panel or Advisory Committee); Self; BMS, Gilead, Janssen, MSD, ViiV. **S. Sanchez:** None. **A. Marcos:** None. **M. Lonca:** None. **B. Torres:** None. **A. Gonzalez-Cordon:** None. **A. Romero:** None. **J.M. Gatell:** C. Consultant; Self; BMS, Gilead, Janssen, MSD, ViiV. E. Grant Investigator; Self; BMS, Gilead, Janssen, MSD, ViiV. J. Scientific Advisor (Review Panel or Advisory Committee); Self; BMS, Gilead, Janssen, MSD, ViiV.

Poster Board Number:

SUNDAY-413

Publishing Title:

GS-9883, a Novel HIV-1 Integrase Strand Transfer Inhibitor (INSTI) with Optimized *In Vitro* Resistance Profile

Author Block:

G. Jones, J. Goldsmith, A. Mulato, K. White, D. Hansen, K. Stray, S. Yant, S. Lazerwith, H. Jin, T. Cihlar, M. Tsiang; Gilead Sci., Inc., Foster City, CA

Abstract Body:

Background: GS-9883 is a potent once-daily unboosted INSTI in clinical development combined with tenofovir alafenamide (TAF) and emtricitabine (FTC) for the treatment of HIV-infection.**Methods:** HIV-1 site-directed mutants with either NRTI, NNRTI, PI, or INSTI resistance mutations as well as a panel of 18 clonal and 47 patient-derived HIV-1 isolates with high-level INSTI resistance were profiled for susceptibility to GS-9883. *In vitro* resistant variants were selected with escalating doses of GS-9883, dolutegravir (DTG), and elvitegravir (EVG), and emergent HIV-1 variants were genotyped and phenotyped. *In vitro* resistance breakthrough studies using clinically relevant fixed doses of GS-9883, EVG and DTG were conducted in MT-2 cells and primary human CD4+ T-cells.**Results:** GS-9883 showed potent antiviral activity (EC_{50} = 1.7 to 3.2 nM) against wild-type virus and mutants resistant to NRTIs, NNRTIs, and PIs. A panel of HIV-1 site-directed mutants with up to 1520-fold change (FC) in susceptibilities to raltegravir (RAL) and EVG remained largely susceptible to GS-9883 (FC = 1 to 9). In addition, GS-9883 had an improved resistance profile (FC = 0.5 to 19; mean and median FC = 2.8 and 2.0, resp.) compared to DTG (FC = 0.64 to 63; mean and median FC = 5.8 and 3.4, resp.) against INSTI-resistant patient-derived isolates, particularly those with high-level INSTI-resistance. Both GS-9883 and DTG displayed a higher barrier to *in vitro* resistance emergence relative to EVG. HIV-1 variants selected *in vitro* by GS-9883 contained the known R263K and M50I integrase mutations and exhibited low level cross-resistance to RAL and DTG (FC = 3 to 8), and intermediate cross-resistance to EVG (FC = 15 to 26) but remained susceptible to other antiretroviral classes. In the viral breakthrough selection studies at clinically relevant concentrations, GS-9883 and DTG, but not EVG or RAL completely suppressed resistance breakthrough in both MT-2 and primary CD4+ T cells.**Conclusions:** GS-9883 is a novel potent INSTI with an *in vitro* resistance profile that is improved compared to RAL and EVG. In addition, GS-9883 was more potent than DTG against a number of patient-derived isolates with high-level INSTI resistance. Overall, these data support further development of GS-9883 as a novel antiretroviral agent.

Author Disclosure Block:

G. Jones: None. **J. Goldsmith:** None. **A. Mulato:** None. **K. White:** None. **D. Hansen:** None. **K. Stray:** None. **S. Yant:** None. **S. Lazerwith:** None. **H. Jin:** None. **T. Cihlar:** None. **M. Tsiang:** None.

Poster Board Number:

SUNDAY-414

Publishing Title:

Discovery of Gs-9883, an Hiv-1 Integrase Strand Transfer Inhibitor (Insti) with Improved Pharmacokinetics and *In Vitro* Resistance Profile

Author Block:

S. Lazerwith[1], R. Cai[1], X. Chen[1], G. Chin[1], M. Desai[1], S. Eng[1], R. Jacques[1], M. Li[1], G. Jones[1], H. Martin[1], C. McMahon[1], M. Mish[1], P. Morganelli[1], J. Mwangi[1], H-J. Pyun[1], G. Stepan[1], J. Szwarcberg[1], J. Tang[1], M. Tsiang[1], J. Wang[1], K. Wang[1], K. White[1], L. Wiser[2], J. Zack[1], H. Jin[1]; [1]Gilead Sci., Foster City, CA, [2]Fibrogen, San Francisco, CA

Abstract Body:

Background: Currently approved INSTI based treatments comprise 5 of 6 DHHS recommended regimens for HIV-1 infection. Limitations of early INSTIs include twice-daily dosing (RAL) and the need for a booster (EVG). DTG exhibits an improved resistance profile compared to RAL and EVG. However, due to dose-limiting exposure above 50 mg, it requires twice-daily dosing in some patients. GS-9883 is a potent, new INSTI with a longer half-life and an improved resistance profile. **Methods:** New INSTIs were synthesized and tested for a wide variety of properties including HIV potency (MT-4 and MT-2 cells), metabolic stability (microsomes), and protein binding (equilibrium dialysis). **Results:** Tetracyclic pyridones containing rigid, sterically-demanding ring systems were synthesized. These tetracycles are potent INSTIs and unexpectedly showed reduced PXR activation. Further work led to the optimization of potency, protein binding, metabolic stability, solubility and an improved resistance profile. This resulted in GS-9883, a potent INSTI (MT-4 EC_{50} = 2.4 nM) with a low potential for drug-drug interactions. GS-9883 shows a remarkable combination of both improved preclinical pharmacokinetics (rat MRT = 45.7 h) and an enhanced resistance profile compared to approved agents (2-fold less susceptible to G140S/Q148R vs. WT). An unusually strong relationship between plasma protein binding and in vivo clearance in preclinical species will be detailed, and the structure of GS-9883 will be disclosed.**Conclusions:** Due to its excellent preclinical PK and its in-vitro potency against many integrase-resistant strains, GS-9883 was selected for development, and is being tested in Phase III studies combined with F/TAF.

Author Disclosure Block:

S. Lazerwith: D. Employee; Self; Gilead Sciences. **R. Cai:** D. Employee; Self; Gilead Sciences. **X. Chen:** D. Employee; Self; Gilead Sciences. **G. Chin:** D. Employee; Self; Gilead Sciences. **M. Desai:** D. Employee; Self; Gilead Sciences. **S. Eng:** D. Employee; Self; Gilead Sciences. **R. Jacques:** D. Employee; Self; Gilead Sciences. **M. Li:** K. Shareholder (excluding diversitied mutual funds); Self; Gilead Scientist. **G. Jones:** D. Employee; Self; Gilead Scientist. **H. Martin:** D. Employee; Self; Gilead Sciences. **C. McMahon:** D. Employee; Self; Gilead

Sciences. **M. Mish:** D. Employee; Self; Gilead Sciences. **P. Morganelli:** D. Employee; Self; Gilead Sciences. **J. Mwangi:** D. Employee; Self; Gilead Sciences. **H. Pyun:** D. Employee; Self; Gilead Sciences. **G. Stepan:** D. Employee; Self; Gilead Sciences. **J. Szwarcberg:** D. Employee; Self; Gilead Sciences. **J. Tang:** D. Employee; Self; Gilead Sciences. **M. Tsiang:** D. Employee; Self; Gilead Sciences. **J. Wang:** D. Employee; Self; Gilead Sciences. **K. Wang:** D. Employee; Self; Gilead Sciences. **K. White:** D. Employee; Self; Gilead Sciences. **L. Wiser:** K. Shareholder (excluding diversitied mutual funds); Self; Gilead Sciences. **J. Zack:** D. Employee; Self; Gilead Sciences. **H. Jin:** D. Employee; Self; Gilead Sciences.

Poster Board Number:

SUNDAY-415

Publishing Title:

Novel Insti Gs-9883 10 Day Monotherapy in Hiv-1 Infected Subjects

Author Block:

J. Gallant[1], M. Thompson[2], T. Mills[3], E. DeJesus[4], G. Voskuhl[5], X. Wei[6], J. Zack[6], K. White[6], H. Martin[6], J. Szwarcberg[6]; [1]Southwest CARE Ctr., Santa Fe, NM, [2]AIDS Res. Consortium of Atlanta, Atlanta, GA, [3]Southern California Men's Med. Group, Los Angeles, CA, [4]Orlando Immunology Ctr., Orlando, FL, [5]AIDS Arms, Inc., Dallas, TX, [6]Gilead Sci., Foster City, CA

Abstract Body:

Background: Integrase strand transfer inhibitor (INSTI)-based regimens comprise 5 of the 6 DHHS recommended regimens for treatment of HIV-1. GS-9883 is a novel, unboosted INSTI with potent in-vitro activity against HIV-1. **Methods:** HIV-1-positive INSTI-naïve subjects were randomized to receive GS-9883 5, 25, 50, or 100 mg, or placebo once daily for 10 days. Primary endpoint was time-weighted average HIV-1 RNA change from baseline on day 11 ($DAVG_{11}$). Mean change in HIV-1 RNA, PK, and safety and laboratory data were analyzed. **Results:** 20 subjects received study treatment and completed the study. Most were male; median age was 29 years; median HIV-1 RNA was 4.32 $\log_{10}$ copies/mL. GS-9883 demonstrated a dose-dependent reduction in HIV-1 RNA, with increased exposures correlating with greater reductions in plasma HIV-1 RNA, up to 2.43 $\log_{10}$ copies/mL at Day 11 (Table). Response was durable through day 17 in the higher dose groups. PK/PD analysis suggested that near maximal efficacy was associated with GS-9883 exposures achieved at doses between 50 and 100 mg. No primary resistance mutations emerged. No serious adverse events (AEs) or clinically significant lab abnormalities were reported; there were no discontinuations due to AEs. **Conclusions:** Daily dosing of GS-9883 for 10 days at 5, 25, 50 and 100 mg was well tolerated and resulted in rapid, dose-dependent decreases in HIV-1 RNA of > 2 log at the higher doses. Based on PK/PD analyses, exposures associated with 75 mg dose of single agent GS-9883 would provide near-maximal virologic response, with a predicted $_{pa}IQ_{95}^{a}$ for wild type HIV of ~20.

	GS-9883 5 mg (n=3)	GS-9883 25 mg (n=4)	GS-9883 50 mg (n=4)	GS-9883 100 mg (n=4)
Δ HIV-1 RNA Day 11 from baseline (log10 copies/mL) Mean (SD)	-1.45 (0.097)	-2.08 (0.209)	-2.06 (0.345)	-2.43 (0.386)
$DAVG_{11}$ (log10 copies/mL) Mean (SD)	-0.92 (0.104)	-1.33 (0.174)	-1.37 (0.310)	-1.61 (0.256)

AUC_{tau} (ng*h/mL) Mean (%CV)	9983.0 (26.7)	48,950.3 (40.0)	87, 538.4 (32.7)	178,901.7 (17.8)
C_{tau} (ng/mL) Mean (%CV)	225.3 (37.5)	1052.3 (54.1)	2053.0 (47.6)	4520.0 (21.9)
t ½ (h) (median, Q1, Q3)	20.79 (17.15, 23.80)	15.86 (14.07, 19.37)	17.84 (15.50, 20.51)	20.88 (17.91, 24.47)
$_{pa}$IQ 95[a] Median (range)	1.3 (0.9 - 2.1)	4.9 (4.4 - 11.7)	13.4 (5.3 - 18.6)	25.9 (23.0 - 36.9)

[a]The protein-adjusted IQ95 value is estimated based on steady state C_{tau} values and the in vitro $_{pa}$IC95 value for wild type HIV-1 (162 ng/mL)

Author Disclosure Block:

J. Gallant: E. Grant Investigator; Self; Gilead, Abbievie, BMS, Janssen, Merck, Sangamo BioSciences, Viiv. J. Scientific Advisor (Review Panel or Advisory Committee); Self; BMS, Gilead, Janssen, Merck, Viiv. **M. Thompson:** H. Research Contractor; Self; Gilead Sciences, BMS, GeoVax, Kowa Research Institute, Merck, Pfizer, Tobira, Viiv, Janssen. **T. Mills:** E. Grant Investigator; Self; Gilead Sciences, Viiv, Merck, BMS, Janssen. J. Scientific Advisor (Review Panel or Advisory Committee); Self; Gilead, Viiv, Merck, Janssen. **E. DeJesus:** E. Grant Investigator; Self; Gilead, Janssen. J. Scientific Advisor (Review Panel or Advisory Committee); Self; Gilead, Janssen. **G. Voskuhl:** H. Research Contractor; Self; Gilead Sciences. **X. Wei:** D. Employee; Self; Gilead Sciences. **J. Zack:** D. Employee; Self; Gilead Sciences. **K. White:** D. Employee; Self; Gilead Sciences. **H. Martin:** D. Employee; Self; Gilead Sciences. **J. Szwarcberg:** D. Employee; Self; Gilead Sciences.

Poster Board Number:

SUNDAY-416

Publishing Title:

Antiviral Activity of GS-9883, a Potent Next Generation HIV-1 Integrase Strand Transfer Inhibitor

Author Block:

M. Tsiang, E. Kan, L. Tsai, G. Jones, R. Phadke, G. Stepan, N. Novikov, S. Eng, G. Lee, R. Gong, E. Aguayo, S. Ahmadyar, Y. Xu, A. Niedziela-Majka, S. Yant, H. Yu, C. Voitenleitner, G. Birkus, M. Perron, J. Feng, S. Lazerwith, H. Jin, T. Cihlar; Gilead Sci., Inc., Foster City, CA

Abstract Body:

Background: GS-9883 is a potent once-daily unboosted integrase strand transfer inhibitor currently in clinical development in combination with tenofovir alafenamide (TAF) and emtricitabine (FTC) for the treatment of HIV-1 infection.**Methods:** The inhibitory activity of GS-9883 was tested against wild-type HIV-1 integrase enzyme. Antiviral potency and cytotoxicity were assessed in MT-2 and MT-4 T-cell lines and primary human CD4+ T-cells and macrophages. Inhibition of integration was assessed by qPCR of 2-LTR circles and integration junctions. A panel of 14 HIV-1 and one HIV-2 clinical isolates was used for antiviral profiling. Antiviral activity of GS-9883 was also tested against several non-HIV viruses. Cytotoxicity of GS-9883 was also tested in four non-target cell lines (Huh-7, HepG2, PC-3, MRC-5) and human primary hepatocytes. Antiviral activity of GS-9883 in pairwise combination with antiretrovirals was also assessed.**Results:** GS-9883 inhibited HIV-1 integrase enzyme strand transfer activity (IC_{50} = 7.5 ± 0.3 nM). Treatment with GS-9883 increased abortive 2-LTR circles and decreased viral-host DNA integration junctions in infected cells. GS-9883 exhibited high potency and selectivity in HIV-1 assays using lymphoblastoid T-cell lines, primary human CD4+T cells, and macrophages with EC_{50} range of 1.5 to 6.6 nM and selectivity indices of 1500 to 8700. GS-9883 was highly potent against all tested HIV-1 subtypes and HIV-2 in human PBMCs (mean EC_{50} = 0.81 nM range of <0.05 to 1.71 nM), and showed no activity against HBV, HCV, Influenza, HRV or RSV. GS-9883 had low cytotoxicity in multiple human cell lines (CC_{50} range from 35 to >44 μM) and in primary human hepatocytes (CC_{50} >100 μM). Highly synergistic *in vitro* antiviral effect was observed for combinations of GS-9883 with TAF, FTC or darunavir.**Conclusions:** GS-9883 is a novel, potent and selective HIV integrase strand transfer inhibitor. GS-9883 was highly synergistic in combinations with TAF, FTC or darunavir. These data support the clinical investigation of GS-9883 for the treatment of HIV-1 infection.

Author Disclosure Block:

M. Tsiang: None. **E. Kan:** None. **L. Tsai:** None. **G. Jones:** None. **R. Phadke:** None. **G. Stepan:** None. **N. Novikov:** None. **S. Eng:** None. **G. Lee:** None. **R. Gong:** None. **E. Aguayo:** None. **S. Ahmadyar:** None. **Y. Xu:** None. **A. Niedziela-Majka:** None. **S. Yant:** None. **H. Yu:**

None. **C. Voitenleitner:** None. **G. Birkus:** None. **M. Perron:** None. **J. Feng:** None. **S. Lazerwith:** None. **H. Jin:** None. **T. Cihlar:** None.

Poster Board Number:

SUNDAY-417

Publishing Title:

Appropriateness of Nrti Dosing During Cvvh at a Large Academic, Medical Center

Author Block:

M. M. McLaughlin[1], I. Masic[1], L. Gerzenshtein[2]; [1]Midwestern Univ., Downers Grove, IL, [2]Northwestern Med. Specialty Pharmacy, Chicago, IL

Abstract Body:

Background: HIV-infected patients may experience renal complications due to the aging process, co-morbid conditions, HIV infection itself, antiretroviral therapy, or a combination of all these factors and require renal replacement therapy. Nucleoside reverse transcriptase inhibitors (NRTIs) must be appropriately dose adjusted for continuous veno-venous hemofiltration (CVVH), due to renal elimination, as higher doses may lead to toxicity and lower doses may lead to decreased drug efficacy. This study sought to determine the percentage of patients that received appropriate doses of NRTIs during CVVH. **Methods:** Patients were considered for inclusion if they were >18 years old, HIV-infected, received an NRTI with concurrent CVVH therapy, and hospitalized from 9/1/2010 through 9/30/2013 at Northwestern Memorial Hospital. Patient demographic information, CVVH characteristics, hospital length of stay, NRTIs, dose of NRTIs, and NRTI dose adjustments were collected. A peer-reviewed dosing table based on pharmacokinetic mathematical calculations was used to determine the percentage of patients that received the appropriate NRTI dose during CVVH. **Results:** A total of 12 patients were included in this study. The median age was 51 years (interquartile range (IQR) 45-52), 11 (92%) patients were male, and 5 (42%) were Caucasian. The median CD4 count and HIV viral load were 174 cells/mm^3 (IQR 87-338) and 44 (IQR 20-48) IU/mL, respectively. Kidney injury (n=2), kidney failure (n=4), and oliguria (n=6) were the indications for CVVH. The median length of CVVH was 6 days (IQR 3-9.25) and median hospital stay was 27 days (IQR 19-31). The 12 patients were on a total of 27 NRTIs (abacavir n=2; emtricitabine n=6; lamivudine n=7; tenofovir n=10; zidovudine n=2). During CVVH therapy 10 (83%) patients had at least one NRTI dose adjustment. Overall, 11 (41%) of NRTIs were dosed correctly. Of the 16 incorrect dosing regimens, 9 (56%) doses were too high and 7 (44%) doses were too low. **Conclusion:** Over half of NRTI doses received during CVVH were incorrect based on pharmacokinetic mathematical calculations. Interventions are needed to alert ID specialists when a patient receiving NRTIs is started on CVVH to aid with appropriate dosing. Future studies with pharmacokinetic sampling are needed to further clarify appropriate dosing of NRTIs during CVVH.

Author Disclosure Block:

M.M. McLaughlin: None. **I. Masic:** None. **L. Gerzenshtein:** None.

Poster Board Number:

SUNDAY-418

Publishing Title:

An Improved Circular Consensus Algorithm to Detect Hiv-1 Drug-Resistance Associated Mutations (Drams)

Author Block:

M. L. Smith, M. P. S. Brown, N. Delany, N. L. Hepler, D. Alexander, E. E. Paxinos; PacBio, Menlo Park, CA

Abstract Body:

Scientists who require confident resolution of heterogeneous populations across complex regions have been unable to transition to short-read sequencing methods. They continue to depend on Sanger sequencing despite its cost and time inefficiencies. Here we present a new redesigned algorithm that allows the generation of circular consensus sequences (CCS) from individual SMRT® Sequencing reads. With this new algorithm, dubbed CCS2, it is possible to reach high quality across longer insert lengths at a lower cost and higher throughput than Sanger sequencing. We applied CCS2 to the characterization of the HIV-1 K103N drug-resistance associated mutation in both clonal and patient samples. This particular DRAM has previously proved to be clinically relevant, but challenging to characterize due to regional sequence context.First, a mutation was introduced into the 3rd position of amino acid position 103 (A>C substitution) of the RT gene on a pNL4-3 backbone by site-directed mutagenesis. Regions spanning ~1.3 kb were PCR amplified from both the non-mutated and mutant (K103N) plasmids, and were sequenced individually and as a 50:50 mixture. Additionally, the proviral reservoir of a subject with known dates of virologic failure of an Efavirenz-based regimen and with documented emergence of drug resistant (K103N) viremia was sequenced at several time points as a proof-of-concept study to determine the kinetics of retention and decay of K103N.Sequencing data were analyzed using the new CCS2 algorithm, which uses a fully-generative probabilistic model of our SMRT Sequencing process to polish consensus sequences to high accuracy. With CCS2, we are able to achieve a per-read empirical quality of QV30 (99.9% accuracy) at 19X coverage. A total of ~5000 1.3 kb consensus sequences with a collective empirical quality of ~QV40 (99.99%) were obtained for each sample. We demonstrate a 0% miscall rate in both unmixed control samples, and estimate a 48:52 frequency for the K103N mutation in the mixed (50:50) plasmid sample, consistent with data produced by orthogonal platforms. Additionally, the K103N escape variant was only detected in proviral samples from time points subsequent (19%) to the emergence of drug resistant viremia. This tool might be used to monitor the HIV reservoir for stable evolutionary changes throughout infection.

Author Disclosure Block:

M.L. Smith: D. Employee; Self; PacBio. **M.P.S. Brown:** D. Employee; Self; PacBio. **N. Delany:** D. Employee; Self; PacBio. **N.L. Hepler:** D. Employee; Self; PacBio. **D. Alexander:** D. Employee; Self; PacBio. **E.E. Paxinos:** D. Employee; Self; PacBio.

Poster Board Number:

SUNDAY-419

Publishing Title:

Renal and Bone Safety with Tenofovir Disoproxil Fumarate-Containing Single Tablet Regimens

Author Block:

E. T. Nkhoma[1], L. Rosenblatt[2], J. Myers[2], A. Villasis-Keever[2], J. Coumbis[3]; [1]Bristol-Myers Squibb, Wallingford, CT, [2]Bristol-Myers Squibb, Plainsboro, NJ, [3]Bristol-Myers Squibb, Hopewell, NJ

Abstract Body:

Objective: To estimate real-world incidence rates (IRs) of renal and bone adverse events (AEs) among patients on tenofovir disoproxil fumarate (TDF)-containing single tablet regimens (STRs). **Methods:** From U.S. health insurance data spanning 2008-2014, we identified HIV-infected patients age ≥18 years and those with ≥6 months of continuous enrolment prior to initiating efavirenz/emtricitabine/TDF (EFV/FTC/TDF), rilpivirine/FTC/TDF (RPV/FTC/TDF) or elvitegravir/cobicistat/FTC/TDF (EVG/c/FTC/TDF). We captured renal events using all renal ICD-9-CM diagnosis codes but excluding nephrolithiasis and infections. We captured bone events in one of two ways: 1) using fracture ICD-9-CM diagnosis codes; and 2) using fracture diagnosis codes or fills for bone-related medications. IRs and associated 95% confidence intervals (CIs) were estimated assuming a Poisson distribution and outcomes between STRs were compared using IR ratios (IRRs) and IR differences (IRDs). **Results;** Observed IRs for renal AEs and fracture varied by STR (Table). The IR for renal events with EFV/FTC/TDF was significantly lower than with EVG/c/FTC/TDF (IRD −3.96; 95% CI: −7.31, −1.06). The IR for fracture with EFV/FTC/TDF was significantly lower than with RPV/FTC/TDF (IRD −1.95; 95% CI: −2.66, −1.31) or than with EVG/c/FTC/TDF (IRD −1.95; 95% CI: −2.89, −1.13). There were no STR differences for the second bone outcome. IRRs showed no differences in any analysis. **Conclusion:** In this large real-world database, observed IRs for renal adverse outcomes and fracture with TDF-containing STRs were lower or similar to those for all HIV patients, with the lowest IRs observed among patients on EFV/FTC/TDF.

PY = person-years IR = incidence rate/1000 PY	**EFV/FTC/TDF**	**RPV/FTC/TDF**	**EVG/c/FTC/TDF**	**All HIV patients**
Renal adverse outcomes				
N	8107	1017	752	126,168
Cases/PY	219/22,677	19/1812	14/1028	5704/317,712

IR (95% CI)	9.7 (8.5, 11.0)	10.5 (6.7, 16.4)	13.6 (8.1, 23.0)	18.0 (17.5, 18.4)
Fracture				
N	8335	1039	773	128,517
Cases/PY	43/23,321	7/1847	4/1054	1209/378,212
IR (95% CI)	1.8 (1.4, 2.5)	3.8 (1.8, 8.0)	3.8 (1.4, 10.1)	3.2 (3.0, 3.4)
Fracture or bone medication				
N	8293	1032	767	124,448
Cases/PY	97/23,186	7/1836	5/1047	2076/356,508
IR (95% CI)	4.2 (3.4, 5.1)	3.8 (1.8, 8.0)	4.8 (2.0, 11.5)	5.8 (5.6, 6.1)

Author Disclosure Block:

E.T. Nkhoma: D. Employee; Self; Bristol-Myers Squibb. K. Shareholder (excluding diversitied mutual funds); Self; Bristol-Myers Squibb. **L. Rosenblatt:** D. Employee; Self; Bristol-Myers Squibb. K. Shareholder (excluding diversitied mutual funds); Self; Bristol-Myers Squibb. **J. Myers:** D. Employee; Self; Bristol-Myers Squibb. K. Shareholder (excluding diversitied mutual funds); Self; Bristol-Myers Squibb. **A. Villasis-Keever:** D. Employee; Self; Bristol-Myers Squibb. K. Shareholder (excluding diversitied mutual funds); Self; Bristol-Myers Squibb. **J. Coumbis:** D. Employee; Self; Bristol-Myers Squibb. K. Shareholder (excluding diversitied mutual funds); Self; Bristol-Myers Squibb.

Poster Board Number:

SUNDAY-420

Publishing Title:

Emergence of HIV-1 Drug Resistance Mutations in Mothers on Treatment with a History of Prophylaxis in Ghana

Author Block:

A. Martin-Odoom[1], E. Delgado[2], M. Lartey[3], T. Adiku[4], W. K. Ampofo[5]; [1]Sch. of BioMed. & Allied Hlth.Sci.,Univ. of Ghana, Accra, Ghana, [2]Univ. of Biology and Variability of HIV, Inst. de Salud Carlos III,, Madrid, Spain, [3]Sch. of Med. and Dentistry,Coll. of Hlth.Sci.,Univ. of Ghana,, Accra, Ghana, [4]Sch. of BioMed. & Allied Hlth.Sci.,Univ. of Ghana, Korle Bu, Accra, Ghana, [5]Noguchi Mem. Inst. for Med. Res.,Coll. of Hlth.Sci., Univ. of Ghana, Legon, Accra, Ghana

Abstract Body:

Antiretroviral therapy (ART) has been available in Ghana since 2003 for HIV-1 positive pregnant women for prevention of mother-to-child transmission (PMTCT) of HIV.For such mothers with a history of prophylaxis for PMTCT, suboptimal responses encountered made it imperative to investigate the pattern of viral mutations generated since it could adversely affect the treatment response.This study investigated HIV-1 drug resistance profiles in women in the PMTCT programme in selected centres in Ghana who were on treatment after a previous exposure to prophylaxis.Genotypic Drug Resistance Testing for HIV-1 was carried out. Subtyping was done by phylogenetic analysis.Participants who had prophylaxis before ART, those who had ART without prophylaxis and those yet to initiate PMTCT showed 32% (8), 5% (3) and 15% (4) HIV-1 drug resistance associated mutations respectively. Thirty-five percent (35%) had resistance associated mutations (RAMs) to nucleoside reverse transcriptase inhibitors (NRTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs) and 1 (3%) had RAMs to the Protease Inhibitors. The most common NRTI mutation found was M184V; K103N and A98G were the most common NNRTI mutations seen. Thymidine Analogue Mutations (TAMs) such as M41L, D67N, K70R, L210W, T215Y/F and K219E were found in all the groups; the most common of the TAMs found was M41L and T215Y. I84V, a major resistance mutation to Protease Inhibitors (PIs), was seen. For the Reverse Transcriptase gene, 33(82%) of samples were of subtypes CRF02_AG, 2(5%) were subtype CRF01_AE, 1(3%) was subtype A, 2 (5%) were subtype B and 2 (5%) were subtype G. With the Protease gene, 32 samples (97%) were subtype CRF02_AG and 1(3%) was subtype A.The study established that in Ghana initiation of uninterrupted treatment upon diagnosis coupled with drug resistance testing would help to produce a better treatment outcome for Ghanaian HIV-1 positive mothers and pregnant women.

Author Disclosure Block:

A. Martin-Odoom: None. **E. Delgado:** None. **M. Lartey:** None. **T. Adiku:** None. **W.K. Ampofo:** None.

Poster Board Number:

SUNDAY-421

Publishing Title:

Correlation of Residual Viremia on Clinical Outcomes and Lipodystrophy Markers in the Kreta Study

Author Block:

J. E. McKinnon[1], J. I. Bernardino[2], J. Y. Zhou[1], F. Pulido[3], R. Delgado Vasquez[3], D. Lucic[4], J. R. Arribas[2]; [1]Henry Ford Hosp., Detroit, MI, [2]Hosp. La Paz. IdiPAZ, Madrid, Spain, [3]Hosp. Univ.rio 12 de Octubre, i+12, Madrid, Spain, [4]Abbott Molecular, Des Plaines, IL

Abstract Body:

Background: The KRETA trial enrolled lipoatrophic (LD) patients on triple nucleoside therapy who were randomized to lopinavir/ritonavir alone (MT) or to LPV/r plus abacavir/lamivudine (TT), to determine the impact of the regimens on LD. We examined the correlations between the randomized regimens and HIV residual viremia (RV) and between RV and LD markers. **Methods:** For RV quantification, we used two modified assays of the Abbot RealTime HIV assay (MA). The ultracentrifugation MA (UMA) assay spins at 170,000xg for 30 minutes to separate the viral pellet. The tabletop centrifugation (TMA) assay uses a new extraction protocol for the *m*2000sp to allow use of 3mL volumes, and a 2 hour 21,000xg centrifugation of the sample. TMA allows for handling of highly lipemic samples. Study plasma samples and data were obtained. Only patients with 4 or more 3mL plasma samples available were analyzed.**Results:** 71 patients were tested using the MA assays. RV was detected in 51 patients and 20 had undetectable samples by MA assays, with only 2 having any detectable clinical viremia (VL) during the study. RV correlated with VL ($p<0.00$), RV and median RV correlated with VL> 50c/ml ($p=0.001$, $p<0.00$). The MT arm trended to have detectable RV a median of 5.5 weeks prior to VL> 50 c/mL, more frequently detectable RV ($p=0.059$), more patients with a VL> 50c/mL ($p=0.014$), and higher RV level ($p=0.001$) than TT patients. In suppressed patients, RV levels were similar across study arms ($p=0.184$). RV detection was not impacted by age, CD4 counts, BMI, HCV status, number of prior ART regimens or duration of HIV diagnosis. Baseline and changes in Fat-Mass ratio (FMR), truncal fat and other LD markers, were not correlated with RV levels. Women had higher FMR levels than men ($p=0.021$) but gender did not impact by RV levels ($p=0.71$).**Conclusions:** RV detection using the modified Abbott assays correlated well with clinical HIV RNA levels and VL> 50 c/mL. RV detection was more prevalent and higher in patients on MT than TT regimens, but suppressed patients had similar levels. RV levels were not impacted by baseline clinical characteristics or LD markers and did not correlate with changes in LD measurements during the study.

Author Disclosure Block:

J.E. McKinnon: E. Grant Investigator; Self; Abbott Molecular. J. Scientific Advisor (Review Panel or Advisory Committee); Self; Gilead Sciences. L. Speaker's Bureau; Self; Abbott Molecular. **J.I. Bernardino:** None. **J.Y. Zhou:** None. **F. Pulido:** None. **R. Delgado Vasquez:** E. Grant Investigator; Self; Abbott, Beckman Coulter, Gilead Sciences, Roche, ViiV Healthcare. **D. Lucic:** D. Employee; Self; Abbott Molecular. **J.R. Arribas:** None.

Poster Board Number:

SUNDAY-422

Publishing Title:

Biofilm Formation by *Candida tropicalis* and Efficacy of Liposomal Amphotericin B for Prevention of Biofilm Development

Author Block:

Y. Yamagishi, A. Kawai, H. Mikamo; Aichi Med. Univ., Aichi, Japan

Abstract Body:

Background: There are an increasing number of cases of infection with non-*albicans Candida*. One of the major non-*albicans* fungi, *Candida tropicalis*, is likely to form biofilms, which cause a poor prognosis. This study was conducted to examine the process of biofilm formation by *C. tropicalis* and the antifungal activity of liposomal amphotericin B (LAB) against biofilm-forming strains, using time-lapse imaging. **Methods:** *C. tropicalis* was inoculated on a silicone tube and media were incubated during perfusion to reproduce biofilm formation in a catheter in clinical practice, with shaking for 90 min to promote adherence of fungi. The silicone tube was incubated at 37°C with perfusion of 5% serum with Sabouraud glucose agar and imaged by time-lapse photography. Biofilm formation was confirmed by observation of a fungi mass on the silicone tube and fluorescent staining with Fin-1 and concanavalin A.**Results:** After 14 h, *C. tropicalis* grew with gas generation, probably because of the high rate of biofilm formation and metabolic activity. After 17 h, the whole tube was covered with thick biofilms of *C. tropicalis*. LAB caused marked degeneration of *C. tropicalis* before biofilm formation and inhibited expansion of mycelia, resulting in blocking of biofilm formation. At 6 h after addition of LAB, the antifungal activity of LAB had reduced the fungi level to below that of the untreated control.**Conclusions:** This is the first study to examine the *in vitro* activity of LAB against *C. tropicalis* biofilms. The results suggest that LAB may be effective for treatment of infections caused by catheter-related non-*albicans* biofilms. This finding requires confirmation in studies including a large number of *C. tropicalis* strains.

Author Disclosure Block:

Y. Yamagishi: None. **A. Kawai:** None. **H. Mikamo:** None.

Poster Board Number:

SUNDAY-423

Publishing Title:

Enhanced Activity of Carbon Nanotubes-Amphotericin B Conjugates Against Mature Biofilms of *Candida albicans*

Author Block:

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Abstract Body:

Background: Amphotericin B (AmB) is commonly used against *Candida* biofilms; however, its application is limited by its toxicity and poor solubility. *Candida* biofilms are resistant to traditional antifungal treatment therefore the need for alternative and new therapeutic approach is urgent. Multiwall carbon-nanotubes (MWNTs) are novel nanomaterials with unique physical properties that can be used to deliver therapeutic agents to the targeted site of the pathogen. Our aim was to evaluate the antifungal activity of AmB conjugated to MWNTs against *Candida albicans* mature BF vs. that by AmB alone. **Methods:** MWNTs were functionalized with poly-ethylene-glycol (PEG) and then chemically linked to AmB by sonication. The stability behavior of the MWNTs-amphotericin B conjugates was evaluated with the zeta potential. Stocks of two BF-producing *C. albicans* strains were grown in YNB medium. For mature-BF formation, 10^6 blastoconidia/mL were added to 96-well plates and incubated at 37oC for 48h. BFs were treated with AmB-PEG-CNTs or AmB at concentrations from 0.007 to 16mg/L for 24 hours. PEG-CNTs, non-functionalized CNTs, were used as controls. Percent BF damage (%BD) was assessed by XTT assay. MICs of BF were determined as the minimum antifungal concentration that caused ≥50% BF damage compared to untreated BF. **Results:** The conjugate efficiency of AmB-MWNTs was 90%, while the zeta potential (a measure of stability behaviour) was -36.1mV. At concentrations of 16mg/L, PEG-MWNTs and pure MWNTs showed minimal antifungal activity against BF of both *Candid*a strains (10.7% and 11.5% respectively), while AmB (99.1%) and AmB-PEG-MWNTs (100%) displayed high antibiofilm activity. The antibiofilm activity of AmB and AmB-PEG-MWNTs was comparable for the concentrations between 4-16mg/L, whereas in lower concentrations (<4 mg/L), AmB-PEG-MWNTs presented significantly stronger activity ($p<0.001$). The MIC50 of pure AmB was 0.25 mg/L, while the MIC50 of the AmB-PEG-MWNTs was 0.007 mg/L, respectively ($p<0.001$). **Conclusions:** AmB-PEG-MWNTs were found to inhibit BF formation to a considerable extent compared to AmB, indicating that nano-formulation of AmB might be a promising treatment for invasive candidiasis.

Author Disclosure Block:

Z.D. Pana: None. **M. Simitsopoulou:** None. **R. Papi:** None. **C. Antachopoulos:** None. **D. Kyriakidis:** None. **E. Roilides:** None.

Poster Board Number:

SUNDAY-424

Publishing Title:

Effective Treatment Of Azole Resistant *candida albicans* in A Murine Ascending Urinary Tract Infection With Liposomal Amphotericin B Delivered By Bladder Lavage

Author Block:

C. Frazier, J. Olson, J. Adler-Moore; Cal Poly Univ., Pomona, CA

Abstract Body:

Background: With the frequent occurrence of urinary tract infections (UTI) in hospital ICUs and assisted-living settings, and the increasing incidence of UTI caused by azole resistant Candida spp., alternative treatments are needed. This study was done to investigate the efficacy of liposomal amphotericin B (L-AmBi) for UTI when it is caused by an azole resistant strain of *Candida albicans*. **Methods:** Mice (n=10/group) were immunosuppressed with 6mg/kg Triamcinolone d-3 and d0, sedated with ketamine/xylazine d0 and challenged transurethrally with 4.7 X 10ex6 azole resistant *C. albicans* (ATCC 62342, L-AmBI MIC = 0.78ug/mL, Fluconazole MIC = 25ug/mL) followed by daily treatment d+1 to d+5 with 7.5mg/kg L-AmBi (AmBisome) given IV, or with 0.2mg L-AmBi by bladder lavage (BLav). Controls received 5% dextrose (D5W) IV or BLav. Kidneys (Kd), bladders (Bd), livers (Lr) and spleen (Sp) were collected d+6, homogenized, and dilutions plated for mean Log_{10}CFU/g (CFU) and mean drug levels of Kd and Bd determined by an agar diffusion bioassay.**Results:** Candida was reduced to undetectable levels in the Bd with L-AmBi IV (10/10 mice) or BLav (9/10 mice) compared to high CFU in Bd of D5W mice (4.04 IV and 3.56 BLav). In Kd, L-AmBi given by IV or BLav significantly reduced the yeast burden versus controls ($p \leq 0.0079$, IV-4.56 vs 5.43; BLav-4.79 vs 5.98). There was no infection in the control livers or spleens. L-AmBi Kd drug levels were 252ug/mL IV and 24ug/mL BLav. L-AmBi Bd drug levels were 41ug/mL IV and 21ug/mL BLav.**Conclusions:** L-AmBi was significantly effective against azole resistant *C. albicans* UTI whether given as IV or bladder lavage with inhibitory drug levels in the kidneys and bladders. With the markedly decreased toxicity of L-AmBi compared to amphotericin B deoxycholate, L-AmBi is a promising alternative polyene treatment for azole resistant *C. albicans* UTI.

Author Disclosure Block:

C. Frazier: None. **J. Olson:** None. **J. Adler-Moore:** E. Grant Investigator; Self; Gilead Sciences, Inc..

Poster Board Number:

SUNDAY-425

Publishing Title:

Selective Photoinactivation of Histoplasma Capsulatum

Author Block:

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Abstract Body:

Histoplasmosis is a fungal infection caused by the dimorphic fungus *Histoplasma capsulatum.* This disease is considered a endemic mycosis that may present asymptomatic infections and disseminated severe form depending of patient immunity. Histoplasmosis treatment can be performed with itraconazole and fluconazole, and in the disseminated forms with amphotericin B. However, it is well-known that amphotericin B presents a high toxicity that may cause, especially, nephrotoxicity and hepatotoxicity. In addition, the development of azole-resistant histoplasmosis has been observed in patients. So, it became necessary new procedures to treat histoplasmosis, and antimicrobial photodynamic therapy (aPDT) seems to be a potential candidate. aPDT involves the synergistic combination of a photosensitizer (PS), molecular oxygen and visible light of appropriate wavelength in order to produce highly reactive oxygen species which lead to the oxidation of several cell components and to cell inactivation. In this study we have investigated a selective photoinactivation of *H. capsulatum* using chalcone water-soluble derivatives as PS. Yeast and cells (NOK, HaCat and MRC-5) were incubated with the same chalcone derivatives concentrations (0.25 - 62.5 μg mL^{-1}) and incubation time (15 and 30 minutes) followed by irradiation with doses of light (12 and 24 J cm^{-2}). The best conditions to kill *H. capsulatum* selectively were 1.95 μg mL^{-1} of PS concentration incubated by 15 min and irradiated with blue LED (450 nm) with 24 J cm^{-2}. This condition promoted a reduction of 95% in the *H. capsulatum* survival without affecting significantly the cells that present a survival index around 95% for NOK, HaCat and MRC-5. It can be concluded that chalcone derivatives have good potential for aPDT application against *H. capsulatum*.**Financial support:** FAPESP and CAPES

Author Disclosure Block:

W. Melo: None. **L. Regasini:** None. **M. Giannini:** None. **A. Fusco:** None.

Poster Board Number:

SUNDAY-426

Publishing Title:

Absent *In Vitro* Interaction between Chloroquine and Antifungals Against *Aspergillus fumigatus*

Author Block:

S. Seyedmousavi[1], H. van der Lee[2], P. Verweij[2], A. Warris[3]; [1]Erasmus Univ. Med. Ctr., Rotterdam, Netherlands, [2]Radboud Univ. Med. Ctr., Nijmegen, Netherlands, [3]Univ. of Aberdeen, Aberdeen, United Kingdom

Abstract Body:

Background: The efficacy of voriconazole, the recommended first choice drug to treat infections caused by *Aspergillus fumigatus*, might be hampered by the emergence of azole resistance. Therefore, it is important to explore alternative treatment strategies. The antimalarial drug chloroquine in combination with antifungals has shown to have a direct antifungal effect on both azole-susceptible and azole-resistant *Candida albicans* isolates. We therefore investigated whether chloroquine potentiate synergistic activity in combination with antifungals for the treatment of *Aspergillus fumigatus*. **Methods:** The *in vitro* interaction between chloroquine and voriconazole or amphotericin B or caspofungin was determined against voriconazole-susceptible and voriconazole-resistant clinical *A. fumigatus* isolates using a checkerboard microdilution method with spectrophotometric analysis and a viability-based XTT {2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino) carbonyl]-2H-tetrazolium hydroxide} assay within 2 h of exposure after 24 and 48 h of incubation at 35°C to 37°C. **Results:** Our studies showed that presence of chloroquine had no growth-inhibitory antifungal activities against clinical *A. fumigatus* isolates, either voriconazole-susceptible or resistant. In addition, no obvious interactions were found *in vitro* for all the combinations tested. **Conclusions:** In conclusion, chloroquine, alone or in combination with different classes of antifungal agents, did not show any *in vitro* inhibitory antifungal activity against *A. fumigatus*. Our previous observation that chloroquine increases the antifungal activity of neutrophils may however suggests that chloroquine exerts different effects in vivo and its role in the treatment of *Aspergillus* infections remains to be further assessed.

Author Disclosure Block:

S. Seyedmousavi: E. Grant Investigator; Self; Astellas Pharma B.V.. H. Research Contractor; Self; Astellas Pharma B.V.. **H. van der Lee:** None. **P. Verweij:** None. **A. Warris:** None.

Poster Board Number:

SUNDAY-427

Publishing Title:

Antifungal Activities and Mode of Action of Amphiphilic Tobramycin Analogues and Their Synergistic Interactions with Azoles Against *Candida albicans*

Author Block:

S. Shrestha; Univ. of Kentucky, Lexington, KY

Abstract Body:

Background: Invasive fungal infections, such as candidiasis, have become a major cause of morbidity and mortality, especially among immunocompromised patients worldwide. Azoles are widely used antifungals to treat these infections in humans. But the increasing frequency of cross-resistance against azoles requires improved therapeutic strategies. Previously, it was shown that amphiphilic tobramycin analogues with the linear alkyl chain of **C_4-C_{14}** exhibited chain-length dependent antibacterial activities but their antifungal profile is unexplored. Besides, combination therapy has become popular in clinical practice as a potential strategy to fight resistant fungal isolates. The aim of this study was to investigate the antifungal properties and mechanism of action of novel amphiphilic tobramycin analogues and their synergistic interactions with four azoles against *C. albicans*. **Methods:** The MICs of TOB analogues against yeast cells were determined as described in CLSI document M27-A3. The fungal membrane-disruptive action of these analogues was studied by using the membrane-impermeable dye propidium iodide. Checkerboard assays were used to assess synergistic, indifferent and antagonistic effects, with **C_{12}** and **C_{14}** used in combination with four azoles by calculating fractional inhibitory concentration indices. Time kill curve assays were performed by colony counting at different time points.**Results:** **C_{12}** and **C_{14}** showed broad-spectrum antifungal activities against yeast and filamentous fungi. with the MIC values ranged from 1.95 to 31.2 μg/mL and 1.95 to 7.8 μg/mL, respectively. Our data suggests that the fungicidal mechanism of action of **C_{12}** and **C_{14}** appears to involve the perturbation of fungal membranes. Strikingly, majority of *C. albicans* strains were synergistically inhibited by **C_{12}** and **C_{14}** when combined with all four azoles (FLC, ITC, POS and VOR) except in few cases. Finally, synergism between **C_{12}** / **C_{14}** and POS were confirmed by time-kill assay.**Conclusions:** In conclusion, **C_{12}** and **C_{14}** showed broad-spectrum antifungal activities against yeasts and filamentous fungi that appear to target fungal plasma membrane. They also showed potent antifungal synergism with azoles which suggest the possibility of combining **C_{12}** or **C_{14}** with azoles to treat invasive fungal infections at lower administration doses or with a higher efficiency.

Author Disclosure Block:

S. Shrestha: None.

Poster Board Number:

SUNDAY-428

Publishing Title:

Screening of Lake Actinomycetes for the Production of Antifungal and Antialgal Compounds

Author Block:

S. Aslam, I. Sajid; Univ. of the Punjab, Lahore, Pakistan, Lahore, Pakistan

Abstract Body:

Background: Although chemical synthesis and engineered synthetic compounds are accelerated in the production process, nature still has an edge for its versatile pharmaceuticals especially from the wetland sediments. The lake sediments for isolating actinobacteria have not been extensively investigated although its ubiquitous presence and diversity remains inexhaustible than its terrestrial counterparts. The present study was designed to screen the lake actinomycetes for the production of antifungal and antialgal compounds.**Methods:** A total of forty strains of actinomycetes were isolated from the water and sediment samples of a saline Kallar Kahar lake of Pakistan. The identified strains were cultivated on small scale as shaking cultures and crude extracts were obtained by ethyl acetate extraction. These crude extracts were screened against *Candida albicans, Candida tropicalis, Chlorella vulgaris* by agar well diffusion method. In chemical screening the crude extracts were analyzed by TLC and HPLC-UV analysis.**Results:** The results revealed that majority of the isolated strains (90%) belong to the genus *Streptomyces* and some of the strains were of *Micromonospora*. Many lake actinomycete isolates showing remarkable antifungal and antialgal activity. However the strains $KL_3$6, KL_{11}7, $KL_3$7, KL_{10}3, $KL_5$1, $KL_7$1, $KL_8$3 and $KL_9$1 exhibited appreciable zones of inhibition of 15mm, 16mm, 17mm, 18mm, 21mm, 23mm, 23mm, 23mm respectively against *Candida tropicalis* while the isolates $KL_2$3, $KL_1$4, $KL_2$12, $KL_7$1, $KL_9$1, KL_{11}2, KL_{11}7, $KL_5$1, $KL_2$9, KL_{10}3 and $KL_3$6 exhibited maximum activity against *Candida albicans*. Approximately 80% of the isolated strains were observed to exhibit the potential antialgal activity against *Chlorella vulgaris*. Among the isolated strains $KL_3$6, $KL_3$7, $KL_4$1, $KL_5$9, $KL_5$2, KL_{11}6 and $KL_9$8 showed maximum zone of inhibition of 16mm, 18mm, 17mm, 19mm, 21mm, 17mm, 15mm respectively. On the other hand, $KL_8$4, $KL_9$4, $KL_4$3, $KL_5$1 and $KL_3$8 gave no zone of inhibition against *Chlorella vulgaris*. In thin layer chromatographic analysis and HPLC-UV analysis, crude extracts of the isolates also showed an impressive chemical diversity of lake actinomycetes.**Conclusions:** Lake actinomycetes are a potential source of antifungal and antialgal compounds which can be exploited for further purification of unique secondary metabolites in drug discovery.

Author Disclosure Block:

S. Aslam: None. **I. Sajid:** None.

Poster Board Number:

SUNDAY-429

Publishing Title:

Antifungal Effect of Soxhlet Extract of *Thymus vulgaris* Leaves on Fungal Isolates from Barbering Equipment

Author Block:

L. C. CHIDI-ONUORAH, C. A. OYEKA; NNAMDI AZIKIWE Univ., AWKA, Nigeria

Abstract Body:

Background: With the aim of ascertaining the efficacy of some plants known to have antifungal properties on fungal organisms and developing new antimicrobial agents, this study was carried out and involved twenty (20) barbershops.**Methods:**Two hundred (200) samples were collected from barbering equipment pre and post sterilization. The equipment included electric clippers, brushes, combs and scissors. The work tops were also sampled. Samples were inoculated onto Sabouraud Dextrose Agar (Micromaster Thane, Maharashta, India) incorporated with 0.5mg/ml of chloramphenicol and incubated at room temperature for 5 - 7days. Subcultures were carried out to purify the isolate. Isolates were identified microscopically and macroscopically. Soxhlet and crude extracts of *Moringa oleifera* seeds and leaves, peels of *Citrus sinensis* (orange) and *C. limon* (lemon) and leaves of *Thymus vulgaris* (thyme) were obtained and the mean zone of inhibition of the isolates to extracts determined at 500mg/ml. The mean minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) values of the effective extracts were also determined.**Results:**A total of one hundred (100) fungal isolates belonging to six (6) genera were recovered. These included *Trichophyton schoenleinii* (20), *Microsporum ferrugineum* (14), *Aspergillus nidulans* (8), *Scedosporium apiospermum* (4), *Rhizopus microsporus* (6), *A. glaucus* (6), *M. nanum* (15), *A. niger* (12), *A. versicolor* (10), *C. albicans* (5). All extracts with the exception of orange peel Soxhlet (OPS), thyme Soxhlet (TS) and thyme crude (TC) extracts were ineffective on the isolates. The MIC and MFC values ranged from 62.5 to 500mg/ml and 250 to >500mg/ml respectively.The antifungal effect of the extracts to the isolates was significantly different ($p < 0.05$). Maximum mean zone of inhibition of 38mm was shown by TS which also had the highest effectiveness of 52%.**Conclusion:**Soxhlet extract of leaves of *Thymus vulgaris* has shown to be a good antifungal agent as it had the highest effectiveness on the isolates.**Key words: *Thymus vulgaris*, antifungal, barbering equipment**

Author Disclosure Block:

L.C. Chidi-onuorah: None. **C.A. Oyeka:** None.

Poster Board Number:

SUNDAY-430

Publishing Title:

Revealing Molecular Mechanisms of Fluconazole (Flc) Resistance in *Candida tropicalis* (Ct) Through Whole Genome Sequencing (Wgs) and Rna-Seq

Author Block:

J. M. Rybak[1], C. M. Dickens[2], P. D. Rogers[1]; [1]Univ. of Tennessee, Memphis, TN, [2]Univ. of Texas A & M, College Station, TX

Abstract Body:

Background: *Candida* spp. are the most common cause of invasive fungal infections in North America and remain associated with mortality rates as high as 40%. Rising rates of resistance to FLC, the most commonly utilized antifungal, remains of clear concern. While much is known about the molecular mechanisms contributing to FLC resistance in *Candida albicans* (Ca), relatively little is known with regards to *Candida tropicalis* (Ct), where rates of FLC resistance are higher. We utilized WGS and RNA-seq to identify novel mutations and altered gene expression in genes related to fluconazole susceptibility.**Methods:** 13 clinical isolates of Ct with reduced susceptibility to FLC (MIC≥1mg/L) and 5 susceptible control clinical isolates were obtained from the University of Iowa repository. FLC MICs for each isolate were determined using Clinical Laboratory and Standards Institute methodology. RNA and genomic DNA were isolated from cultures of each isolate in mid-log growth phase. WGS and RNA-seq was performed using the Ion Proton System and aligned to the Ct MYA-3404 reference genome. RNA-seq analysis was performed using edgeR, and gene expression was compared to a composite of the 5 control isolates.**Results:** MIC ranged from 0.5 to ≥256mg/L. 12 isolates were observed to be FLC resistant (MIC 8 to ≥256mg/L). WGS revealed numerous novel mutations in several ergosterol biosynthetic genes including *ERG3* and *ERG11*, as well as transcription factor genes *UPC2, TAC1*, and *MRR1*. RNA-seq results were consistent with increased transcription factor activity in Ct isolates with potential gain- of- function mutations in *UPC2, TAC1*, and *MRR1*. Overexpression of genes associated with reduced FLC susceptibility in Ca, *ERG11* (>3 fold), *CDR1* (≥10 fold), and *MDR1* (20 fold) was observed in FLC resistant Ct isolates. Overexpression (>10 fold) of the efflux pump genes *SNQ2* and *CDR11*, not previously reported in Ca, was also observed in FLC resistant isolates.**Conclusions:** These data reveal potential molecular mechanisms of FLC resistance in Ct which parallel mechanisms previously identified in Ca. Additional mechanisms of resistance such as greatly increased expression of the efflux pump genes *SNQ2* and *CDR11* were also identified. Further research is needed to definitively identify mechanisms of FLC resistance among Ct.

Author Disclosure Block:

J.M. Rybak: None. **C.M. Dickens:** None. **P.D. Rogers:** None.

Poster Board Number:

SUNDAY-431

Publishing Title:

Metal Chelation as a Powerful Strategy to Probe Cellular Circuitry Governing *C. albicans* Drug Resistance and Morphogenesis

Author Block:

E. J. Polvi, L. E. Cowen; Univ. of Toronto, Toronto, ON, Canada

Abstract Body:

Fungal pathogens have evolved diverse strategies to sense host relevant cues and coordinate cellular responses, which enable virulence and drug resistance. Defining circuitry controlling drug resistance and virulence opens new opportunities for chemical diversity in therapeutic drugs, as the cognate inhibitors are not typically explored by conventional screening approaches. This has great potential to address the pressing need for new strategies to treat invasive fungal infections, which have a staggering impact on human health. To explore this approach, we focused on a leading fungal pathogen of humans, *Candida albicans*, and performed a screen of 1,280 pharmacologically active compounds to identify those that abrogate resistance to the echinocandins, which are the newest class of antifungal and target synthesis of the fungal cell wall. We identified 13 compounds that abolished echinocandin resistance of a clinical isolate, with the broad spectrum chelator DTPA having the most potent synergistic activity. Depletion of metals individually revealed that DTPA modulates echinocandin resistance via chelation of magnesium. Whole genome sequencing of mutants resistant to the combination of DTPA and echinocandin identified mutations in the histidine kinase gene, *NIK1*. Furthermore, *NIK1* mutations are sufficient to confer resistance to the combination. Functional analyses demonstrated that DTPA activates the mitogen-activated protein kinase Hog1, and that *NIK1* mutations block activation of Hog1 in response to both echinocandin and DTPA. This suggests that DTPA may modulate Hog1 signaling through Nik1, in order to enhance the efficacy of echinocandins. We found that DTPA not only abrogates drug resistance but also modulates morphogenesis, a key virulence trait that is normally regulated by environmental cues. DTPA induced filamentation via depletion of zinc, in a manner that is contingent upon Ras1-protein kinase A (PKA) signaling, as well as the transcription factors Brg1 and Rob1. Thus, we establish a new mechanism by which metal chelation modulates morphogenetic circuitry and echinocandin resistance, and illuminate a new facet to metal homeostasis at the host-pathogen interface.

Author Disclosure Block:

E.J. Polvi: None. **L.E. Cowen:** None.

Poster Board Number:

SUNDAY-432

Publishing Title:

The Influence of Ergosterol Biosynthesis Inhibition on the Mycelial Growth in *Aspergillus flavus*

Author Block:

E. Mizusawa, K. Hashimoto, H. Maki, A. Naito; Shionogi & Co., Ltd., Toyonaka, Japan

Abstract Body:

Background: Invasive aspergillosis (IA) contributes to significant mortality in immunosuppressed patients. Azoles, known as Cyp51 inhibitors, are used to treat IA. The majority of IA is caused by *Aspergillus fumigatus*, followed by *Aspergillus flavus*. The effect of these inhibitors on ergosterol biosynthesis pathway has been studied mostly in *A. fumigatus*, whereas little has been reported in *A. flavus*. In this study, we compared the influence of Cyp51 inhibition by voriconazole (VRCZ) on the mycelial growth in *A. fumigatus* and *A. flavus*. **Methods:** *A. fumigatus* and *A. flavus* were grown by shaking overnight in YNB broth at 35°C, then VRCZ and 2-^{13}C-acetate were added to the cultures. After incubation at 35°C for 16 hr, mycelia pellets were harvested by vacuum filtration. Sterols were extracted by n-heptane from the cell pellets. From them, ergosterol, eburicol and abnormal C_{14}-methylated sterols such as obtusifoliol were individually analyzed by liquid chromatography-mass spectrometry. *De novo* synthesized sterols were detected as the [^{13}C] labeled sterols. Growth rates were estimated by the ratios of the dry weight of mycelia against drug free control. **Results:** The MICs of VRCZ against the species were both 0.5 µg/mL. In both species, VRCZ-treated cells showed dose-dependent decreases in growth rates and ergosterol biosynthesis rates until almost complete inhibitions. There was little difference between the 80% inhibition concentration of growth rate [$IC_{80\ grow}$] and that of ergosterol biosynthesis rate [$IC_{80\ erg}$] in *A. fumigatus* ($IC_{80\ grow}$: 0.25 µg/mL, $IC_{80\ erg}$: 0.25 µg/mL). In contrast, $IC_{80\ grow}$ was 4 times higher than $IC_{80\ erg}$ ($IC_{80\ grow}$: 0.5 µg/mL, $IC_{80\ erg}$: 0.13 µg/mL) in *A. flavus*. VRCZ treatment showed the accumulation of eburicol and abnormal sterols, and abnormal sterols accumulation per mg-mycelia was similar in both species. In *A. fumigatus*, the total ergosterol amount per mg-mycelia was not decreased at the $IC_{80\ erg}$, where the mycelial growth was not observed. On the other hand, in *A. flavus*, it was decreased to the half of the control at the $IC_{80\ erg}$ of VRCZ, where the mycelial growth was observed. **Conclusions:** *In vitro* VRCZ exposure revealed that the contribution of ergosterol biosynthesis inhibition on the mycelial growth in *A. flavus* is less than that in *A. fumigatus*. The ergosterol metabolisms may be different between these two species.

Author Disclosure Block:

E. Mizusawa: D. Employee; Self; Shionogi & Co., Ltd. **K. Hashimoto:** D. Employee; Self; Shionogi & Co., Ltd. **H. Maki:** D. Employee; Self; Shionogi & Co., Ltd. **A. Naito:** D. Employee; Self; Shionogi & Co., Ltd..

Poster Board Number:

SUNDAY-433

Publishing Title:

Differential Contribution of ROS Production to the Fungicidal Action of Amphotericin B and Micafungin in *Candida albicans*

Author Block:

J. Argüelles; Univ. de Murcia, Murcia, Spain

Abstract Body:

As well acting according to the well established mechanism, recent evidence suggests that the lethal effect of some clinical antifungals depends on previously unknown targets. In this study, we have examined the hypothetical role played by the intracellular formation of ROS in the fungicidal action carried out by Amphotericin B (AMB) and Micafungin (MF) on the opportunistic yeast *Candida albicans*, which remains the most prevalent fungal pathogen. The clinical MIC_{90} for MF and AMB were 0.016 and 0.12 mg/L, respectively. The ROS levels were measured by flow cytometry with dihydrofluorescein diacetate and the level of survival was assessed by counting viable cells. Whereas AMB (0.5-1.0 x MIC) induced a marked production of ROS in parallel with a severe degree of cell killing in the *C. albicans* SC5314 strain, the fungicidal effect of MF was still effective with only a slight degree of ROS generation. Preincubation with rotenone or thiourea suppressed the generation of ROS and caused a marked increase of cell viability regardless of the antifungal supplied. Simultaneous measurement of a set of well established antioxidant enzymes (catalase, glutathione reductase and superoxide dismutase) revealed a strong AMB-induced activation of the three activities, whereas MF only had a weak stimulating effect. Likewise, AMB but not MF promoted a conspicuous intracellular synthesis of trehalose, the non-reducing disaccharide which acts as a specific protector against oxidative stress in *C. albicans*. Taken together, our results strongly point to that the induction of an internal oxidative stress in *C. albicans* through the release of ROS is a contributory factor to the antifungal action of polyenes (AMB) but not echinocandins (MF). Acknowledgements. The experimental work reported here was supported by financial contracts provided by Vitalgaia España, S.L. and Cespa, Servicios Auxiliares de Murcia, S.A. The authors declare that they have no disclosures.

Author Disclosure Block:

J. Argüelles: None.

Poster Board Number:

SUNDAY-434

Publishing Title:

HXP124 as a Novel Treatment for Onychomycosis

Author Block:

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Abstract Body:

Background: Onychomycosis, or fungal infection of the nail, affects about 14% of the population. These infections are long-term, hard to treat, can be painful and have a cosmetic impact to the patient. Treatment options include systemic and topical therapies. Although effective, systemic therapies commonly have adverse effects, such as liver toxicity. Current topical have low cure rates due to poor nail penetration, and as a result typically require long treatment times. We are developing a novel topical treatment for onychomycosis infections, using a naturally occurring cationic peptide from plants. This molecule, HXP124, belongs to a family of innate immunity peptides called plant defensins, which have potent antifungal activity against a wide range of fungal pathogens. In this study, we evaluated the suitability of HXP124 in the treatment of onychomycosis by assessing its activity against the causative fungal pathogens and analysing its ability to penetrate human nails.**Method:** The antifungal activity of HXP124 was tested against fungal pathogens commonly associated with onychomycosis (*T. rubrum, T. interdigitale* and *C. albicans*) using an *in vitro* antifungal susceptibility assay. The ability of HXP124 to penetrate human cadaver toenails was assessed in an *in vitro* system using Franz cell nail adapters and In-Line cells (PermeGear) using a method modified from Hui et al, (2002). In brief, human nails were hydrated on PBS-soaked kimwipe, screwed tightly into a nail adapter and PBS-soaked cotton wool placed underneath. HXP124 was added to the top surface of the nail every 24H and penetration was assessed by analysing the contents of the cotton wool by RP-HPLC.**Results:** HXP124 has antifungal activity against fungal species most commonly associated with onychomycosis, and this activity is comparable to the activity of currently available topical treatments. HXP124 efficiently penetrates cadaver toenails with up to 60% passing through within 24 h.**Conclusions:** The plant defensin HXP124 has potent activity against the causative agents of onychomycosis and has the potential to be a novel topical onychomycosis treatment with superior nail penetration compared to current therapies.

Author Disclosure Block:

B. Hayes: I. Research Relationship; Self; Hexima Ltd. **M. Bleackley:** I. Research Relationship; Self; Hexima Ltd. **M. Baker:** I. Research Relationship; Self; Hexima Ltd. **P. Quimbar:** I. Research Relationship; Self; Hexima Ltd. **M. Anderson:** A. Board Member; Self; Hexima Ltd..

I. Research Relationship; Self; Hexima Ltd. **N. van der Weerden:** A. Board Member; Self; Hexima Ltd.. I. Research Relationship; Self; Hexima Ltd..

Poster Board Number:

SUNDAY-435

Publishing Title:

Nanosystems Containing Cationic Lipids as Effective Candidacidal Agents for Topical Application

Author Block:

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Abstract Body:

Background: Growing numbers of infections caused by antibiotic resistant fungi require increasing efforts to develop new treatment and prevention strategies.**Methods:** In this study, we assessed the physicochemical properties and fungicidal activity of magnetic nanoparticles (MNP) coated with selected ceragenins CSA-13 and CSA-131 (MNP@CSA-13; MNP@CSA-131) against different *Candida* spp., including fluconazole-resistant strains. Fourier transform infrared spectroscopy (FTIR), thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) were employed to confirm the chemical structure of the obtained nanoparticles. To determine the candidacidal ability of tested agents, MIC/MFC/MBIC measurements were performed in the presence of DNase I, 50 % human blood plasma or pus. Biofilm mass was evaluated using crystal violet (CV) staining, and the morphology of the treated cells was assessed using atomic force microscopy (AFM).**Results:** Ceragenins in the presence of DNase I demonstrated an increased ability to kill DNA-induced *Candida* biofilms. Microscopy studies show that treatment with the tested nanosystems causes *Candida* cells to undergo extensive surface changes, indicating surface membrane damage.**Conclusion:** Magnetic nanoparticles functionalized by antimicrobial cationic lipids might serve as drug carriers in drug delivery system for topical application.

Author Disclosure Block:

R.A. Bucki: None. **K. Niemirowicz:** None. **B. Durnaś:** None. **K. Pogoda:** None. **P. Deptuła:** None. **M. Wątek:** None. **T. Wollny:** None. **E. Pktel:** None. **U. Wnorowska:** None. **X. Gu:** None. **P. Savage:** None.

Poster Board Number:

SUNDAY-436

Publishing Title:

Molecular Characterization of *CYP*51 Gene Coding for Cytochrome P450 14α-Sterol Demethylase from a Posaconazole-Resistant, Voriconazole-Susceptible *Scedosporium apiospermum* Isolated from a Cystic Fibrosis Patient

Author Block:

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Abstract Body:

Background: *Scedosporium apiospermum* (*Sa*) complex rank second among the filamentous fungi colonizing the airways of cystic fibrosis (CF) patients. Clinical isolates of *Sa* show varying degree of susceptibility to antifungal drugs. *Sa* IHEM 14462 (*Sa*14462) isolated from a CF patient showed high level resistance (MIC 16µg/ml) to posaconazole (PCZ), but remained susceptible (MIC 0.5 µg/ml) to voriconazole (VCZ). On the other hand, *Sa* ATCC MYA-3634 (*Sa*3634) was susceptible to PCZ and VCZ. Since azole resistance in fungi is largely due to drug target mutation(s), we characterized *CYP51* coding for the target enzyme P450 14α-sterol demethylase from *Sa*14462 and *Sa*3634.**Methods:** Genomic DNA was purified from *Sa*14462 and *Sa*3634, and a 2.4-kb DNA fragment containing *CYP51* was amplified by PCR. The DNA sequence of the protein coding and non-coding strands of the amplicon was determined. The DNA and the deduced amino acid sequences were analyzed using Analytical Tools available from NCBI and EMBL.**Results:** The protein coding sequence of *Sa CYP*51 gene was contained in a 1751-bp DNA fragment with a 75-bp and a 92-bp introns located at the 5'-end. The *Sa* Cyp51 protein consists of 528 amino acid (aa) residues. Amino acid alignment of *Sa* Cyp51 with those from five other fungi showed six highly conserved regions (CR). A comparison of the deduced Cyp51 aa sequences of *Sa*3634 and *Sa*14462 revealed 98.5% identity and the latter showed I34V, K74R, D416E, I434L, E439D (conservative), G152A, V485A (semi-conservative) and D446A (non-conservative) aa substitutions within or close to the CRs. The D446A change is located in the heme binding region which could affect the inhibitory effect of triazole(s) on the enzyme molecule.**Conclusions:** In vitro antifungal drug susceptibility studies showed that *Sa*14462 is highly resistant to PCZ, while remaining susceptible to VCZ, whereas *Sa*3634 is highly susceptible to both PCZ and VCZ. Characterization of Cyp51 from *Sa*14462 and *Sa*3634 showed several aa changes, including D446A at the critical heme binding region of the enzyme. One or more of these aa changes could be at least partly responsible for the differential susceptibility of *Sa*14462 to the triazoles PCZ and VCZ.

Author Disclosure Block:

E. Manavathu: None. **S. Wakade:** None. **A. Rao:** None. **J. Bouchara:** None. **J. Vazquez:** None.

Poster Board Number:

SUNDAY-437

Publishing Title:

***In Vitro* Activity of Cefepime-Zidebactam [WCK 5222, A Combination of Cefepime with the Dual-action Bicyclo-Acyl Hydrazide β-Lactam Enhancer Zidebactam (WCK 5107)]**

Author Block:

M. R. Jacobs[1], S. Bajaksouzian[1], K. M. Papp-Wallace[2], R. A. Bonomo[2]; [1]CWRU,UHCMC, Cleveland, OH, [2]Cleveland VAMC, CWRU, Cleveland, OH

Abstract Body:

Background: Zidebactam (ZID) is a β-lactam enhancer inhibiting PBP2 of Gram negative species as well as a broad-spectrum β-lactamase inhibitor with activity against class A and C enzymes. These activities are targeted at overcoming resistance to β-lactams, including carbapenems. ZID with cefepime (FEP) is currently being studied in a Phase 1 clinical trial. **Methods:** In vitro susceptibilities of FEP-ZID, ZID (Wockhardt Research Center, India) and comparator agents piperacillin-tazobactam (PIP-TAZO), ceftazidime (TAZ), ceftazidime-avibactam (TAZ-AVI), cefepime (FEP), imipenem (IMI), meropenem (MER) were tested by CLSI broth microdilution. ZID was tested alone and combined with FEP at fixed concentrations of 4 and 8 mg/L. Isolates tested were from a genetically well-characterized collection of strains that included Enterobacteriaceae and non-fermenters expressing i) class A wild-type and ESBLs (CTX-M, SHV and TEM types), ii) KPC, OXA 23/24, NDM, VIM and L1 β -lactamases and iii) OMP deletions (Omp35, -36 and OprD). PIP-TAZO resistant ESBL-producing Enterobacteriaceae were specifically selected for inclusion in this study.**Results:** PIP-TAZO, FEP and CAZ were only active against the NSBL group, while IMI and MER were active against the NSBL and 92% of the ESBL isolates. TAZ-AVI was active against these groups as well as KPC and OMP. ZID alone was active at 4 mg/L against 30.7% of the isolates, including 51% of the strains containing *bla*$_{NDM}$. None of the agents tested showed good activity against *bla*$_{OXA}$ containing strains. FEP-ZID was active against all NSBL, ESBL, OMP and KPC containing isolates as well as 87.7% of *bla*$_{NDM}$, 80-100% of *bla*$_{L1}$ strains and 50% of *bla*$_{VIM}$. Overall, FEP-ZID inhibited the highest percentage of strains.**Conclusions:**In this study FEP-ZID demonstrated activity against all KPC and most NDM isolates with a broad spectrum of activity especially against NDM and some VIM producing strains.

Percent of isolates inhibited by resistance mechanism at breakpoints shown									
Agent	PIP-TAZO	FEP	IMI	MERO	TAZ	TAZ-AVI	ZID	FEP-ZID (ZID at 4 mg/L)	FEP-ZID (ZID at 8 mg/L)
Breakpoint MIC (mg/L)	16	8	4	4	8	8	4	8	8

Resistance mechanism (number)									
Narrow spectrum β-lactamase (NSBL) (17)	88.2	94.1	100.0	100.0	94.1	94.1	52.9	100.0	100.0
Extended-spectrum β-lactamase (ESBL) (37)	18.9	43.2	91.9	91.9	13.5	100.0	29.7	100.0	100.0
ESBL/OMP (10)	10.0	10.0	70.0	20.0	10.0	100.0	20.0	100.0	100.0
KPC (53)	0.0	20.8	26.4	22.6	7.5	100.0	22.6	100.0	100.0
NDM (57)	10.5	0.0	22.8	21.1	0.0	14.0	50.9	87.7	87.7
OMP (8)	12.5	12.5	25.0	25.0	0.0	100.0	37.5	100.0	100.0
OXA (18)	5.6	5.6	16.7	16.7	11.1	11.1	0.0	11.1	11.1
L1 (5)	20.0	0.0	0.0	0.0	80.0	80.0	0.0	80.0	100.0
VIM (10)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	50.0
All (215)	14.9	21.4	41.9	38.1	14.9	64.2	30.7	84.2	87.0

Author Disclosure Block:

M.R. Jacobs: E. Grant Investigator; Self; Actavis, Wockhardt. **S. Bajaksouzian:** None. **K.M. Papp-Wallace:** E. Grant Investigator; Self; Actavis, AstraZeneca, Wockhardt, Merck. **R.A. Bonomo:** E. Grant Investigator; Self; Actavis, AstraZeneca, Merck, Wockhardt, GSK.

Poster Board Number:

SUNDAY-438

Publishing Title:

WCK 5107 (Zidebactam, ZID): A Pan Gram-Negative β-Lactam Enhancer Augmenting β-Lactam Pharmacodynamics in Wild Type and Carbapenemase Producers (CP)

Author Block:

S. R. Palwe, P. R. Joshi, H. N. Khande, S. S. Biniwale, **S. S. Bhagwat**, M. V. Patel; Wockhardt Res. Ctr., Aurangabad, India

Abstract Body:

Background: ZID is a β-lactam (BL) enhancer with pan Gram-negative high affinity PBP2 binding and Class A and C βlactamase inhibition. We describe β-lactam enhancer-based approach leading to enhanced potency and cidality against strains expressing ZID non-inhibitable carbapenamases as well as those lacking significant β-lactamase expression. **Methods:** Microscopy was performed to observe PBP-binding-dependent specific morphological changes induced by ZID and other BLs. MICs were determined as per CLSI recommendation for 12 ZID-resistant (ZID-R) β-lactamase-negative (BLN, confirmed by nitrocefin assay) Enterics (Proteus -9, *K. pneumoniae* (KP)-3). Time-kill studies comparing BL-BLIs [clavulanic acid (CLA) and tazobactam (TAZ)] and BL-ZID combinations involving six PBP3 binding BLs [cefepime (FEP), piperacillin (PIP), ceftriaxone (CRO), ceftazidime (CAZ), aztreonam (ATM) and sulbactam (SUL)] were undertaken employing nine BLN Gram-negatives (E. coli (EC)-7, Enterobacter-2) and 1 wild type strain each of *P. aeruginosa* (PA) and *A. baumannii* (AB). Time-kill studies were undertaken with CP Gram-negatives [EC (NDM), KP (NDM), PA (VIM-10) and AB (OXA 23 and 25)]. Time-kill studies were conducted in CA-MHB with starting inoculum of 10^6-10^7 CFU/mL and periodic viable count enumeration.**Results:** ZID (0.12-16 µg/mL) efficiently induced spheroplast formation indicating effective PBP2 binding. For 12/12 ZID-R (>16 µg/mL) BLN strains, MICs of FEP lowered by 4-32x and 8-64x with the addition of ZID at 0.25 and 0.5 µg/mL, respectively. Such lowering of FEP MICs was not brought about by CLA and TAZ (2x lowering in 3/12 strains). In time-kill studies, employing BLN/WT strains neither standalone BLs (1x MIC) nor ZID (2-4µg/mL) provided any cidal effect. However, unlike BL-BLIs, BLs (0.25x MIC)-ZID (2 µg/mL) combination, induced rapid 2->3 log kill. For CP strains, standalone BLs (4-16 µg/mL) or ZID (2-8 µg/mL) did not provide killing. However, ZID combination with FEP or ATM brought about 1.78->3 $\log_{10}$ kill in EC, KP and PA while its combination with FEP or SUL was effective against AB.**Conclusion:** ZID enhances the antibacterial effects of PBP3 binding BLs owing to complementary PBP binding in BLN/WT strains and broadens the therapeutic coverage of BLs to CP pathogens.

Author Disclosure Block:

S.R. Palwe: D. Employee; Self; Wockhardt Research Center. **P.R. Joshi:** D. Employee; Self; Wockhardt Research Center. **H.N. Khande:** D. Employee; Self; Wockhardt Research Center. **S.S. Biniwale:** D. Employee; Self; Wockhardt Research Center. **S.S. Bhagwat:** D. Employee; Self; Wockhardt Research Center. K. Shareholder (excluding diversitied mutual funds); Self; Wockhardt Ltd.,. **M.V. Patel:** D. Employee; Self; Wockhardt Research Center. K. Shareholder (excluding diversitied mutual funds); Self; Wockhardt Ltd.,.

Poster Board Number:

SUNDAY-439

Publishing Title:

Activity of Combinations of Cefepime with Zidebactam (WCK 5107), a Novel Triple-Action Diazabicyclooctane

Author Block:

D. M. Livermore[1], S. Mushtaq[2], M. Warner[2], A. Vickers[2], N. Woodford[2]; [1]Univ. of East Anglia, Norwich, United Kingdom, [2]Publ. Hlth.England, London, United Kingdom

Abstract Body:

Background. Diazabicyclooctanes (DBOs) inhibit Class A, C and some Class D β-lactamases. A few, e.g. OP0595/RG6080, also bind PBP2, giving direct antibacterial activity together with a β-lactamase–inhibition-independent 'enhancement' of the activity of β-lactams binding to other PBPs. We characterised the behaviour of a novel acyl hydrazide-DBO, zidebactam. **Methods**. CLSI agar dilution MICs of cefepime were determined with zidebactam (0.06-8 mg/L) in a chequerboard format for bacteria with known resistance genotypes and phenotypes. **Results**. MICs of zidebactam alone were ≤1 mg/L (mostly 0.12-0.5 mg/L) for 49/50 *E. coli*, 39/55 *Klebsiella*, 43/45 *Citrobacter* and *Enterobacter*, but were >32 mg/L for 19/19 Proteeae, 7/10 *Serratia* and for 14/150 *E. coli*, *Klebsiella* and *Enterobacter/Citrobacter*. At subinhibitory concentrations, zidebactam potentiated cefepime strongly against Enterobacteriaceae with class A, C and OXA-48 enzymes. Overall, 33/33 ESBL Enterobacteriaceae (6 with zidebactam MICs >32 mg/L) were susceptible to cefepime + zidebactam 1+1 mg/L, as were 35/35 AmpC producers (7 with zidebactam MICs >32 mg/L), 29/30 with KPC enzymes (2 with zidebactam MICs >32 mg/L), 15/15 with OXA-48 (3 with zidebactam MICs >32 mg/L) and 30/35 with MBLs (7 with zidebactam MICs >32 mg/L). Among 8 MBL Enterobacteriaceae with zidebactam MICs ≥16 mg/L, 4 showed strong enhancer-effect cefepime-zidebactam synergy whereas 4 -one *E. coli* and 3 *P. rettgeri* - did not. MICs of zidebactam for 36/50 *P. aeruginosa* were 4-16 mg/L whereas all MICs for *A. baumannii* and *S. maltophilia* exceeded 32 mg/L; synergy with cefepime was frequent for AmpC-derepressed *P. aeruginosa* and for cystic fibrosis isolates that were resistant to meropenem, cefepime and multiple other antibiotics but rare for those with efflux or MBLs; synergy was frequent also for *S. maltophilia*, but absent for *A. baumannii* regardless of resistance type. **Conclusion**. Zidebactam represents a second, triple action DBO, after OP0595, with β-lactamase-inhibitory activity, direct antibacterial activity and a potential to enhance cefepime activity independently of β-lactamase inhibition. MICs of zidebactam were lower than those previously found for OP0595 for Enterobacteriaceae and *P. aeruginosa*,

Author Disclosure Block:

D.M. Livermore: C. Consultant; Self; Accelerate, Achaogen, Adenium, Alere, Allecra, Altermune, AstraZeneca, Auspherix, Basilea, BioVersys, Cubist, Cycle, Discuva, GSK, Meiji,

Nordic, Pfizer, Roche, Shionogi, Tetraphase, VenatoRx, Wockha. H. Research Contractor; Self; Melinta, Wockhardt, AstraZeneca, Roche, Tetraphase. K. Shareholder (excluding diversitied mutual funds); Self; Dechra, GSK, Merck, Perkin Elmer, Pfizer. L. Speaker's Bureau; Self; AstraZeneca, Merck, Nordic, Pfizer,. **S. Mushtaq:** None. **M. Warner:** None. **A. Vickers:** None. **N. Woodford:** H. Research Contractor; Self; Achaogen, Allecra Antiinfectives, Amplex, AstraZeneca, Becton Dickinson, Cepheid, Check-Points, Cubist, Wockhardt, Roche, Meiji.

Poster Board Number:

SUNDAY-440

Publishing Title:

Wck 5222 [Cefepime (FEP)-WCK 5107 (Zidebactam, Zid)]: *In Vitro* and *In Vivo* Coverage of OXA-Carbapenemases Expressing-*Acinetobacter* (OXA-Ab)

Author Block:

P. R. Joshi, H. N. Khande, S. S. Takalkar, A. M. Kulkarni, S. R. Palwe, S. S. Biniwale, **S. S. Bhagwat**, M. V. Patel; Wockhardt Res. Ctr., Aurangabad, India

Abstract Body:

Background: Though preferred, β-lactam-based therapies for OXA-AB are currently ineffective due to unavailability of β-lactam inhibitors (BLIs) inhibiting OXA carbapenemases. ZID is a novel β-lactam enhancer with selective, high-affinity pan Gram-negative (including AB) PBP2 binding, and its combination with FEP provides a novel approach in tackling these pathogens. Herein, we describe potent FEP-ZID activity against AB through a range of *in vitro* and *in vivo* studies.**Method:***Acinetobacter* PBP IC_{50}s for FEP and ZID were determined by Bocillin FL competition assay. The OXA-carbapenemase inhibition (IC_{50}) by ZID was assessed by nitrocefin as substrate. MICs of FEP-ZID for 208 Indian OXA-AB strains were determined as per CLSI recommended agar dilution method. Time-kill studies employing reference OXA-AB (AB NCTC 13301 and AB NCTC 13303) were conducted in CA-MHB (starting inoculums: 6.5-6.8 log_{10} CFU/mL). *In vivo* ED_{90} doses were determined through systemic infection studies. Mice were intraperitonially infected with OXA-AB (AB NCTC 13301 and AB NCTC 13303, 6.5-6.69 log_{10} CFU/mouse) and treatment was initiated 1h post-infection (BID, 3h apart for 1 day) and survival was monitored till 7 days.**Results:**ZID showed potent PBP2 affinity (IC_{50}: 0.01 µg/mL) while FEP showed complementary dual binding to PBP1a, 1c (IC_{50}: 0.05, 0.84 µg/mL) and PBP3 (IC_{50}: 0.08µg/mL). OXA IC_{50} of ZID for OXA-23, 24, 25, 26, and 27 was ≥2.0 µM. $MIC_{50/90}$ for standalone FEP or ZID were 64/128, >1024 µg/mL. Addition of ZID to FEP (1:1) lowered the $MIC_{50/90}$ to 16/32 µg/mL [$MIC_{50/90}$: imipenem (IPM) and meropenem -16/64 µg/mL; ceftazidime (CAZ)-avibactam (AVI) -64/128 µg/mL]. FEP-ZID (sub-MIC concentrations, 8-16 + 4-8 µg/mL) time-kill studies showed 2 log_{10} kill while standalone FEP, ZID, IPM and CAZ-AVI failed to show any cidality. *In vivo* systemic infection studies employing OXA-AB strains (FEP-ZID MICs, 16-32 µg/mL), provided FEP-ZID ED_{50} of (FEP) 50–100 mg/kg - (ZID) 12.21–28.60 mg/kg and ED_{90} of (FEP) 50–100 mg/kg - (ZID) 25.28–53.42 mg/kg. Even at higher doses, FEP and IPM even at higher doses failed to provide protection.**Conclusion:**Despite high MICs, FEP-ZID combination provided therapeutically promising activity against OXA-AB through β-lactam enhancer mechanism.

Author Disclosure Block:

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Poster Board Number:

SUNDAY-441

Publishing Title:

WCK 5222 [Cefepime (Fep)-WCK 5107 (Zidebactam, Zid)]: Thigh and Lung Pk/Pd Studies against Higher MIC OXA Carbapenemase-Expressing *A. baumannii* (Ab)

Author Block:

S. S. Takalkar, R. P. Chavan, A. M. Patel, K. V. Umarkar, J. S. Satav, A. P. Udaykar, A. M. Kulkarni , V. S. Zope, S. S. Bhagwat, **M. V. Patel**; Wockhardt Res. Ctr., Aurangabad, India

Abstract Body:

Background: FEP-ZID combination is based on β-lactam enhancer, ZID, a pan Gram-negative PBP2 binder, irrespective of associated β-lactamases. In combination with FEP, ZID evokes a strong cidal response against AB. *In vivo* thigh and lung PK/PD studies in neutropenic mouse were undertaken for identifying the efficacy driving ZID threshold concentrations (C_T) and associated FEP exposures for three OXA-23 carbapenemase-expressing AB strains with FEP-ZID (1:1) MICs of 16-64 µg/mL.**Methods:** Mouse PK data was obtained by administering subcutaneous q2h doses of FEP-ZID (2:1) ranging from 12.5/6.25-200/100 mg/kg. Serum was collected over 24h and assayed by LC-MS/MS. Neutropenic mice were infected intra-nasally or intramuscularly in thigh (10^7 CFU), to achieve bacterial burden of 6.13-7.02 $\log_{10}$ CFU/thigh at the initiation of treatment (2h post-infection). 27h post-infection bacterial lung/thigh load were determined. To arrive at the optimal dosing regimen and ZID C_T, total daily doses (100-550 mg/kg) and multiple regimens (q2h, q4h, q6h, q12h and q24h) were employed in combination with FEP doses (50 and 75 mg/kg, q2h). Imipenem (IPM) was administered 20 mg/kg, q2h. Exposure-response analysis to arrive at ZID C_T was undertaken using non-linear regression using Graph Pad 6.0.**Results:** Standalone FEP, ZID and IPM did not provide bactericidal effect in lung and thigh infection studies. In lung infection caused by AB SL46 (FEP-ZID MICs 64 µg/mL), combination of FEP 50 mg/kg q2h and ZID 8.33-45.83 mg/kg q2h provided 2.91-3.41 log kill from the base line. In thigh AB 13301 infection (FEP-ZID MICs 16 µg/mL), AB SL 71 and SL46 (FEP-ZID MICs 64 µg/mL), combination of FEP 50 mg/kg q2h and ZID 16.66-91.66 mg/kg q4h provided 1.15-1.31 log kill. Against AB 13301, FEP 75 mg/kg q2h in combination with ZID 8.33-45.83 mg/kg q2h, provided 1.14-2.74 log kill. The lowest total ZID dose of 100 mg/kg (AUC_{0-24} 90-100 µg.h/mL) provided effective eradication. Dosing regimen q2h and q4h were more efficacious. For three CHDL OXA-producers, exposure-response analysis showed that ZID C_T in combination with FEP was 2-8 µg/mL.**Conclusions:** FEP-ZID showed therapeutically promising PK/PD for high MIC carbapenemase-expressing AB.

Author Disclosure Block:

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Research Center. **A.M. Patel:** D. Employee; Self; Wockhardt Research Center. **K.V. Umarkar:** D. Employee; Self; Wockhardt Research Center. **J.S. Satav:** D. Employee; Self; Wockhardt Research Center. **A.P. Udaykar:** D. Employee; Self; Wockhardt Research Center. **A.M. Kulkarni :** D. Employee; Self; Wockhardt Research Center. **V.S. Zope:** D. Employee; Self; Wockhardt Research Center. **S.S. Bhagwat:** D. Employee; Self; Wockhardt Research Center. K. Shareholder (excluding diversitied mutual funds); Self; Wockhardt Ltd.,. **M.V. Patel:** D. Employee; Self; Wockhardt Research Center. K. Shareholder (excluding diversitied mutual funds); Self; Wockhardt Ltd.,.

Poster Board Number:

SUNDAY-442

Publishing Title:

WCK 5222 [Cefepime (FEP)-WCK 5107 (Zidebactam, ZID)]: Activity against ESBL, Class C, and KPC-Expressing Enterics and *Pseudomonas* (PA) Expressing AmpC (PA AmpC) or OXA β-Lactamases (PA OXA)

Author Block:

H. N. Khande, P. R. Joshi, S. R. Palwe, **S. S. Bhagwat**, M. V. Patel; Wockhardt Res. Ctr., Aurangabad, India

Abstract Body:

Background:β-lactam-β-lactamase inhibitor (BL-BLI) combinations rely on β-lactamase inhibition and restoration of BL activity. However, FEP-ZID confers activity by dual mechanism of action based on ZID's PBP2 binding and β-lactamase inhibition. The *in vitro* and *in vivo* activity of FEP-ZID was explored by multi-pronged analysis to measure the additional benefits conferred by β-lactam enhancer, ZID.**Method:**β-lactamase inhibition was determined by nitrocefin assay. MICs were determined against 194 enterics [ESBL (96), Class C (76), KPC (22)] and 225 *Pseudomonas* (PA) [AmpC (153), OXA (72)] strains as per the reference CLSI agar dilution method. Time-kill studies employed initial inoculum 6.0–7.0 $\log_{10}$ CFU/mL. Mutant prevention concentration (MPC) were determined for KPC and Class C enteric strains (MHA, inoculum: 10^9–10^{10} CFU/plate, 48h of incubation).**Results:**IC_{50} of ZID against ESBL and KPC enzymes ranged from 0.16–1.21 µM and against Class C IC_{50} was 0.01–0.21 µM. For PA AmpC and PA OXA, ZID IC_{50} was 0.16–0.21 µM and 0.76–0.82 µM, respectively. For enterics, FEP-ZID MIC_{90}s were 4–16 times lower than ceftazidime (CAZ)-avibactam (AVI) and >16 times lower than ceftolozane-tazobactam (CXA-201). For PA AmpC, CXA 201 was 1–2 times more active than FEP-ZID and CAZ-AVI, however for PA OXA, FEP-ZID was more active (table). In time-kill studies, at comparable concentrations [FEP-ZID and CAZ-AVI (4+4 µg/mL)], FEP-ZID brought about 1–2 $\log_{10}$ additional kill over CAZ-AVI within 2–4h for ESBL and Class C enterics. Unlike CXA 201 and CAZ-AVI, FEP-ZID (all at 8+8 µg/mL) showed >2 $\log_{10}$ kill for PA OXA. For KPC and Class C enteric strains, FEP-ZID MPCs were 4 +1 µg/mL and for CAZ-AVI MPCs were 4–8 + 4 µg/mL. CXA 201 did not attain MPC.**Conclusion:**WCK 5222 through its dual action imparts *in vitro* features superior than existing BL-BLIs against ESBL and carbapenem-resistant organisms.

Organisms, β-lactamase	$MIC_{50/90}$ (µg/mL)					
	FEP	ZID	FEP-ZID (1:1)	CAZ-AVI	CXA-201	Imipenem
Enteric, ESBL	32/>64	4/>32	0.25/2	1/8	32/>64	0.5/4

Enteric, Class C	8/>32	0.5/>32	0.12/1	0.5/4	16/>64	0.5/4
Enteric, KPC	16/>32	1/>32	0.25/0.5	1/2	>32/>32	8/32
PA AmpC	4/32	8/16	2/8	2/8	<0.5/4	2/4
PA OXA	256/>256	8/16	4/8	32/>128	64/128	2/16

Author Disclosure Block:

H.N. Khande: D. Employee; Self; Wockhardt Research Center. **P.R. Joshi:** D. Employee; Self; Wockhardt Research Center. **S.R. Palwe:** D. Employee; Self; Wockhardt Research Center. **S.S. Bhagwat:** D. Employee; Self; Wockhardt Research Center. K. Shareholder (excluding diversitied mutual funds); Self; Wockhardt Ltd.,. **M.V. Patel:** D. Employee; Self; Wockhardt Research Center. K. Shareholder (excluding diversitied mutual funds); Self; Wockhardt Ltd.,.

Poster Board Number:

SUNDAY-443

Publishing Title:

WCK 5222 [Cefepime (Fep)-WCK 5107 (Zidebactam, ZID)]: Lung Pk/Pd Studies for Carbapenamase-Expressing *K. pneumoniae* (KP) and *P. aeruginosa* (Pa)

Author Block:

S. S. Takalkar, R. P. Chavan, A. M. Patel, K. V. Umarkar, J. S. Satav, A. P. Udaykar, A. M. Kulkarni , J. U. Shaikh, **S. S. Bhagwat**, M. V. Patel; Wockhardt Res. Ctr., Aurangabad, India

Abstract Body:

Background: WCK 5222 is based on β-lactam enhancer - ZID, a pan Gram-negative PBP2 binder, irrespective of associated β-lactamases. In combination with FEP, ZID evokes a strong cidal response against carbapenemase-producers. *In vivo* PK/PD studies aimed at identifying the efficacy driving ZID threshold concentrations (C_T) and associated FEP exposures were undertaken employing KP strains expressing KPC (FEP-ZID MIC 0.5 µg/mL) and NDM (FEP-ZID MIC 8 µg/mL) and PA expressing VIM (FEP-ZID MIC 16 µg/mL).**Methods:** Mouse PK data for subcutaneous FEP-ZID (2:1) q2h doses (12.5/6.25–200/100 mg/kg) were determined. Serum was collected over 24h and assayed by LC-MS/MS. Neutropenic mice were infected intra-nasally (10^7–10^8 CFU), with bacterial burden at the initiation of treatment (2h post-infection) in the range of 5.6–7.11 $\log_{10}$ CFU/lung. 27h post-infection bacterial lung load was enumerated. For determination of optimal dosing regimen and ZID C_T, total daily doses (100–550 mg/kg) and regimens (q2h, q4h, q6h, q12h and q24h) were employed in combination with FEP (50 and 75 mg/kg, q2h). Imipenem (IPM) was administered 20 mg/kg, q2h. Exposure-response analysis to arrive at ZID C_T was undertaken using nonlinear regression using Graph Pad 6.0.**Results:** Standalone FEP, ZID and IPM did not provide bactericidal effect. Combination of FEP 50 mg/kg q2h and ZID 8.33–45.83 mg/kg q2h provided 0.94–3.97 log kill for the three strains studied. Similarly, higher FEP dose of 75 mg/kg q2h in combination with ZID, provided 1.66–4.0 log kill. Thus, lowest total ZID dose of 100 mg/kg ($AUC_{0–24}$: 90–100 µg.h/mL) provided effective eradication of all the three carbapenamase-producing strains. Dosing regimen q2h was more efficacious. For three carbapenemases-producers, exposure-response analysis showed that in combination with FEP, ZID C_T was 1–8 µg/mL.**Conclusion:** FEP-ZID showed therapeutically promising PK/PD for carbapenemase-expressing KP and PA.

Author Disclosure Block:

S.S. Takalkar: D. Employee; Self; Wockhardt Research Center. K. Shareholder (excluding diversitied mutual funds); Self; Wockhardt Ltd.,. **R.P. Chavan:** D. Employee; Self; Wockhardt Research Center. **A.M. Patel:** D. Employee; Self; Wockhardt Research Center. **K.V. Umarkar:** D. Employee; Self; Wockhardt Research Center. **J.S. Satav:** D. Employee; Self; Wockhardt Research Center. **A.P. Udaykar:** D. Employee; Self; Wockhardt Research Center. **A.M.**

Kulkarni : D. Employee; Self; Wockhardt Research Center. **J.U. Shaikh:** D. Employee; Self; Wockhardt Research Center. **S.S. Bhagwat:** D. Employee; Self; Wockhardt Research Center. K. Shareholder (excluding diversitied mutual funds); Self; Wockhardt Ltd.,. **M.V. Patel:** D. Employee; Self; Wockhardt Research Center. K. Shareholder (excluding diversitied mutual funds); Self; Wockhardt Ltd.,.

Poster Board Number:

SUNDAY-444

Publishing Title:

WCK 5222 [(Cefepime (FEP)-WCK 5107 (Zidebactam, ZID)]: *In Vitro* and *In Vivo* Activity against Indian Metallo-β-Lactamase (MBL)-Producing *Enterobacteriaceae* (ENT) and *Pseudomonas* (PA)

Author Block:

S. S. Takalkar, A. M. Kulkarni, P. R. Joshi, H. N. Khande, J. S. Satav, S. S. Bhagwat, **M. V. Patel**; Wockhardt Res. Ctr., Aurangabad, India

Abstract Body:

Background:MBL-expressing pathogens are currently untreatable by β-lactam (BL)-based antibacterial agents, necessitating the use of compromised therapies such as colistin and tigecycline. FEP-ZID exerts its antibacterial activity against MBL ENT and PA isolates by virtue of complementary PBP binding of the two constituents. Here, we describe the *in vitro* and *in vivo* activity of FEP-ZID against MBL ENT and PA.**Method:**MICs of FEP-ZID (1:1 and 2:1**)** were determined by reference CLSI agar dilution method against 106 MBL enterics (*E. coli* (EC)-39, *K. pneumoniae* (KP)-57, *Citrobacter*-2, *Enterobacter*-1, Proteae-6 and unidentified strain-1) and 91 PA isolates. *In vitro* cidal activity of FEP-ZID was established through time-kill studies (starting inoculum: 10^6-10^7 CFU/mL). $ED_{50/90}$ doses of FEP-ZID were determined through systemic infection employing eight strains (6 ENT: EC S30, EC S35, EC S33, KP B88, KP 13443, PA13437 and PA 32). Mice were intraperitonially infected with 6.5-6.69 $\log_{10}$ CFU/mouse and treatment was initiated 1h post-infection (BID/TID for 1 day).**Results:**Standalone ZID $MIC_{50/90}$ was 0.25/2 µg/mL. FEP-ZID (1:1 and 2:1) was highly active against MBL enterics with $MIC_{50/90}$ of 0.25/1 and 0.5/2 µg/mL, respectively. Meropenem (MEM), and ceftazidime (CAZ)-avibactam (AVI) $MIC_{50/90}$ for enterics were >32 µg/mL. FEP-ZID inhibited 73.62% and 96.7% of the PA MBL strains at ≤ 8 and ≤ 16 µg/mL, respectively. $MIC_{50/90}$ for FEP and MEM were 128/>256 µg/mL and for CAZ-AVI >128/>256 µg/mL. Time-kill studies showed that, unlike standalone agents, FEP-ZID at 8+8 µg/mL caused 1.5–>3 $\log_{10}$ CFU kill, while MEM and CAZ-AVI showed no cidal activity. *In vivo* systemic infection efficacy studies for enteric MBL provided ED_{90} of FEP 50–100 mg/kg + ZID 7.12–50.57 mg/kg. Similarly for PA MBL strains, ED_{90} was achieved at FEP 50 mg/kg + ZID 21.20–25.28 mg/kg. Carbapenems and CAZ-AVI failed to protect animals at significantly higher doses.**Conclusion:**WCK 5222 demonstrates promising *in vitro* and *in vivo* activity against MBL ENT and PA strains based on ZID's β-lactam enhancer action.

Author Disclosure Block:

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Poster Board Number:

SUNDAY-445

Publishing Title:

WCK 5222 (Cefepime-Zidebactam) Antimicrobial Activity Tested against *Enterobacteriaceae* Clinical Isolates Collected Worldwide (2015)

Author Block:

H. S. Sader, M. Castanheira, R. K. Flamm, D. J. Farrell, R. N. Jones; JMI Lab., Inc., North Liberty, IA

Abstract Body:

Background: Zidebactam (ZID), a bicyclo-acyl hydrazide, is a β-lactam-enhancer with a dual mechanism of action involving selective and high binding affinity to Gram-negative PBP2 and β-lactamase inhibition. We evaluated the *in vitro* activity of cefepime (FEP) combined with ZID against contemporary clinical isolates of Enterobacteriaceae (ENT).**Methods:** 1,404 isolates from USA (452), Europe (645), Asia-Pacific (257) and Latin America (50) were collected in 2015 by the SENTRY Antimicrobial Surveillance Program and susceptibility (S) tested by a reference broth microdilution method against FEP-ZID (1:1 and 2:1 ratios) and comparator agents.**Results**: FEP-ZID was the most active compound with $MIC_{50/90}$ of ≤0.03/0.12 and 0.06/0.25 μg/mL, and highest MIC values of 4 (1:1 ratio) and 8 μg/mL (2:1). Amikacin ($MIC_{50/90}$, 2/4 μg/mL; 98.1% S) and meropenem (MEM; $MIC_{50/90}$, 0.03/0.06 μg/mL; 97.4% S; Table) were also very active. FEP-ZID was active against all ENT species ($MIC_{50/90}$, ≤0.03-0.06/0.12-0.5 μg/mL [1:1 ratio]) and retained potent activity against MEM-non-S *K. pneumoniae* (KPN; $MIC_{50/90}$, 0.5/2 μg/mL; highest MIC, 4 μg/mL [1:1]) and ceftazidime-non-S *Enterobacter* spp. ($MIC_{50/90}$, 0.12/0.5 μg/mL; highest MIC, 1 μg/mL [1:1]). FEP-ZID activity was consistent among geographic regions and only 2 isolates showed MIC values of >2 μg/mL for FEP-ZID (1:1 ratio), 2 KPN (Russia and Taiwan), both with a MIC of 4 μg/mL. S rates for MEM among KPN was lower in the USA (86.0%) compared to the other regions (94.4-97.0%).**Conclusion:** FEP-ZID (WCK 5222) was very active against this worldwide collection of ENT, including isolates resistant to broad-spectrum cephalosporins and/or carbapenems. These results support the further clinical development of WCK 5222.

	MIC_{50}/MIC_{90} (% susceptible[a])			
Organism (n)	FEP-ZID (1:1)	FEP	PIP-TAZ[b]	Merpoenem
Enterobacteriaceae (1,404)	≤0.03/0.12 (99.9/100.0)[c]	0.06/32 (82.9)	2/32 (87.2)	0.03/0.06 (97.4)
E. coli (561)	≤0.03/0.12 (100.0/100.0)[c]	0.06/32 (83.5)	2/8 (92.7)	≤0.015/0.03 (99.6)
Klebsiella spp. (383)	≤0.03/0.5 (99.5/100.0)[c]	0.06/>64 (72.6)	2/>128 (79.6)	0.03/0.12 (93.5)

MEM-NS KPN (23)	0.5/2 (91.3/100.0)[c]	>64/>64 (0.0)	>128/>128 (4.3)	8/>32 (0.0)
Enterobacter spp. (219)	≤0.03/0.25 (100.0/100.0)[c]	0.06/4 (86.8)	2/64 (77.6)	0.03/0.12 (96.3)
P. mirabilis (85)	0.06/0.12 (100.0/100.0)[c]	0.06/16 (88.2)	0.25/1 (98.8)	0.06/0.12 (100.0)
S. marcescens (66)	0.06/0.25 (100.0/100.0)[c]	0.06/0.25 (93.9)	2/8 (95.5)	0.06/0.06 (97.0)
Indole-positive Proteae (40)	≤0.03/0.06 (100.0/100.0)[c]	≤0.03/0.12 (100.0)	0.25/1 (100.0)	0.06/0.12 (100.0)
a. According to CLSI breakpoints; b. PIP-TAZ = piperacillin-tazobactam; c. % inhibited at ≤2/≤8 μg/mL (low/high dose, CLSI).				

Author Disclosure Block:

H.S. Sader: H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Wockhardt. **M. Castanheira:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Wockhardt. **R.K. Flamm:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Wockhardt. **D.J. Farrell:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Wockhardt. **R.N. Jones:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Wockhardt.

Poster Board Number:

SUNDAY-446

Publishing Title:

***In Vitro* activity of WCK 5222 (Cefepime-Zidebactam) Tested against Clinical Isolates of Antimicrobial-Resistant Gram-Negative Bacilli**

Author Block:

H. S. Sader, R. K. Flamm, D. J. Farrell, M. Castanheira, R. N. Jones; JMI Lab., Inc., North Liberty, IA

Abstract Body:

Background: Zidebactam (ZID) is a bicyclo-acyl hydrazide with a dual mechanism of action: selective binding to Gram-negative PBP2 and β-lactamase inhibition. We evaluated the *in vitro* activity of cefepime (FEP) combined with ZID against contemporary clinical isolates of Enterobacteriaceae (ENT) and *P. aeruginosa* (PSA) with various resistant (R) phenotypes.**Methods:** Isolates were collected from 55 medical centers from 19 countries worldwide in 2015 by the SENTRY Antimicrobial Surveillance Program. Susceptibility testing was performed in a central laboratory by a reference broth microdilution method against FEP-ZID (1:1 and 2:1 ratios) and comparator agents.**Results:** FEP-ZID (1:1 ratio) was very active against all R subsets (see Table). The highest FEP-ZID (1:1) MIC values among ENT was only 4 μg/mL; thus, 100.0% of strains would be considered susceptible (S) when the CLSI high dose FEP breakpoint (≤8 μg/mL) is applied. Further, 93.8-100.0% of ENT were inhibited at FEP-ZID (1:1) MIC of ≤2 μg/mL (low dose FEP breakpoint, CLSI). MIC values for FEP-ZID at a 2:1 ratio were slightly higher (less than one doubling dilution overall) compared to the 1:1 ratio. Among ESBL-phenotype *E. coli* (EC) / *Klebsiella* spp. (KSP), meropenem (MEM) and amikacin (AMK) were active against 98.3/80.5% and 98.3/83.7%, respectively. Only 78.7-84.6% of multidrug-R (MDR) ENT were S to MEM, AMK or colistin (COL). Carbapenem-R ENT (CRE) and extensively drug R (XDR) ENT exhibited low S to AMK (71.9 and 77.8% S, respectively), COL (87.5 and 72.2% S, respectively) and all antimicrobials tested except for FEP-ZID. COL and the FEP-ZID combinations were the most active compounds tested against MDR and XDR PSA. ZID tested alone was also active *in vitro* against MDR and XDR PSA ($MIC_{50/90}$, 8/16 μg/mL for both); whereas only 59.6 and 50.0% of strains were S to AMK, respectively.**Conclusion:** FEP-ZID (WCK 5222) showed potent *in vitro* activity against R subsets of Gram-negative bacilli with high rates of R to most antimicrobial agents currently available for clinical use.

Resistant subset (no.)	**MIC_{50}/MIC_{90} (% susceptible[a])**				
	FEP-ZID (1:1)	**ZID**	**Meropenem**	**Amikacin**	**Colistin**
ESBL-phenotype EC (120)	0.12 / 0.25/ (100.0 / 100.0)[b]	0.12 / 0.25	0.03 / 0.06 (98.3)	4 / 8 (98.3)	0.12 / 0.25 (100.0)[c]

ESBL-phenotype KSP (129)	0.25 / 1 (98.4 / 100.0)[b]	0.5 / >64	0.06 / 8 (80.5)	4 / 32 (83.7)	0.12 / 0.25 (94.6)[c]
Ceftazidime-non-SEBS (74)	0.12 / 0.5 (100.0 / 100.0)[b]	0.25 / 4	0.06 / 2 (89.2)	1 / 4 (100.0)	0.12 / >8 (86.3)[c]
CRE (32)	0.5 / 2 (93.8 / 100.0)[b]	2 / >64	8 / >32 (0.0)	4 / 32 (71.9)	0.12 / >8 (87.5)[c]
MDR ENT (169)	0.25 / 1 (98.8 / 100.0)[b]	0.5 / >64	0.06 / 8 (78.7)	4 / 32 (84.6)	0.12 / >8 (81.1)[c]
XDR ENT (18)	0.5 / 2 (94.4 / 100.0)[b]	4 / >64	4 / 32 (5.6)	4 / 32 (77.8)	0.12 / >8 (72.2)[c]
MDR PSA (57)	4 / 8 (98.2)[d]	8 / 16	16 / >32 (5.3)	16 / >32 (59.6)	≤0.5 / 1 (100.0)
XDR PSA (42)	4 / 8 (97.6)[d]	8 / 16	16 / >32 (2.4)	16 / >32 (50.0)	≤0.5 / 1 (100.0)
a. According to CLSI breakpoints; b. % inhibited at ≤2/≤8 μg/mL (low/high dose, CLSI). c. According to EUCAST breakpoints;d. CLSI and EUCAST S breakpoints for FEP					

Author Disclosure Block:

H.S. Sader: H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Wockhardt. **R.K. Flamm:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Wockhardt. **D.J. Farrell:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Wockhardt. **M. Castanheira:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Wockhardt. **R.N. Jones:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Wockhardt.

Poster Board Number:

SUNDAY-447

Publishing Title:

WCK 5222 (Cefepime-Zidebactam) Antimicrobial Activity Tested against Gram-Negative Organisms Producing Clinically Relevant β-Lactamases

Author Block:

H. S. Sader, P. R. Rhomberg, R. N. Jones, M. Castanheira; JMI Lab., Inc., North Liberty, IA

Abstract Body:

Background: Zidebactam (ZID) is a β-lactam enhancer with dual mechanism of action involving binding to Gram-negative (GN) PBP2 and β-lactamase (BL) inhibition. Cefepime (FEP) combined with ZID is under clinical development.**Methods:** 193 clinical GN strains producing the most clinically relevant BL types plus 71 wild type (WT) strains were tested for susceptibility (S) by a reference broth microdilution method against FEP-ZID (1:1 and 2:1 ratios tested at 0.06-128 μg/mL), FEP and ZID. BL encoding genes were evaluated by a microarray-based assay.**Results:** FEP-ZID (1:1) was very active against Enterobacteriaceae (ENT) producing CTX-M-15 (21; $MIC_{50/90}$, 0.25/1 μg/mL), SHV (20; $MIC_{50/90}$, 0.12/0.25 μg/mL), other extended-spectrum BLs (ESBLs; 20, including GES-18; OXA-1/30, OXY-, PER-, TEM- and VEB-like; $MIC_{50/90}$, 0.25/1 μg/mL), plasmidic AmpC (10; $MIC_{50/90}$, ≤0.06/≤0.06 μg/mL), derepressed AmpC (23; $MIC_{50/90}$, 0.12/0.5 μg/mL), KPC (35; $MIC_{50/90}$, 0.25/1 μg/mL), metallo-BL (MBL; 20 including VIM, IMP and NDM; $MIC_{50/90}$, 0.5/8 μg/mL; Table). WT ENT had $MIC_{50/90}$ values of ≤0.06/≤0.06, ≤0.06/0.12 and 0.25/>128 μg/mL for FED-ZID (1:1), FEP and ZID, respectively. FEP-ZID was also active against *P. aeruginosa* (PSA) producing derepressed AmpC (21; $MIC_{50/90}$, 4/8 μg/mL) and MBL (12 [VIM and IMP]; $MIC_{50/90}$, 4/8 μg/mL). FEP-ZID 1:1 ratio was slightly (2-fold) more active than FEP-ZID 2:1 ratio when tested against BL-producing ENT and PSA; and ZID alone exhibited potent *in vitro* activity against some ENT and PSA, including BL-producing strains. FEP-ZID 1:1 ratio ($MIC_{50/90}$, 32/32 μg/mL) showed only moderate activity against OXA-23/24-producing *Acinetobacter* spp., but it was >4-fold more active than FEP or ZID tested alone.**Conclusion:** FEP-ZID (1:1 and 2:1 ratios) showed potent in vitro activities against ENT and PSA producing various clinically relevant BLs, including ESBLs, KPCs, AmpC and MBLs for which limited treatment options are currently available. These *in vitro* results support further clinical development of FEP-ZID (WCK 5222).

β-lactamase (organism; no. tested)[a]	**No. of isolates (cumulative %) inhibited at FEP-ZID (1:1 ratio) MIC (μg/mL) of:**								
	≤0.12	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**
CTX-M-15 (ENT; 21)	6 (28.6)	8 (66.7)	4 (85.7)	1 (90.5)	1 (95.2)	1 (100.0)	--	--	--

SHV (ENT; 20)	14 (70.0)	5 (95.0)	0 (95.0)	1 (100.0)	--	--	--	--	--
Other ESBLs (ENT; 20)	9 (45.0)	6 (75.0)	2 (85.0)	3 (100.0)	--	--	--	--	--
Plasmidic AmpC (ENT; 10)	10 (100.0)	--	--	--	--	--	--	--	--
Derepressed AmpC (ENT; 23)	15 (65.2)	4 (82.6)	2 (91.3)	2 (100.0)	--	--	--	--	--
KPC (ENT; 35)	7 (20.0)	12 (54.3)	11 (85.7)	4 (97.1)	1 (100.0)	--	--	--	--
MBL (ENT; 20)	1 (5.0)	6 (35.0)	3 (50.0)	1 (55.0)	4 (75.0)	1 (80.0)	2 (90.0)	1 (95.0)	1 (100.0)
Derepressed AmpC (PSA; 21)	--	--	1 (4.8)	1 (9.5)	4 (28.6)	7 (61.9)	6 (90.5)	2 (100.0)	--
MBL (PSA; 12)	--	--	1 (8.3)	0 (8.3)	2 (25.0)	6 (75.0)	2 (91.7)	1 (100.0)	--
OXA-23/24 (ASP; 11)	--	--	--	--	--	--	1 (9.1)	3 (36.4)	6 (90.9)

Author Disclosure Block:

H.S. Sader: H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Wockhardt. **P.R. Rhomberg:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Wockhardt. **R.N. Jones:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Wockhardt. **M. Castanheira:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Wockhardt.

Poster Board Number:

SUNDAY-448

Publishing Title:

Cefepime (FEP) and Zidebactam (ZID) Mediated Dual Pbp Engagement at Sub-Mic Concentrations Drive Cidality against Diverse β-Lactamase Expressing Gram-Negatives

Author Block:

S. R. Palwe, S. S. Biniwale, H. N. Khande, P. R. Joshi, **S. S. Bhagwat**, M. V. Patel; Wockhardt Res. Ctr., Aurangabad, India

Abstract Body:

Background: β-lactams induce morphological changes based on their preferential binding to Gram-negative PBPs thereby causing elongation (PBP3 binders) or spheroplastation (PBP2 binders). Multiple PBP binders with preferential PBP2 affinity - such as carbapenems, are rapidly cidal. However, amidst carbapenemases, they lose their spheroplast inducing activity and therefore cidality. ZID is a pan β-lactamase stable, spheroplasting agent for pan Gram-negatives. Herein, we describe the functional basis of cidal synergy exerted by FEP-ZID combination against pathogens expressing various β-lactamases including carbapenemases.**Methods**: Microscopic observation inducing morphological changes were performed to identify minimum elongation concentration (MEC) and minimum spheroplast forming concentrations (MSC) required for elongation and spheroplastation respectively. Time-kill studies were undertaken by employing log phase cultures (10^6-10^7 CFU/mL) in CA-MHB. Strains and associated β-lactamases employed in the study are listed in the table. The viable counts were enumerated periodically by plating aliquots of serially diluted culture exposed to FEP, ZID and FEP-ZID.**Results**: MICs of FEP and ZID were in the range of 0.03-256 µg/mL and 0.25->256 µg/mL, respectively. Elongation and spheroplastation were attained at sub-MIC concentrations. MEC and MSC for FEP and ZID, were 0.015-16 µg/mL and 0.12-8 µg/mL, respectively. Time-kill studies (n=13) **i**nvolving combinations of ZID at its MSC and FEP at its MEC brought about 0.31->3 and 1.29->3 $\log_{10}$ CFU/mL kill at 4 and 6h, respectively.**Conclusion:** Combination of MEC and MSC, the functional attributes of PBP2 and PBP3, confers cidality to FEP-ZID combination even in the presence of carbapenamases.

Organisms	β-lactamases	MIC (µg/mL)		MEC (µg/mL)	MSC (µg/mL)
		FEP	ZID		
E. coli 25922	None	0.03	0.25	0.015	0.12
K. pneumoniae J111	None	0.06	0.25	0.015	0.12
P. aeruginosa PAO1	None	2	4	0.5	2

E. coli 7MP	SHV+TEM+CMY	32	1	4	0.5
K. pneumoniae H521	SHV+TEM+KPC	64	0.5	4	0.25
K. pneumoniae NCTC 13443	SHV+TEM+NDM	>32	>32	8	4
K. pneumoniae B88	ESBL+NDM	>32	>32	16	0.25
K. pneumoniae S 215	ESBL+NDM	64	>32	16	0.12
K. pneumoniae S35	ESBL+NDM	>32	0.5	8	0.5
P. aeruginosa NCTC 13437	VIM-10+VEB	256	16	16	8
P. aeruginosa Q119	VIM	64	8	8	8
A. baumannii NCTC13302	OXA-25	256	>32	16	0.5
A. baumannii NCTC 13304	OXA-27	256	>32	16	0.5

Author Disclosure Block:

S.R. Palwe: D. Employee; Self; Wockhardt Research Center. **S.S. Biniwale:** D. Employee; Self; Wockhardt Research Center. **H.N. Khande:** D. Employee; Self; Wockhardt Research Center. **P.R. Joshi:** D. Employee; Self; Wockhardt Research Center. **S.S. Bhagwat:** D. Employee; Self; Wockhardt Research Center. K. Shareholder (excluding diversitied mutual funds); Self; Wockhardt Ltd.,. **M.V. Patel:** D. Employee; Self; Wockhardt Research Center. K. Shareholder (excluding diversitied mutual funds); Self; Wockhardt Ltd.,.

Poster Board Number:

SUNDAY-449

Publishing Title:

WCK 5107 (Zidebactam, ZID) and WCK 5222 [Cefepime (FEP)-ZID]: Repeated-Dose Toxicity in Rat and Dog

Author Block:

M. B. Nandanwar, A. J. Kansagara, S. Satale, M. I. Patel, S. R. Gupta, R. P. Chavan, A. M. Patel, R. D. Yeole, M. V. Patel; Wockhardt Res. Ctr., Aurangabad, India

Abstract Body:

Background:ZID is a Gram-negative antibacterial agent acting both as a pan Gram-negative β-lactam enhancer and a β-lactamase inhibitor. In order to establish the toxicity, 28-day repeated-dose toxicity studies were performed with both ZID and FEP-ZID in Wistar rats and Beagle dogs.**Method:**ZID was administered intravenously (i.v.) at 200, 400 and 800 mg/kg/day in rat and at 200, 300 and 750 mg/kg/day in dog. FEP-ZID was administered at 300+150 and 300+300 mg/kg/day in dogs (i.v.). Control group included standalone i.v. FEP administration at 300 mg/kg/day in the dog study. In parallel, corresponding recovery groups were also maintained for 14 days for control, ZID (800 mg/kg/day) in rat, ZID (750 mg/kg/day) and FEP-ZID (300+300 mg/kg/day) in dog. In both rat and dog, the doses were equally divided and administered twice-daily. For toxicokinetic profiling, blood was sampled at multiple time points (on 1, 14 and 28 days) in both rat and dog. Clinical signs, body weight, food consumption, ophthalmoscopy, clinical pathology, ECG, organ weight, gross and histopathology evaluations were performed.**Results:**No major or significant changes were observed in both rat and dog studies even at the highest doses tested for ZID and FEP-ZID. In rat, a mild increase in AST and ALT values was observed at 400 and 800 mg/kg/day doses with no changes in bilirubin levels. Histopathological investigations did not reveal any changes either in liver or any other vital organ. The mild elevation of AST and ALT values were fully normalized during the 14 day recovery period. In dog, mild increase in AST (males) was observed at all doses of ZID on day 29 with no histopathological alterations in liver. This AST level was normalized by the end of 14-day reversal period. ZID stand-alone or in combination with FEP elicited reaction at injection site. No Observable Adverse Effect Level (NOAEL) of WCK 5107 in rat and dog was found to be 800 and 750 mg/kg/day, respectively. The highest AUC achieved at NOAEL doses in rat and dog were 1948 and 1962 μg.h/mL, respectively. For WCK 5222, 300+300 mg/kg/day dose was estimated as NOAEL in dog and the AUC achieved was 850 μg.h/mL (FEP) + 1660 μg.h/mL (ZID).**Conclusions:**Both WCK 5107 and WCK 5222 showed a promising safety profile in rat and dog.

Author Disclosure Block:

M.B. Nandanwar: D. Employee; Self; Wockhardt Research Center. K. Shareholder (excluding diversitied mutual funds); Self; Wockhardt Ltd.,. **A.J. Kansagara:** D. Employee; Self; Wockhardt Research Center. **S. Satale:** D. Employee; Self; Wockhardt Research Center. **M.I. Patel:** D. Employee; Self; Wockhardt Research Center. **S.R. Gupta:** D. Employee; Self; Wockhardt Research Center. **R.P. Chavan:** D. Employee; Self; Wockhardt Research Center. **A.M. Patel:** D. Employee; Self; Wockhardt Research Center. **R.D. Yeole:** D. Employee; Self; Wockhardt Research Center. K. Shareholder (excluding diversitied mutual funds); Self; Wockhardt Ltd.,. **M.V. Patel:** D. Employee; Self; Wockhardt Research Center. K. Shareholder (excluding diversitied mutual funds); Self; Wockhardt Ltd.,.

Poster Board Number:

SUNDAY-450

Publishing Title:

WCK 5107 [Zidebactam, (ZID)] and WCK 5222 [Cefepime (FEP)-Zidebactam (ZID)] Intravenous Pharmacokinetics (PK) in Beagle Dogs

Author Block:

R. P. Chavan, A. M. Patel, V. S. Zope, J. U. Shaikh, A. D. Patil, R. D. Yeole, **M. V. Patel**; Wockhardt Res. Ctr., Aurangabad, India

Abstract Body:

Background: ZID is a novel β-lactam enhancer currently undergoing Phase I study in combination with FEP. ZID is a bicyclo-acyl hydrazide with a pan Gram-negative PBP2 binding as well as potent β-lactamase inhibition. **Methods:** Intravenous PK of standalone ZID, FEP and FEP-ZID (2:1) was evaluated in beagle dogs to investigate PK interactions. The doses were administered as 0.25h constant infusion. Other BLIs- avibactam (AVI) and relebactam (REL) were also studied. Plasma concentrations were determined by validated LC/MS-MS method. PK parameters were calculated by non-compartmental analysis (Phoenix WinNonlin).**Results:** Standalone ZID at 15 and 30 mg/kg, yielded plasma C_{max} of 61.2 and 138.5 µg/mL, and AUC of 78.6 and 200.4 µg.h/mL, respectively. ZID (15 and 30 mg/kg) administered as FEP-ZID provided plasma C_{max} of 76.6 and 99.4 µg/mL, and AUC of 100.5 and 157.9 µg.h/mL, respectively. Standalone FEP at 30, and 60 mg/kg, yielded plasma C_{max} of 137.6 and 251.6 µg/mL, and AUC of 181.6 and 358.5 µg.h/mL, respectively whereas FEP (30 and 60 mg/kg) in FEP-ZID yielded plasma C_{max} of 134.9 and 208.8 µg/mL, and AUC of 174.3 and 327.0 µg.h/mL, respectively. Plasma elimination half-life of FEP and ZID was in the range of 1.0 to1.5h and 1.3 to 1.7h, respectively. AVI and REL at 15, 30 mg/kg, achieved plasma C_{max} of 33.28, 100.12 µg/mL and 79.57, 141.91 µg/mL, respectively. Similarly, plasma AUCs of AVI and REL (both at 15, 30 mg/kg) were 35.83, 79.11 µg.h/mL and 98.35, 188.55 µg.h/mL, respectively. Elimination half-life of AVI and REL was observed to be 1.0 and 1.5h, respectively.**Conclusions:**No PK interaction between WCK 5107 and WCK 5222 was observed. WCK 5107 showed higher exposures than AVI and comparable to REL.

Author Disclosure Block:

R.P. Chavan: D. Employee; Self; Wockhardt Research Center. **A.M. Patel:** D. Employee; Self; Wockhardt Research Center. **V.S. Zope:** D. Employee; Self; Wockhardt Research Center. **J.U. Shaikh:** D. Employee; Self; Wockhardt Research Center. **A.D. Patil:** D. Employee; Self; Wockhardt Research Center. **R.D. Yeole:** D. Employee; Self; Wockhardt Research Center. K. Shareholder (excluding diversitied mutual funds); Self; Wockhardt Ltd.,. **M.V. Patel:** D. Employee; Self; Wockhardt Research Center. K. Shareholder (excluding diversitied mutual funds); Self; Wockhardt Ltd.,.

Poster Board Number:

SUNDAY-451

Publishing Title:

Ceftazidime-Avibactam (CAZ-AVI) Activity Tested against Eleven *Enterobacteriaceae* (ENT) Species Producing KPC Enzymes

Author Block:

M. Castanheira, R. E. Mendes, R. N. Jones, H. S. Sader; JMI Lab., North Liberty, IA

Abstract Body:

Background: KPC enzymes are usually detected in *K. pneumoniae* (KPN); however other ENT species often carry these carbapenemases. We evaluated the activity of CAZ-AVI and comparators tested against KPC-producing ENT belonging to 11 bacterial species.**Methods:** 662 KPC-producing ENT clinical isolates collected worldwide from 2009-2014 were susceptibility (S) tested by reference broth microdilution methods using CAZ-AVI (AVI at fixed 4 µg/ml) and comparators. CLSI, US-FDA (tigecycline [TIG] and CAZ-AVI) and EUCAST interpretative criteria were applied. KPC and metallo-β-lactamase (MBL) genes were detected by PCR/sequencing.**Results:** Overall, CAZ-AVI inhibited 98.8% of the isolates at the breakpoint recently established by the US-FDA. Among other antimicrobial agents, TIG (97.4 and 90.8% S by US-FDA and EUCAST criteria, respectively) and colistin (COL; 82.1% S [EUCAST]) were the only agents with S rates >80.0%. Amikacin inhibited 56.2 and 40.2% of the isolates according to CLSI and EUCAST S criteria, respectively. Isolates displaying elevated CAZ-AVI (>8 µg/ml) were one *E. cloacae* (ECL; Poland) and seven KPN isolates from China, Greece, USA (one each) and Italy (n=4). These 8 isolates co-produced MBL: five VIM-1-producers and one each of IMP-4, VIM-4 and VIM-26. KPN isolates displayed higher resistance rates for comparators; CAZ-AVI, TIG and COL were the agents with higher S rates (98.7, 97.5/91.1 and 81.9% S, respectively). KPC-producing ECL isolates had higher S rates to amikacin (95.2/85.7% S) compared to KPN and CAZ-AVI displayed the highest S rates (97.6% S). CAZ-AVI was active against all isolates belonging to other ENT species (100.0% S; Table).**Conclusions:** CAZ-AVI was very active against this large collection of KPC-producing isolates, inhibiting >97.0% of the isolates at the US-FDA breakpoint criteria. Eight isolates in this collection displaying CAZ-AVI MIC values >8 µg/ml and co-produced MBLs that are not inhibited by AVI.

KPC-producing species(no. tested)	% susceptible CLSI (US-FDA for CAZ-AVI and tigecycline)/EUCAST				
	CAZ-AVI	Meropenem	Amikacin	Tigecycline	Colistin
K. pneumoniae (554)	98.7/-	1.8/6.0	49.5/32.5	97.5/91.1	-/81.9
E. cloacae (42)	97.6/-	4.8/11.9	95.2/85.7	95.2/81.0	-/83.3
E. coli (24)	100.0/-	12.5/37.5	83.3/62.5	100.0/100.0	-/95.8

K. oxytoca (12)	100.0/-	0.0/33.3	91.7/83.3	100.0/100.0	-/91.7
C. freundii (10)	100.0/-	10.0/30.0	90.0/90.0	100.0/90.0	-/100.0

Author Disclosure Block:

M. Castanheira: H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Allergan. **R.E. Mendes:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Allergan. **R.N. Jones:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Allergan. **H.S. Sader:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Allergan..

Poster Board Number:

SUNDAY-452

Publishing Title:

Meropenem/Vaborbactam (MER/VAB) Tested Against Contemporary *Enterobacteriaceae* (ENT) Isolates from USA Hospitals

Author Block:

M. Castanheira, M. D. Huband, R. K. Flamm, R. N. Jones; JMI Lab., North Liberty, IA

Abstract Body:

Background: Carbapenem-resistant ENT (CRE) have been detected in various USA hospitals and most of these isolates produce KPC enzymes. We evaluated the activity of MER/VAB (formerly RPX7009), that has enhanced activity against KPC-producing isolates, and comparators tested against Enterobacteriaceae (ENT) isolates collected during 2015 in 29 USA hospitals.**Methods:** 3995 ENT clinical isolates consecutively collected were susceptibility (S) tested by reference broth microdilution methods for MER ± VAB (at fixed 8 µg/ml) and comparators. CRE was defined as an isolate non-S (CLSI criteria) to imipenem and/or meropenem. CLSI, US-FDA (tigecycline) and EUCAST interpretative criteria were applied.**Results:** MER/VAB ($MIC_{50/90}$, 0.03/0.06 µg/ml) was very active against ENT isolates and 99.8% of the isolates were inhibited at ≤1 µg/ml (MER S CLSI breakpoint) and only one isolate (*K. pneumoniae* [KPN] from Texas) had a MER/VAB MIC >4 µg/ml. MER alone inhibited 98.4% of the isolates at the same concentration. Among 839 KPN tested, 94.9% were S to MER and 98.2% of the isolates were inhibited by MER/VAB at ≤1 µg/ml. MER/VAB was very active against other ENT species and inhibited all 1621 *E. coli* and 121 *C. freundii* at ≤0.06 µg/ml and all 371 *E. cloacae*, 232 *P. mirabilis* and 68 *M. morgannii* isolates tested at ≤0.12 µg/ml. One *S. marcescens* displayed a MER/VAB MIC result at 0.5 µg/ml but the other 180 isolates tested had MIC results at ≤0.12 µg/ml and 97.8% were S to MER using the CLSI criteria. MER/VAB was very active against ESBL-phenotype isolates (n=387 [9.7% overall]; $MIC_{50/90}$, 0.03/0.06 µg/ml) and CRE isolates (n=59 [1.5% overall]; $MIC_{50/90}$, 0.06/2 µg/ml) that included 38 KPN and six other species. MER alone inhibited 87.9% of the ESBL, but only 3.4% of CRE isolates using CLSI breakpoints. CRE isolates were resistant to β-lactams (S rates ranged from 0.0 to 3.4%) and more resistant to other antimicrobial classes when compared to overall ENT isolates. Minocycline, colistin and tigecycline displayed the highest S rates (81.4, 82.4 and 100.0% S by CLSI, EUCAST and US-FDA criteria) against CRE.**Conclusions:** MER/VAB was very active against ENT isolates recently collected in USA hospitals and the advantage of this combination over MER alone was demonstrated in CRE isolates that are less susceptible to other clinically available antimicrobials.

Author Disclosure Block:

M. Castanheira: H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from The Medicines Co. **M.D. Huband:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from The Medicines Co. **R.K. Flamm:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from The Medicines Co. **R.N. Jones:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from The Medicines Co..

Poster Board Number:

SUNDAY-453

Publishing Title:

Using Tetrazole-Based Compounds to Study the Function and Inhibition of Beta-Lactamases in Beta-Lactam Resistance

Author Block:

Y. CHEN[1], D. A. Nichols[1], N. J. Torelli[1], X. Zhang[1], O. A. Pemberton[1], P. Jaishankar[2], K. Defrees[2], A. R. Renslo[2], R. Bonnet[3], R. Sanishvili[4]; [1]Univ. of South Florida, Tampa, FL, [2]Univ. of California San Francisco, San Francisco, CA, [3]Clermont Univ´., Clermont-Ferrand, France, [4]Advanced Photon Source, Argonne, IL

Abstract Body:

Beta-lactamases are the main resistance mechanism against beta-lactam antibiotics in Gram-negative bacteria. Inhibitor discovery against these enzymes has mainly focused on covalent ligands, while the development of novel chemotypes remains challenging. Using a fragment-based structural approach, we recently uncovered a tetrazole-based non-covalent inhibitor displaying a Ki of 89 nM against CTX-M-9 Class A beta-lactamase, one of the most common clinically observed extended spectrum beta-lactamase. Here we demonstrate that this compound is able to inhibit a range of serine beta-lactamases including different CTX-M subtypes and some enzymes from other classes, reducing the MIC of cefotaxime by 2-8 fold in bacteria expressing these proteins. The same inhibitor also allowed us to capture a special short hydrogen bond (2.53 Angstrom) between the catalytic Ser70 and the purported general base Lys73, where the hydrogen is equi-distant to the two heteroatoms. This observation sheds new light on the catalytic mechanism by Class A beta-lactamases and potentially by many other enzymes as well. Additionally, through virtual screening, we have developed new tetrazole-based inhibitors displaying activity against both KPC-2 and NDM-1, two of the most problematic carbapenemases, in biochemical testing. These studies demonstrate that tetrazole-based compounds can provide new scaffolds in developing novel beta-lactamase inhibitors as well as unique chemical probes in studying the catalytic mechanisms of these proteins.

Author Disclosure Block:

Y. Chen: J. Scientific Advisor (Review Panel or Advisory Committee); Self; Gordian Biotechnologies. K. Shareholder (excluding diversitied mutual funds); Self; Gordian Biotechnologies. **D.A. Nichols:** None. **N.J. Torelli:** None. **X. Zhang:** None. **O.A. Pemberton:** None. **P. Jaishankar:** None. **K. Defrees:** None. **A.R. Renslo:** J. Scientific Advisor (Review Panel or Advisory Committee); Self; Gordian Biotechnologies. K. Shareholder (excluding diversitied mutual funds); Self; Gordian Biotechnologies. **R. Bonnet:** None. **R. Sanishvili:** None.

Poster Board Number:

SUNDAY-454

Publishing Title:

Easy Detection of Bacterial Tolerance to Antibiotics in Clinical Isolates by a Modified Disk-Diffusion Assay

Author Block:

O. Gefen[1], **J. Strahilevitz**[2], N. Q. Balaban[1]; [1]Hebrew Univ., Jerusalem, Israel, [2]Hadassah Med. Ctr., Jerusalem, Israel

Abstract Body:

Background: Antibiotic drug tolerance - the ability to survive longer under bactericidal treatments - is a potentially clinically significant phenomenon that is overlooked because time-kill assays are too labor intensive to perform routinely in clinical microbiology labs. We developed the TDtest, a simple modification of the standard disk-diffusion assay, that allows the semi-quantitative evaluation of survival rate and killing dynamics. **Methods:** The TDtest consists of a regular disk-diffusion test modified by the replacement of the antibiotic disk with a nutrient-rich disk, and incubation for additional 7 hours. Tolerant bacteria were detected by the growth of colonies within the inhibition zone. Tolerance levels were categorized according to the ratio of surviving colonies to the number of plated cells within the zone of inhibition; low tolerance ($<10^{-5}$), medium tolerance (10^{-5} to 10^{-3}) and high tolerance ($>10^{-3}$). Tolerance detected by the TDtest was correlated with time-kill curves.**Results:** Time-kill curves showed a 2 to 3-log increased survival to ampicillin for the previously defined *tolerance by lag E.coli* mutants (*metG* and *vapB*) versus *wt E.coli* K-12 after 10 hours incubation. With the TDtest, the level of tolerance to ampicillin of *metG* and *vapB* mutants and *wt E.coli* K-12 was high, high, and low, respectively. Using the TDtest, we were able to detect similar differences in the levels of antibiotic tolerance in clinical isolates of *E.coli*. Furthermore, the TDtest identified antibiotics that effectively eliminate ampicillin tolerant bacteria, as further confirmed by time-kill curves.**Conclusions:** The additional information on drug susceptibility provided by the TDtest should enable the tailoring of better treatment regimens for antibiotic tolerant pathogenic bacteria.

Author Disclosure Block:

O. Gefen: None. **J. Strahilevitz:** None. **N.Q. Balaban:** None.

Poster Board Number:

SUNDAY-455

Publishing Title:

Identification of Novel Fluoroquinolones, Wfq-101 and Its Analogues, Circumventing Bacterial Efflux Pumps

Author Block:

D. Kazamori, T. Kinoshita, A. Sasaki, S. Inoue, T. Hirano, H. Amano, Y. Kuramoto, A. Yazaki; Wakunaga Pharmaceutical Co., Ltd., Hiroshima, Japan

Abstract Body:

Background: Spread of drug-resistant bacteria has been a serious and global concern. Over-expression of efflux pumps is one of the major resistant mechanisms in Gram-negative bacteria. In this study, we evaluated antibacterial activity of novel fluoroquinolones of WFQ-101 and its analogues against Gram-negative bacteria, focusing on the impact of the bacterial efflux pumps.**Methods:** Clinical isolates of bacteria were collected from hospitals, universities, and bacteria suppliers. MICs were determined according to standard agar dilution method described by the Japanese Society of Chemotherapy. The effect of efflux pumps on the antibacterial activities was evaluated by comparing the MIC values with and without efflux pump inhibitor of PAβN.**Results:** WFQ-101 and its most potent analogue of WFQ-228 displayed highly potent antibacterial activities against clinical isolates of *P. aeruginosa* (133 strains) and *E. coli* (111 strains); MIC_{90} values of WFQ-101, WFQ-228, and levofloxacin were 8, 2, and 64 mg/L for *P. aeruginosa* and 4, 2, 32 mg/L for *E. coli*, respectively. Compared to levofloxacin, WFQ-228 exerted 32 and 16 times more potent activities against *P. aeruginosa* and *E. coli*, respectively. In addition, the mean MIC value of WFQ-101 was only 3.2-fold decreased against *P. aeruginosa* (3 strains) by co-treatment with PAβN, whereas that of levofloxacin was drastically decreased (26-fold). Furthermore, the treatment of PAβN had little impact on the antibacterial activities of WFQ-101 analogues.**Conclusions:** WFQ-101 analogues demonstrated the superior antibacterial activities against quinolone-resistant Gram-negative bacteria, including *P. aeruginosa* and *E. coli*. This high potency of the compounds would be attributed to the distinct property being poor substrates for efflux pumps of Gram-negative bacteria. These findings indicate that this series of compounds could overcome the drug resistance conferred by the over-expression of bacterial efflux pumps.

Author Disclosure Block:

D. Kazamori: None. **T. Kinoshita:** None. **A. Sasaki:** None. **S. Inoue:** None. **T. Hirano:** None. **H. Amano:** None. **Y. Kuramoto:** None. **A. Yazaki:** None.

Poster Board Number:

SUNDAY-456

Publishing Title:

***In Vitro* Activity of WCK 771, a Benzoquinolizine Fluoroquinolone (Levonadifloxacin) When Tested against Contemporary Gram-Positive and -Negative Bacteria from a Global Surveillance Program**

Author Block:

R. K. Flamm, H. S. Sader, D. J. Farrell, P. R. Rhomberg, R. N. Jones; JMI Lab., North Liberty, IA

Abstract Body:

Background: Intravenous (WCK 771) and oral (WCK 2349) formulations of the anti-MRSA fluoroquinolone, levonadifloxacin (LND) are in clinical development and were awarded QIDP status. LND has a broad spectrum of activity including fluoroquinolone- and methicillin-resistant *Staphylococcus aureus* (MRSA). In this study, LND was tested against Gram-positive and -negative clinical isolates collected in medical centers worldwide as part of the 2014 SENTRY Antimicrobial Surveillance Program.**Methods: 4,276 isolates from United States, 4,264 (Europe), 1,975 (Asia-Pacific), and 1,959 (Latin America) were susceptibility (S) tested against LND and multiple comparators by reference broth microdilution (CLSI).Results:** 96.8% of *S. aureus* (SA) isolates were inhibited at a LND concentration of ≤2 µg/mL (n=4,077; MIC_{50}/MIC_{90}, 0.015/1 µg/mL). MICs for LND were elevated for MRSA (MIC_{50}/MIC_{90}, 0.5/2 µg/mL) compared to MSSA (MIC_{50}/MIC_{90}, 0.015/0.015 µg/mL) and for levofloxacin (LEV)-R (MIC_{50}/MIC_{90}, 0.5/4 µg/mL) compared to LEV-S (MIC_{50}/MIC_{90}, 0.015/0.015 µg/mL) isolates. However 91.5 and 88.8% of MRSA and LEV-R SA were inhibited at LND MICs ≤2 µg/mL, respectively. All SA were S to vancomycin, tigecycline, and linezolid. Daptomycin S was 99.9% and LEV S 71.7% (MRSA LEV S, 30.8%). The MIC_{50}/MIC_{90} for LND against *Streptococcus agalactiae* (n=353) and *S. pyogenes* (n=352) was 0.25/0.5 µg/mL; against viridans group streptococci (n=438) the MIC_{50}/ MIC_{90}, was 0.5/1 µg/mL. Against enterococci (n=535), LND MICs were elevated for *E. faecium* (MIC_{50}/MIC_{90}, >8/>8 µg/mL) compared to *E. faecalis* (MIC_{50}/MIC_{90}, 0.25/8 µg/mL). LND MIC_{50}/MIC_{90} values were 0.015/0.03 µg/mL for *Haemophilus influenzae* (n=1,002) and 0.015/0.015 µg/mL for *Moraxella catarrhalis* (n=504). Against *S. pneumoniae* the MIC_{50}/MIC_{90} for LND was 0.25/0.5 µg/mL and for LEV 1/1 µg/mL (n=1,196). The LND MIC_{50}/MIC_{90} against 3,000 Enterobacteriaceae was 0.5/>8 µg/mL with 73.2% of isolates inhibited at ≤4 µg/mL (LEV-S, 78.1/76.9% [CLSI/EUCAST]) and for *Pseudomonas aeruginosa* was 2/>8 µg/mL and 67.4% inhibited at ≤4 µg/mL (LEV-S, 71.2/62.7% [CLSI/EUCAST]).**Conclusions:** WCK 771 activity, including potent activity against MRSA supports the potential value of further development studies to define its clinical use.

Author Disclosure Block:

R.K. Flamm: H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Wockhardt. **H.S. Sader:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Wockhardt. **D.J. Farrell:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Wockhardt. **P.R. Rhomberg:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Wockhardt. **R.N. Jones:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Wockhardt.

Poster Board Number:

SUNDAY-457

Publishing Title:

***In Vitro* Activity of Delafloxacin (DLX) When Tested against Contemporary Bacterial Pathogens from the USA (2014)**

Author Block:

R. K. Flamm, D. J. Farrell, H. S. Sader, P. R. Rhomberg, R. N. Jones; JMI Lab., North Liberty, IA

Abstract Body:

Background: DLX is an anionic fluoroquinolone in clinical development (oral and intravenous routes) for the treatment of acute bacterial skin and skin structure infections and community acquired bacterial pneumonia. In this study, DLX was tested against clinical isolates collected in USA medical centers as part of the 2014 SENTRY Antimicrobial Surveillance Program.**Methods:** A total of 4,410 USA clinical isolates were tested for susceptibility (S) to DLX and comparators by reference broth microdilution.**Results:** DLX was the most potent ($MIC_{50/90}$, ≤0.004/0.03 μg/mL) agent tested against methicillin-susceptible *Staphylococcus aureus* (MSSA) and based on MIC_{90} was eight- and 128-fold more potent than ceftaroline (CPT) and levofloxacin (LEV). Tigecycline ($MIC_{50/90}$, 0.06/0.06 μg/mL), DLX ($MIC_{50/90}$, 0.06/0.5 μg/mL), trimethoprim-sulfamethoxazole (SXT, $MIC_{50/90}$, ≤0.5/≤0.5 μg/mL), and daptomycin ($MIC_{50/90}$, 0.25/0.5 μg/mL) were the most potent agents tested against MRSA. MRSA exhibited high levels of resistance (R) against LEV (68.4%) and erythromycin (82.9%). DLX ($MIC_{50/90}$, 0.06/1 μg/mL), linezolid ($MIC_{50/90}$, 1/1 μg/mL) and SXT ($MIC_{50/90}$, ≤0.5/≤0.5 μg/mL) were the most active agents against *Enterococcus faecalis.* Against *S. pneumoniae* ($MIC_{50/90}$, 0.008/0.015 μg/mL), DLX was eight-fold more active than CPT ($MIC_{50/90}$, ≤0.015/0.12 μg/mL; 99.7% S), 16-fold more active than moxifloxacin ($MIC_{50/90}$, ≤0.12/0.25 μg/mL; 98.3% S), and 64-fold more active than LEV ($MIC_{50/90}$, 1/1 μg/mL; 98.3% S). All DLX MIC values for *S. pyogenes* were ≤0.015 μg/mL and for *S. dysgalactiae* ≤0.03 μg/mL. For *S. agalactiae*, 98.0% of isolates were ≤0.03 μg/mL; the highest MIC was only 0.5 μg/mL. Against Enterobacteriaceae, the DLX $MIC_{50/90}$ was 0.06/2 μg/mL with 82.3% of isolates at ≤1 μg/mL. Ciprofloxacin (CIP) and LEV S were 82.8 and 84.3%, respectively. DLX inhibited 75.0% of *P. aeruginosa* at ≤1 μg/mL; CIP and LEV exhibited S at 76.0 and 75.0%, respectively. DLX inhibited 59.0% of *Acinetobacter* spp. at ≤1 μg/mL. CIP S and LEV S were poor (48.0 and 50.0%, respectively).**Conclusions:** DLX offers advantages in potency and spectrum *in vitro* when compared to currently marketed fluoroquinolone agents, especially with its enhanced activity against *S. aureus* including methicillin-resistant strains, and improved potency against *S. pneumoniae* and β-hemolytic streptococci.

Author Disclosure Block:

R.K. Flamm: H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Melinta Therapeutics. **D.J. Farrell:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Melinta Therapeutics. **H.S. Sader:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Melinta Therapeutics. **P.R. Rhomberg:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Melinta Therapeutics. **R.N. Jones:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Melinta Therapeutics.

Poster Board Number:

SUNDAY-458

Publishing Title:

***In Vitro* Efficacy of Am-1977, a New Fluoroquinolone, Against *Streptococcus pneumoniae* with Fluoroquinolone Resistance-determining Regions (Qrdrs) Mutations**

Author Block:

M. Murata, K. Kosai, S. Yamauchi, D. Sasaki, N. Kaku, Y. Morinaga, K. Yanagihara; Nagasaki Univ. Hosp., Nagasaki, Japan

Abstract Body:

Background: Fluoroquinolone resistance in *Streptococcus pneumoniae* is caused by gradual accumulation of DNA gyrase (GyrA) and topoisomerase IV (ParC) mutations in the fluoroquinolone resistance-determining regions (QRDRs). In this study, we evaluated relations between QRDR mutations, drug susceptibility and frequencies of appearance of resistant strains against fluoroquinolones including AM-1977, which was a newly developed by Kyorin Pharmaceutical Co., Ltd.**Methods:** We evaluated minimum inhibitory concentrations (MICs) for seven fluoroquinolones and the presences of *gyr*A and *par*C mutations by pyrosequencing method in 34 clinical isolates. Further, we evaluated frequencies of appearance of resistant strains against levofloxacin (LVX) and AM-1977 for two strains which had wild-type *gyr*A and *par*C mutation as a first step mutation.**Results:** The MICs for LVX were 1 or 2 μg/mL in 19 strains with neither *gyr*A nor *par*C mutation, 2 μg/mL in 14 strains with either mutation and 4 μg/mL in one strain with both mutations. All strains had the MICs of 0.06 or 0.12 μg/mL for AM-1977. Table showed the frequencies of appearance of resistant strains selected by multiple concentrations of fluoroquinolones. GyrA mutation was additionally detected in resistant strains generated by exposure to 2×, 4×MIC of LVX, but not to 2×MIC of AM-1977. Those resistant strains, which were generated by LVX exposure and had both *gyr*A and *par*C mutations, had MICs of 16 or 32 μg/mL for LVX and 0.12 to 0.5 μg/mL for AM-1977.**Conclusions:** Both the MICs and the frequencies of appearance of resistant strains were lower for AM-1977 than for LVX. In addition to low MICs, AM-1977 was superior to LVX with respect to preventing resistance induction when the strains had a single step mutation and were exposed to each fluoroquinolone.

The frequencies of appearance of resistant strains selected by fluoroquinolones

Mutation in ParC	Drug	Frequency at the following drug concentration			
		2×MIC	4×MIC	8×MIC	16×MIC
Ser79Phe	LVX	1.08×10^{-7}	4.66×10^{-8}	$<1.27\times10^{-9}$	$<1.27\times10^{-9}$
	AM-1977	5.52×10^{-9}	$<1.27\times10^{-9}$	$<1.27\times10^{-9}$	$<1.27\times10^{-9}$
Asp83Tyr	LVX	1.90×10^{-7}	3.47×10^{-8}	$<2.59\times10^{-8}$	$<2.59\times10^{-8}$

	AM-1977	3.90×10^{-9}	$<2.59\times10^{-8}$	$<2.59\times10^{-8}$	$<2.59\times10^{-8}$

Author Disclosure Block:

M. Murata: None. **K. Kosai:** None. **S. Yamauchi:** None. **D. Sasaki:** None. **N. Kaku:** None. **Y. Morinaga:** None. **K. Yanagihara:** I. Research Relationship; Self; Kyorin Pharmaceutical Co., Ltd..

Poster Board Number:

SUNDAY-459

Publishing Title:

Mechanism for High Pulmonary Distribution of Lascufloxacin (Am-1977), a New Fluoroquinolone Antibiotic

Author Block:

K. Ohya, S. Manita; Kyorin Pharmaceutical Co., Ltd., tochigi, Japan

Abstract Body:

Background: Lascufloxacin is highly active especially against respiratory tract pathogens. Moreover, clinical pharmacological study (Phase I) revealed that the tissue-to-plasma concentration ratio (T/P) of lascufloxacin was 21 in the lung epithelial lining fluid (ELF). Generally, a high tissue distribution of drugs can be attained through higher active transport or tissue binding. In this study, therefore, several *in vitro* assays were carried out to clarify the mechanisms governing higher distribution of lascufloxacin into the alveolar compartment.**Methods:** Transcellular transport of lascufloxacin and the effects of various inhibitors on this transport were both evaluated using Calu-3 cell monolayers, known as a human lung epithelial cell model. Substrate recognition of lascufloxacin mediated by various drug transporters was assessed using each of the transporter-expressing cells.**Results:** Vectorial transport of lascufloxacin to the secretory direction was observed in the transcellular transport assay across Calu-3 cell monolayer. The concentration-dependent inhibition of lascufloxacin transport by valspodar and Ko143 resulted from involvements of P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP) in to this transport. In addition, each of the transporter-expressing cells showed that lascufloxacin is a substrate for P-gp and BCRP.**Conclusions:** This result clearly indicates that lascufloxacin is subject to P-gp- and BCRP-mediated active transport in the Calu-3 model. This transporter-dependent secretion may lead to higher lascufloxacin concentrations in ELF than those in plasma. Furthermore, we now assume that lascufloxacin might bind to some component(s) of ELF.

Table. Substrate recognition of lascufloxacin mediated by MDR1 and BCRP

Transporter	Control cells			Transporter-expressing cells			Corrected P_{app} ratio
	P_{app} ($\times 10^{-6}$cm/sec)		P_{app} ratio	P_{app} ($\times 10^{-6}$cm/sec)		P_{app} ratio	
	AP to BL	BL to AP		AP to BL	BL to AP		
MDR1	14.7	21.0	1.4	7.08	31.1	4.4	3.1
BCRP	18.1	49.0	2.7	18.6	80.9	4.3	1.6

P_{app}: apparent permeability, AP: apical, BL: basal

Author Disclosure Block:

K. Ohya: D. Employee; Self; Kyorin Pharmaceutical Co., Ltd. **S. Manita:** D. Employee; Self; Kyorin Pharmaceutical Co., Ltd..

Poster Board Number:

SUNDAY-460

Publishing Title:

Gepotidacin (GSK2140944) *In Vitro* Activity against Gram-Positive and Gram-Negative Bacteria (MBC/MIC, Kill Kinetics, Checkerboard, PAE/SME Tests)

Author Block:

R. K. Flamm[1], H. S. Sader[1], P. R. Rhomberg[1], N. E. Scangarella-Oman[2], D. J. Farrell[1]; [1]JMI Lab., North Liberty, IA, [2]GSK, Collegeville, PA

Abstract Body:

Background: Gepotidacin (GEP) is a novel triazaacenaphthylene antibiotic, which inhibits bacterial DNA replication and has *in vitro* activity against susceptible and drug-resistant pathogens associated with a range of conventional and biothreat infections.**Methods:** Reference *in vitro* methods were used to evaluate the MIC/MBC activity of GEP and comparators against *S. aureus* (SA), *S. pneumoniae* (SPN) and *Escherichia coli* (EC). GEP *in vitro* activity was also evaluated using time-kill kinetics (KK), broth microdilution checkerboard methods (CM) and for post-antibiotic (PAE) and sub-inhibitory (PAE-SME) effects.**Results:** MIC_{90} values for GEP against 50 SA (including MRSA) and 50 SPN (including penicillin-intermediate and -resistant) isolates were 0.5 µg/mL and for EC (n=25) was 4 µg/mL. GEP was bactericidal against the tested strains of SA, *SPN*, and EC. GEP had MBC/MIC ratios of ≤4 against 98, 98, and 88% of isolates tested, respectively. KK indicated that bactericidal activity for GEP was generally observed at 4 or 10x MIC concentrations at 24 hours. In a few instances, regrowth was observed in the presence of GEP and in the presence of levofloxacin. CM experiments demonstrated no occurrences of antagonism when testing GEP in combination with a variety of currently used antimicrobial agents including aztreonam, ceftriaxone, tetracycline and trimethoprim-sulfamethoxazole. The most common interaction when testing GEP was indifference (82.7% for Gram-positive and 82.0% for Gram-negative). The PAE for GEP against SA was short (≤0.6 hours against MRSA and MSSA) and the PAE-SME was extended in length (>8 hours; 3 isolates at ½x MIC). Against the levofloxacin-susceptible *SA* isolate tested, the PAE for levofloxacin was modest (0.1-2.4 hours) and an extended PAE-SME was observed (>9 hours at ½x MIC).**Conclusions:** GEP demonstrated bactericidal activity against the majority of Gram-positive and Gram-negative isolates tested. For the isolate and drug combinations tested, *in vitro* checkerboard studies showed that interactions were generally indifferent and no antagonism was seen. The PAE for GEP was of short to modest duration, with an extended PAE-observed in the presence of sub-MIC concentrations. These *in vitro* data indicate that further study of GEP is warranted.

Author Disclosure Block:

R.K. Flamm: H. Research Contractor; Self; This work was supported by GSK and funded with Federal funds from ASPR, BARDA, under Contract No. HHSO100201300011C. **H.S. Sader:** H. Research Contractor; Self; This work was supported by GSK and funded with Federal funds from ASPR, BARDA, under Contract No. HHSO100201300011C. **P.R. Rhomberg:** H. Research Contractor; Self; This work was supported by GSK and funded with Federal funds from ASPR, BARDA, under Contract No. HHSO100201300011C. **N.E. Scangarella-Oman:** D. Employee; Self; This work was supported by GSK and funded with Federal funds from ASPR, BARDA, under Contract No. HHSO100201300011C. **D.J. Farrell:** H. Research Contractor; Self; This work was supported by GSK and funded with Federal funds from ASPR, BARDA, under Contract No. HHSO100201300011C..

Poster Board Number:

SUNDAY-461

Publishing Title:

Gepotidacin (GSK2140944) *In Vitro* Activity against *Neisseria gonorrhoeae* (MIC/MBC, Kill Kinetics, Checkerboard, PAE/SME Tests)

Author Block:

D. J. Farrell[1], H. S. Sader[1], P. R. Rhomberg[1], N. E. Scangarella-Oman[2], R. K. Flamm[1]; [1]JMI Lab., North Liberty, IA, [2]GSK, Collegeville, PA

Abstract Body:

Background: Gepotidacin (GEP) is a novel triazaacenaphthylene antibiotic, which inhibits bacterial DNA replication and has *in vitro* activity against susceptible and drug-resistant pathogens associated with a range of conventional and biothreat infections, including *Neisseria gonorrhoeae* (GC).**Methods**: Broth microdilution using fastidious broth was used to evaluate the MIC/MBC activity of GEP and comparator agents against 25 GC strains (including 5 ciprofloxacin [CIP] non-susceptible [NS] strains). GEP *in vitro* activity was also evaluated against 3 GC strains (including ATCC 49226 and two tetracycline [TET]- and azithromycin [AZI]-NS strains) using time-kill kinetics and checkerboard methods, and against 2 GC strains for the investigation of post-antibiotic (PAE) and sub-inhibitory (PAE-SME) effects.**Results**: The MIC_{50} and MIC_{90} for GEP against 25 GC isolates were 0.12 and 0.25 µg/mL, respectively. The highest GEP MIC value was 0.25 µg/mL. The MBC_{50} and MBC_{90} for GEP were 0.25 and 0.5 µg/mL, respectively, and the highest MBC value was 1 µg/mL (2 isolates). GEP was bactericidal when tested against GC with 25/25 of isolates exhibiting a MBC/MIC ratio of ≤4 (≤2 for 20/25 of the isolates). GEP demonstrated bactericidal activity in time-kill curves against the 3 GC strains tested. For all the combinations of GEP and comparators tested against GC, using checkerboard methods, there were no instances where antagonism occurred and only one instance where synergy occurred (with moxifloxacin; FIC index, 0.375), but this was not confirmed by *in vitro* time-kill studies. An extended PAE for GEP against the wild-type GC strain (0.5->2.5 hours) and an extended PAE-SME (>2.5 hours) occurred. The GEP PAE (0.7 hours at all exposures) and PAE-SME (1.2-2.7 hours) was shorter with the TET- and AZI-NS GC strain.**Conclusions**: Using MBC and time-kill kinetics, GEP demonstrated bactericidal activity against the *N. gonorrhoeae* isolates tested. *In vitro* checkerboard studies showed interactions were generally additive/indifferent and no antagonism was seen. The PAE for GEP was generally short to modest duration, with an extended PAE-observed in the presence of sub-MIC concentrations. These *in vitro* data indicate that further study of GEP is warranted for potential use in treating infections caused by GC.

Author Disclosure Block:

D.J. Farrell: H. Research Contractor; Self; This work was supported by GSK and funded with Federal funds from ASPR, BARDA, under Contract No. HHSO100201300011C. **H.S. Sader:** H. Research Contractor; Self; This work was supported by GSK and funded with Federal funds from ASPR, BARDA, under Contract No. HHSO100201300011C. **P.R. Rhomberg:** H. Research Contractor; Self; This work was supported by GSK and funded with Federal funds from ASPR, BARDA, under Contract No. HHSO100201300011C. **N.E. Scangarella-Oman:** D. Employee; Self; This work was supported by GSK and funded with Federal funds from ASPR, BARDA, under Contract No. HHSO100201300011C. **R.K. Flamm:** H. Research Contractor; Self; This work was supported by GSK and funded with Federal funds from ASPR, BARDA, under Contract No. HHSO100201300011C..

Poster Board Number:

SUNDAY-462

Publishing Title:

Analysis of Agar Dilution Mic Testing Methods and Variables and *In Vitro* activity of Gepotodacin (Gsk2140944) Against *Neisseria gonorrhoeae*

Author Block:

N. E. SCANGARELLA-OMAN[1], P. Dixon[2], L. M. Koeth[3], J. DiFranco-Fisher[3], L. A. Miller[1]; [1]GSK, COLLEGEVILLE, PA, [2]UAB, Birmingham, AL, [3]LSI Inc., Westlake, OH

Abstract Body:

Background: Gepotidacin (GEP), a novel triazaacenaphthylene antibiotic which inhibits bacterial DNA replication, has *in vitro* activity against susceptible and drug-resistant pathogens associated with a range of conventional and biothreat infections, including *Neisseria gonorrhoeae* (GC).**Methods**: 145 GC isolates selected from the University of Alabama at Birmingham's isolate collection were tested by agar dilution according to Gonococcal Isolate Surveillance Program and CLSI methodology. The effect of Mueller-Hinton agar manufacturer, temperature, incubation time, atmospheric conditions, inoculum concentration and pH on the *in vitro* activity of GEP were also studied.**Results**: The MIC_{50} and MIC_{90} for GEP against the 145 GC isolates tested were 0.25 and 0.5 µg/mL, respectively (highest GEP MIC was 1 µg/mL). Against 108 ciprofloxacin-susceptible (CIP-S) and 37 non-susceptible (CIP-NS) isolates, GEP MIC_{90}s were 0.25 and 1 µg/mL, respectively. A 2-dilution higher shift in GEP MICs was noted for the CIP-NS subset when compared with the CIP-S isolates. Variables that affected GEP MICs were media, low inoculum concentrations, pH levels and atmospheric conditions, Media had the most effect, but the effect was not specific to GEP. Remel GC agar supplemented with GCHI enrichment did not support the growth of any study isolate. Lower MIC results and poor/no growth were observed with all agents tested with Oxoid GC agar supplemented with GCHI enrichment. Inoculum concentration also affected and caused lower GEP MICs. Use of an organism suspension equivalent to a 0.5 McFarland (10^5 CFU/spot) was necessary to obtain sufficient growth of all study strains and acceptable quality control results. GEP MICs were affected by pH and by 10% CO_2 (29/30 were within +/-2 dilutions and 25/30 were lower than the reference method MIC).**Conclusions**: GEP was active *in vitro* with MICs 1 µg/mL against the 145 strains of GC tested. Further evaluation is recommended to understand the mechanism causing the 2-dilution higher GEP MICs against CIP-NS GC isolates. When performing MIC testing with GEP, it is important to control media manufacturer, inoculum, CO_2 and pH. These *in vitro* data indicate that further study of GEP is warranted for potential use in treating infections caused by GC.

Author Disclosure Block:

N.E. Scangarella-oman: D. Employee; Self; GSK. K. Shareholder (excluding diversitied mutual funds); Self; GSK. **P. Dixon:** I. Research Relationship; Self; GSK. **L.M. Koeth:** H. Research Contractor; Self; GSK. **J. DiFranco-Fisher:** I. Research Relationship; Self; GSK. **L.A. Miller:** D. Employee; Self; GSK. K. Shareholder (excluding diversitied mutual funds); Self; GSK.

Poster Board Number:

SUNDAY-463

Publishing Title:

***In Vitro* Biology and Pharmacokinetics of Novel Bacterial Topoisomerase Inhibitors**

Author Block:

C. Charrier, A-M. Salisbury, V. Savage, E. Moyo, N. Ooi, N. Chaffer-Malam, H. Forward, J. Cheung, R. Metzger, M. Pichowicz, R. Sigerson, I. Cooper, P. Vince, I. Morrison, H. Butler, S. Best, A. Ratcliffe, N. Stokes; Redx Pharma Plc, Alderley Edge, United Kingdom

Abstract Body:

Background: The bacterial type II topoisomerases, DNA gyrase and topoisomerase IV (topo IV), are validated antibiotic targets. Owing to the emergence of quinolone resistance, structurally-novel inhibitors of these targets are sought. The objective of this study was to profile a new series of Novel Bacterial Topoisomerase Inhibitor (NBTI) type compounds.**Methods:** MICs and MBCs were determined according to CLSI guidelines M07-A10, M11-A8 and M26-A. Inhibition of purified DNA gyrase, topo IV and human topoisomerase II was evaluated using supercoiling, decatenation or DNA cleavage complex formation assays. Frequency of resistance was determined with *Escherichia coli* ATCC 25922. Serial passage experiments were carried out at 0.25 × MIC. Mutants were characterised by whole genome sequencing. Cytotoxicity was evaluated using the HepG2 cell line. hERG inhibition was determined using IonWorks patch clamp electrophysiology. Pharmacokinetics (PK) were measured in male CD-1 mice following intravenous dosing at 2 and 20 mg/kg.**Results:** Representative compounds REDX06213, REDX06276 and REDX07623, showed broad-spectrum activity with MICs from 0.12 to 8 µg/mL against the ESKAPE pathogens. These compounds demonstrated MIC_{90} values of 4-8 µg/mL against *Acinetobacter baumannii* and *E. coli* compared to 16 µg/mL for levofloxacin. REDX06213 had IC_{50} values of 1.66 and 0.17 µM against *E. coli* and 0.14 and 0.37 µM against *S. aureus* DNA gyrase and topo IV, respectively and an IC_{50} of ~100 µM against human topoisomerase II. REDX06213 did not stabilise double-strand DNA cleaved complexes. The mutation rate of *E. coli* against this series was $<4.2 \times 10^{-9}$ at 4 × MIC. Unique mutations were identified in the mutants. HepG2 IC_{50} values were typically 32 - >128 µg/mL and hERG inhibition IC_{50} values >33 µM. PK profiling showed good tolerability and exposure for REDX06213.**Conclusions:** Compounds of this NBTI series demonstrated broad-spectrum potency against clinically-important pathogens, a low potential for resistance development and a mechanism-of-action distinct from the quinolones. The biological properties of these novel dual-targeting NBTIs merit further exploration of their potential as new antibacterial agents.

Author Disclosure Block:

C. Charrier: D. Employee; Self; Redx Pharma Plc. **A. Salisbury:** D. Employee; Self; Redx Pharma Plc. **V. Savage:** D. Employee; Self; Redx Pharma Plc. **E. Moyo:** D. Employee; Self;

Redx Pharma Plc. **N. Ooi:** D. Employee; Self; Redx Pharma Plc. **N. Chaffer-Malam:** D. Employee; Self; Redx Pharma Plc. **H. Forward:** D. Employee; Self; Redx Pharma Plc. **J. Cheung:** D. Employee; Self; Redx Pharma Plc. **R. Metzger:** D. Employee; Self; Redx Pharma Plc. **M. Pichowicz:** D. Employee; Self; Redx Pharma Plc. **R. Sigerson:** D. Employee; Self; Redx Pharma Plc. **I. Cooper:** D. Employee; Self; Redx Pharma Plc. **P. Vince:** D. Employee; Self; Redx Pharma Plc. **I. Morrison:** D. Employee; Self; Redx Pharma Plc. **H. Butler:** D. Employee; Self; Redx Pharma Plc. **S. Best:** D. Employee; Self; Redx Pharma Plc. **A. Ratcliffe:** D. Employee; Self; Redx Pharma Plc. **N. Stokes:** D. Employee; Self; Redx Pharma Plc.

Poster Board Number:

SUNDAY-464

Publishing Title:

Novel and Potent Dual Bacterial Dna Topoisomerase Inhibitors Active Against Gram-positive and Gram-negative Bacteria: from Target to Lead

Author Block:

R. Ombrato, C. Apicella, A. Capezzone De Joannon, G. Corso, N. D'Atanasio, G. Furlotti, B. Garofalo, G. Mangano, G. Magarò, C. Milanese, S. Tongiani; Angelini Res. Ctr., S Palomba-Pomezia Rome, Italy

Abstract Body:

DNA gyrase and topoisomerase IV (topo IV) are clinically validated bacterial targets. Our team is involved in the discovery and development of Novel Bacterial Topoisomerase Inhibitors (NBTIs) acting concurrently on both DNA gyrase and topo IV. This program started with the identification of a series of hit compounds following a structure-based screening campaign. The X-ray structure of *S. aureus* DNA gyrase with the DNA and co-crystallized with one of our hits was used to further optimize the new NBTIs as more potent dual enzyme inhibitors. The *in vitro* results together with the knowledge of the 3D-structure supported an extensive hit to lead program around the hit core scaffolds generating a patentable series. Our NBTIs were assessed for antibacterial activity against key Gram-positive, Gram-negative strains and clinical isolates and found to possess broad-spectrum activity, especially against antibiotic-resistant strains, with MIC ranges of 0.016-4 mg/l. The bactericidal activities against *S. aureus* ATCC 29213 and *S. aureus* BAA1720 was recorded by time-kill experiments at concentrations equal to 4× MIC. Presently, the identified compounds show an exquisite antibacterial profile with a selective DNA Synthesis inhibition while having a low to moderate *in vitro* frequency resistance. To our knowledge, in comparison with NBTI currently in development, Angelini's proprietary compounds showed an improved antibacterial profile against selected panels of both Gram-positive and -negative bacteria. The ADME *in vitro* profile was investigated and the most promising compounds show low *in vivo* clearance, slow elimination rate, rapid absorption and a high distribution volume which make them potentially suitable for oral administration. So, these compounds are going to be evaluated for *in vivo* efficacy in the murine sepsis model to assess their efficacy in comparison with quinolone treatment. Ongoing, *in vivo* PK/PD studies in mice are planned to support *in vitro* results. Angelini's new proprietary bacterial topoisomerase inhibitors show very promising properties to be transformed into clinically useful antibacterial agents.

Author Disclosure Block:

R. Ombrato: None. **C. Apicella:** None. **A. Capezzone De Joannon:** None. **G. Corso:** None. **N. D'Atanasio:** None. **G. Furlotti:** None. **B. Garofalo:** None. **G. Mangano:** None. **G. Magarò:** None. **C. Milanese:** None. **S. Tongiani:** None.

Poster Board Number:

SUNDAY-465

Publishing Title:

Drug Candidate Discovery: Targeting Bacterial Topoisomerase I Enzymes for Novel Antibiotic Leads

Author Block:

S. Sandhaus; Florida Intl. Univ., Miami, FL

Abstract Body:

Introduction: The global community is facing a crisis—antibiotics are often ineffective due to the emergence of multi-drug resistant bacterial pathogens. The need for new antibiotics acting against novel bacterial cell targets is dire. Bacterial topoisomerase I (TopoI) is an attractive target for new antibiotics, since it should be vulnerable to bactericidal topoisomerase poison inhibitors in every bacterium, and its function is known to be required for the survival of certain bacterial pathogens including *Mycobacterium tuberculosis*. Selective and potent inhibitors of bacterial TopoI can be useful as new antibiotic leads. Bacterial TopoI relaxes supercoiled DNA by using its active-site tyrosine residue to attack the phosphodiester backbone of the DNA, forming a covalent intermediate and cleaving one strand of the DNA. It then passes the other strand through the break and rejoins the DNA to increase the DNA linking number by one. Catalytic inhibitors of topoisomerase I may prevent the enzyme from binding or cleaving the DNA, while poison inhibitors can stabilize the DNA-enzyme covalent intermediate, thus causing the accumulation of DNA breaks, leading to bacterial cell death. This project seeks novel inhibitors of bacterial topoisomerase I in various bacterial strains such as *E. coli*, *M. tuberculosis*, and *Y. pestis*. **Methods:** Two main assays are used to find antibacterial compounds that target TopoI—an enzyme inhibition assay (a gel-based assay that monitors the formation of relaxed DNA in the presence of inhibiting compounds), and a growth inhibition assay (an assay that monitors the growth of bacteria in the presence of topoisomerase inhibitors). **Results:** Several promising compounds have been found from various screens—an *in silico* study has uncovered several small molecule inhibitors, and a mixture-based compound screen has revealed several polyamine inhibitors—that inhibit bacterial TopoI well, and are able to prevent bacterial cell growth as well. Specifically, many of the discovered compounds are effective against *M. tuberculosis* topoisomerase I, and can prevent the growth of *M. smegmatis*, a non-pathogenic homolog of *M. tuberculosis*. **Conclusions:** The use of diverse approaches such as *in silico* docking studies and mixture-based compound screening is effective at finding novel inhibitors of bacterial topoisomerase I, and may bring us one step closer to new and effective antibiotics.

Author Disclosure Block:

S. Sandhaus: None.

Poster Board Number:

SUNDAY-466

Publishing Title:

Targeting Bacterial Topoisomerase I for Selective Anti-streptococcal Drug Design

Author Block:

J. A. Jones, E. Price, K. E. Hevener; Idaho State Univ., Meridian, ID

Abstract Body:

Background: Viridans Group Streptococci (VGS) are increasingly implicated in a variety of diseases such as endocarditis, pneumonia and bacteremia. Furthermore, VGS predominates the oropharynx and a primary representative of the group, *Streptococcus mutans*, is the principal cause of costly dental cariogenesis. With drug-resistant VGS infections on the rise and the dangers associated with the collateral eradication of beneficial bacteria within the oropharynx and gastrointestinal tract via the use of wide-spectrum antibacterials, the need for novel and selective VGS-directed antibacterial targets has become more apparent. Bacterial topoisomerase I may constitute such a potential target as studies have arisen showcasing the essentiality of topoisomerase I in bacterial species lacking the only other type IA topoisomerase, topoisomerase III. Accordingly, the goals of these studies were to develop a simplified purification protocol for topoisomerase I from *S. mutans* that would result in a significant increase in pure protein suitable for crystallography and assay development; and to develop and optimize a high-throughput assay suitable for screening and identification of selective anti-streptococcal agents. **Methods and Results:** Via the systematic analysis of different target protein affinity and fusion tag constructs utilizing immobilized metal affinity chromatography (IMAC) and gel filtration techniques, as well as auto-induction, we successfully developed a simplified, rapid expression and two-step purification protocol resulting in >20 mg/L of high-purity *S. mutans* topoisomerase I—a significantly higher yield than previously reported bacterial topoisomerase I purifications. Furthermore, we have also developed and optimized a microplate-based, fluorescence intensity assay for use in high-throughput compound screening against bacterial topoisomerase I. **Conclusion:** With the ease and speed afforded by the protocol, a considerable amount of bacterial topoisomerase I can be obtained without difficulty. As successful drug discovery efforts require substantial amounts of target protein for use in reliable high-throughput assays and structural studies, these results are anticipated to aid in the discovery and development of novel inhibitors of bacterial topoisomerase I, a potentially promising selective antibacterial drug target.

Author Disclosure Block:

J.A. Jones: None. **E. Price:** None. **K.E. Hevener:** None.

Poster Board Number:

SUNDAY-467

Publishing Title:

Phase I Study to Determine the Safety and Pharmacokinetics (Pk) of Single and Multiple Oral Doses of Lascufloxacin (Am-1977) in Healthy Subjects

Author Block:

K. Totsuka[1], M. Odajima[2], M. Nakauchi[2], S. Sesoko[3], M. Nakashima[3]; [1]Tokyo Women's Med. Univ., Tokyo, Japan, [2]Kyorin Pharmaceutical. Co. Ltd., Tokyo, Japan, [3]Maruyama Hosp., Shizuoka, Japan

Abstract Body:

Background: Lascufloxacin is a new generation fluoroquinolone with superior activity against respiratory tract pathogens, especially Gram-positives and anaerobes. A combined Phase I study protocol was designed to evaluate the safety and PK of single and multiple ascending oral doses of lascufloxacin in healthy adult subjects.**Methods:** In the single-dose study, 46 Japanese healthy adult males were included, 6 of which received single oral dose of 20 or 50 mg lascufloxacin under open-label design, 40 of which received single oral doses of 100-800 mg lascufloxacin/placebo under single-blind design. After safety was confirmed in the single-dose study, single-blind, placebo-controlled, multiple-dose study was performed. 16 subjects (in groups of 6 active and 2 placebo) received daily oral doses of 200 or 400 mg lascufloxacin for 7 consecutive days.**Results:** Lascufloxacin rapidly appeared in plasma with mean Tmax values ranged from 1.50 to 2.58 hours, and was slowly eliminated with mean t1/2 values ranged from 15.6 to 18.2 hours at 100-800 mg single doses. Mean Cmax and AUClast for lascufloxacin increased linear and approximately dose-proportional manner (0.732-6.00 μg/mL and 12.1-135 μg*h/mL respectively). %UR and CLr values were ranged from 11.7 to 15.0%, and 0.899 to 0.987 L/h, respectively. In the multiple dose study, mean Tmax value was 2.50 hours, and mean t1/2 values ranged from 22.8 to 24.2 hours on Day 7. Day 7 mean Cmax and AUCt for lascufloxacin 200 and 400 mg administration reached 2.61-6.55 μg/mL and 46.3-113 μg*h/mL respectively. Lascufloxacin was well tolerated at all doses. No serious adverse events (AEs) were observed, all AEs were mild to moderate in severity and disappeared without treatment.**Conclusions:** Overall safety and tolerability of lascufloxacin were excellent up to a single dose of 800mg and a repeated dose of 400mg. Lascufloxacin is expected to be clinically useful due to its favorable pharmacokinetics.

Author Disclosure Block:

K. Totsuka: J. Scientific Advisor (Review Panel or Advisory Committee); Self; Kyorin Pharmaceutical Co. Ltd. **M. Odajima:** D. Employee; Self; Kyorin Pharmaceutical. Co. Ltd. **M. Nakauchi:** D. Employee; Self; Kyorin Pharmaceutical. Co. Ltd.. **S. Sesoko:** None. **M. Nakashima:** F. Investigator; Self; Kyorin Pharmaceutical. Co. Ltd..

Poster Board Number:

SUNDAY-468

Publishing Title:

Pharmacodynamics of a Novel Fluoroquinolone, Lascufloxacin (Am-1977) in Polymicrobial Murine Pneumonia Model Caused By *Streptococcus pneumoniae* and *Prevotella intermedia*

Author Block:

M. Hagihara, H. Kato, N. Nishiyama, Y. Koizumi, D. Sakanashi, H. Suematsu, Y. Yamagishi, H. Mikamo; Aichi Med. Univ., Nagakute, Aichi, Japan

Abstract Body:

Background: Lascufloxacin (AM-1977) is a novel fluoroquinolone antimicrobial agent which has antimicrobial activity against aerobes and anaerobes, and could be administered orally or intravenously. *Streptococcus pneumoniae* (*Sp*) and *Prevotella intermedia* (*Pi*) are major aerobic and anaerobic pathogens of RTI, respectively. The purpose of this study is to compare the antimicrobial activities of lascufloxacin and levofloxacin (LVX) for the treatment of polymicrobial pneumonia caused by *Sp* and *Pi* in the neutropenic mice model.**Methods:** We used clinical isolates of *Pi* (MICs of lascufloxacin and LVX were 0.5 and 4 mg/L, respectively) and *Sp* (MICs of lascufloxacin and LVX were 0.06 and 1 mg/L, respectively) in neutropenic pneumonia polymicrobial infection model. Lascufloxacin and LVX were administered at the single doses of 25 or 50 mg/kg 1h after inoculation. 50 mg/kg was determined according to the AUC in mouse, which was nearly equivalent to the AUC of the clinical dose of lascufloxacin in human (i.e. 75mg q.d.). Efficacy was evaluated after 24h of therapy for the change of bacterial quantity in treated animals as compared with the 24h control animals.**Results:** At the start of the antimicrobial treatments (0h), bacterial quantities of *Sp* and *Pi* were 7.7 and 7.8 $\log_{10}$CFU, respectively. After 24h incubation, bacterial densities of *Sp* and *Pi* were 8.5 and 6.4 $\log_{10}$CFU, respectively. Lascufloxacin showed good *in vivo* activity against *Sp* (-1.7~-4.2 $\Delta\log_{10}$CFU) and *Pi* (-6.0~-6.5 $\Delta\log_{10}$CFU). On the other hand, the treatment with LVX resulted in lower activity against *Sp* isolates tested, while *in vivo* activity of lascufloxacin against *Sp* showed dose dependency. When comparing the antimicrobial activity of LVX at 50mg/kg, lascufloxacin revealed significantly higher bacterial density reduction against *Sp* (-4.2±0.3 vs. 0.7±0.1 $\Delta\log_{10}$CFU, $p<0.001$) and *Pi* (-6.9±0.7 vs. -4.7±0.8 $\Delta\log_{10}$CFU, $p=0.002$).**Conclusions:** Lascufloxacin showed greater *in vivo* activity not only for *Sp*, but also *Pi* in mouse mixed-infection model. Therefore, our results suggested that lascufloxacin would be potent candidate for the therapy of polymicrobial pneumonia caused by aerobes and anaerobes.

Author Disclosure Block:

M. Hagihara: None. **H. Kato:** None. **N. Nishiyama:** None. **Y. Koizumi:** None. **D. Sakanashi:** None. **H. Suematsu:** None. **Y. Yamagishi:** None. **H. Mikamo:** None.

Poster Board Number:

SUNDAY-469

Publishing Title:

Intrapulmonary Pharmacokinetics of Lascufloxacin (Am-1977), a New Generation Fluoroquinolone, in Healthy Japanese Subjects

Author Block:

H. Furuie[1], H. Yoshida[2], K. Kume[2], Y. Owada[1], M. Yagi[1], H. Mikami[1], M. Nishimura[3]; [1]Osaka Pharmacology Clinical Res. Hosp., Osaka, Japan, [2]Kyorin Pharmaceutical Co., Ltd., Tokyo, Japan, [3]Hokkaido Univ. Sch. of Med., Sapporo, Japan

Abstract Body:

Background: Lascufloxacin (AM-1977) is a new generation fluoroquinolone with superior activity against respiratory tract pathogens, especially Gram-positives and anaerobes which causes a variety of infections including bacterial pneumonia in the elderly. This study was to evaluate the drug concentration in epithelial lining fluid (ELF) and alveolar macrophages (AMs) adding to plasma as a surrogate marker for lung penetration in order to assess the bronchopulmonary drug disposition of orally administered lascufloxacin 75 mg in healthy volunteers.**Methods:** Thirty never-smoked healthy Japanese male subjects were randomly allocated to 5 groups of 6 subjects each according to the time of bronchoalveolar lavage (BAL) at 1, 2, 4, 6, and 24 hours after dose. Blood samples were also taken at the corresponding time. Lascufloxacin was measured in plasma, ELF and AMs, and the pharmacokinetics analysis was performed.**Results:** Lascufloxacin was rapidly distributed to plasma and ELF while it slowly distributed to AMs. Tmax of ELF and plasma were 1 hour, and Tmax of AMs was 6 hour. Cmax of plasma, ELF and AMs were 0.576 μg/mL, 12.3 μg/mL and 21.8 μg/mL, respectively. The mean drug concentrations in ELF and AMs were much higher than those in plasma at all sampling points. The average drug concentration ratios of ELF/plasma and AMs/plasma were in the range of 15.0-22.4 and 18.5-56.4, respectively. And AUC of plasma, ELF and AMs were 7.67 μg*h/mL, 123 μg*h/mL, and 325 μg*h/mL, respectively.**Conclusions:** These excellent penetrations of lascufloxacin into the lung together with the activity against respiratory tract pathogens suggest that lascufloxacin is a promising new agent for treatment of respiratory tract infections.

Author Disclosure Block:

H. Furuie: None. **H. Yoshida:** D. Employee; Self; Kyorin Pharmaceutical Co., Ltd. **K. Kume:** D. Employee; Self; Kyorin Pharmaceutical Co., Ltd.. **Y. Owada:** None. **M. Yagi:** None. **H. Mikami:** None. **M. Nishimura:** J. Scientific Advisor (Review Panel or Advisory Committee); Self; Kyorin Pharmaceutical Co., Ltd..

Poster Board Number:

SUNDAY-470

Publishing Title:

Phase I Study to Determine the Safety and Pharmacokinetics (Pk) of Single and Multiple Intravenous (Iv) Infusion of Lascufloxacin (Am-1977) in Healthy Subjects

Author Block:

K. Totsuka[1], M. Odajima[2], M. Nakauchi[2], S. Sesoko[3], M. Nakashima[3]; [1]Tokyo Women's Med. Univ., Tokyo, Japan, [2]Kyorin Pharmaceutical. Co. Ltd., Tokyo, Japan, [3]Maruyama Hosp., Shizuoka, Japan

Abstract Body:

Background: Lascufloxacin is a new generation fluoroquinolone with superior activity against respiratory tract pathogens, especially Gram-positives and anaerobes. The primary objective of this study was to evaluate the safety and PK of IV doses of lascufloxacin in healthy adult subjects.**Methods:** This presentation includes 2 studies. (1) Single-does study: 43 Japanese healthy adult males were received single IV doses of 50-800mg lascufloxacin. In the open-label arm, 3 subjects received 50mg lascufloxacin. In the single-blind arm, each cohort of 8 subjects (6 active and 2 placebo) received 100, 200, 400, 600, 800 mg lascufloxacin. (2) Multiple-dose study: single-blind, multiple-dose study was performed in each cohort of 8 subjects (6 active and 2 placebo) received daily 200, 400 and 400 mg loading doses (400 mg LD; 400 mg BID on Day1, 400 mg QD on Day 2-7) of lascufloxacin for 7 consecutive days.**Results:** In a single IV infusion over 1 hour of 100, 200, and 400 mg lascufloxacin, mean Cmax (1.09, 2.91, and 3.45 μg/mL) and AUClast values (12.7, 39.8, and 68.8 μg*h/mL) were dose proportional. In a repeated dose of 200 or 400mg infusion for 7 days, Accumulation ratio (Racc) values of Cmax and AUCt on Day7/Day1 were 1.32 -1.84 and 1.91-2.22, respectively. In the 400 mg LD arm, Racc values of Cmax and AUCt on Day7/Day1 were 1.13 and 1.34, respectively. Lascufloxacin was well tolerated at all doses. No serious adverse events (AEs) were observed, all AEs were mild to moderate in severity and disappeared without treatment.**Conclusions:** Overall safety and tolerability of intravenous lascufloxacin were excellent up to a single dose of 800mg, a repeated dose of 400mg and 400 mg LD. Plasma concentration with LD regimen was reached on steady level from the first day. The LD regimen is expected clinically useful.

Author Disclosure Block:

K. Totsuka: J. Scientific Advisor (Review Panel or Advisory Committee); Self; Kyorin Pharmaceutical. Co. Ltd. **M. Odajima:** D. Employee; Self; Kyorin Pharmaceutical. Co. Ltd. **M. Nakauchi:** D. Employee; Self; Kyorin Pharmaceutical. Co. Ltd.. **S. Sesoko:** None. **M. Nakashima:** F. Investigator; Self; Kyorin Pharmaceutical. Co. Ltd..

Poster Board Number:

SUNDAY-471

Publishing Title:

In Vivo* Bactericidal Activity of Lascufloxacin (Am-1977), a Newly Developed Fluoroquinolone, Against Mouse Pulmonary Infection Model Caused by *Streptococcus pneumoniae

Author Block:

R. Kishii, N. Yamamuro, Y. Yamaguchi, H. Abukawa, M. Takei; Kyorin Pharmaceutical Co., Ltd., Tochigi, Japan

Abstract Body:

Background: Quinolones are known to possess the bactericidal property, therefore, they are expected to eradicate pathogenic bacteria quickly from the patients. Lascufloxacin (AM-1977) is a novel fluoroquinolone in clinical phase with potent antibacterial activity especially against respiratory tract pathogens. In this study, *in vitro* and *in vivo* bactericidal activity of lascufloxacin was evaluated.**Methods:** *In vivo* bactericidal activity was evaluated against mouse model of pulmonary infection caused by *Streptococcus pneumoniae*. The dose of each drug for *in vivo* model was determined according to the AUC in mouse, which was equivalent to the AUC of the clinical dose in human (i.e. lascufloxacin, 75 mg q.d.; levofloxacin, 500 mg q.d.).**Results:** *In vitro* time-kill study showed that lascufloxacin had rapid bactericidal activity with dose dependent manner. The *in vitro* activity of lascufloxacin was similar to that of other quinolones tested, however, lascufloxacin had more potent activity against *S. pneumoniae* pulmonary infection model in mouse than levofloxacin. 24 h after drug administration, 2.0 log cfu/lung of bacterial reduction from first inoculums were observed in the mouse treated with levofloxacin, whereas bacteria was decreased close to the assay limit (4.2 log cfu/lung reduction) in the mouse treated with lascufloxacin. Lung-to-plasma concentration ratio of lascufloxacin was 3.7 in rodent and it was higher than that of levofloxacin (0.9, reported data).**Conclusions:** Lascufloxacin showed potent bactericidal activity both *in vitro* and *in vivo*. In particular, lascufloxacin showed significant bacterial killing in mouse when the AUC in the model was same as that in the clinical dose, although the estimated clinical dose of lascufloxacin is the lowest level in the marketed quinolones. Good distribution of lascufloxacin in lung is likely to contribute to the potent efficacy *in vivo*. Clinical pharmacological study revealed that lascufloxacin showed excellent tissue distribution to lung. The results in this study suggest that lascufloxacin possesses rapid bactericidal activity and highlight the potential of lascufloxacin as a potent bactericidal agent for the treatment of respiratory tract infections.

Author Disclosure Block:

R. Kishii: D. Employee; Self; Kyorin Pharmaceutical Co., Ltd. **N. Yamamuro:** D. Employee; Self; Kyorin Pharmaceutical Co., Ltd. **Y. Yamaguchi:** D. Employee; Self; Kyorin

Pharmaceutical Co., Ltd. **H. Abukawa:** D. Employee; Self; Kyorin Pharmaceutical Co., Ltd. **M. Takei:** D. Employee; Self; Kyorin Pharmaceutical Co., Ltd..

Poster Board Number:

SUNDAY-472

Publishing Title:

Bactericidal Activity of Delafloxacin Against Recent Isolates of *Staphylococcus aureus*

Author Block:

J. Remy, A. Marra, E. Duffy; Melinta Therapeutics, New Haven, CT

Abstract Body:

Background: Delafloxacin, an anionic fluoroquinolone antimicrobial agent, demonstrates excellent *in vitro* activity against Gram-positive and Gram-negative pathogens and is currently undergoing evaluation as a potential treatment for acute bacterial skin and skin structure infections. Despite increased infection control efforts in the clinic, MRSA remains an important pathogen in skin infections. MRSA isolates from a recently completed delafloxacin Phase 3 clinical trial for the treatment of skin infections were selected for experiments to determine the bactericidal activity of delafloxacin by time-kill methodology. **Methods:** Delafloxacin, levofloxacin, vancomycin, daptomycin, and linezolid MICs were determined by the broth microdilution method against MRSA isolates that were identified as harboring zero (MRSA 110), three (MRSA 124), or four (MRSA 165) mutations in the QRDR. MBCs were determined after sampling from sub-MIC and subsequent concentrations. Time-kill experiments were performed at MIC multiples for each antibiotic to include human free C_{max} concentrations, with an inoculum of ~$5x10^5$ CFU/mL of each organism. At 0, 1, 2, 4, 6, 8, and 24 hours, a sample was removed from each flask, centrifuged, washed and diluted in PBS, and plated to determine the number of viable cells. Plates were incubated overnight at 35°C in ambient air. Colonies were counted and kill-curves were plotted using GraphPad Prism. Bactericidal activity was interpreted as a 3-$\log_{10}$ or greater decrease in CFU/mL. **Results:** At 16X MIC, delafloxacin killed MRSA 124 more rapidly than the same concentration of levofloxacin, and was bactericidal at 16X and 32X MIC against MRSA 165 at 24 hours. Against all three strains, daptomycin at 8X MIC and above demonstrated the most rapid killing of all agents tested. Vancomycin was bactericidal at 24 hours against these 3 isolates at concentrations above 8X MIC. Linezolid was bactericidal against MRSA 165 at 24 hours but was bacteriostatic against MRSA 110 and 124 at all concentrations tested. Bactericidal activity was observed for delafloxacin and levofloxacin against MRSA 110 at six hours for most concentrations. **Conclusions:** Delafloxacin, with MICs and MBCs more potent than those of levofloxacin, demonstrates excellent cidal activity in vitro against MRSA, including those isolates that are levofloxacin-resistant.

Author Disclosure Block:

J. Remy: D. Employee; Self; Melinta Therapeutics. **A. Marra:** D. Employee; Self; Melinta Therapeutics. **E. Duffy:** D. Employee; Self; Melinta Therapeutics.

Poster Board Number:

SUNDAY-473

Publishing Title:

***In Vivo* Pk/Pd of a Delafloxacin Against *Staphylococcus aureus* (Sa), *Streptococcus pneumoniae* (Spn), and *Klebsiella pneumoniae* (Kpn) in the Murine Lung Infection Model**

Author Block:

A. Lepak, **D. R. Andes**; Wisconsin Univ., Madison, WI

Abstract Body:

Background: Delafloxacin is a broad-spectrum anionic fluoroquinolone under development for the treatment of pneumonia. The goal of the study was to determine the PK/PD targets in the murine lung infection model for SA, SPN, and KPN. **Methods:** 4 isolates of each species were utilized for in vivo studies: SA (1 MSSA, 3 MRSA), SPN (2 PCN-S, 2 PCN-R), KPN (3 ESBL, 1 WT). MICs were determined using CLSI methods. A neutropenic murine lung infection model was utilized for all studies. Single dose plasma PK was determined in the mouse model after SC administration of 2.5, 10, 40 and 160 mg/kg. For in vivo studies, four fold increasing doses of delafloxacin (range 0.03 to 160 mg/kg) were administered q6h. Outcome was measured by determining organism burden in the lung (CFU) at 24 h. The Emax Hill equation was used to model the dose-response data. The magnitude of the PK/PD index AUC/MIC associated with net stasis and 1-log kill were determined in the lung model for all isolates. **Results:** MICs ranged from 0.06-1 mg/L. Single dose PK parameter ranges include: Cmax 2-70.7 mg/L, $AUC_{0-\infty}$ 2.8-152 mg*h/L, $T_{1/2}$ 0.7-1 h. At the start of therapy mice had 6.3 ± 0.09 $\log_{10}$ CFU/lung. In control mice the burden increased 2.1 ± 0.44 $\log_{10}$ CFU/lung over the study period. There was a relatively steep dose-response relationship observed with escalating doses of delafloxacin. Maximal organism reductions ranged from 2- to more >4-$\log_{10}$. The median AUC/MIC magnitude associated with each endpoint for each species group is shown in the table.

Organism	Stasis	1-log kill				
Dose (mg/kg/24h)	24h AUC/MIC	24h free drug AUC/MIC	Dose (mg/kg/24h)	24h AUC/MIC	24h free drug AUC/MIC	
SA Median	0.73	1.64	0.04	1.74	3.90	0.09
SPN Median	1.36	16.7	0.40	4.97	89.2	2.14
KPN Median	106	403	9.68	217	752	18.1

Conclusion: Delafloxacin demonstrated in vivo potency against a diverse group of pathogens including those with phenotypic drug-resistance to other classes. Median free drug AUC/MIC targets associated with net stasis were very low for all pathogen groups: SA 0.04, SPN 0.4, and KPN 9.68. 1-log kill targets were 2- to 5-fold higher. These results have potential relevance for

clinical dose selection and evaluation of susceptibility breakpoints for delafloxacin for the treatment of lower respiratory tract infections involving these pathogens

Author Disclosure Block:

A. Lepak: None. **D.R. Andes:** E. Grant Investigator; Self; Melinta.

Poster Board Number:

SUNDAY-474

Publishing Title:

Wck 4873 (Nafithromycin): Preclinical Cardiovascular Safety Assessment of a Novel Lactone-Ketolide Antibiotic

Author Block:

A. M. Patel, R. P. Chavan, A. D. Patil, V. S. Zope, **M. V. Patel**; Wockhardt Res. Ctr., Aurangabad, India

Abstract Body:

Background: WCK 4873 is a novel lactone-ketolide antibiotic currently under development and has completed Phase 1 - single, multiple ascending dose and intra-pulmonary pharmacokinetic (PK) studies, following oral administration in US and Europe. QT prolongation risk has been associated with certain macrolide and ketolide antibiotics. Therefore, we tested the preclinical cardiovascular safety of WCK 4873 by both *in vitro* and *in vivo* methods.**Methods:***In vitro* inhibition of potassium selective IKr current by WCK 4873 was investigated using Chinese Hamster Ovary (CHO) cells stably transfected with hERG, using whole cell patch-clamp technique. *In vivo*, cardiovascular safety and PK profile of WCK 4873 (single intravenous dose of 7.5, 15 and 25 mg/kg) were assessed in six conscious telemetered Beagle dogs employing a cross-over study design. Haemodynamic parameters including heart rate, arterial blood pressure, body temperature and electrocardiogram were recorded before and after treatment up to a period of 24h. Serum WCK 4873 levels were analysed using LC-MS/MS and the PK parameters were calculated by non-compartmental analysis (WinNonlin).**Results:**WCK 4873 showed 50% inhibition of hERG current at a concentration of 36.39 µM (31.26 µg/mL). This concentration was 78 times higher than the intended therapeutic *free* plasma C_{max} (0.4 µg/mL). In dog telemetry study, up to maximum dose of 25 mg/kg, no changes were observed in haemodynamic and electrocardiographic parameters including PR and PQ interval duration, QRS complex duration, QT interval duration and QT interval corrected for heart rate using Bazette's formula, in comparison to vehicle group. The *free* serum C_{max} at 25 mg/kg (No observed effect level) dose in dog was 11 times higher than the intended free therapeutic C_{max} (0.4 µg/mL), suggesting a good cardiac safety margin.**Conclusion:**WCK 4873 (Nafithromycin) showed favourable cardiac safety as evident by both *in vitro* and *in vivo* studies undertaken at significantly supra-therapeutic concentrations, and therefore is potentially unlikely to cause prolongation of QT interval.

Author Disclosure Block:

A.M. Patel: D. Employee; Self; Wockhardt Research Center. **R.P. Chavan:** D. Employee; Self; Wockhardt Research Center. **A.D. Patil:** D. Employee; Self; Wockhardt Research Center. **V.S. Zope:** D. Employee; Self; Wockhardt Research Center. **M.V. Patel:** D. Employee; Self;

Wockhardt Research Center. K. Shareholder (excluding diversitied mutual funds); Self; Wockhardt Ltd.,.

Poster Board Number:

SUNDAY-475

Publishing Title:

In Vitro* Activity of a Novel Lactone Ketolide WCK 4873 against Resistant *Streptococcus pneumoniae* and *Haemophilus influenzae

Author Block:

J. Dubois, M. Dubois, J-F. Martel; M360, Sherbrooke, QC, Canada

Abstract Body:

Background: WCK 4873 is a lactone ketolide antimicrobial agent possessing potent activity against macrolide and ketolide resistant strains of *S. pneumoniae* due to its dual mechanism of action targeting both Domain V and Domain II of 23S rRNA. To determine if this compound could be used as monotherapy for the treatment of LRTI pathogens, *in vitro* activity against a variety of resistant *S. pneumoniae* and *H.* was investigated.**Methods:** The *in vitro* activity of **WCK 4873** was compared with that of telithromycin (TEL), azithromycin (AZ), erythromycin (ER) and levofloxacin (LE) against a total of 199 resistant *S. pneumoniae* and 190 *H. influenzae* by standard broth microdilution MIC determination. The tested strains included macrolide or ketolide-resistant (*erm* B (109) and *mef*E (52)) and ciprofloxacin (CIP)-resistant (gyrA and *par*C (38) *S. pneumoniae* and macrolide (*erm* A, B, C (137)) and CIP-resistant (gyrA and *par*C (53) *H. influenzae*.**Results:** Against ER-resistant *S. pneumoniae* strains (*ermB* genotype), the MIC of **WCK 4873** ranged from equal or less than 0.004 to 0.25 mg/L and the activity of **WCK 4873** (MIC_{90} 0.25mg/L) was superior to LE (MIC_{90} 1mg/L), TEL (MIC_{90} 2mg/L), AZ (MIC_{90} 128 mg/L or ER (MIC_{90} 128 mg/L). Against the 45 ER-resistant (*mef*E genotype*)*, strains of *S. pneumoniae*, **WCK 4873** (MIC_{90} 0.12 mg/L) was the most active agent followed by TEL (MIC_{90} 1 mg/L), LE (MIC_{90} 2mg/L), AZ (MIC_{90} 8 mg/L) and ER (MIC_{90} 32 mg/L). Against the CIP-resistant (gyrA & *par*C genotype) 38 strains of *S. pneumoniae*, **WCK 4873** (MIC_{90} 0.016 mg/L) was the most active among tested agents, followed by TEL (MIC_{90} 1 mg/L), LE (MIC_{90} 2mg/L), AZ (MIC_{90} 128 mg/L) and ER (MIC_{90} 128 mg/L). Against the ER-resistant (*erm* A, B, C genotype) 137 strains of *H. influenzae* the MIC of **WCK 4873** ranged from equal to or less than 0.25 to 16 mg/L and the activity of **WCK 4873** (MIC_{90} 2mg/L) was significantly superior to ketolide (TEL: MIC_{90} 16mg/L) or macrolides tested (AZ: MIC_{90} 16 mg/L, ER: MIC_{90} 32 mg/L). Against the 53 tested strains of CIP-resistant *H. influenzae* (gyrA & *par*C genotype), the MIC_{50} and MIC_{90} of **WCK 4873**, LE and TEL were comparable, whereas AZ and ER MIC50/90 were 1 fold lower: 2 and 4 mg/L, 2 and 4 mg/L, 2 and 4 mg/L, 1 and 2 mg/L and 1 and 2 mg/L respectively.**Conclusions:** This data confirms the interesting *in vitro* activity of this new lactone ketolide, **WCK 4873** against macrolide and ciprofloxacin resistant *S. pneumoniae* and *H. influenzae*.

Author Disclosure Block:

J. Dubois: H. Research Contractor; Self; Wockhardt. **M. Dubois:** None. **J. Martel:** None.

Poster Board Number:

SUNDAY-476

Publishing Title:

***In Vitro* Activity of Lactone Ketolide WCK 4873 When Tested against Contemporary Community-Acquired Bacterial Pneumonia Pathogens from a Global Surveillance Program**

Author Block:

D. J. Farrell, H. S. Sader, P. R. Rhomberg, R. K. Flamm, R. N. Jones; JMI Lab., North Liberty, IA

Abstract Body:

Background: WCK 4873 is a novel antimicrobial agent of the lactone ketolide class currently in clinical development for the treatment of community acquired bacterial pneumonia (CABP). It has completed SAD and MAD Phase 1 studies in Europe and intra-pulmonary pharmacokinetic study in US. WCK 4873 was awarded QIDP status in 2015. In this study, WCK 4873 was tested against contemporary CABP clinical isolates collected in medical centers worldwide as part of the 2014 SENTRY Antimicrobial Surveillance Program.**Methods**: A total of 1,512 contemporary (2014) CABP clinical isolates from the United States, 1,505 from Europe, 558 from Asia-Pacific, and 558 from Latin America, as part of the SENTRY Program, were susceptibility (S) tested against WCK 4873 and multiple comparator agents by reference broth microdilution methods and interpretive criteria.**Results**: WCK 4873 was very active ($MIC_{50/90}$, 0.015/0.06 µg/mL) against 1,911 *Streptococcus pneumoniae* (SPN) and inhibited all strains at MIC values ≤0.25 µg/mL. Telithromycin (TEL) S (CLSI) was 99.9% against SPN, and WCK4873 was up to eight-fold more potent than TEL ($MIC_{50/90}$, 0.015/0.5 µg/mL). Overall, 37.9% of SPN were resistant (R) to erythromycin (ERY) and 19.7% were R to clindamycin (CLI). WCK 4873 retained good activity against ERY-R/CLI-S strains ($MIC_{50/90}$, 0.03/0.06 µg/mL) and ERY-R/CLI-R strains ($MIC_{50/90}$, 0.03/0.12 µg/mL). Against 716 *Staphylococcus aureus* (SA), 88.5% of isolates were inhibited by WCK 4873 at the CLSI TEL S breakpoint of ≤1 µg/mL ($MIC_{50/90}$, 0.06/>2 µg/mL). MIC_{90} values for WCK 4873 were elevated for methicillin-resistant (MR) compared to methicillin-susceptible (MS) SA (MRSA, $MIC_{50/90}$, 0.06/>2 µg/mL; MSSA, $MIC_{50/90}$, 0.06/0.06 µg/mL). WCK 4873 ($MIC_{50/90}$, 4/4 µg/mL) demonstrated similar activity to TEL ($MIC_{50/90}$, 2/4 µg/mL; 97.9% S, CLSI) against 1,002 *Haemophilus influenzae* (HI) isolates. WCK 4873 ($MIC_{50/90}$, 0.12/0.25 µg/mL) exhibited similar activity to TEL ($MIC_{50/90}$, 0.12/0.12 µg/mL; 99.6% S, EUCAST) against 504 *M. catarrhalis* (MC) isolates.**Conclusions**: WCK 4873 showed a broad range of potent *in vitro* activity against contemporary (2014) global CABP pathogens (SPN, SA, HI and MC). These results support the continued clinical development of WCK 4873 for CAPB.

Author Disclosure Block:

D.J. Farrell: H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Wockhardt. **H.S. Sader:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Wockhardt. **P.R. Rhomberg:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Wockhardt. **R.K. Flamm:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Wockhardt. **R.N. Jones:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Wockhardt.

Poster Board Number:

SUNDAY-477

Publishing Title:

In Vitro* Activity of a Novel Lactone Ketolide WCK 4873 against *Legionella pneumophila

Author Block:

J. Dubois, M. Dubois, J-F. Martel; M360, Sherbrooke, QC, Canada

Abstract Body:

Background: WCK 4873 is a lactone ketolide antimicrobial agent possessing potent activity against macrolide and ketolide resistant strains of *S. pneumoniae*, *S. pyogenes* and other *Streptococci* due to its dual mechanism of action targeting both Domain V and Domain II of 23S rRNA in these organisms. **WCK 4873** also demonstrates potent activity against penicillin, macrolide and quinolone resistant strains of *S. pneumoniae*, *S. pyogenes* and other *Streptococci*. To determine if this compound could be used as monotherapy for the treatment of community-acquired bacterial pneumonia involving typical and atypical RTI pathogens, *in vitro* activity against a variety of *L. pneumophila* was investigated.**Methods:** The *in vitro* activity of **WCK 4873** was compared with that of telithromycin, azithromycin, erythromycin and levofloxacin against a total of 150 *L. pneumophila* isolates (serogroup 1 (n=125), 2 (n=5), 3 (n=5), 4 (n=5), 5 (n=5) and 6 (n=5)) by a standard microdilution procedure using buffered yeast extract broth containing *Legionella* growth supplement (BYE). A pre-test to determine if antibiotic activity was impacted artificially by BYE supplement or iron was done by testing three ATCC quality control isolates on BYE, BYE without iron and cation-adjusted Mueller-Hinton Broth.**Results:** Only BYE supported *L. pneumophila* growth. Pilot tests indicated that BYE broth do not affect the MIC result of **WCK 4873** against *L. pneumophila* even if *L. pneumophila* strains are incubated during 48 hours.Regardless, the $MIC_{50/90}$ values of **WCK 4873**, levofloxacin, telithromycin, azithromycin and erythromycin against all *L. pneumophila* strains were 0.008/0.03, 0.016/0.016, 0.03/0.06, 0.06/0.5 and 0.25/1 mg/L respectively.Against *L. pneumophila* serogroup 1, usually the most frequently recovered serogroup, the $MIC_{50/90}$ of **WCK 4873**, levofloxacin, telithromycin, azithromycin and erythromycin was 0.008/0.03, 0.016/0.016, 0.03/0.06, 0.12/0.5 and 0.25/1 mg/L.**Conclusions:** This data confirms the interesting *in vitro* activity of this new lactone ketolide, **WCK 4873** against *Legionella pneumophila*.

Author Disclosure Block:

J. Dubois: H. Research Contractor; Self; Wockhardt. **M. Dubois:** None. **J. Martel:** None.

Poster Board Number:

SUNDAY-478

Publishing Title:

Determination of Disk Mass and Correlations Between Disk Diffusion Zones and Broth Microdilution MIC's for Lactone Ketolide Nafithromycin (WCK 4873)

Author Block:

M. Hackel[1], S. Bhagwat[2], M. Patel[2], D. Sahm[1]; [1]IHMA, Inc., Schaumburg, IL, [2]Wockhardt Res. Ctr., Aurangabad, India

Abstract Body:

Background: Nafithromycin **(**WCK 4873) is a new lactone ketolide discovered at Wockhardt with potent activity against macrolide / ketolide resistant *S. pneumoniae*. WCK 4873 has completed Phase 1 studies in US and Europe. As part of this drug's development it is important to determine the optimal disk mass (content) for WCK 4873 necessary to achieve high correlation with broth microdilution MICs. This study determined the optimal disk mass and the relative correlation between disk inhibitory zone diameters and broth dilution MICs for WCK 4873.**Methods:** To determine optimal disk mass, three concentrations of disk mass (10 µg, 15 µg, and 20 µg) were tested in triplicate against three ATCC quality control (QC) strains (*Streptococcus pneumoniae* ATCC 49619, *Haemophilus influenzae* ATCC 49247, and *Staphylococcus aureus* ATCC 25923). To determine correlation between disk zone and MIC, a common inoculum was used to concurrently test 511 clinical isolates selected to represent the key target pathogens against WCK 4873 by disk diffusion (10 µg, 15 µg, and 20 µg disks) and broth microdilution according to CLSI guidelines. Correlation coefficients (R^2 values) were determined using the 15 µg disk. Telithromycin was included as a control for all testing.**Results:** All WCK 4873 disk masses showed an increase in zones of inhibition in a concentration dependent manner. WCK 4873 exhibited a high correlation between zone size and MIC for *S. aureus* with an R^2 value of 0.93. WCK 4873 exhibited potent *in vitro* activity against *S. pneumoniae*, *S. pyogenes*, *H. influenzae* and *Moraxella catarrhalis* resulting in lower R^2 values as no isolates with high MICs were found (R^2 values 0.37, 0.67, 0.49, 0.0, respectively).**Conclusions:**All WCK 4873 disk masses showed an increase in zones of inhibition in a concentration dependent manner. The 15 µg disk was chosen as the optimal disk mass. The high R^2 value for *S. aureus* indicates that a disk test for the determination of susceptibility is quite feasible for this organism group. The high potency of WCK 4873 against *S. pneumoniae*, *S. pyogenes*, *H. influenzae* and *M. catarrhalis* precludes a final status of its use for disk testing against these organisms.

Author Disclosure Block:

M. Hackel: M. Independent Contractor; Self; IHMA, Inc. **S. Bhagwat:** D. Employee; Self; Wockhardt. **M. Patel:** D. Employee; Self; Wockhardt. **D. Sahm:** M. Independent Contractor; Self; IHMA, Inc..

Poster Board Number:

SUNDAY-479

Publishing Title:

In Vitro* Intracellular Activity of Novel Lactone Ketolide WCK 4873 against *Legionella pneumophila

Author Block:

J. Dubois, M. Dubois, J-F. Martel; M360, Sherbrooke, QC, Canada

Abstract Body:

Background: WCK 4873 is a lactone ketolide antimicrobial agent possessing potent activity against macrolide and ketolide resistant strains of *S. pneumoniae*, *S. pyogenes* and other *Streptococci* due to its dual mechanism of action targeting both Domain V and Domain II of 23S rRNA. **WCK 4873** also demonstrates potent activity against penicillin, macrolide and quinolone resistant strains of *S. pneumoniae*, *S. pyogenes* and other *Streptococci*. *In vitro* intracellular activities using human monocytes against a variety of *L. pneumophila* were investigated.**Methods:** The intracellular activity of **WCK 4873** was compared with that of telithromycin (TE), azithromycin (AZ), erythromycin (ER) and levofloxacin (LE) against a total of 3 erythromycin-resistant strains of *L. pneumophila* serogroup 1. The intracellular activity was determined by exposing human monocytes, U937 cell line, infected with intracellular *Legionella* at the 1 or 2XMIC of antibiotic for each strain during 6 days exposure. After 2 days of antibiotic exposure, the infected cell line was split into two groups; one group was kept drug-free and the other group was treated with the same antibiotic as before till 7 days. Viable bacterial cells (CFU/mL) were enumerated for all groups at time zero, after 24h before and after washing, at 48h, then at 72h before and after washing, and every day till next 4 days, by plating on the Buffer Charcoal Yeast Extract agar in duplicate.**Results:** A significant reduction of >3 $\log_{10}$ CFU/mL or >99.9% of ER-resistant *L. pneumophila* grown in monocytes was observed after 3 days of antibiotic exposure until day 7 by **WCK 4873** (1 or 2XMIC) and TE. A regrowth of *L. pneumophila* in monocytes was observed after 1 day of ER exposure, after 3 days of AZ exposure and after 4 days of LE exposure, however such regrowth was not observed with **WCK 4873** and TE. After drug wash-out at day 3, rapid regrowth of *L. pneumophila* was observed in ER in contrast to substantially delayed regrowth observed with **WCK 4873** and that too at just 1XMIC. However **WCK 4873** concentration at 2XMIC completely prevented regrowth. Significant decrease of *L. pneumophila* count (reduction of >4 $\log_{10}$ CFU/ml or >99.99%) in human monocytes was observed with WCK 4873 until day 7 at 2XMIC.**Conclusions: WCK 4873** shows good human monocytes penetration features and demonstrates potent bactericidal effect for intracellular ER-resistant *L. pneumophila* serogroup 1.

Author Disclosure Block:

J. Dubois: H. Research Contractor; Self; Wockhardt. **M. Dubois:** None. **J. Martel:** None.

Poster Board Number:

SUNDAY-480

Publishing Title:

***In Vitro* Activities of Investigational Ketolide Wck 4873 (Nafithromycin) and Other Antimicrobial Agents Against Human Mycoplasmas and Ureaplasmas**

Author Block:

K. B. Waites, D. M. Crabb, L. B. Duffy; Univ. of Alabama at Birmingham, Birmingham, AL

Abstract Body:

WCK 4873 (INN: Nafithromycin, Phase 1 completed, Wockhardt)) is a lactone ketolide being developed for treatment of community-acquired bacterial pneumonia. We determined MICs for WCK 4873 (WCK) in comparison to azithromycin (AZI), clarithromycin (CLA), telithromycin (TEL), clindamycin (CLI), levofloxacin (LEV), and tetracycline (TET) by broth microdilution for reference strains and clinical isolates of *Mycoplasma pneumoniae* (n = 20), *Mycoplasma hominis* (n = 21), *Mycoplasma genitalium* (8), and *Ureaplasma* species (n = 23). Isolates tested included organisms known to be resistant to tetracyclines, macrolides, and/or fluoroquinolones. Results are shown in the table below.

	AZI	CLA	TEL	WCK	LEV	TET	CLI
M. genitalium (n = 8)							
Range (µg/ml)	≤0.000125-0.005	≤0.000125-0.002	≤0.000125-0.005	≤0.000125	0.25-1	0.125-4	--
M. hominis (n = 21)							
Range (µg/ml)	1-8	8-32	0.25-8	0.002-.25	0.125-1	0.063->32	0.032-0.5
MIC50	4	16	1	0.032	0.25	0.5	0.063
MIC90	8	32	8	0.125	0.5	>32	0.125
M. pneumoniae (n = 20)							
Range (µg/ml)	≤0.000125-16	0.005->32	0.00025-16	≤0.000125-16	0.5-1	0.25-1	--
MIC50	≤0.000125	0.001	0.0005	≤0.000125	1	0.5	--
MIC90	0.001	0.004	0.001	≤0.000125	1	1	--

Ureaplasma sp. *(n = 23)*							
Range (µg/ml)	0.25->256	0.008->256	0.016->256	0.001-256	0.25->32	0.125-128	--
MIC50	1	0.032	0.063	0.008	1	1	--
MIC90	2	0.125	0.25	0.032	4	128	--

WCK 4873 was uniformly active against all 8 *M. genitalium* isolates with MICs comparable to AZI, CLA, and TEL. WCK 4873 demonstrated good activity against *M. hominis,* with all MICs $\leq$ 0.25 µg/ml, including 2 strains with elevated TEL MICs ($\geq$4 µg/ml). WCK 4873 and CLI had the lowest MIC_{90} (0.125 µg/ml) for this species. WCK 4873 was active against 18 macrolide-susceptible *M. pneumoniae* with all MICs $\leq$.000125 µg/ml. However, WCK 4873 MICs for 2 strains known to be macrolide-resistant were also elevated (16µg/ml). WCK 4873 was very active against *Ureaplasma* species and had the lowest MIC_{90} (0.032 µg/ml) of all drugs tested. However, its MICs were 256 µg/ml for 2 strains that were known to have high level macrolide resistance. Tetracycline and levofloxacin resistance had no effect on WCK 4873 MICs for *M. hominis* and *Ureaplasma* spp. These data suggest that ACK 4873 may be useful to treat infections due to mycoplasmas and ureaplasmas, unless there is macrolide-resistance, thus meriting further clinical development..

Author Disclosure Block:

K.B. Waites: E. Grant Investigator; Self; Wockhardt. **D.M. Crabb:** None. **L.B. Duffy:** None.

Poster Board Number:

SUNDAY-481

Publishing Title:

Comparative *In Vitro* Activities of Ketolides Wck4873, Wck4897 and Wck4918 Against Macrolide Resistant *Streptococcus pneumoniae*

Author Block:

M. R. Jacobs[1], S. Bajaksouzian[1], S. Bhagwat[2], M. Patel[2]; [1]Case Western Reserve Univ., Cleveland, OH, [2]Wockhardt Res. Ctr., Aurangabad, India

Abstract Body:

Background: Ketolides have been developed to overcome macrolide resistance in pneumococci, with telithromycin in clinical use and others in clinical study. Three new ketolides, WCK4873, WCK4897 and WCK4918, have been produced and are being evaluated for clinical development. **Methods:** In vitro susceptibilities of WCK4873, WCK4897 and WCK4918, Wockhardt Research Center, Aurangabad, India) and comparator agents azithromycin and telithromycin were determined by CLSI broth microdilution. Isolates tested included 63 macrolide resistant (33 clindamycin susceptible and 30 clindamycin resistant) and 27 macrolide susceptible strains.**Results:** MIC ranges and $MIC_{50/90}$ values for the 90 isolates tested are shown in the table. MIC distributions of the four ketolides were similar against macrolide susceptible and resistant strains. WCK4897 was the most potent of the agents tested (MIC_{90} 0.12 mg/L), followed by WCK4873 and 4918 (MIC_{90}s 0.25 mg/L) and telithromycin (MIC_{90} 0.5 mg/L). Azithromycin was active against macrolide susceptible (MIC_{90} 0.12 mg/L) but nor resistant isolates (MIC_{90} >2 mg/L).**Conclusion:** Among the three new ketolides evaluated, all were active against macrolide resistant pneumococci, with activity similar to that against macrolide susceptible strains. WCK4897 was the most potent of the new ketolides as well as more potent than telithromycin.

	MIC values (mg/L)		
Agent	Range	MIC50 values	MIC90 values
WCK4873	≤0.008-0.25	0.06	0.25
WCK4897	≤0.004-0.25	0.06	0.12
WCK4918	≤0.008-0.5	0.12	0.25
Telithromycin	≤0.004-2	0.25	0.5
Azithromycin (macrolide susceptible strains)	0.03-0.25	0.12	0.12

Azithromycin (macrolide resistant strains)	1->2	>2	>2

Author Disclosure Block:

M.R. Jacobs: F. Investigator; Self; Wockhardt Pharmaceuticals. J. Scientific Advisor (Review Panel or Advisory Committee); Self; Wockhardt Pharmaceuticals. **S. Bajaksouzian:** None. **S. Bhagwat:** D. Employee; Self; Wockhardt Pharmaceuticals. **M. Patel:** D. Employee; Self; Wockhardt Pharmaceuticals.

Poster Board Number:

SUNDAY-482

Publishing Title:

Solithromycin, a Novel Fluoroketolide, *In Vitro* Antibacterial Activity against Anaerobes Isolated in Japan

Author Block:

Y. Yamagishi, Y. Matsukawa, H. Suematsu, H. Mikamo; Aichi Med. Univ., Aichi, Japan

Abstract Body:

Background: Anaerobes invade the tissue due to a failure of defense mechanisms or through infection from the environment and can causes various infectious diseases. Solithromycin (SOL) is a novel fluoroketolide with reported high potency against diverse groups of Gram-positive and Gram-negative bacteria, including mycoplasma and ureaplasma. In this study, we evaluated the antibacterial activities of solithromycin against clinical isolates of anaerobic pathogens recently collected in Japan. **Methods:** MICs of SOL and comparator agents, including erythromycin (EM), clarithromycin (CAM), azithromycin (AZM), levofloxacin (LVFX), moxifloxacin (MFLX), sitafloxacin (STFX) and meropenem (MEPM), against clinical isolates (20 *S. anginosus*, 20 *S. constellatus*, 20 *S. intermedius*, 23 *F. magna*, 19 *P. asaccharolyticus*, 23 *P. micra*, 50 *B. fragilis*, 40 *B. thetaiotaomicron*, 16 *F. nucleatum*, 5 *F. necrophorum*, 47 *P. bivia*, 20 *P. melaninogenica*, 38 *P. intermedia*, 20 *Veillonella* species and 48 *C. difficile*), were determined by the CLSI broth dilution method.**Results:** SOL showed broad and potent anti-anaerobic activity. MIC_{90}s of SOL against Gram-positive and Gram-negative anaerobic species were <0.015-32 µg/mL and 0.06->32 µg/mL, respectively.**Conclusions:** SOL demonstrated the most potent Gram-positive anti-anaerobic activity compared to the other macrolides and quinolones tested. These results suggest SOL has a potency that is promising for the alternative treatment of anaerobic infections caused by Gram-positive bacteria.

MIC90					
Organism (No. of strains)	MIC_{90}	MIC_{90}		MIC_{90}	MIC_{90}
	SOL	LVFX		SOL	LVFX
C. difficile (48)	>32	>64	*B. fragilis* (50)	>32	64
S. anginosus (20)	0.25	2	*B. thetaiotaomicron* (40)	32	>64
S. constellatus (20)	0.5	2	*F. nucleatum* (16)	8	16
S. intermedius (20)	0.25	2	*P. bivia* (47)	2	16
F. magna (23)	0.12	64	*P. melaninogenica* (20)	0.25	>16
P. asaccharo;yticus (19)	0.25	64	*P. intermedia* (38)	0.06	8
P. micra (23)	<0.015	4	*Veillonella* spp. (20)	8	8

Author Disclosure Block:

Y. Yamagishi: None. **Y. Matsukawa:** None. **H. Suematsu:** None. **H. Mikamo:** None.

Poster Board Number:

SUNDAY-483

Publishing Title:

Comparative Evaluation of Oritavancin, Dalbavancin, and Tedizolid *In Vitro* Antimicrobial Activity Against Clinical Isolates of *Enterococcus*

Author Block:

F. Foolad, C. Young, D. Newton, A. Smith, T. S. Patel; Univ. of Michigan Hlth.System, Ann Arbor, MI

Abstract Body:

Background: New antimicrobial agents with activity against *Enterococcus*, including some with activity against vancomycin-resistant and daptomycin non-susceptible isolates, have been introduced. However, there are minimal data comparing the in vitro activities of oritavancin, dalbavancin, tedizolid, vancomycin, and daptomycin for clinical isolates using a custom TREK Sensititre panel.**Methods:** All cultures from patient infections with isolated *Enterococcus* from February 2015 to November 2015 were included. Organisms were identified by MALDI-TOF mass spectrometry (Bruker Daltonik, Bremen, Germany). Minimum inhibitory concentrations (MICs) for all antimicrobials were determined using broth microdilution methods (TREK Diagnostic Systems, Cleveland, Ohio). The percentage of isolates within the susceptible range was determined using Clinical and Laboratory Standards Institute (CLSI) and US Food and Drug Administration (FDA) breakpoint criteria.**Results**: A total of 1,186 *Enterococcus* isolates from clinical specimens were included. Higher MIC_{50} and MIC_{90} were determined for *E. faecium* than *E. faecalis* (table). Using published interpretive criteria, 97.6%, 80.6%, and 99% of all *Enterococcus* isolates were considered susceptible to oritavancin, dalbavancin, and tedizolid, respectively as compared to 79.9% and 98.1% susceptible to vancomycin and daptomycin. Susceptibility to oritavancin, dalbavancin, and tedizolid was lower among vancomycin-resistant isolates (n=236; 88.6%, 3.4%, and 98.3%) and daptomycin non-susceptible isolates (n=22; 86.4%, 40.9%, and 90.9%).**Conclusions**: Oritavancin, dalbavancin, and tedizolid demonstrated good in vitro activity against *Enterococcus* isolated from a variety of clinical infections with comparatively lower activity in *E. faecium* than *E. faecalis* and even lower activity in multi-drug resistant strains. Oritavancin and tedizolid may be useful for vancomycin-resistant and daptomycin non-susceptible *Enterococcus* infections.

	Range (µg/ml)	**MIC_{50} (µg/ml)**	**MIC_{90} (µg/ml)**
Enterococcus faecalis (n=908)			
Oritavancin	≤0.004 - >0.5	0.015	0.06
Dalbavancin	≤0.06 - >0.5	≤0.06	≤0.06

Tedizolid	≤0.12 - >4	0.05	0.05
Enterococcus faecium (n=248)			
Oritavancin	≤0.004 - 0.5	0.06	0.12
Dalbavancin	≤0.06 - >0.5	>0.5	>0.5
Tedizolid	≤0.12 - 2	0.25	0.5

Author Disclosure Block:

F. Foolad: None. **C. Young:** None. **D. Newton:** None. **A. Smith:** None. **T.S. Patel:** None.

Poster Board Number:

SUNDAY-484

Publishing Title:

Wck 4873 (Nafithromycin): *In Vivo* lung Infection Studies against Macrolide-Resistant (Mr) and Telithromycin-Non-Susceptible (Tel-Ns) Pneumococci

Author Block:

S. S. Bhagwat, S. S. Takalkar, J. S. Satav, A. M. Kulkarni , A. P. Udaykar, **M. V. Patel**; Wockhardt Res. Ctr., Aurangabad, India

Abstract Body:

Background: WCK 4873 is a lactone-ketolide (Phase 1 studies completed in Europe) with a broad-spectrum activity against respiratory tract pathogens. WCK 4873 is characterized by potent *S. pneumonia*e (SPN) activity (global SPN n=1911, MIC_{90}: 0.06 µg/mL) including MR (SPN n=394, MIC_{90}: 0.12 µg/mL) and TEL-NS SPN (n=204, MIC_{90}: 0.12 µg/mL) possibly due to its high affinity binding to domain II and V of 23S rRNA. Herein, we describe *in vivo* efficacy of WCK 4873 against MR and TEL-NS SPN pathogens.**Method:**Neutropenic (intraperitonial cyclophosphamide on day -4 and -1) murine lung infection studies were performed employing four strains of SPN [Two MR: SPN 6683 (L4/L22 mutations) and SPN 1179 (*mef* + *ermB*); two TEL-NS: SPN 5032 and SPN 3773 (both expressing high-level *ermB*)]. MIC was determined by agar dilution method (MHA +5% sheep blood). Oral treatment was initiated 2h post-infection (initial inoculum: 6.5-7.2 $\log_{10}$ CFU/mL in lungs) administered BID/TID (3h apart) for 3 days. Bacterial enumeration in lungs was undertaken 18–20h post last dose.**Results:**MICs of WCK 4873 and TEL for SPN 6683, 1179, 5032 and 3773 were 0.06, 0.12, 0.25, 0.5 and 0.12, 1.0, 2.0, 4.0 µg/mL, respectively. Sustained progression of infection was noted with bacterial load at the start of treatment being 6.5–7.0 $\log_{10}$ CFU/lung increasing up to 8.0–9.0 $\log_{10}$ CFU/lung by the end of 3 days, resulting in mortality among untreated animals. In strains SPN 6683 and SPN 1179, WCK 4873 caused $\geq$2 $\log_{10}$ kill in lung at 6.25 and 25 mg/kg dose, respectively. While, TEL at the doses of 6.25 and 50 mg/kg doses provided mere bacteriostatic effect in the two strains, respectively. In TEL-NS SPN 5032 and SPN 3773 strains, WCK 4873 doses of 12.5 mg/kg BID and 12.5 mg/kg TID resulted in 2.87 $\log_{10}$ and 1.8 $\log_{10}$ kill in lung, respectively. Organisms recovered post 2–3 days therapy did not show any shift in WCK 4873 MICs.**Conclusion**: WCK 4873 (Nafithromycin) provided unique coverage of MR and TEL-NS pneumococci which is not evident with any other ketolides to date.

Author Disclosure Block:

S.S. Bhagwat: D. Employee; Self; Wockhardt Research Center. K. Shareholder (excluding diversitied mutual funds); Self; Wockhardt Ltd.,. **S.S. Takalkar:** D. Employee; Self; Wockhardt Research Center. K. Shareholder (excluding diversitied mutual funds); Self; Wockhardt Ltd.,. **J.S. Satav:** D. Employee; Self; Wockhardt Research Center. **A.M. Kulkarni :** D. Employee;

Self; Wockhardt Research Center. **A.P. Udaykar:** D. Employee; Self; Wockhardt Research Center. **M.V. Patel:** D. Employee; Self; Wockhardt Research Center. K. Shareholder (excluding diversitied mutual funds); Self; Wockhardt Ltd.,.

Poster Board Number:

SUNDAY-485

Publishing Title:

Improved Antimicrobial Activity of Fully Synthetic Macrolides Against Macrolide Resistant Gram-positive Isolates

Author Block:

S. D. Lahiri, R. A. Alm; Macrolide Pharmaceuticals, Watertown, MA

Abstract Body:

Background: Rapid emergence of resistance by multiple drug resistant (MDR) pathogens has intensified the search for new antimicrobial agents that are safe and efficacious. Methicillin-resistant *Staphylococcus aureus* (MRSA) and MDR *Streptococcus pneumoniae* remain a clinical concern for a variety of community-associated and hospital-acquired infections. Macrolides are a class of known antibiotics that are safe and effective. However, the macrolide class has limited activity against Gram-negative pathogens, as well as against Gram-positive isolates carrying macrolide resistance determinants. In particular, the utility of macrolides against MDR gram-positive pathogens, including MRSA, has been hampered primarily by various changes to the binding pocket, including the methylation of the macrolide binding nucleotide by erm methylases.**Methods:** Novel macrolides were synthesized via a fully synthetic, chemistry platform. These compounds were tested against characterized clinical isolates following CLSI methods.**Results:** These new compounds demonstrated improved activity against strains resistant to older generations of macrolides. They do not induce the *ermA* methylase gene in *S. aureus* or the *ermB* methylase gene in *S. pneumoniae*. They were also tested against MRSA strains that contained a variety of alterations in the leader peptide, resulting in constitutive expression of the *ermA* gene. The novel macrolides described here showed a >16-fold improvement in MIC compared to in-class comparators which had MIC values of >256 μg/mL. Further, these compounds were not affected by efflux-based resistance mechanisms, such as Mef(A) or Msr(A), found in *S. pneumoniae* and *S. aureus*, with MIC values of ≤ 1 μg/mL against these isolates. Lastly, *S. pneumoniae* isolates carrying 23S rRNA and ribosomal protein mutations that result in reduced susceptibility to macrolides remain susceptible to these novel compounds.**Conclusions:** These data demonstrate that leveraging the flexibility of the synthetic chemistry platform should identify promising candidate molecules that circumvent the common pre-existing resistance mechanisms.

Author Disclosure Block:

S.D. Lahiri: None. **R.A. Alm:** None.

Poster Board Number:

SUNDAY-486

Publishing Title:

***in Vitro* Activity Of Lefamulin Against Macrolide-Susceptible (Msmp) And Macrolide-Resistant *mycoplasma Pneumoniae* (Mrmp) From The United States, Europe, And China**

Author Block:

K. Waites[1], D. M. Crabb[1], L. B. Duffy[1], Y. Liu[2], S. Paukner[3]; [1]Univ. of Alabama at Birmingham, Birmingham, AL, [2]Fudan Univ., Shanghai, China, [3]Nabriva Therapeutics AG, Vienna, Austria

Abstract Body:

Background: Pleuromutilin (PM) antibiotics bind to the peptidyl transferase center of the 50S ribosomal subunit, blocking protein synthesis. Lefamulin (LMU, Nabriva Therapeutics) is a novel semi-synthetic PM for systemic human use with potent activity against a variety of gram-positive and gram-negative bacteria, including multi-drug resistant strains, in Phase 3 trials for the treatment of community-acquired bacterial pneumonia. In previous *in vitro* surveillance studies cross-resistance between LMU and commonly used respiratory antibiotics was not observed. Macrolide-resistance in *Mycoplasma pneumoniae* (MPN) has become increasingly prevalent globally. We performed an *in vitro* evaluation of respiratory isolates of macrolide-susceptible (MSMP) and macrolide-resistant MRMP *M. pneumoniae* from the US, Europe, and China. **Methods:** The broth microdilution MIC assay per CLSI guidelines (2011) was used to test strains against LMU and 4 comparators (see Table).**Results:** LMU was highly active against all strains, with MICs $\leq$ 0.004 µg/ml. The LMU MIC_{90} for MRMP (0.002 µg/ml) was the lowest among all 5 drugs tested. Additionally, for LMU, minimum bactericidal concentrations (MBCs) were determined for 2 macrolide-susceptible and 6 macrolide-resistant isolates and all MBCs were 2 to 4 times the MIC values, indicating a bactericidal effect.**Conclusions:** LMU has potent in vitro activity against MPN, including macrolide resistant strains. LMU may be a promising therapeutic option for infections due to MPN.

	Lefamulin (LMU)	Azithromycin (AZI)	Erythromycin (ERY)	Tetracycline (TET)	Moxifloxacin (MOX)
MSMP(n = 14) Range (µg/ml)	≤0.00025-0.001	≤0.00025-0.001	0.004-0.008	0.25-1	0.063-0.25
MIC_{50}	≤0.00025	0.0005	0.008	0.5	0.125
MIC_{90}	0.001	0.001	0.008	1	0.25
MRMP(n = 36) Range (µg/ml)	0.0005-0.004	16 - >32	>32	0.25-1	0.125-0.25

MIC_{50}	0.002	32	>32	0.5	0.125
MIC_{90}	0.002	>32	>32	1	0.25

Author Disclosure Block:

K. Waites: E. Grant Investigator; Self; Ken Waites. **D.M. Crabb:** None. **L.B. Duffy:** None. **Y. Liu:** None. **S. Paukner:** D. Employee; Self; Nabriva Therapeutics AG.

Poster Board Number:

SUNDAY-487

Publishing Title:

Activity of Cadazolid against Recent Clinical Isolates of *Staphylococcus aureus* and *Enterococcus* spp.

Author Block:

I. Morrissey[1], S. Hawser[1], E. Genet[1], P. Jeandey[1], T. W. Morris[2], H. H. Locher[2]; [1]IHMA Sàrl, Epalinges, Switzerland, [2]Actelion Pharmaceuticals Ltd, Allschwil, Switzerland

Abstract Body:

Objectives: Cadazolid (CDZ) is a novel quinolonyl-oxazolidinone antibacterial currently in phase III clinical trials for the treatment of *C. difficile*-associated diarrhoea. This study was undertaken to determine CDZ activity against recent clinical isolates of *S. aureus* and *Enterococcus* spp and evaluate varying test parameters.**Methods:** The minimum inhibitory concentration (MIC) for CDZ and comparators was determined by CLSI aerobic broth microdilution methodology [CLSI M07-A10]. Test isolates included *S. aureus* from 2014 and enterococci mainly from between 2012 and 2014. Varying testing parameters against CLSI quality control strains were: anaerobic conditions; addition of Ca^{2+} (50 or 100 µg/ml), Mg^{2+} (25µg/ml), human serum (25 or 50%), human plasma (50%), bovine serum albumin (0.001 - 0.1%) or tween 80 (0.002%); pH 5.8, 6.5 & 8.5 and inocula of 10^4 - 10^7 cfu/ml.**Results**: CDZ MICs were 0.12 - 0.5 µg/ml against *S. aureus* and ≤0.03 - 1 µg/ml against *Enterococcus* spp. Linezolid-resistant (LZD-R) enterococci were at the higher end of the CDZ MIC range but vancomycin-resistance (VAN-R) did not affect activity. Methicillin-resistant *S. aureus* (MRSA) and susceptible strains (MSSA) were equally inhibited by CDZ (See Table). Based on MIC_{50} or MIC_{90}, CDZ was more active than LZD, VAN or fidaxomicin (FDX). This included Van-R, LZD-R and MRSA strains. Anaerobic conditions and low pH lead to lower MICs (2-4-fold) whereas varying the other parameters had no effect.

	CDZ		**LZD**		**VAN**		**FDX**	
Organism	**MIC_{50}**	**MIC_{90}**	**MIC_{50}**	**MIC_{90}**	**MIC_{50}**	**MIC_{90}**	**MIC_{50}**	**MIC_{90}**
E. faecalis (n=108)	0.25	0.5	2	2	1	>32	4	8
E. faecialis VAN-R (n=43)	0.25	0.25	1	1	>32	>32	4	4
E. faecium (n=114)	0.5	0.5	2	2	1	>32	4	8
E. faecium VAN-R (n=42)	0.5	0.5	1	1	>32	>32	4	8
Enterococci LZD-R (n=8, range)	0.5-1		16-32		0.5->32		4-8	
S. aureus (n=106)	0.25	0.25	2	2	1	1	8	8

MSSA (n=55)	0.25	0.25	2	2	1	1	8	8
MRSA (n=51)	0.25	0.25	2	2	1	1	8	8

Conclusions: CDZ shows potent *in vitro* activity against *S. aureus* and enterococci, including resistant strains. This activity is retained or even increased under anaerobic conditions and at low pH. CDZ was more active against *S. aureus* and enterococci than current agents used to treat *C. difficile* infections, such as VAN or FDX.

Author Disclosure Block:

I. Morrissey: M. Independent Contractor; Self; IHMA Sàrl. **S. Hawser:** M. Independent Contractor; Self; IHMA Sàrl. **E. Genet:** M. Independent Contractor; Self; IHMA Sàrl. **P. Jeandey:** M. Independent Contractor; Self; IHMA Sàrl. **T.W. Morris:** D. Employee; Self; Actelion Pharmaceuticals Ltd. **H.H. Locher:** D. Employee; Self; Actelion Pharmaceuticals Ltd.

Poster Board Number:

SUNDAY-488

Publishing Title:

Pharmacokinetics of Linezolid in Critically Ill Patients

Author Block:

S. Corcione, L. Baietto, A. d'Avolio, V. Fanelli, M. V. Ranieri, G. di Perri, F. de Rosa; Univ. of Turin, Turin, Italy

Abstract Body:

Background: Therapeutic drug monitoring (TDM) of linezolid (LZD) could be helpful in severe ill patients. The higher success occur at AUC_{0-24}/MIC values of 80-12, Cmin ≥2 mg/L or AUC_{24} > 160-200 mg h/L. Aim of this study was to evaluated the pharmacokinetic of LZD in patients admitted to Intensive care Unit (ICU).**Methods:** All patients treated with intravenous LZD (600mg twice daily), admitted to ICU at City of Science and Health, Molinette Hospital, Turin (Italy), were enrolled between 2011-2012. Plasma LZD concentrations were determined at the steady-state by 1 h-infusion. AUC_{0-24} of LNZ was calculated with blood samples collected before (time 0) and after 2.5, 4 and 8hs intravenous drug administration. Drug concentrations were determined using an ultra performance liquid chromatography (UPLC-PDA). Pharmacokinetic data were studied using Kinetica software. Simulated data for LNZ pharmacokinetic parameters were calculated with *S. aureus* MICs corresponding to 0.5, 1, 2 and 4 mg/L.**Results:** 16 patients were enrolled in the study. The mean days of ICU stay at time of diagnosis were 21.6 (SD ±14.6), mean days duration of treatment 11 (SD ± 3.9). Two patients were on ECMO and 5 on CVVH while they were treated. 21 day-mortality was 19% (3).In Table 1, the main LNZ pharmacokinetic parameters are reported. 62.5% (10) patients achieved C_{min} >2 mg/L and 31.6% (5) had AUC_{0-24} > 160-200.At univariate analysis, mortality was associated with high APACHE score (p=0.012) but PK parameters were not associated with mortality. Simulated data calculated showed that Pk parameters are satisfactory when the MIC is 0.5 mg/L.**Conclusion:**The peculiar pathophysiological conditions of these patients, might have a considerable impact on the therapeutic effect of LNZ, even when PK/PD parameters fall in the favourable range.

P.	**Age, SEX**	**BMI**	**APACHE SCORE**	**organism**	**MIC LZD**	**Cmin (mg/L)**	**Cmax (mg/L)**	**AUCss (mg/L*h)**	**CLss (L/h)**	**Vss (L)**	**Thalf (h)**
1	83, M	25	25	MRSA	2	11,6	20,1	182,1	3,3	79,2	16,7
2	62, M	23	34	MRSA	1	4,2	15,6	105,5	5,7	50,1	6,1
3	53,M	24	31			8,6	29,2	197,3	3,0	32,7	7,5
4	33,F	21	19			14,2	25,9	229,7	2,6	49,5	13,1
5	66,F	20	20	MRSA	2	4,6	15,8	114,7	5,2	35,3	4,7

6	24,F	25	17			2,4	22,2	79,6	7,5	36,2	3,3
7	77,F	24	27			1,7	17,1	74,8	8,0	42,1	3,6
8	72,M	24	34			4,9	27,5	146,7	4,1	26,9	4,6
9	41,F	17	24	MRSA	1	0,5	18,6	76,1	7,9	19,7	1,7
10	26,F	18	19	MRSA	1	0,2	5,4	17,2	34,8	93,1	1,9
11	74,M	24	18			0,8	12,9	55,5	10,8	39,3	2,6
12	74,M	26	38	MRSA	2	2,4	12,5	75,4	8,0	38,7	2,5
13	79,M	28	36	Coag neg	4	14,3	23,2	203,6	2,9	112,5	26,5
14	44,M	26	21	MRSA	1	0,4	10,9	45,7	13,1	35,1	1,9
15	64,M	33	16			0,9	9,6	39,7	15,1	81,4	3,7
16	73,M	31	24	MRSA	1	22,5	29,8	298,1	2,0	130,5	44,9

Author Disclosure Block:

S. Corcione: None. **L. Baietto:** None. **A. d'Avolio:** None. **V. Fanelli:** None. **M.V. Ranieri:** N. Other; Self; ASPIRE 2010. **G. di Perri:** L. Speaker's Bureau; Self; pfizer. **F. de Rosa:** N. Other; Self; aspire 2010.

Poster Board Number:

SUNDAY-489

Publishing Title:

Patients in the Intensive Care Unit Exhibit a Higher Target Attainment for β-Lactam Antibiotics When Actual Compared to Estimated Minimum Inhibitory Concentrations Are Applied

Author Block:

H. Woksepp[1], A. Hällgren[2], S. Borgström[1], F. Kullberg[3], A. Wimmerstedt[3], A. Oscarsson[2], P. Nordlund[4], M-L. Lindholm[1], J. Bonnedahl[1], B. Carlsson[5], T. Schön[1]; [1]County Hosp., Kalmar, Sweden, [2]Linköping Univ., Linköping, Sweden, [3]Central Hosp., Växjö, Sweden, [4]Ryhov Hosp., Jönköping, Sweden, [5]County Council of Östergötland, Linköping, Sweden

Abstract Body:

ICU patients are at risk for low antibiotic levels which may lead to poor treatment efficacy. Our aim was to investigate if empirical treatment with β-lactams in ICU patients results in sufficient serum drug levels during the first three days of treatment to cover 100% time over the minimum inhibitory concentration (MIC) (100 % *f*T>MIC), both in relation to the estimated and actual MIC. In a prospective, multicenter study, 111 consecutive ICU patients, treated with piperacillin-tazobactam (PIP n=49), meropenem (MER n=24) or cefotaxime (CTX n=38) were included. Clinical and laboratory data including MIC testing by E-test for relevant bacteria (MIC_a) were recorded. The MIC_a was compared to the highest estimated MIC (MIC_e) for susceptible pathogens commonly used for defining targets in clinical studies. Blood samples were collected as through levels three consecutive days and analyzed by LC-MS. Based on the MIC_e 54% (60/111) of patients reached the target. A higher rate 78% (31/40) reached 100% *f*T>MIC_a compared to 50 % for the same patients using MIC_e. Drug levels were stable over time within patients but varied extensively between patients (Figure 1). The actual MIC, when available, results in higher target attainment for broad spectrum β-lactams in ICU patients. Considering the variability in the serum drug levels between ICU patients, the dosage should be individualized based on the actual MIC and antibiotic concentrations to maximize clinical efficacy.

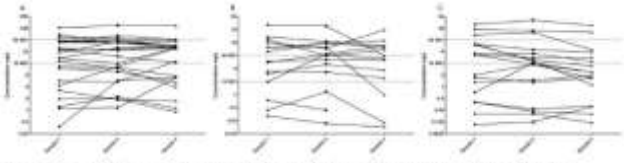


Figure 1. Plot over individual concentrations from three consecutive samples (day 1-3) of PIP (A), MER (B) and CTX (C). Mean concentrations day 1-3 were 10.1, 10.5 and 11.8 mg/L for PIP, 9.93, 9.34 and 8.44 mg/L for MER and 6.38, 6.66 and 5.02 mg/L for CTX. Dotted lines indicate 1x MIC equal to 100% T>MIC, (16, 2 and 4 mg/L for PIP, MER and CTX respectively) and 4x MIC, (64, 8 and 16 mg/mL).

Author Disclosure Block:

H. Woksepp: None. **A. Hällgren:** None. **S. Borgström:** None. **F. Kullberg:** None. **A. Wimmerstedt:** None. **A. Oscarsson:** None. **P. Nordlund:** None. **M. Lindholm:** None. **J. Bonnedahl:** None. **B. Carlsson:** None. **T. Schön:** None.

Poster Board Number:

SUNDAY-490

Publishing Title:

Improving the Bayesian Prediction of Vancomycin Exposure in Obese Adults with Trough Only Measurements

Author Block:

L. C. Krop[1], J. Hong[1], M. P. Pai[2]; [1]Morton Plant Hosp.; BayCare Hlth.System, Clearwater, FL, [2]Albany Coll. of Pharmacy and Hlth.Sci., Albany, NY

Abstract Body:

Background: Bayesian modeling can predict vancomycin area under the concentration-time curve over 24 hours (AUC_{24}) using trough only measurements and a reliable population pharmacokinetic model. The standard model estimates central compartment volume of distribution (V) with total body weight (TBW) and clearance (CL) with estimated creatinine clearance (CLcr) that may be biased in obese patients.**Objective:** To compare the precision and bias of vancomycin AUC_{24} estimates using a TBW independent model (Model-1) to the standard TBW dependent model (Model-2) in obese patients. **Methods:** Vancomycin plasma concentration-time data from 75 obese adults (BMI≥30 kg/m^2) with peak and trough measurements were best modeled by a 1-compartment system (PMID 26011138), that served as the Bayesian prior for Model-1. The Bayesian prior for Model-2 was 0.7 L/kg for V, (0.79 x CLcr + 15.7) x 0.06 for CL (L/h) (PMID 3415206), and a covariance matrix based on a 50% coefficient of variation (CV) for both system parameters. Reference AUC_{24} values were computed for each model based on 2-4 plasma samples per patient by individual model-based integration (ADAPT 5, BMSR, Los Angeles, CA). The analyses were repeated for each model after inclusion of trough only data (peak data removed). External validation was performed in a similar manner using data from an additional 31 obese patients not included in the original model. Bias (percent difference) and precision (R^2) were determined for models with trough only data compared to their respective base models (peak and trough data).**Results:** The mean (CV) age, TBW, and CLcr for the base data set was 57.9 (26.1%) years, 112 (22.4%) kg, and 101 (42.3%) mL/min/1.73m^2. The mean (CV) for V and CL was 71.9 (24.4%) L and 5.18 (40.4%) L/h, respectively. The precision of AUC_{24} estimation with trough only data was higher with Model-1 (R^2= 0.820) compared to Model-2 (R^2 = 0.537). The mean [95% CI] %bias of AUC_{24} estimation was lower with Model-1 (2.79 [0.452, 5.12]) compared to Model-2 (12.5 [8.01, 17.0]). The external validation data set had similar demographics and revealed higher precision and lower %bias with Model-1 compared to Model-2 with use of trough only data.**Conclusions:** Use of a TBW independent model improves estimation of AUC_{24} with trough only data in obese patients compared to the standard model.

Author Disclosure Block:

L.C. Krop: None. **J. Hong:** None. **M.P. Pai:** None.

Poster Board Number:

SUNDAY-491

Publishing Title:

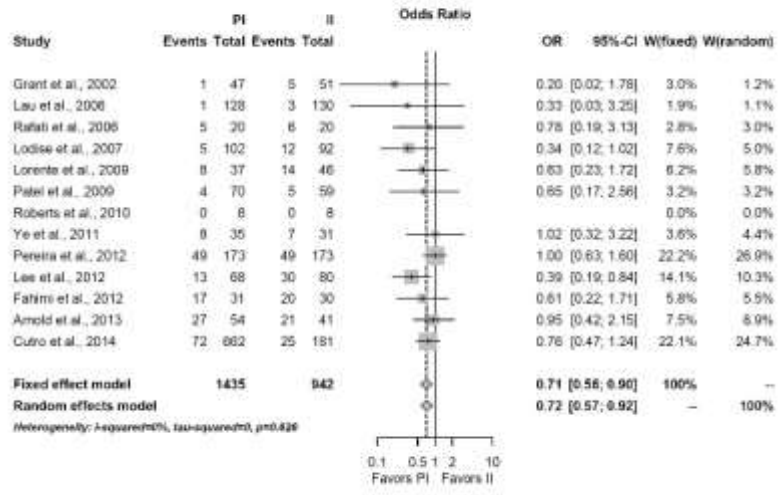
Impact of Piperacillin-Tazobactam Prolonged Infusion Schemes on Clinical Outcomes: A Meta-Analysis and Meta-Regression

Author Block:

N. J. Rhodes[1], J. Liu[1], B. Nadler[2], J. O'Donnell[1]; [1]Midwestern Univ., Downers Grove, IL, [2]Midwestern Univ., Glendale, AZ

Abstract Body:

Background: While prolonged-infusion (PI) dosing of piperacillin-tazobactam (TZP) has been found beneficial compared to intermittent infusion (II), few studies have examined the impact of covariates on observed mortality. **Methods:** MEDLINE (Pubmed) was searched for studies of TZP or piperacillin alone PI v. II. The following terms were searched: "piperacillin AND (infusion OR infusions OR infusion*) AND (outcome OR outcomes OR outcom* OR mortality)". Studies were screened by title and abstract to determine if inclusion criteria were met. Severity of illness scores (e.g., APACHE II, SAPS II) were converted to % predicted mortality using published regression equations. Other covariates were directly extracted. Fixed and random-effects meta-analysis of included studies were conducted using the *meta* package for R (v.4.3-0). Meta-regressions of the log-odds ratio (PI v. II) were carried out for covariates if ≥10 studies reported both the outcome and the covariate value and the covariate had a plausible biologic relationship to the outcome. **Results:** The search identified 31 studies, of which 13 were included. Sample sizes ranged from n=8 per group (PI v. II) to greater than 150 patients in each group. The results of the meta-analysis are shown in Figure 1. Both the fixed (P=0.005) and random-effects (P=0.008) models found a protective effect of PI v. II TZP against the study-defined mortality outcome. Severity of illness scores, transformed to % predicted mortality, had a borderline significant (P=0.07) impact on the log-odds ratio.



Conclusions: PI was significantly protective against mortality in published studies. The impact of patient covariates on this benefit warrants additional study.

Author Disclosure Block:

N.J. Rhodes: None. **J. Liu:** None. **B. Nadler:** None. **J. O'Donnell:** None.

Poster Board Number:

SUNDAY-492

Publishing Title:

Antibioperf: A Nationwide Survey on Beta-Lactams Administration and Therapeutic Drug Monitoring Practices in Critically Ill Patients in France

Author Block:

A. Charmillon[1], E. Novy[1], N. Agrinier[2], M. Leone[3], A. Kimmoun[1], B. Levy[1], B. Demoré[1], J. Dellamonica[4], **C. Pulcini**[1]; [1]CHU de Nancy, Vandœuvre-lès-Nancy, France, [2]Université de Lorraine, Vandœuvre-lès-Nancy, France, [3]CHU de Marseille, Marseille, France, [4]CHU de Nice, Nice, France

Abstract Body:

Background: Recent studies have suggested that continuous or extended infusions with beta-lactams may be associated with improved clinical outcomes, especially in critically ill patients. However, not much is known on how these studies translate into practice. Our objective was to assess current practices concerning the administration (intermittent, extended or continuous infusions) and therapeutic drug monitoring (TDM) of beta-lactam antibiotics and vancomycin in France. **Methods:** We conducted a nationwide cross-sectional survey between May and August 2015, using an online questionnaire, sent by e-mail to infectious diseases specialists (IDS) and intensivists through national mailing lists. We used clinical vignettes of critically ill patients to assess physicians' practices regarding administration and TDM practices for amoxicillin, cloxacillin, piperacillin/tazobactam, cefotaxime, ceftazidime, cefepime, meropenem and vancomycin. **Results:** Five-hundred and seven physicians participated (response rate 42%), 71% intensivists and 20% IDS. TDM of beta-lactams was rarely available (ranging from 16.5% for cloxacillin to 30% for ceftazidime), whereas vancomycin TDM was available in 97% of the cases. In the clinical vignettes, ceftazidime and piperacillin/tazobactam were the beta-lactams the most frequently administered using extended or continuous infusions respectively in 76% and 57% of cases. Gaps in knowledge regarding the duration of stability of intravenous beta-lactams were common (correct answers ranging from 8% for cloxacillin to 33% for ceftazidime). The majority of physicians (76%) were convinced of the interest of extended or continuous infusions for beta-lactams in critically ill patients, but 45% declared that having no easy access to practical guidelines was a barrier. **Conclusions:** Our survey found that most IDS and intensivists are favourable to optimised administration of beta-lactams in critically ill patients. But the lack of guidelines and limited TDM availability of beta-lactams in hospitals are potential barriers to its implementation.

Author Disclosure Block:

A. Charmillon: None. **E. Novy:** None. **N. Agrinier:** None. **M. Leone:** None. **A. Kimmoun:** None. **B. Levy:** None. **B. Demoré:** None. **J. Dellamonica:** None. **C. Pulcini:** None.

Poster Board Number:

SUNDAY-493

Publishing Title:

Pharmacokinetic/Pharmacodynamic (PK/PD) Determinants of Vancomycin Efficacy in Enterococcal Bacteremia

Author Block:

M. Bin Jumah[1], S. Vasoo[2], S. R. Menon[1], P. P. De[1], **C. B. Teng**[3]; [1]Tan Tock Seng Hosp., Singapore, Singapore, [2]Inst. of Infectious Diseases and Epidemiology, Tan Tock Seng Hosp., Singapore, Singapore, [3]Natl. Univ. of Singapore, Singapore, Singapore

Abstract Body:

Background: PK/PD determinants of vancomycin efficacy are unclear for enterococcal infections. We studied vancomycin PK/PD and clinical outcomes in a cohort of patients with *Enterococcus* bacteremia.**Methods:** Laboratory and clinical records from 1st January 2009 to 31st May 2015 were reviewed for blood cultures positive for *Enterococcus* sp. Subjects were excluded if they received < 72 hours of I.V. vancomycin, had polymicrobial bacteremia, had vancomycin-resistant *Enterococcus* (VRE), or if vancomycin concentrations were not performed. The average vancomycin $AUC_{0\text{-}24hrs}$ was computed using a Bayesian approach based on patient-specific creatinine clearance, vancomycin dosing and concentrations available in the first 72 hours of therapy. MIC was determined by E-test (bioMerieux) and the average $AUC_{0\text{-}24hrs}$/MIC over the 72 hours was calculated. Primary outcomes examined were: 30-day all-cause mortality and treatment failure (defined as 30-day mortality, persistent bacteremia $\geq$ 5 days or relapse at $\leq$ 90 days).**Results:** There were 417 index cases of *Enterococcus sp*. bacteremia; 174 with polymicrobial bacteremia, 145 with $\leq$ 3 days of vancomycin therapy, 23 VRE, 7 without vancomycin concentrations, and 5 with isolates unavailable for MIC testing were excluded. Thus, 63 cases (34 *E. faecium*, 25 *E. faecalis*, 4 other *Enteroccocus* sp.) were included in the analysis. Median vancomycin MIC was 0.75 mcg/ml (range 0.38-3 mcg/ml). Univariate analysis revealed that the median $AUC_{0\text{-}24hrs}$/MIC was significantly associated with treatment failure [failure, 479 (IQR 324-714) vs success, 723 (IQR 484-873); P = 0.03] and mortality, [death, 449 (IQR 296-714) vs survival, 662 (IQR 465-873); P = 0.04]. In the multivariate analysis with a model that also included APACHE II scores, and creatinine clearance, the association of $AUC_{0\text{-}24hrs}$/MIC with mortality approached but did not achieve statistical significance (P = 0.06).**Conclusions:** Lower vancomycin $AUC_{0\text{-}24hrs}$/MIC may be associated with increased mortality and treatment failure in enterococcal bacteremia. Further studies are needed to define the breakpoints that will optimize patient outcomes.

Author Disclosure Block:

M. Bin Jumah: None. **S. Vasoo:** None. **S.R. Menon:** None. **P.P. De:** None. **C.B. Teng:** None.

Poster Board Number:

SUNDAY-494

Publishing Title:

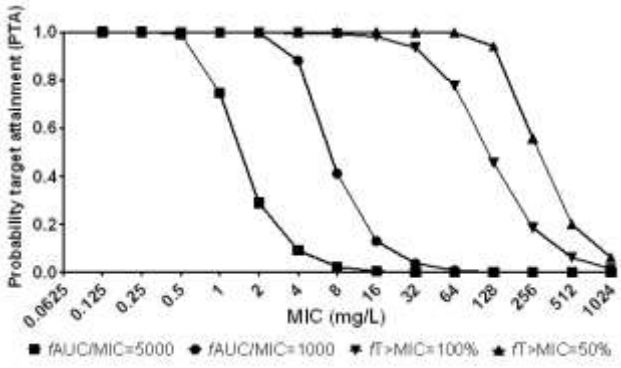
Population Pharmacokinetic of Fosfomycin in Non-Critically Ill Patients with Bacteremic Urinary Infection Caused by Multidrug-Resistant *Escherichia coli*

Author Block:

V. Merino-Bohórquez[1], F. Docobo-Pérez[2], M. Cameán[1], J. Sojo-Dorado[1], P. Retamar[1], L. López-Cortés[1], I. Morales[1], I. López-Hernández[1], A. Pascual[2], J. Rodríguez-Baño[1]; [1]Hosp. Univ.rio Virgen Macarena, Sevilla, Spain, [2]Univ. de Sevilla, Sevilla, Spain

Abstract Body:

Background: Fosfomycin (FOS) is a potential treatment of invasive infections caused by MDR Enterobacteriaceae. Scarce data are available on the PK/PD parameters of I.V. FOS in non-critically ill patients. PD target is not clear, due to the appearance of resistant bacteria. The aim is to develop a population PK model to describe the FOS concentrations variability in bacteremic patients with UTI by MDR *E. coli* and exploring its efficacy using *in vitro* PD targets. **Methods:** 10 patients with bacteremic UTI due to MDR *E. coli* (FOS MIC=0.5-8 mg/L) were included. 8 received FOS 4g/6h, and 2 with renal impairment (CrCl <40 mL/min) 4g/12h. Serum samples obtained from 4 time-points within the interval dose were analyzed by LC-MS/MS. A population PK model was built (Pmetrics). *f*T>MIC was used as PD efficacy target predictor and $fAUC_{48\text{-}72h}$/MIC for resistance suppression. The probability to reach $fAUC_{48\text{-}72h}$/MIC=1000 or 5000 and *f*T>MIC of 50% or 100% were explored by Monte Carlo simulations. **Results:** 7 patients were men. Median (IQR) age was 66 years (61-72.8), weight 74 kg (65.8-76.3), BMI 26 kg/m^2 (23.8-31.4), and CrCl 76 mL/min (54.3-107.8). A two-compartment model with CrCl as covariate (R^2=0.977) was used. The probabilities for target attainments are in the Figure. All the patients were cured. **Conclusion:** With a dosage of FOS 4g/6h, efficacy would be achieved for *E. coli* strains with MIC of 32-256 mg/L. However, resistance suppression prevention would be reached for low FOS MICs (0.5-2 mg/L). Higher doses should be explored to increase the therapeutic range of FOS.



Author Disclosure Block:

V. Merino-Bohórquez: None. **F. Docobo-Pérez:** None. **M. Cameán:** None. **J. Sojo-Dorado:** None. **P. Retamar:** None. **L. López-Cortés:** E. Grant Investigator; Spouse/Life Partner; NOVARTIS. L. Speaker's Bureau; Spouse/Life Partner; MSD AND NOVARTIS. **I. Morales:** None. **I. López-Hernández:** None. **A. Pascual:** None. **J. Rodríguez-Baño:** None.

Poster Board Number:

SUNDAY-495

Publishing Title:

Maximizing Efficiency Of Vancomycin Dosing And Monitoring

Author Block:

K. R. Beaulac, F. Massaro, T. Chang, S. Doron; Tufts Med. Ctr., Boston, MA

Abstract Body:

Background: Pharmacists often manage dosing and monitoring of vancomycin (VAN), due to their expertise in attaining target levels and limiting nephrotoxicity. At our institution, the VAN per pharmacy (VPP) program was successful, but labor intensive requiring 1.2 pharmacist full-time equivalents (FTE). Upon discontinuation of VPP, the Infectious Diseases Pharmacist (ID Pharm) provided didactic training to house officers and performed targeted surveillance and intervention for high-risk patients on VAN.**Methods:** A quasi-experimental study was performed to assess the rate of nephrotoxicity (NT) for 4 months periods during VPP (pre), immediately after VPP (post), and eight months after VPP (late post). NT was defined as serum creatinine (SCr) increase 0.5 mg/dL or 50% from baseline. Additionally, NT recovery (SCr 10% from baseline) and VAN troughs (tr) were assessed. Patients were excluded if they had kidney disease (eGFR<30 mL/min), were on a pediatric unit, or had no SCr measured. In patients with NT, concomitant nephrotoxins (CNs) were documented. Student t-test and χ^2 test were used compare the post and late post to the pre cohort.**Results:** There were 640, 648, and 767 patients (pts) included in the pre, post, and late post cohorts respectively. NT occurred in 48 patients (7.5%) in the pre phase, with 35% recovering and 40 (83%) pts receiving CNs. In the post phase, NT increased to 10.5% (72 patients) (p=0.05), with recovery in 39 (54%) and all 72 receiving CNs. After months of didactic training of housestaff and targeted interventions, the NT incidence returned 7.6% (61 patients) (p>0.05), with 57% recovering and 90% receiving CNs. Pts with a VAN tr >20 mg/dL were more likely to develop NT than pts with a tr ≤20, 29% vs. 15% (p=0.001). The frequency of VAN tr >20 and tr 10-20 were not different in the 3 phases (p>0.05), although trs were obtained in 37%, 22% and 40% during the 3 phases respectively. During the late post phase, 0.125 ID Pharm FTE was required to maintain the surveillance and interventions.**Conclusions:** Though pharmacist-guided dosing and monitoring of VAN can optimize target attainment and lower rates of NT, this goal can be efficiently attained with targeted surveillance and intervention by an ID Pharm.

Author Disclosure Block:

K.R. Beaulac: None. **F. Massaro:** None. **T. Chang:** None. **S. Doron:** None.

Poster Board Number:

SUNDAY-496

Publishing Title:

Validation Of A Bayesian Approach To Estimate Vancomycin Exposure In Obese Patients With Limited Pk Sampling

Author Block:

J. J. Carreno[1], B. Lomaestro[2], J. Tietjan[2], T. Lodise[1]; [1]Albany Coll. of Pharmacy and Hlth.Sci., Albany, NY, [2]Albany Med. Ctr. Hosp., Albany, NY

Abstract Body:

Background: Vancomycin (VAN) area under the curve (AUC) can be accurately estimated using a Bayesian approach with limited PK sampling. However, the validity of this approach in obese subjects is not well described. This study evaluates the validity of a Bayesian approach for AUC estimation in obese subjects. **Methods:** This was an IRB-approved prospective PK study. Inclusion criteria: inpatients age ≥ 18 y, IV VAN for treatment and weight ≥ 110 kg. Exclusion criteria: ANC < 1000/mm^3, pregnancy, SCr ≥ 2.0 mg/dL and CrCL <50 mL/min. Five serum VAN levels were obtained from each subject. VAN 24-hour AUC (AUC_{24}) was estimated using the MAP procedure within ADAPT 5 using two vancomycin PK models as priors: (1) Rodvold population PK model (non-obese PK mode) and (2) population PK model derived from this dataset (obese PK model). AUC_{24} estimates from data depleted subsets (trough only: AUC_T; peak and trough: AUC_{PT}; midpoint and trough: AUC_{MT}) were compared to AUC estimates from full data (AUC_{FULL}). **Results:** 12 patients were enrolled. Median (IQR) age, CrCL, and BMI were 61 y (39 - 71), 86 mL/min (75 - 120), and 45 kg/m^2 (40 - 52) respectively; 7 (58%) were male. The obese PK model derived from this dataset had a mean (SD) V_D, K_{12}, K_{21}, CL_{INT} and CL_{SLOPE} of 25.76 L (11.68), 2.29 h^{-1} (2.42), 1.44 h^{-1} (1.73), 3.054 L/h (0.71) and 0.036 (0.02) respectively. AUC estimates and comparisons are shown in Table 1. For both priors, the AUC with trough only data (AUC_T) tended to overestimate AUC_{FULL}. Use of 2 levels to estimate the AUC_{FULL} was associated with greater precision and less bias relative to trough only data. **Conclusions:** The data from this study suggests that obtaining multiple levels improves the ability to accurately dose vancomycin in obese patients.

Table 1. Comparison of AUC estimates using two Bayesian priors and data subsets

	Median (range) AUC (mg-h/L)	Computed AUC to AUC_{FULL} Ratio (95% CI)	R^2
Non-Obese Prior			
Full Data Set (AUC_{FULL})	493 (280 - 756)	1.00 (N/A)	N/A
Trough Only (AUC_T)	687 (399 - 1046)	1.43 (1.33 - 1.53)	0.986
Peak and Trough (AUC_{PT})	463 (243 - 702)	0.93 (0.90 - 0.96)	0.998

Midpoint and Trough (AUC_{MT})	584 (359 - 850)	1.21 (1.14 - 1.27)	0.995
Obese Prior			
Full Data Set (AUC_{FULL})	483 (257 - 767)	1.00 (N/A)	N/A
Trough Only (AUC_{T})	549 (174 - 1121)	1.12 (0.81 - 1.44)	0.851
Peak and Trough (AUC_{PT})	441 (241 - 698)	0.93 (0.84 - 1.01)	0.981
Midpoint and Trough (AUC_{MT})	478 (217 - 653)	1.01 (0.91 - 1.10)	0.983

Author Disclosure Block:

J.J. Carreno: None. **B. Lomaestro:** None. **J. Tietjan:** None. **T. Lodise:** None.

Poster Board Number:

SUNDAY-497

Publishing Title:

Adequacy of Recommended Cefazolin (Cfz) Prophylaxis in Cardiac Surgery: A Prospective Pharmacokinetic (Pk) Study

Author Block:

D. Calic, R. E. Ariano, R. C. Arora, H. P. Grocott, T. M. Lakowski, R. G. Lillico, S. A. Zelenitsky; Univ. of Manitoba, Winnipeg, MB, Canada

Abstract Body:

Background: Although practice guidelines recommend the standard CFZ dosing for antimicrobial prophylaxis (AP) in cardiac surgery (1), there is limited study as to whether adequate concentrations (concs) are achieved in this population. The purpose of this PK study of CFZ AP in patients undergoing cardiac surgery was to determine the incidence of sub-therapeutic intra-op concs and to identify risk factors for failure to maintain target concs.**Methods:** The study was conducted with consent in adults undergoing cardiac surgery with cardiopulmonary bypass and receiving CFZ AP as per institutional protocol (1 or 2 g IV pre-op based on body weight, repeated every 4 h during surgery). Blood samples were collected 30 min after the pre-op dose, prior to intra-op doses and within 15 min of wound closure. A quantitative liquid chromatography-tandem mass spectrometry assay was developed to measure total CFZ concs. Based on pharmacodynamic (PD) principles for AP (1), the therapeutic target was defined as maintaining total CFZ concs ≥ 40 mg/L during surgery (≥ 8 mg/L free concs, assuming 80% protein binding).**Results:** Fifty-five subjects (69% male) with a mean age of 65 ± 10 yrs, weight of 90 ± 17 kg and Clcr of 80 ± 19 mL/min/72 kg completed the study. Twelve (22%) subjects received 1 g CFZ doses and 43 (78%) were given 2 g doses. Total CFZ concs at closure ($C_{closure}$) were < 40 mg/L in 5 (9.6%) of the 52 evaluable cases, whereas levels below the target were observed at some point during surgery in 30.9% (17/55). A sub-therapeutic $C_{closure}$ was more frequently observed with 1 g doses for CFZ (P = 0.009), most often used in females (P=0.001) and those with increased age (P=0.037) and lower body weight (P < 0.001). Further analysis identified a critical dose threshold of > 24 mg/kg for maintaining target CFZ conc during surgery (P=0.035).**Conclusion:** Under current dosing guidelines sub-therapeutic CFZ concs occur frequently during cardiac surgery. As antimicrobial concs at wound closure are a risk factor for surgical site infections (2), current dosing guidelines may not be adequate. In addition, we demonstrate how PK-PD directed targets might be used to optimize AP and thus potentially reduce post-op infections.

Author Disclosure Block:

D. Calic: None. **R.E. Ariano:** None. **R.C. Arora:** None. **H.P. Grocott:** None. **T.M. Lakowski:** None. **R.G. Lillico:** None. **S.A. Zelenitsky:** None.

Poster Board Number:

SUNDAY-498

Publishing Title:

Development of a Population Pharmacokinetic Model of Cefepime

Author Block:

A. Muller[1], S. Bhagwat[2], M. Patel[2], J. W. Mouton[3]; [1]Med. Ctr. Haaglanden, The Hague, Netherlands, [2]Wockhardt Res. Ctr., Aurangabad, India, [3]ErasmusMC, Rotterdam, Netherlands

Abstract Body:

Background: Cefepime is a cephalosporin with broad spectrum activity including Amp-C producing Gram-negatives. Cefepime is being developed as a high -proportion combination with tazobactam as an extended infusion (WCK 4282), to extend its coverage against ESBL, Class C β-lactamases, and KPC producing micro-organisms. Given the relatively poor availability of pharmacokinetic data and population models to perform Monte Carlo simulations and determine target attainment for various potential dosing schedules, pharmacokinetic studies were performed over one week of exposure.**Methods:** 44 healthy volunteers received dosing regimens of cefepime (in combination with tazobactam) of 1 gr or 2 gr given either bid or tid with infusion times of 0.5-1.5 hour for 6 days. Creatinine clearance ranged from 85-181.4 ml/min and weight 53-91 kg respectively. Blood samples were collected just before administration and after infusion at 0.08, 0.5, 1, 1,5, 2, 4, 6.5 or 7 h, and trough during the whole first day and whole 6^{th} day; peak and trough concentrations were determined on day 1-5. 2155 concentrations were analyzed with nonlinear mixed effect modeling (NONMEM, version 7.2) and the ADVAN5 subroutine. Different one- two-and three compartment disposition models were evaluated and the impact of covariates were determined. Model selection criteria were decrease in objective function, diagnostic plots and visual predictive checks.**Results:** The data were best described by a two-compartment model. In the analysis of covariates, it appeared that there was a slight, but significant difference in V1 and V2 between groups receiving different dosing schedules. However, the variability was similar for these schedules for V1 and V2, respectively. The final estimates were 5.86 L/h for clearance, 9.67-11.6 L for V1, 3.97 -5.69 L for V2 and 4.38 L/h for Q. Creatinine clearance and body weight were significant covariates for Cl and V1 respectively with estimates of 0.00384 and 0.011.**Conclusions:** Cefepime pharmacokinetics was well described by a two-compartment population model. The model allows Monte Carlo simulations to be performed and determine target attainments, including longer infusion duration.

Author Disclosure Block:

A. Muller: None. **S. Bhagwat:** D. Employee; Self; Wockhardt Research Center. **M. Patel:** D. Employee; Self; Wockhardt Research Center. **J.W. Mouton:** E. Grant Investigator; Self; see general statement of the author.

Poster Board Number:

SUNDAY-499

Publishing Title:

Comparison and Evaluation of Vancomycin Trough Levels in Critically Ill Obese Pediatric Patients

Author Block:

D. Boeck[1], B. Duhon[2], K. Reveles[3], E. Hand[1]; [1]Univ. Hlth.System, San Antonio, TX, [2]Univ. of Texas Coll. of Pharmacy, Austin, TX, [3]Univ. of Texas Hlth.Sci. Ctr. at San Antonio, San Antonio, TX

Abstract Body:

Background: Vancomycin is the drug of choice for invasive methicillin-resistant *Staphylococcus aureus* infections in pediatric patients. Although data pertaining to dosing in adults is well-documented, appropriate weigh-based pediatric dosing recommendations, specifically in critically ill overweight and obese children, is lacking. The purpose of this study is to compare vancomycin serum concentrations in critically ill pediatric patients of varying body mass index percentiles (BMI%).**Methods:** This was a single-center, retrospective chart review of pediatric patients in the pediatric intensive care unit receiving scheduled vancomycin between July 1, 2009 and August 31, 2015. Patients were differentiated based upon BMI%. **Results:** A total of 130 patients met inclusion criteria, 76 with normal body weight (NBW), 21 considered overweight (85^{th}-94^{th} percentile), and 33 deemed obese ($\geq 95^{th}$ percentile). Median vancomycin doses were 50 mg/kg/day for NBW patients and 45 mg/kg/day for overweight and obese patients. There was a trend toward higher initial trough levels in overweight (10.2 mg/L,) and obese children (9.3 mg/L) when compared the NBW cohort (7.7 mg/L)($p = 0.18$ and $p = 0.08$, respectively). Significantly more overweight patients (52.4%) achieved initial troughs within the therapeutic range (10 - 20 mcg/mL) when compared to those with NBW (23.7%) ($p = 0.01$). More NBW patients obtained subtherapeutic troughs than overweight patients ($p = 0.03$). Supratherapeutic concentrations were more common in obese (18.2%) compared to NBW (7.9%) children, although not statistically significant ($p = 0.11$). **Conclusions:** Critically ill overweight and obese pediatric patients may obtain higher initial vancomycin concentrations compared with NBW patients. However, these patients may also be more likely to achieve supratherapeutic vancomycin concentrations with more aggressive dosing, increasing the risk for nephrotoxicity. Further investigation into the optimal dosing strategy for this patient population should be undertaken.

Author Disclosure Block:

D. Boeck: None. **B. Duhon:** None. **K. Reveles:** None. **E. Hand:** None.

Poster Board Number:

SUNDAY-500

Publishing Title:

Development of a Population Pharmacokinetic Model of Tazobactam Including High Doses

Author Block:

A. E. Muller[1], S. Bhagwat[2], M. Patel[2], J. W. Mouton[3]; [1]Med. Ctr. Haaglanden, The Hague, Netherlands, [2]Wockhardt Res. Ctr., Aurangabad, India, [3]ErasmusMC, Rotterdam, Netherlands

Abstract Body:

Background: Tazobactam is a β-lactamase inhibitor that is used in combination with several β-lactams to extend coverage against ESBL producing micro-organisms. It is being developed in combination with cefepime with larger doses, up to 2 gr of tazobactam (WCK 4282) to include coverage of KPC producing bacteria. Given the relatively poor availability of pharmacokinetic data and population models, as well as the unavailability of data at high dosages of tazobactam, pharmacokinetic studies were performed over one week of exposure.**Methods:** 44 healthy volunteers received dosing regimens of tazobactam of 1 gr or 2 gr given either bid or tid with infusion times of 0.5-1.5 hour for 6 days. Creatinine clearance ranged from 85-181.4 ml/min and weight 53-91 kg respectively. Blood samples were collected just before administration and after infusion at 0.08, 0.5, 1, 1,5, 2, 4, 6.5 or 7 h, and trough during the whole first day and whole 6th day; peak and trough concentrations were determined on day 1-5. 1615 concentrations were analyzed with nonlinear mixed effect modeling (NONMEM, version 7.2) and the ADVAN5 subroutine. Different one- two-and three compartment disposition models were evaluated and the impact of covariates were determined. Model selection criteria were decrease in objective function, diagnostic plots and visual predictive checks.**Results:** The data were best described by a three-compartment model. In the analysis of covariates, there were some interoccasion effects but not significantly for the full sampling days 1 and 6. The final estimates were 19.8 L/h for clearance, 5.45 L for V1, 7.44 L for V2, 3.92 L for V3, 33.4 L/h for Q and 1.89 L/H for Q1. Creatinine clearance and bodyweight were significant covariates for Cl and V2 respectively with estimates of 0.00617 and 0.051.**Conclusions:** Tazobactam pharmacokinetics was well described by a three-compartment population model. The model allows Monte Carlo simulations to be performed and determine target attainments, including longer infusion duration and doses up to 2 gr.

Author Disclosure Block:

A.E. Muller: None. **S. Bhagwat:** D. Employee; Self; Wockhardt Research Center. **M. Patel:** D. Employee; Self; Wockhardt Research Center. **J.W. Mouton:** E. Grant Investigator; Self; see general statement of the author.

Poster Board Number:

SUNDAY-501

Publishing Title:

Association between Initial Vancomycin (Van) Exposure Profile and Nephrotoxicity (Nt) among Hospitalized Patients with Bacteremia

Author Block:

E. J. Zasowski[1], K. P. Murray[2], T. D. Trinh[1], N. A. Finch[2], R. P. Mynatt[2], M. J. Rybak[1]; [1]Wayne State Univ., Detroit, MI, [2]Detroit Med. Ctr., Detroit, MI

Abstract Body:

Data indicate VAN area under the curve (AUC) predicts efficacy while troughs do not. AUC monitoring may maintain efficacy while reducing the # of patients requiring troughs associated with NT (>15 mg/L), yet data for AUC ranges associated with NT in adults are limited. This study examines the association between initial VAN exposure profiles & NT.Single center retrospective cohort, 6/2014 - 5/2015. Inclusion: age ≥ 18 y; ≥ 72 h VAN for bacteremia; ≥ 1 serum concentration during 1st 96 h. Exclusion: baseline serum creatinine (SCr) ≥ 2 mg/L; renal replacement therapy during 1st 96 h; cystic fibrosis; concomitant TZP. NT defined as > of SCr increase of 0.5 mg/L or 50% measured consecutively from initiation to 72 h after VAN. Bayesian estimated AUCs & troughs on days 1 & 2. Thresholds of VAN exposure variables strongly associated with NT were identified via classification & regression tree analysis. Exposure thresholds & characteristics were compared between NT & non-NT groups. Poisson regression was used to adjust for confounders & quantify association between VAN exposure thresholds & NT.74 patients included: mean (SD) age 62.7 (17.2) y; 54.1% male; median (IQR) wt & APACHE II score 80 (65, 108) kg & 14.5 (10, 20) respectively. NT occurred in 10.8%, median (IQR) time to NT 83 (759, 200) h. 67.6% received ≥ 1 nephrotoxin (24.3% intravenous contrast, 39.2% furosemide, 18.9% lisinopril (LIS), 4.1% aminoglycoside). Median (IQR) VAN duration, $AUC_{0\text{-}24}$, $AUC_{24\text{-}48}$, Cmin D1 & Cmin D2 were 6 (4, 8.25) days, 564 (383, 746) mg*h/L, 578 (441, 831) mg*h/L, 12.1 (8, 15.4) mg/L & 14.8 (9.7, 20) mg/L respectively. Following table shows results of the final Poisson regression models. There was no significant threshold in Cmin D2.

Variable	Non-NT (N=66) n (%)	NT (N=8) n (%)	RR (95%CI)	aRR (95% CI)
$AUC_{0\text{-}24} \geq 857$ mg*h/L	11 (16.7)	4 (50)	3.93 (1.11, 13.93)	3.90[a] (1.06, 16.06)
$AUC_{24\text{-}48} \geq 730$ mg*h/L	20 (30.3)	6 (75)	5.54 (1.20, 25.51)	6.33[b] (1.28, 31.40)

Cmin D1 ≥ 15.9 mg/L	11 (16.7)	5 (62.5)	6.04 (1.61, 22.62)	4.48[c] (1.06, 21.30)

[a] Liver disease & concomitant LIS retained[b] APACHE II score retained[b] Liver disease, concomitant LIS & APACHE II score retainedPreliminarily, daily VAN AUCs > 730 – 857 mg*h/L in 1st 48 h were associated with increased NT. Further analyses are needed to define therapeutic VAN AUC range.

Author Disclosure Block:

E.J. Zasowski: None. **K.P. Murray:** None. **T.D. Trinh:** None. **N.A. Finch:** None. **R.P. Mynatt:** None. **M.J. Rybak:** C. Consultant; Self; Allergan, Bayer, Cempra, Merck, Sunovian, Theravance, The Medicines Company. F. Investigator; Self; Allergan, Bayer, Cempra, Merck, Sunovian, Theravance, The Medicines Company, National Institutes of Health. J. Scientific Advisor (Review Panel or Advisory Committee); Self; Allergan, Bayer, Cempra, Merck, Sunovian, Theravance, The Medicines Company. L. Speaker's Bureau; Self; Allergan, Bayer, Cempra, Merck, Sunovian, Theravance, The Medicines Company.

Poster Board Number:

SUNDAY-502

Publishing Title:

Genetic Polymorphism Relating to the Concentration and Adverse Drug Reactions of Sulfamethoxazole- Trimethoprim and Metabolite

Author Block:

T-L. Chien[1], C-Y. Wang[1], C-C. Hung[2], S-Y. Chang[1], J-H. Yang[1], C-H. Kuo[1], Y-T. Lin[1], W-C. Liu[2], **S-W. Lin**[1]; [1]Natl. Taiwan Univ., Taipei, Taiwan, [2]Natl. Taiwan Univ. Hosp., Taipei, Taiwan

Abstract Body:

Background: Combination of sulfamethoxazole-trimethoprim (SMX-TMP) is the drug of choice in the treatment of *Pneumocystis jirovecii* pneumonia (PJP). Limited studies suggested peak plasma levels of SMX and TMP should target 100-200 and 3-10 μg/mL, respectively. SMX is mainly metabolized to N-acetyl SMX via N-acetyltranferase (NAT). The other pathway is CYP2C9-mediated bioactivation to a reactive, toxic metabolite. In Asian population, 20~30% are slow acetylators (SA) who may be more vulnerable to adverse drug reactions (ADRs). This study aimed to investigate single nucleotide polymorphism (SNP) of NAT and its correlation with levels of SMX-TMP and N-acetyl SMX, and ADRs.**Methods:** Adult inpatients receiving SMX-TMP ≥ 5 mg/kg/day (TMP) were enrolled in this prospective study. Blood samples of peak levels were drawn 3 hrs after oral dose or 1 hr after intravenous infusion at steady state. Trough levels were collected just before the next dose. The plasma levels of SMX-TMP and N4-acetyl SMX were assayed using a validated HPLC-UV method. SNP were determined by PCR amplification and restriction fragment-length polymorphism method. Chi-square test and Mann-Whitney U test were used to compare the differences between two groups. A p-value of <0.05 was considered statistically significant.**Results:** A total of 82 inpatients with a median age of 45 years (range, 23-87) were enrolled between January 2014 and December 2015. Thirty-six patients (43.9%) had HIV infection and 53 (64.6%) had cancers. Fifty-two patients (70.2%) had confirmed or presumed PJP. Approximately 70% of the patients achieved peak levels within therapeutic range for treating PJP. We found 14 patients (22.2%) were SAs in NAT1 and 22 (32.4%) were SAs in NAT2. Despite no significant differences between the ratio of N4-acetyl SMX/SMX (N/S) and NAT SNPs, lower ratios were observed in SAs. Of the 50 ADRs such as hepatotoxicity, nausea and vomiting, thrombocytopenia, skin rash, and psychosis, patients with definite or probable ADRs had lower ratio of N/S (p<0.05), suggesting the metabolism of SMX prone to CYP2C9 toxic pathway.**Conclusions:** A correlation between ratio of N/S and SMX-associated ADR indicated the critical role of NAT enzymes. More studies are needed to confirm this finding.

Author Disclosure Block:

T. Chien: None. **C. Wang:** None. **C. Hung:** None. **S. Chang:** None. **J. Yang:** None. **C. Kuo:** None. **Y. Lin:** None. **W. Liu:** None. **S. Lin:** None.

Poster Board Number:

SUNDAY-503

Publishing Title:

The Combination of Polymyxin B, Ampicillin/Sulbactam, and Meropenem Combats Polymyxin-Resistant *Acinetobacter baumannii* Over 14 Days

Author Block:

J. R. Lenhard[1], N. M. Smith[1], Z. P. Bulman[1], J. B. Bulitta[2], C. B. Landersdorfer[3], V. Thamlikitkul[4], P. N. Holden[1], R. L. Nation[5], J. Li[5], B. T. Tsuji[1]; [1]SUNY, Buffalo, NY, [2]Florida Univ., Orlando, FL, [3]Monash Univ., Melboure, Australia, [4]Mahidol Univ., Bangkok, Thailand, [5]Monash Univ., Melbourne, Australia

Abstract Body:

Background: The emergence of polymyxin-resistant *A. baumannii* (PRAB) has forced clinicians to use empiric, non-optimized triple antibiotic combinations; however, combinatorial pharmacodynamics (PD) of such regimens are poorly defined. Our objective was to characterize the PD of single, double, and triple combinations of polymyxin B (PB), ampicillin/sulbactam (A/S), and meropenem (MERO) against PRAB in a hollow fiber infection model (HFIM).**Methods:** Two extensively-resistant *A. baumannii* isolates resistant to PB (MIC 32 mg/L for 03-149-2, MIC 64 mg/L for N5406), A/S (MIC both strains 32/16 mg/L), and MERO (MIC both strains 64 mg/L) were investigated in a HFIM over 14 days at a 10^8 CFU/mL inoculum. PB (3.33 mg/kg x 1 dose, then 1.43 mg/kg q12h [fAUC$_{0\text{-}24h}$ 48.2 mg*h/L, fAUC$_{ss}$ 35.9 mg*h/L], $t_{1/2}$ 8h), A/S (8/4g q8h, $t_{1/2}$ 1.5h), and MERO (2g q8h, $t_{1/2}$ 1.5h, 3h prolonged infusion) were all administered alone and in double/triple combinations. Viable counts and PB population analysis profiles were conducted.**Results:** Against strain 03-149-2, single agents and double combinations were unable to achieve sustained killing. PB + MERO was the only double combination that resulted in early bactericidal activity, with a maximal reduction of 4.2 $\log_{10}$CFU/mL at 6h; however, drastic regrowth occurred by 72h and the percentage of the population capable of growing on 10 mg/L of PB was amplified 55,932 times higher compared to 0h. The triple combination also achieved a 4.2 log reduction at 6h, followed by eradication at 96h. Against strain N5406, PB or MERO alone and the combination of both agents achieved maximal reductions of 0, 0.6, and 1.9 logs, respectively, followed by regrowth. In contrast, A/S alone and in combination eradicated N5406. The triple combination displayed the most rapid killing with a 3.2 log reduction at 6h, while A/S alone, A/S + PB, and A/S + Mero resulted in 0.1, 0.6, and 1.9 log reductions at 6h, respectively, followed by sustained killing.**Conclusions:** The triple combination was the most active regimen against both *A. baumannii* strains and may offer a promising countermeasure for combating PRAB.

Author Disclosure Block:

J.R. Lenhard: None. **N.M. Smith:** None. **Z.P. Bulman:** None. **J.B. Bulitta:** None. **C.B. Landersdorfer:** None. **V. Thamlikitkul:** None. **P.N. Holden:** None. **R.L. Nation:** None. **J. Li:** None. **B.T. Tsuji:** None.

Poster Board Number:

SUNDAY-504

Publishing Title:

Optimization of Synergistic Combinations Against Carbapenem- and Aminoglycoside (Ags)-Resistant Clinical Isolates of *Pseudomonas* a*eruginosa* (Pa) *via* Mechanism-based Modeling

Author Block:

R. Yadav[1], J. Bulitta[2], R. L. Nation[1], C. B. Landersdorfer[1]; [1]Monash Univ, Melbourne, Australia, [2]Univ of Florida, Orlando, FL

Abstract Body:

Background: Carbapenem-resistant Pa are highly challenging clinically and effective early therapy is likely critical for treatment success. We aimed to systematically evaluate synergistic killing and prevention of resistance of Pa by carbapenem + AGS combinations and to rationally optimize combination dosage regimens *via* MBM.**Methods:** We studied imipenem (IPM) in monotherapy & combination with tobramycin (TOB) or amikacin (AMK) *vs.* 3 carbapenem- & AGS-resistant clinical Pa isolates. Serial viable counts of total & resistant populations (at 3×MIC) were determined in 48-h static concentration time-kill studies (inoculum $10^{7.5}$ CFU/mL). MBM characterized the time-course of bacterial killing & resistance. Optimized clinically relevant combination dosage regimens *vs.* a Pa isolate representing the 98th percentile of the EUCAST MIC distributions for IPM (MIC 16 mg/L) & TOB (MIC 32 mg/L) were proposed *via* Monte Carlo simulations.**Results:** Monotherapies provided little bacterial killing, except for < 2 $\log_{10}$ killing at 1-3h by high (24 & 36 mg/L) IPM concentrations followed by extensive regrowth with resistance. Clinically relevant IPM + AGS concentrations achieved synergistic killing (>2 $\log_{10}$ *vs.* the most active monotherapy at 24 h and 48h) and suppression of resistance for all isolates (MIC: IPM 16 to 32 mg/L & AGS 4 to 32 mg/L). MBM revealed an up to 3-fold decrease in the IPM conc. required for half-maximal bacterial killing with increasing AGS conc. Synergy was likely caused by the AGS enhancing target site penetration of IPM. Lower TOB (1/32th of MIC) than AMK (1/4th of MIC) conc. were sufficient for synergy with IPM, possibly due to the higher outer membrane affinity of TOB *vs.* AMK. An optimized combination regimen (IPM continuous infusion at 16.7 [9.63 - 29.1] mg/L (median [5th - 95th percentile]) + TOB 7 mg/kg body weight q24h) was predicted to achieve >2 $\log_{10}$ killing & prevent regrowth over 48 h in 90.3% of simulated critically-ill patients *vs.* the double-resistant isolate.**Conclusion:** The optimized IPM + TOB regimen is highly promising against Pa strains resistant to both carbapenems and AGS. Future evaluation in dynamic infection models will provide guidance on effective early therapy *vs.* infections by these extremely difficult-to-treat Pa.

Author Disclosure Block:

R. Yadav: None. **J. Bulitta:** E. Grant Investigator; Self; Trius, Cempra. H. H. Research Contractor; Self; Pfizer, Cubist. **R.L. Nation:** None. **C.B. Landersdorfer:** None.

Poster Board Number:

SUNDAY-505

Publishing Title:

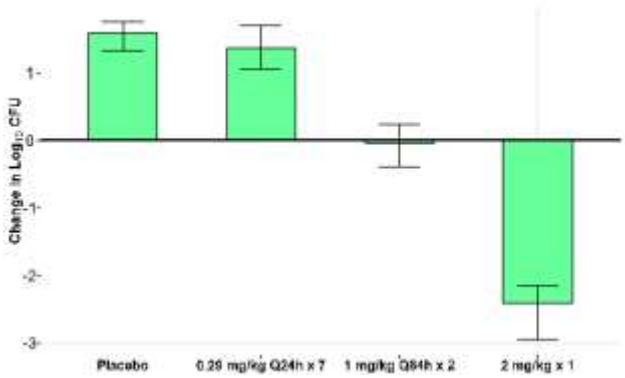
Pharmacological Basis of Cd101 Efficacy: Exposure Shape Matters

Author Block:

E. A. Lakota[1], C. M. Rubino[1], V. Ong[2], K. Bartizal[2], L. Miesel[3], S. M. Bhavnani[1], P. G. Ambrose[1]; [1]ICPD, Latham, NY, [2]Cidara Therapeutics, San Diego, CA, [3]Europhins Panlabs, Ltd., St. Charles, MO

Abstract Body:

Introduction: All commercially available echinocandin antifungal agents are administered once daily, with efficacy driven by AUC. CD101 is a novel echinocandin with a concentration-dependent pattern of fungicidal activity *in vitro* and a long half-life (up to 150 h in humans, approximately 70-80 h in mice). Given these distinct characteristics, it is likely that the shape of the CD101 AUC greatly influences efficacy. **Methods:** To test this hypothesis, we administered the same total AUC to groups (n=5) of neutropenic ICR mice infected with *Candida albicans* using 3 different schedules. A total CD101 dose of 2 mg/kg was administered as a single IV dose or in equal divided doses of either 1 mg/kg twice weekly or 0.29 mg/kg/d over 7 days. The studies included a no-treatment control group. Animals were rendered neutropenic by 2 IP cyclophosphamide doses (150 mg/kg and 100 mg/kg, administered at -4 and -1 days) and inoculated with *C. albicans* R303 (1×10^3 CFU/mouse) 24 h prior to treatment. Animals were euthanized at 192 h post-inoculation. Paired kidneys were harvested, homogenized, serially diluted and plated for CFU determination. **Results:** As shown in the figure, fungi grew well in the no-treatment control group with variable activity in treatment groups. When the CD101 $AUC_{0\text{-}168h}$ was administered as a single dose, there was a >2 $\log_{10}$ CFU reduction from baseline at 192 h. When that same AUC was administered in 7 equal divided daily doses, there was an increase by >1 log10 CFU from baseline at 192 h. **Conclusions:** These data support the hypothesis that the shape of the CD101 AUC greatly influences efficacy. CD101 was significantly more effective when given once per week compared to the same dose divided into twice weekly or daily regimens.



Author Disclosure Block:

E.A. Lakota: I. Research Relationship; Self; Cidara. **C.M. Rubino:** I. Research Relationship; Self; Cidara. **V. Ong:** D. Employee; Self; Cidara. **K. Bartizal:** D. Employee; Self; Cidara. **L. Miesel:** D. Employee; Self; Eurofins Panlabs. H. Research Contractor; Self; Cidara. **S.M. Bhavnani:** I. Research Relationship; Self; Cidara. **P.G. Ambrose:** I. Research Relationship; Self; Cidara.

Poster Board Number:

SUNDAY-506

Publishing Title:

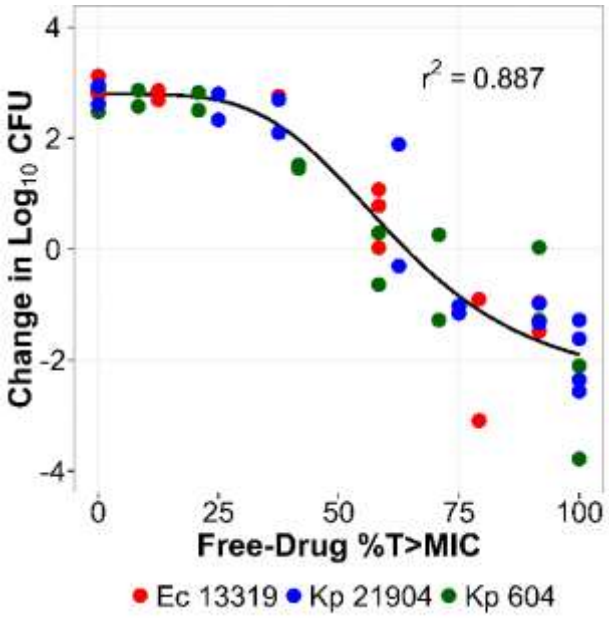
Determination of the Tazobactam (Taz) Exposure Required for Piperacillin (Pip) Efficacy Using a One-compartment *In Vitro* Infection Model (Ivim)

Author Block:

B. D. VanScoy, C. M. Rubino, J. McCauley, J. Adams, C. Jones, S. M. Bhavnani, P. G. Ambrose; ICPD, Latham, NY

Abstract Body:

The PK-PD index describing TAZ efficacy in combination with PIP was recently determined to be % time > a TAZ threshold concentration when examined against a panel of isogenic laboratory-derived isolates. In the studies described herein, we identified a translational relationship for TAZ threshold concentrations which describes PIP/TAZ efficacy for a panel of Enterobacteriaceae (ENT) clinical isolates. A series of 24 h dose range studies were completed using a one compartment IVIM and 3 clinical ENT isolates known to produce extended spectrum beta-lactamase enzymes. Challenge inoculums of 10^6 were exposed to PIP and TAZ concentrations using a $t_{1/2}$ = 1h for both compounds. A 4 g PIP regimen was administered in combination with a range of TAZ doses every 6 h, using a 30 min infusion. The data from the dose range studies were pooled and an evaluation of %T>TAZ threshold values equivalent to 0.5, 1, 2, and 4 times the PIP/TAZ potentiated MIC (with TAZ at a fixed concentration of 4 mg/L) were completed. Discrimination of potential translational relationships for TAZ threshold concentrations were evaluated by dispersion of data across the X-axis and optimization of r^2 values. As evidenced by r^2=0.887, the relationship between change in $\log_{10}$ CFU from baseline at 24 h and %T>TAZ threshold concentration, the translational relationship best describing the efficacy of TAZ in combination with PIP was the %T>PIP/TAZ MIC (figure). The %T>PIP/TAZ MIC required for net bacterial stasis as well as a 1- and 2-$\log_{10}$ CFU reduction from baseline was determined to be 63.9, 77.4, and 100, respectively. The identified translational relationship allows for the evaluation and of PIP/TAZ regimens currently utilized in the clinic to treat ESBL-producing ENT.



Author Disclosure Block:

B.D. VanScoy: D. Employee; Self; ICPD. **C.M. Rubino:** D. Employee; Self; ICPD. K. Shareholder (excluding diversitied mutual funds); Self; ICPD. **J. McCauley:** D. Employee; Self; ICPD. **J. Adams:** D. Employee; Self; ICPD. **C. Jones:** D. Employee; Self; ICPD. **S.M. Bhavnani:** D. Employee; Self; ICPD. K. Shareholder (excluding diversitied mutual funds); Self; ICPD. **P.G. Ambrose:** D. Employee; Self; ICPD. K. Shareholder (excluding diversitied mutual funds); Self; ICPD.

Poster Board Number:

SUNDAY-507

Publishing Title:

Anti-staphylococcal Activity Resulting from Achievable Epithelial Lining Fluid (Elf) Concentrations of Amikacin Inhalation Administered via the Pulmonary Drug Delivery System

Author Block:

I. M. Ghazi, M. Grupper, D. P. Nicolau; Hartford Hosp., Hartford, CT

Abstract Body:

Background: Amikacin Inhale (BAY41-6551), a unique drug-device combination of a specially formulated drug solution and a Pulmonary Drug Delivery System device (AMK-I) is currently under phase III study as an adjunctive therapy to IV antibiotics for the treatment of Gram-negative pneumonia in mechanically ventilated patients. While the epidemiology of nosocomial pneumonia is predominated by Gram-negative pathogens such as *P. aeruginosa* and the Enterobacteriaceae, *Staphylococcus aureus* is increasingly recognized as a pathogen of concern for these pulmonary based infections. Since the aminoglycosides are historically quite active against *S. aureus* the use of adjunctive AMK-I may enhance bacterial eradication. Herein, we aimed to characterize the *in vitro* pharmacodynamic (PD) profile of human-simulated ELF exposures of AMK-I against both methicillin-sensitive (MSSA) and -resistant (MRSA) *S. aureus*.**Methods:** An *in vitro* model was used to simulate the resultant ELF pharmacokinetic profile of amikacin after the administration of AMK-I 400mg q12h. The antibacterial activity of this regimen was tested against 7 *S. aureus* isolates that display MIC profiles encountered clinically (4 MRSA; MIC range 4-64, 3 MSSA; MIC range 8-16 mg/L). Experiments were conducted over 24 h and samples were taken throughout this period to assess the bacterial density in both control and treatments.**Results:** The mean ± SD inoculum 0h bacterial density was 6.4 ± 0.09 which increased to 8.6 ± 0.19 $\log_{10}$ CFU/mL in the control models by the end of 24 h experiments. Simulated ELF concentrations of AMK-I resulted in a rapid, 5 $\log_{10}$ declined in CFU over the initial 12 h for all MRSA and MSSA isolates. After 12 h, all bacterial counts remained below the limit of detection (LOD, 1.7 $\log_{10}$ CFU/mL) and no regrowth was evident at the end of the study.**Conclusions:** AMK-I produced an ELF exposure profile that was rapidly bactericidal against *S. aureus* displaying typical MICs to amikacin irrespective of their phenotypic profile to methicillin. While the Gram-negative organisms are the target pathogens for AMK-I in the ongoing clinical trials, these data suggest that this adjunctive regimen may also have the potential to eradicate both MSSA and MRSA from lower airway which needs to be further evaluated in randomized-controlled clinical trials.

Author Disclosure Block:

I.M. Ghazi: None. **M. Grupper:** None. **D.P. Nicolau:** C. Consultant; Self; Bayer. E. Grant Investigator; Self; Bayer. L. Speaker's Bureau; Self; Bayer.

Poster Board Number:

SUNDAY-508

Publishing Title:

Hollow Fiber *In Vitro* infection Model (Hfim) Simulating Pharmacokinetics (Pk) in Critically Ill (Icu) Patients: Substantial Impacts on Anti-Bacterial Effects of Meropenem

Author Block:

P. J. Bergen[1], J. B. Bulitta[2], C. M. J. Kirkpatrick[1], M. McGregor[1], K. Rogers[1], S. C. Wallis[3], D. L. Paterson[3], J. Lipman[3], J. A. Roberts[3], **C. B. Landersdorfer**[1]; [1]Monash Univ., Melbourne, Australia, [2]Univ. of Florida, Orlando, FL, [3]The Univ. of Queensland, Brisbane, Australia

Abstract Body:

Background: ICU patients can have severely altered PK leading to widely varying antibiotic exposures. This study aimed to characterize the impact of altered plasma PK on bacterial killing and resistance for meropenem in the dynamic hollow fiber infection model (HFIM).**Methods:** A clinical *Pseudomonas aeruginosa* isolate (MIC 0.25 mg/L) was studied in the HFIM (inoculum $10^{7.5}$ cfu/mL). PK profiles of 3 meropenem dosing regimens (0.5g, 1g and 2g q8h, administered as 30 min intravenous infusions) as observed in ICU patients with augmented renal clearance (ARC), normal or impaired renal function (creatinine clearances 250, 120 or 30 mL/min) were simulated in the HFIM over 10 days. The time-courses of viable counts for the total bacterial population and less-susceptible populations (at 5× and 10×MIC) were determined.**Results:** For all regimens with ARC, initial bacterial killing of 2.5 $\log_{10}$ cfu/mL at 7 h was followed by regrowth to 10^9-10^{10} cfu/mL at 72 h; less susceptible bacteria (on 10xMIC plates) amplified by 5 $\log_{10}$ compared to controls. The 0.5g q8h regimen at normal renal function provided 3.4 $\log_{10}$ killing at 24 h followed by regrowth to 10^9 cfu/mL at 72 h; less susceptible bacteria (10x MIC) almost completely replaced the susceptible population (7 $\log_{10}$ increase *vs.* control). The 2g q8h regimen at normal renal function and the 1g q8h and 2g q8h regimens at impaired renal function, *i.e.* regimens that provided 100% $fT_{>MIC}$, suppressed regrowth over 10 days.**Conclusions:** Standard meropenem dosing regimens led to extensive emergence of resistance in the hollow fiber system when simulating patients with ARC. High meropenem concentrations were required to prevent regrowth of *P. aeruginosa*. Individualized dosing regimens that account for altered plasma PK in ICU patients and aim for higher than standard antibiotic exposures were necessary to maximize bacterial killing and suppress emergence of resistance.

Author Disclosure Block:

P.J. Bergen: None. **J.B. Bulitta:** None. **C.M.J. Kirkpatrick:** None. **M. McGregor:** None. **K. Rogers:** None. **S.C. Wallis:** None. **D.L. Paterson:** None. **J. Lipman:** None. **J.A. Roberts:** None. **C.B. Landersdorfer:** None.

Poster Board Number:

SUNDAY-509

Publishing Title:

Evaluation of Daptomycin (DAP) Combined with ß-Lactams (Bl) and ß-Lactamase Inhibitors (BLI) against Methicillin-Resistant *Staphylococcus aureus* (MRSA)

Author Block:

K. R. Henson[1], J. Yim[1], J. R. Smith[2], G. Sakoulas[3], M. J. Rybak[1]; [1]Wayne State Univ., Detroit, MI, [2]High Point Univ., High Point, NC, [3]Univ. of California San Diego, La Jolla, CA

Abstract Body:

Background: The management of serious MRSA infections presents therapeutic challenges to clinicians and is compounded by the emergence of DAP-nonsusceptible (DNS) MRSA strains during therapy. Recent studies demonstrate that DAP+nafcillin or ceftaroline is effective in the treatment of prolonged bacteremia and infections caused by DNS MRSA. There are limited data on alternative combination therapy using DAP + BL/BLI. **Methods:** Eight clinical strains of MRSA were used, 4 of which were DNS. ß-lactamase production was tested using nitrocefin disks. Minimum inhibitory concentrations (MICs) to DAP, ampicillin (AMP), piperacillin (PIP), AMP+sulbactam (SUL), and PIP+tazobactam (TAZ) were obtained by broth microdilution using standard techniques. MICs to DAP in the presence of BL and BL/BLI were also obtained. Time kill experiments (TKEs) were used to evaluate synergy between DAP+BL and DAP+BL/BLI using DAP and BL concentrations at 0.5xMIC; BLIs were given at free peak concentrations. Samples were obtained at 0, 4, 8, and 24 h of incubation. Synergy was defined using standard definitions. The ΔT24 log CFU/mL was obtained as the difference between the log CFU/mL of DAP and DAP+BL or DAP+BL/BLI at 24 h. **Results:** The DAP MICs ranged from 0.125 to 4 µg/mL. DAP MICs decreased further in the presence of BL and BL/BLI in all 8 isolates. All strains produced ß-lactamase. Synergy between DAP and BL/BLIs was demonstrated in 7 out of 8 strains. In 5 out of 8 strains, synergy was demonstrated only in the presence of the BLI. Doubling the BLI concentration had little effect on bacterial killing in 3 of 3 isolates tested.

Isolate	**675**	**R8845**	**494**	**67**	**684**	**R8846**	**R6253**	**R9024**
DAP MIC (µg/mL)	0.25	0.125	0.25	0.25	2	4	2	2
ΔT24 log CFU/mL$_{DAP+PIP}$	-0.93	-0.65	-0.19	+0.12	-6.94	-4.64	-1.64	+0.04
ΔT24 log CFU/mL$_{DAP+PIP+TAZ}$	-6.31	-5.72	-2.37	-1.53	-6.66	-4.63	-7.42	-2.88
ΔT24 log CFU/mL$_{DAP+AMP}$	-0.86	-0.35	-0.14	-0.04	-1.94	-4.55	+0.48	-0.03
ΔT24 log CFU/mL$_{DAP+AMP+SUL}$	-6.95	-6.53	-4.7	-1.53	-7.22	-4.67	-8.31	-3.04

Conclusions: This preliminary *in vitro* study demonstrated the synergy between DAP and BL/BLI. In the majority of the isolates tested, the BLI demonstrated a significant effect in enhancing bacterial killing. Further *in vitro* modeling and clinical trials are needed to evaluate these combinations.

Author Disclosure Block:

K.R. Henson: None. **J. Yim:** None. **J.R. Smith:** None. **G. Sakoulas:** C. Consultant; Self; The Medicines Company. I. Research Relationship; Self; Allergan. L. Speaker's Bureau; Self; Allergan, Merck, Sunovian, The Medicines Company. **M.J. Rybak:** C. Consultant; Self; Allergan, Bayer, Cempra, Merck, Sunovian, The Medicines Company. E. Grant Investigator; Self; NIAID R01AI12400-01, NIAID R21 AI109266-01. I. Research Relationship; Self; Allergan, Bayer, Cempra, Merck, Sunovian, The Medicines Company. L. Speaker's Bureau; Self; Allergan, Bayer, Cempra, Merck, Sunovian, The Medicines Company.

Poster Board Number:

SUNDAY-510

Publishing Title:

The Antibacterial Effects of Two Dosing Regimens of Ceftolozane (TOZ) in Combination with Tazobactam (TAZ) in Comparison to Meropenem (MER) against *Pseudomonas aeruginosa* (PA)

Author Block:

K. E. Bowker, A. R. Noel, S. Tomaselli, M. Attwood, A. P. MacGowan; BCARE, North Bristol NHS Trust, Bristol, United Kingdom

Abstract Body:

Background: TOL/TAZ is an anti-pseudomonal cephalosporin (TOL) combined with a B.lactamase inhibitor (TAZ). TOL has marked in vitro potency against PA being active against many multi-drug resistant and extensively drug resistant strains. With anti-pseudomonal antibiotics, emergence of resistance (EoR) is a significant issue, hence it is important to optimise dosing regimens to limit this risk. We used an in vitro pharmacokinetic model (IVPKM) to simulate two human dose regimens of TOL/TAZ and measured their effect on 3 strains of PA and potential EOR over 7 day exposure.**Methods:** A one compartment IVPKM was used to simulate free drug serum concentrations associated with 1G TOL/0.5TAZ (Cmax 46/16mg/L) and 2G TOL/IG TAZ (Cmax 112/32); TOL t½ 2.5h, TAZ t½ 1h. Dosing was q8hly for 7d (168h). MER 1G (Cmax 80mg/L); t½ 1h was also simulated against one strain. 3 strains of PA were used, one wild type strain, one with AmpC hyper-expression and the other with a meropenem MIC 6mg/L: TOL/TAZ MICs were 0.5, 1 and 2mg/L. The inoculum was 10^6 CFU/ml and simulations were performed in triplicate. ABE was measured by log change in viable count and area-under-the-bacterial-kill-curve (AUBKC) over 168h. EOR was assessed by growth on nutrient agar plates containing x2, x4 MIC of the test antibiotic 24hly over 7 days..**Results:** Both TOL/TAZ regimens produced a >4 log reduction in viable count (below the limit of detection) by 12h for all three PA strains. Some regrowth occurred with all three strains after 24h with the TOL/TAZ 1G/0.5G regimen resulting in a log drop of 0-1 log at 168h. Regrowth also occurred with all three strains with the 2G/1G regimen after 48h with a 1-2 log drop at 168h. The 1G/0.5G was less effective at producing pathogen clearance than the 2G/1G regimen against all strains (AUBKC 168h 1G/0.5G 320±90; 2G/1G 172±117 log CFU/mL.h $p<0.01$). Against a single strain MER 1g q8h (MER MIC 1mg/L) was less effective than either TOL/TAZ regimen (ANOVA $p<0.01$). EOR on MICx2 plates was present with TOL/TAZ from 144h, and meropenem at 96h.**Conclusions:** TOL/TAZ was effective at reducing PA bacterial load. The 2G/1G regimen was more effective than the 1G/0.5G and EOR delayed until 144h. TOL/TAZ appeared more effective than MER in reducing bacterial load and preventing EOR.

Author Disclosure Block:

K.E. Bowker: None. **A.R. Noel:** None. **S. Tomaselli:** None. **M. Attwood:** None. **A.P. MacGowan:** E. Grant Investigator; Self; Cubist/Merck, The Medicines Company, Bayer Healthcare, Achaogen, Tetraphase. L. Speaker's Bureau; Self; Astellas.

Poster Board Number:

SUNDAY-511

Publishing Title:

Aminoglycoside Resistance Prevention by Short-Term, High Concentration Exposure in *Pseudomonas aeruginosa* Assessed *via* Novel Mechanism-Based Modeling (MBM)

Author Block:

V. E. Rees[1], J. B. Bulitta[2], B. T. Tsuji[3], A. Oliver[4], R. L. Nation[1], C. B. Landersdorfer[1]; [1]Monash Univ., Melbourne, Australia, [2]Univ. of Florida, Orlando, FL, [3]Univ. of Buffalo, Buffalo, NY, [4]Hosp. Son Espases, Palma, Spain

Abstract Body:

Background: Hypermutable *Pseudomonas aeruginosa* (Pa) are highly problematic. For aminoglycosides (AGS) the area under the free concentration-time curve divided by the MIC (*f*AUC/MIC) is used to predict bacterial killing and clinical success. We hypothesized that delivering the same *f*AUC over short *vs.* long durations of exposure would provide better killing and minimize AGS resistance in both hypermutable and non-hypermutable Pa. **Methods:** *P. aeruginosa* PAO1 and PAOΔ*mutS* (hypermutable) were studied in 24 h *in vitro* static time-kills with tobramycin (TOB; MIC=0.5 mg/L for both). This was carried out at *f*AUC/MIC of 36, 72 and 168 with initial inocula (CFU_o) of $\log_{10}$ 4 and 6, in duplicate. Antibiotic concentrations were calculated by the exposure durations and targeted *f*AUC/MIC. Tobramycin was added at 0 h and removed at 1, 4, 10 or 24 h *via* multiple centrifugation and re-suspension of bacteria in antibiotic-free broth. Viable counts of the total and resistant populations (mutation frequencies, MF) were determined and then characterized by novel MBM. MIC were assessed at 0 & 24 h.**Results:** For both strains at the same *f*AUC/MIC more rapid and extensive killing was observed with high concentrations over 1 and 4 h exposures (4 to 6 $\log_{10}$) compared to 10 and 24 h exposures (<4 $\log_{10}$). Regrowth by 24 h was extensive after 1 and 4h exposures, but the regrowing bacteria had unchanged MICs and MF compared to 0 h. While regrowth was less pronounced for 24 and 10 h exposure, susceptible bacteria had been completely replaced by AGS-resistant bacteria at CFU_o 6 and MICs increased up to 8-fold. The hypermutable PAOΔ*mutS* revealed higher MF at 0 and 24 h. Viable counts were well described by MBM (r=0.97).**Conclusions:** The AGS concentration profile shape was vital for resistance prevention. Short durations of exposure yielded extensive killing without resistance, whereas resistance was substantial for 24 and 10 h durations of exposure. High dose, short durations of exposure of AGS antibiotics are promising for innovative combination regimens to combat the susceptible regrowth.

Author Disclosure Block:

V.E. Rees: None. **J.B. Bulitta:** E. Grant Investigator; Self; Trius, Cempra. H. Research Contractor; Self; Pfizer, Cubist. **B.T. Tsuji:** None. **A. Oliver:** None. **R.L. Nation:** None. **C.B. Landersdorfer:** None.

Poster Board Number:

SUNDAY-512

Publishing Title:

Pharmacodynamics of Combination Therapy with Colistin and Meropenem Against Carbapenemase Producing *Klebsiella pneumoniae* Isolates in an *In Vitro* Pk-Pd Model

Author Block:

M. Tsala[1], S. Vourli[1], V. Miriagou[2], L. Tzouvelekis[1], L. Zerva[1], G. Daikos[1], J. Mouton[3], **J. Meletiadis**[3]; [1]Univ. Athens, Athens, Greece, [2]Hellenic Pasteur Inst., Athens, Greece, [3]Erasmus MC, Rotterdam, Netherlands

Abstract Body:

Background: As the last resort, colistin is often used for the management of carbapenamase producing *Klebsiella pneumoniae (CP-Kp)* infections. Since meropenem possesses some activity against these isolates, combination therapy may enhance efficacy of monotherapy regimens. We therefore assessed the combination of standard dosing regimens of colistin and meropenem against CP-Kp in an *in vitro* PK-PD model. **Methods:** Three clinical isolates with colistin and meropenem CLSI MIC's 0.5, 0.25, 0.5 mg/l and 0.06, 16 (CP-Kp1), 256 (CP-Kp2) mg/L, respectively were used at 10^7CFU/ml in an in vitro PK-PD model. The human plasma concentration-time profiles of 4.5MU q12 colistin and 1g q8 of meropenem was simulated for 48h targeting colistin and meropenem free serum maximum concentrations of 1.6 and 60 mg/L with half-lives 12h and <2h, respectively. Drug levels were determined with microbiological assays and bacterial growth by quantitative cultures estimating the CFU/ml at regular time points. **Results:** The pharmacokinetics of both drugs were well simulated in the model and within the target concentrations of mean±SD colistin and meropenem fC_{max} 1.86±0.27 mg/l and 47.82±11.56 mg/l and $t_{1/2}$ of 8-12h and 2-3h, respectively. Drug concentrations remained above the MIC for all three isolates for colistin and for 100%, 38% and 0% of the dosing interval for isolates with MICs 0.06, 16, 256 mg/L, respectively for meropenem. Colistin monotherapy exhibited rapid killing (>5$\log_{10}$CFU/ml reduction) within 2h exposure against all three isolates followed by regrowth after 12h reaching 9$\log_{10}$CFU/ml at 48h. Meropenem monotherapy exhibited rapid killing (4-5$\log_{10}$CFU/ml reduction) within 2h followed by regrowth within 24h only for the isolates with MIC 16 and 256 mg/l reaching 9$\log_{10}$CFU/ml at 48h. The combination regimen resulted in complete killing of the isolates with meropenem MICs 0.06 and 16 mg/l and 1.3$\log_{10}$CFU/ml reduction of the isolate with meropenem MIC 256 mg/l at 48h. **Conclusions:** Combination therapy with standard dosing regimens of colistin and meropenem enhanced efficacy of monotherapy regimens against CP-Kp but did not prevent regrowth of the highly meropenem resistant isolate.

Author Disclosure Block:

M. Tsala: None. **S. Vourli:** None. **V. Miriagou:** None. **L. Tzouvelekis:** None. **L. Zerva:** None. **G. Daikos:** None. **J. Mouton:** None. **J. Meletiadis:** E. Grant Investigator; Self; Astellas, Gilead, MSD, Pfizer.

Poster Board Number:

SUNDAY-513

Publishing Title:

***In Vitro* Evaluation of Resistance Selection in *Shigella flexneri* by Azithromycin, Ceftriaxone, Ciprofloxacin, Levofloxacin, and Moxifloxacin**

Author Block:

G. P. Allen, K. Harris; Univ. of New England Coll. of Pharmacy, Portland, ME

Abstract Body:

Background: *Shigella flexneri* has developed resistance to many antimicrobials. Alternate agents with superior pharmacodynamics are thus needed. The mutant prevention concentration (MPC) is a susceptibility parameter that measures the selection of mutant bacterial subpopulations. The MPC is the MIC of the most resistant mutant in a heterogeneous inoculum. Resistance is promoted when antimicrobial concentrations fall in the mutant selection window (MSW), the concentration range between the MIC and MPC. We used MSW testing and time-kill assays to compare the activity of azithromycin (AZM), ceftriaxone (CRO), ciprofloxacin (CIP), levofloxacin (LVX), and moxifloxacin (MXF) against *S. flexneri*.**Methods:** We studied ATTC 12022 and an isogenic *gyrA* mutant (m-12022). MPCs were determined by culturing 10^{10} CFU of *S. flexneri* on agar with increasing supra-MIC concentrations of each antimicrobial. Pharmacokinetic parameters attained by therapeutic doses of AZM (500 mg PO q24h; *f*C_{max} 0.28 mg/L, $T_{1/2}$ 68 h), CRO (250 mg x1 IM; *f*C_{max} 0.95 mg/L, $T_{1/2}$ 8 h), CIP (500 mg PO q12h; *f*C_{max} 2.08 mg/L, $T_{1/2}$ 4 h), LVX (500 mg PO q24h; *f*C_{max} 3.93 mg/L, $T_{1/2}$ 7 h), and MXF (400 mg PO q24h; *f*C_{max} 2.7 mg/L, $T_{1/2}$ 12 h) were used to assess the relationship between antimicrobial concentrations and the MSW. A time-kill assay was used to evaluate bacterial killing over 24 h.**Results:** For ATCC 12022, MIC/MPC (mg/L) were: AZM 4/>64, CRO 0.0625/0.5, CIP 0.03125/0.25, LVX 0.0625/0.5, and MXF 0.0625/0.5. For m-12022, MIC/MPC (mg/L) were: AZM 8/>64, CRO 0.125/2, CIP 0.25/2, LVX 0.25/2, and MXF 0.5/2. Concentrations of CRO, CIP, LVX, and MXF exceed the MPC for ATCC 12022, although concentrations of CRO and LVX fall in the MSW for 69.1% and 13.2% of the dosage interval, respectively. For m-12022, concentrations of CRO, CIP, LVX, and MXF fall in the MSW for 97.5%, 98.1%, 71.6%, and 78.3% of the dosage interval, respectively. Concentrations of AZM fall below the MIC, MPC, and MSW for both strains. In time-kill assays, CRO, CIP, LXV, and MXF were bactericidal (> 3 $\log_{10}$ CFU/mL kill) against both strains, while AZM was bacteriostatic.**Conclusion:** As *S. flexneri* resistance continues to emerge, new antimicrobial therapies are needed. The tested agents pose a variable risk of resistance selection, particularly in a *gyrA* mutant. The development of novel antimicrobials for shigellosis is needed.

Author Disclosure Block:

G.P. Allen: None. **K. Harris:** None.

Poster Board Number:

SUNDAY-514

Publishing Title:

Exploring the Activity of Tedizolid in Combination with Other Orally Bioavailable Antimicrobials Against *Staphylococcus aureus* and *Staphylococcus epidermidis*

Author Block:

B. J. Werth; Univ. of Washington Sch. of Pharmacy, Seattle, WA

Abstract Body:

Background: Tedizolid (TDZ) is an oxazolidinone, with high oral bioavailability, once-daily dosing, and broad-spectrum activity against gram-positives. Unlike linezolid, TDZ carries a reduced risk of hematologic toxicities with prolonged use and may fill a therapeutic gap where long-term, oral therapy against MDR gram-positives is desirable. Combination therapy is commonly indicated to improve efficacy against difficult to treat pathogens and biofilms. *S. aureus* and *S. epidermidis* are among the most commonly identified pathogens in orthopedic infections requiring prolonged therapy. To date there are no studies describing the pharmacodynamic interactions between TDZ and other orally bioavailable antimicrobials.**Methods:** MICs of TDZ, rifampin (RIF), trimethoprim/sulfamethoxazole (TMP/SMX), doxycycline (DOX), and moxifloxacin (MOX), were determined by broth microdilution against a convenience sample of 45 staphylococcal isolates. 7 MRSA isolates including 2 hVISA, 1 VISA and 4 VSSA, and 3 *S. epidermidis* were evaluated by time-kill using concentrations equal to 0.5 x the MIC. These strains had variable susceptibility to study antimicrobials. Synergy was defined as a $\geq$2 $\log_{10}$CFU/mL reduction of the combination over the most active single agent, antagonism was defined as $\geq$1 $\log_{10}$CFU/mL growth compared to the most active single agent, and other interactions were indifferent.**Results:**

Susceptibilities n=45 strains				
TDZ (MIC50)	**DOX (MIC50)**	**TMP/SMX (MIC50)**	**MOX (MIC50)**	**RIF (MIC50)**
0.25mg/L	0.25mg/L	0.125/2.38mg/L	2mg/L	0.02mg/L
TDZ (MIC90)	**DOX (MIC90)**	**TMP/SMX (MIC90)**	**MOX (MIC90)**	**RIF (MIC90)**
0.5mg/L	8mg/L	≥4/76mg/L	16mg/L	4mg/L
Time kills: interactions with tedizolid n=10 strains				
	Synergy	**Synergy**	**Synergy**	**Synergy**
	2/10	0/10	0/10	2/10
	Antagonism	**Antagonism**	**Antagonism**	**Antagonism**
	0/10	0/10	3/10	0/10

Conclusions: Pharmacodynamic interactions between TDZ and other agents were largely indifferent. Antagonism with MOX has been seen with linezolid, which may be evident with TDZ. MOX antagonism was observed in MOX susceptible and resistant strains. TDZ plus DOX or RIF had the most favorable activity but further research is warranted to assess the therapeutic potential of these combinations.

Author Disclosure Block:

B.J. Werth: E. Grant Investigator; Self; Merck, Allergan.

Poster Board Number:

SUNDAY-515

Publishing Title:

The Affect of Timing of Synergistic Antimicrobials on Bacterial Killing Against Methicillin Resistant *Staphylococcus aureus*

Author Block:

S. N. Leonard[1], T. A. Arriola[2]; [1]Ohio Northern Univ., Ada, OH, [2]Northeastern Univ., Boston, MA

Abstract Body:

Background: When considering combination antimicrobials for synergy it is thought that those drugs should and will be administered at the same time. However, in reality many issues complicate this notion. There may be more drugs than IV lines available for administration, there may be incompatibilities, the nursing staff may not know to administer them at the same time, and more. The goal of this project was to evaluate the effect of different administration times of two synergistic drugs on bacterial killing against methicillin resistant *Staphylococcus aureus* (MRSA) using an in vitro PK/PD model with simulated human pharmacokinetics. **Methods:** 2 isolates of MRSA were evaluated using a PK/PD model with simulated doses of vancomycin (VAN) q12h with a trough of 15 mg/L combined with gentamicin (GEN) 5 mg/kg daily, nafcillin (NAF) 2 g q4h, or cefazolin (CFZ) 1 g q8h with a starting inoculum of ~10^7 CFU/mL over 24 h in duplicate. VAN was always administered at T = 0 h while GEN, NAF, and CFZ were started at T=0, 1, 2, 3, 4, 5, or 6 h so that the effect of different start times could be evaluated. Total reduction in $\log_{10}$ CFU/mL over 24 h was determined by plotting time kill curves. Time to bactericidal activity was defined as 99.9% kill from initial inoculum ($T_{99.9}$) and determined by linear regression. Differences in CFU/mL at 24 h as well as $T_{99.9}$ were evaluated by using ANOVA with Tukey's post-hoc test. $P \leq 0.05$ was considered significant.**Results:** For both isolates there was no difference in the amount of kill at 24 h between the different administration times of each respective synergistic agent ($P \geq 0.24$). For CFZ and NAF combinations there were no differences in $T_{99.9}$ between administration times. For GEN combinations there was a difference in $T_{99.9}$ with later administration time having a longer $T_{99.9}$ (1.75-6.4 h, $P \leq 0.01$).**Conclusions:** Altering the administration time of GEN can increase the time to bactericidal activity when given in combination with VAN against MRSA. This effect was not observed with beta lactams (NAF or CFZ). There were no differences in magnitude of kill at 24 hours for any combination based on administration time of each respective synergistic agent.

Author Disclosure Block:

S.N. Leonard: None. **T.A. Arriola:** None.

Poster Board Number:

SUNDAY-516

Publishing Title:

Synergy Between Beta-Lactams (Bls) and Daptomycin (Dap) Against Vancomycin-resistant *Enterococci* (Vre) in an *In Vitro* Pharmacokinetic/Pharmacodynamic (Pk/Pd) Biofilm Model: Does This Apply to All Vre?

Author Block:

J. Yim[1], J. R. Smith[1], J. A. Hallesy[1], N. Singh[1], C. A. Arias[2], M. J. Rybak[1]; [1]Wayne State Univ., Detroit, MI, [2]Univ. of Texas Med. Sch. at Houston, Houston, TX 77030, TX

Abstract Body:

Background: *Enterococcus faecium (Efm)* may cause biofilm-associated infections. *Efm* strains are often resistant to vancomycin and BLs, posing important clinical challenges. We previously reported data supporting a potential role for the DAP+ ampicillin (AMP) and DAP+ ertapenem (ERT) combinations against *Efm* in an *in vitro* PK/PD biofilm model. Here, we further investigate the ability of AMP, ERT and ceftaroline (CPT) to enhance DAP activity against *Efm*. **Methods:** Treatment of *Efm* S447 was evaluated in a PK/PD biofilm models over 7 days, simulating human PK of DAP 14 mg/kg/day, DAP 12, DAP 12 + AMP 2g q6h, DAP 12 + CPT 600 mg q8h, DAP 12 + ERT 1g q24h and drug-free control. *Efm* biofilm was developed on titanium coupons using the CDC biofilm reactor. Synergy of these combinations in the absence of biofilm was further evaluated in a 1-compartment PK/PD model for 96 h and by time-kill analysis (TKA) methods over 48h.**Results:** DAP, AMP, CPT and ERT MICs for *Efm* S447 were 2 µg/mL, 32 µg/mL, > 16 µg/mL and > 64 µg/ml, respectively. DAP 14 mg/kg/day achieved bactericidal activity at 168h in S447 biofilm (-Δ3.5 $\log_{10}$CFU/cm^2 from 0h). Addition of ERT enhanced DAP activity, allowing DAP 12+ERT to produce bactericidal activity at 168h (-Δ3.87 $\log_{10}$ CFU/cm^2). DAP 12+AMP and DAP 12+CPT failed to demonstrate synergy in comparison to DAP 12 alone (DAP 12, -Δ2.4 $\log_{10}$CFU/cm^2; DAP 12+AMP, -Δ0.47 $\log_{10}$CFU/cm^2; DAP12+CPT, +Δ0.13 $\log_{10}$CFU/cm^2). While both DAP+AMP and DAP+CPT were synergistic at 48h in TKA at an initial inoculum of ~6.5 $\log_{10}$CFU/mL, no synergy was found at an initial inoculum of ~8 $\log_{10}$CFU/mL. In a 1-compartment PK/PD model at ~10^7 CFU/ml, both combinations were synergistic at 96h compared to DAP 12 alone (DAP 12, -Δ 0.08 CFU/mL; DAP12+AMP, -Δ 5.02 CFU/mL; DAP12+CPT, -Δ 4.8 CFU/mL).**Conclusions:** Our data provide further support for the potential use of DAP+ERT against biofilm-associated infections by *Efm*. Enhanced DAP activity by AMP or CPT against *Efm* may be both organism and inoculum dependent, and potentially affected by biofilm presence. Further research is warranted to examine the impact of these conditions on DAP + BL combinations against *Efm*.

Author Disclosure Block:

J. Yim: None. **J.R. Smith:** None. **J.A. Hallesy:** None. **N. Singh:** None. **C.A. Arias:** C. Consultant; Self; Theravance, Cubist, Bayer. E. Grant Investigator; Self; Theravance. F. Investigator; Self; Forest. J. Scientific Advisor (Review Panel or Advisory Committee); Self; Cubist, Bayer. L. Speaker's Bureau; Self; Forest, Theravance, Pfizer, AstraZeneca, Cubist, The Medicines Company, Norvartis. **M.J. Rybak:** C. Consultant; Self; Actavis, Bayer, Cempra, Cubist (now Merck), The Medicine Company, Sunovian, Theravance. E. Grant Investigator; Self; Actavis, Bayer, Cempra, Cubist (now Merck), The Medicine Company, Sunovian, Theravance, NIH. L. Speaker's Bureau; Self; Actavis, Bayer, Cempra, Cubist (now Merck), The Medicine Company, Sunovian, Theravance.

Poster Board Number:

SUNDAY-517

Publishing Title:

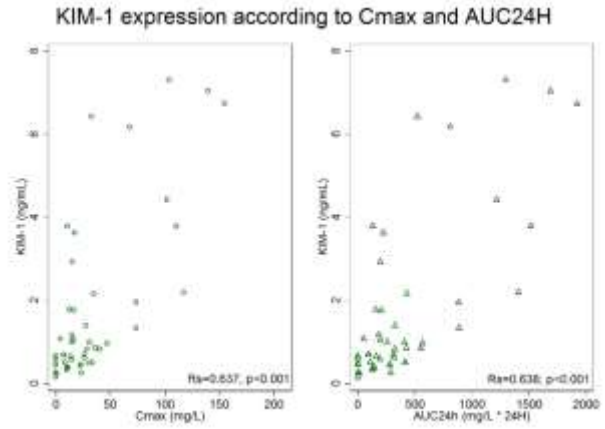
24-Hour Pharmacokinetic Relationships for Vancomycin (Van) and Novel Urinary Biomarkers of Acute Kidney Injury (Aki)

Author Block:

J. O'Donnell[1], N. J. Rhodes[1], T. Lodise[2], W. Prozialeck[1], C. M. Miglis[1], V. Natarajan[3], M. Joshi[1], G. Pais[1], P. Lamar[1], C. Cluff[1], A. Gulati[1], M. H. Scheetz[1]; [1]Midwestern Univ., Downers Grove, IL, [2]Albany Coll. of Pharmacy, Albany, NY, [3]TesoRx Pharma LLC, Pomona, CA

Abstract Body:

Background: VAN has been associated with AKI in both laboratory and clinical settings, however the precise pharmacokinetic (PK) exposures associated with the toxicodynamics (TD) have not been defined. We sought to determine exposures associated with the development of AKI using highly sensitive novel biomarkers. **Methods:** Male Sprague-Dawley rats received clinical grade VAN or normal saline as an intraperitoneal injection for 24 hours. Total daily doses ranging from 0 to 400 mg/kg/day were administered either as single or 2 divided doses. At least 5 rats were utilized for each dosing protocol. A max of 7 plasma samples per rat were obtained. 24-hour urine was collected and KIM-1, clusterin and osteopontin were determined using MILLIPLEX MAP Rat Kidney Panels. VAN plasma concentrations were determined via HPLC. PK analyses were conducted using Pmetrics for R and a 3-compartment model. Bayesian maximal a posteriori concentrations were fit to calculate 24-hour AUC, Cmax, and Cmin. PK/TD relationships were determined with non-parametric Spearman's rho (rs).**Results:** 47 rats contributed PK/TD data. Observed vs. predicted plots demonstrated a R2 of 0.833 for VAN model fit. VAN Cmax and AUC were most predictive, demonstrating Rs values of 0.637, 0.414, and 0.579, and 0.638, 0.418, and 0.567 for KIM-1, clusterin, and osteopontin respectively. All P-values for these relationships were </= 0.01. Cmin was less predictive with Rs values of 0.308, 0.040, 0.361 respectively.



Conclusions: Novel urinary biomarkers demonstrate that kidney injury occurs early with VAN and as a function of AUC or Cmax.

Author Disclosure Block:

J. O'Donnell: None. **N.J. Rhodes:** None. **T. Lodise:** None. **W. Prozialeck:** None. **C.M. Miglis:** None. **V. Natarajan:** None. **M. Joshi:** None. **G. Pais:** None. **P. Lamar:** None. **C. Cluff:** None. **A. Gulati:** None. **M.H. Scheetz:** None.

Poster Board Number:

SUNDAY-518

Publishing Title:

Probability of Target Attainment for Meropenem (Mero) and Polymyxin B (Pb) Combinations Against Carbapenem-Resistant *Acinetobacter baumanii* (Crab)

Author Block:

N. M. Smith[1], J. R. Lenhard[1], M. Trang[2], Z. Bulman[1], J. Bulitta[3], C. Landersdorfer[4], R. L. Nation[5], J. Li[4], A. Forrest[6], B. T. Tsuji[1]; [1]SUNY Buffalo, Buffalo, NY, [2]ICPD, Latham, NY, [3]Univ. of Florida, Orlando, FL, [4]Monash Univ., Parkville, Australia, [5]Monash Univ., Parkville, Austria, [6]UNC, Chapel Hill, NC

Abstract Body:

Background: Although the PK/PD targets for MERO and PB are well defined individually, there is a paucity of data on targets for combination, especially against carbapenem-resistant strains. Our objectives were to determine the likelihood of attaining desired bacterial reduction targets for MERO in combination with PB . **Methods:** AB strain N16870 (PB MIC 0.5 mg/L, MERO MIC 16 mg/L) was previously studied over 14 days in a hollow fiber infection model (HFIM) at a starting inoculum of 10^8 CFU/mL (Lenhard et. al., ICAAC 2015 A-938). The HFIM regimens included a growth control, a PB loading-dose (LD) monotherapy (2.22 mg/kg x1, then 1.43 mg/kg q12h [$fAUC_{0\text{-}24}$ 35.9 mg*h/L]), and PB LD plus dose escalated MERO (2, 4, 6, 8 g doses 3h prolonged infusion (PI) q8h [$fAUC_{24}$ 337, 673, 1010, 1347 mg*h/L, respectively]). Monte Carlo simulations (MCS) for 1000 patients was used to determine MERO concentrations, along with resulting reductions in area under the bacterial CFU/mL vs. time curve (AUCFU). The resulting patients were divided into groups by MERO AUC_{24} of <632, 687, 758, 824, and >824 mg*h/L, which were associated with <0.5, 1, 2, 3, >3 log decreases in AUCFU, respectively.**Results:** In the HFIM, PB monotherapy produced modest changes in AUCFU, whereas the PB-MERO combo showed maximal reductions in AUCFU (4.85 CFU*hr/mL). The AUCFU vs. MERO-fAUC24 relationship was described by a Hill-function with (E_{max}: 4.87 CFU*hr/mL, E0: 0 [fixed], EC50: 786 mg*hr/L, and Hill: 10). In presence of PB. The MCS predicted that, for a 2g regimen, 0% of patients would achieve >0.5 log decrease in AUCFU. The 4g regimen displayed a distribution of target attainment with 0.70%, 11.8%, 53.8%, 27.8%, and 5.9% of the patients seeing decreases in bacterial AUCFU of <0.5, 1, 2, 3, and >3 log reductions, respectively. Both the 6g and 8g regimens showed that all patients would respond with >3 log decreases in AUCFU.**Conclusion:** In the face of carbapenem resistance, escalation of meropenem ≥ 4g PI q8h in the presence of PB was necessary to achieve adequate target attainment in patients, which may provide insights to the benefits-risks of increasing MERO dose.

Author Disclosure Block:

N.M. Smith: None. **J.R. Lenhard:** None. **M. Trang:** None. **Z. Bulman:** None. **J. Bulitta:** None. **C. Landersdorfer:** None. **R.L. Nation:** None. **J. Li:** None. **A. Forrest:** None. **B.T. Tsuji:** None.

Poster Board Number:

SUNDAY-519

Publishing Title:

Combination of Daptomycin (Dap) and Ceftaroline (Cpt) or Gentamicin (Gen) Against *Streptococcus mitis* in an *In Vitro* Model of Simulated Endocardial Vegetations (Sevs)

Author Block:

J. Yim[1], J. R. Smith[1], N. Singh[1], J. A. Hallesy[1], C. Garcia de la Mària[2], A. S. Bayer[3], N. N. Mishra[4], J. M. Miro[5], C. A. Arias[6], T. T. Truc[6], P. Sullam[7], M. R. Rybak[1]; [1]Wayne State Univ., Detroit, MI, [2]Hosp. Clinic-IDIBAPS, Univ. Barcelona, Barcelona, Spain, [3]David Geffen Sch. of Med. at UCLA, Los Angeles, CA, [4]LA BioMed. Res. Inst., Torrance, CA, [5]Hosp. Clinic-IDIBAPS, Univ. Barcelona, Spain, Spain, [6]Univ. of Texas Med. Sch. at Houston, Houston, TX, [7]UCSF and VA Med. Ctr, San Francisco, CA

Abstract Body:

Background:S. mitis is a frequent cause of streptococcal endocarditis. Management of S. mitis infective endocarditis (IE) poses a clinical challenge, as strains are frequently resistant to β-lactams. Our study objective was to evaluate the efficacy of DAP alone and in combination with CPT or GEN against S. mitis in an in vitro SEV model.**Methods:**DAP, CPT, and GEN MICs were determined for S. mitis 351 and SF 100 by broth microdilution. DAP MICs were re-measured in presence of 0.5 x MIC of CPT or GEN to determine the DAP 'MIC lowering effect' of each agent. Both isolates were evaluated in a 96 h SEV model, mimicking human pharmacokinetics of DAP 6 mg/kg q24h, CPT 600 mg q8h, and GEN 3 mg/kg q24h alone and in combination. Emergence of resistance to DAP (DAP-R) was evaluated at 96 h.**Results:**CPT reduced the DAP MIC against S. mitis 351 and SF100 by more than 8-fold, whereas GEN decreased the DAP MIC only against *S. mitis* 351 and by 2-fold. In SEVs infected with either isolate, DAP alone was not bactericidal at 96h (351, $+\Delta 0.17$ $\log_{10}$ CFU/ml from 0h; SF100, $-\Delta 0.07$ $\log_{10}$ CFU/ml), and DAP-R was detected at 96h. GEN alone demonstrated little activity against these strains at 96h. CPT alone was bactericidal as soon as 24h and 8h for the two strains, and maintained its efficacy at 96h (351, $-\Delta 5.18$ $\log_{10}$ CFU/ml; SF 100, $-\Delta 6.57$ $\log_{10}$ CFU/ml). Addition of GEN to DAP was not synergistic in either strain at 96h. (351, $-\Delta 0.77$ $\log_{10}$ CFU/ml; SF100, $-\Delta 0.42$ $\log_{10}$ CFU/ml), and not prevent DAP-R. DAP+CPT was synergistic and rapidly bactericidal at 8h and 4h, and showed sustained efficacy at 96h. (351, $-\Delta 8.17$ $\log_{10}$ CFU/ml; SF100, $-\Delta 6.57$ $\log_{10}$ CFU/ml). DAP + CPT prevented emergence of DAP-R in both strains.**Conclusions:**DAP+CPT is a promising therapeutic option for treatment of S. mitis IE both in terms of efficacy and prevention of DAP-R. DAP + GEN appears to possess limited synergistic activity in this regard. Further investigation in animal IE models is warranted.

Author Disclosure Block:

J. Yim: None. **J.R. Smith:** None. **N. Singh:** None. **J.A. Hallesy:** None. **C. Garcia de la Mària:** None. **A.S. Bayer:** None. **N.N. Mishra:** None. **J.M. Miro:** None. **C.A. Arias:** C. Consultant; Self; Theravance, Cubist, Bayer. E. Grant Investigator; Self; Theravance. F. Investigator; Self; Forest. J. Scientific Advisor (Review Panel or Advisory Committee); Self; Cubist, Bayer. L. Speaker's Bureau; Self; Forest, Theravance, Pfizer, AstraZeneca, Cubist, The Medicines Company, Norvartis. **T.T. Truc:** None. **P. Sullam:** None. **M.R. Rybak:** C. Consultant; Self; Actavis, Bayer, Cempra, Cubist (now Merck), The Medicine Company, Sunovian, Theravance. E. Grant Investigator; Self; Actavis, Bayer, Cempra, Cubist (now Merck), The Medicine Company, Sunovian, Theravance, NIH. L. Speaker's Bureau; Self; Actavis, Bayer, Cempra, Cubist (now Merck), The Medicine Company, Sunovian, Theravance.

Poster Board Number:

SUNDAY-520

Publishing Title:

Histamine-Induced Toxicity in Rats with Antibiotic Compounds

Author Block:

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Abstract Body:

During drug discovery rat PK/TK studies with antibiotic compounds, it was observed that certain compounds would cause some rats to experience adverse effects often resulting in death. It was thought that perhaps the release of histamine could be attributed to these effects. Histamine has a large part in the inflammatory process and is released by mast cells in response to foreign pathogens.To correlate the release of histamine with the compound concentration, a LC/MS/MS quantitative method was developed to monitor histamine.A Histamine working standard of 0.01 mg/ml was prepared in which serially diluted calibration curve spiking solutions were generated. Because histamine is endogenous, calibration curves were prepared in water. 5 µL of standard working solutions were spiked into 25 µL of water to generate a calibration curve ranging from 0.1 ng/ml to 2000 ng/ml. 25 µL of histamine-d4 solution (500 ng/ml) were added to all samples, including calibration curves. 50 µL of ammonium hydroxide (5%) was added to all samples. The samples were vortexed, transferred to an Isolute SLE+ plate, and extracted with 900 µL of ethyl acetate using positive pressure. PK plasma extracts were then analyzed using a Shimadzu Nexera system coupled to a Sciex 5500 Qtrap mass spectrometer. A Waters BEH Amide (50x2 mm, 1.7 µm) column with a generic HILIC gradient was used. Mobile phase A consisted of 25mM ammonium formate in 95:5 ACN:water with 0.1% formic acid and mobile phase B was 25 mM ammonium formate in 50:50 ACN:water.For all rat PK/TK studies, histamine concentrations were measured along with quantitation of the test article. Of the over 100 compounds dosed, at least 50% of the compounds showed histamine-induced effects. The baseline levels for histamine in rats ranged between 5-10 ng/ml. However, when particular compounds were dosed, as the drug concentrations increased, histamine concentrations also increased, sometimes to even tenfold of the baseline concentrations. This result proved that there was a positive correlation between increasing drug concentration in plasma and histamine release.Our findings indicated that these antibiotic compounds often triggered histamine release in rats. Compound optimization mitigating these histamine-induced toxicological effects is imperative to producing a safe, efficacious and reliable antibacterial drug.

Author Disclosure Block:

S.S. Ubhayakar: None. **X. Liang:** None. **Y. Chen:** None.

Poster Board Number:

SUNDAY-521

Publishing Title:

High Rate of Carbapenemase-Producing Enterobacteriaceae in a Hospital in Angola; The Oxa-181 Threat

Author Block:

L. Poirel[1], N. Kieffer[1], M. Aires de Sousa[2], P. Nordmann[1]; [1]Univ. of Fribourg, Fribourg, Switzerland, [2]Univ. of Fribourg, Lisbon, Portugal

Abstract Body:

Background: The class B carbapenemase NDM-1 and the class D carbapenemase OXA-48 are increasingly identified worldwide, being mostly found in Enterobacteriaceae. No epidemiological data are available regarding the occurrence of carbapanemases in Angola. We conducted a prospective study in order to evaluate the rate of colonization of patients by carbapenemase producers at a Pediatric hospital in Luanda. **Methods**: Rectal swabs were collected from children being either inpatients or outpatients during a one-week screening period, May 2015. After a pre-enrichement in broth supplemented with ertapenem 0.25 µg/ml, samples were screened for the occurrence of carbapenem-resistant enterobacterial isolates using Chrom ID Carba SMART agar plates (bioMérieux). Colonies were tested with the Rapidec Carba NP test (bioMérieux) for detection of carbapenemase production. PCR experiments were further performed using primers specific for all carbapenemase genes (KPC, NDM and OXA-48-like). Conjugation experiments were realized by broth mating method in order to detect the possible transfer of the carbapenemase genes. Genotyping was performed by PFGE analysis and MLST. **Results:** A total of 157 samples were collected from children being 3 months to 13 years-old. A total of 90 carbapenem-resistant Gram negative isolates were recovered, the majority being *K. pneumoniae* (n=34) and *E. coli* (n=37) isolates. A total of 74 carbapenemase producers were identified, including 34 *K. pneumoniae*, 33 *E. coli*, and 4 *A. baumannii*. The most common carbapenemase identified was OXA-181 (a derivative of OXA-48) found in 32 *K. pneumoniae*, 29 *E. coli*, and a single *E. cloacae*. Nine NDM-1-producing isolates were identified, being 4 *E. coli*, 2 *K. pneumoniae*, a single *P. stuartii*, a single *P. retgeri*, and a single *A. baumannii*. PFGE analysis indicated that eleven different clones were identified among the 32 *K. pneumoniae* isolates. Seventeen different clones were identified among the 31 *E. coli* isolates. MLST indicated that Among the *E. coli* isolates, the most frequent Sequence Type was ST5692.**Conclusion:** This study identified for the first time carbapenemase producers in Angola. Noteworthy, those isolates were recovered from children. There was a significant number of NDM-1 producers, there was a large majority of OXA-181-producing isolates.

Author Disclosure Block:

L. Poirel: None. **N. Kieffer:** None. **M. Aires de Sousa:** None. **P. Nordmann:** None.

Poster Board Number:

SUNDAY-522

Publishing Title:

Characterization of Three *Escherichia coli* Clinical Isolates Carrying Plasmid-Mediated *oqxab* Genes

Author Block:

G. Rincon, M. Papalia, M. Rolan, **G. Gutkind**, M. Radice, J. Di Conza; UBA, BA, Argentina

Abstract Body:

Background: *oqxAB* genes encoding a multidrug efflux pump that commonly confer low resistance to quinolones and other antibiotics, but its presence may contribute to the selection of quinolone resistant mutants. *oqxAB* was initially detected in *E. coli* isolated from swine manure. However, it is infrequently reported in clinical isolates. The aim of this study was to characterize three clinical isolates of *oqxAB*-harboring *E. coli*, evaluate the inhibitory effect of phenylalanylarginine-β-naphthylamide (PAβN) and reserpine, and describe the genetic environment of this resistance marker. **Methods:** *E. coli* isolates (ER2, ER3 and DAC3) were collected in the metropolitan area in Lima, Peru. They were categorized as quinolone resistant and CTX-M-65 producers. Antimicrobial susceptibility and inhibitory effects of PAβN (50 mg/L) and reserpine (20 mg/L) were evaluated by agar dilution method according to CLSI. PMQR genes and QRDR mutations were determined by PCR and sequencing. *XbaI*-PFGE and MLST were used to characterize the isolates. *oqxAB*-containing plasmids were transferred to *E. coli* DH5α by electroporation. The genetic context of *oqxAB* was determined by PCR mapping and sequencing. **Results:** Isolates were resistant to nalidixic acid (NAL ≥1024 μg/ml), ciprofloxacin (32 μg/ml), levofloxacin (LVX 32 μg/ml), gatifloxacin (≥ 32 μg/ml), norfloxacin (≥128 μg/ml) and also trimethoprim- sulfamethoxazole (TMS ≥ 32/608 μg/ml) and chloramphenicol (CHL ≥128 μg/ml). In addition of *oqxAB*, ER3 also harbored *aac(6')-Ib-cr.* All of them carried two mutations in GyrA (S83L, D87N) and one in ParC (S80I). ER2 and ER3 strains were clonally related and were typified as ST602. DAC3 displayed a different banding pattern and was identified as ST448. The transfer of plasmids carrying *oqxAB* from ER2 and ER3 displayed an increase in TMS and CHL MICs (≥ 4 fold dilutions), however, quinolone MICs increased slightly. NAL, LVX and CHL MICs were reduced only in the presence of PAβN. The *oqxA1* and *oqxB3* alleles were identified in these isolates and they were always flanked downstream by IS*26* and upstream by a *tnpA* which displayed 99% identity with IS*15DI*. **Conclusions:** *E. coli* harboring *oqxAB* displayed the same allelic variants which were surrounded by insertion sequences belonging to IS*26* family. This operon has lightly modified the MICs of quinolones when it was transferred

Author Disclosure Block:

G. Rincon: None. **M. Papalia:** None. **M. Rolan:** None. **G. Gutkind:** None. **M. Radice:** None. **J. Di Conza:** None.

Poster Board Number:

SUNDAY-523

Publishing Title:

***klebsiella Pneumoniae* Viability And Antibiotic Resistance Reduced By Peptide Phosphorodiamidate Morpholino Oligomers (Ppmos)**

Author Block:

E. Sully[1], L. Li[1], S. Bailey[2], M. Wong[2], D. E. Greenberg[3], **B. L. Geller**[1]; [1]Oregon State Univ., Corvallis, OR, [2]Sarepta Therapeutics, Cambridge, MA, [3]Univ. of Texas Southwestern Med. Ctr., Dallas, TX

Abstract Body:

Peptide phosphorodiamidate morpholino oligomers (PPMOs) are antisense nucleic acid analogs designed silence expression of specific genes through sequence specific binding to the coded mRNA resulting in translation inhibition. PPMOs targeted to essential genes inhibit growth of many Gram-negative bacteria, including *Escherichia coli* and *Acinetobacter*. It is now shown for the first time that PPMOs targeted to essential genes of *Klebsiella pneumoniae* inhibited growth and were bactericidal. In another strategy, PPMOs were targeted to the bla_{KPC} carbapenemase, and were found to restore susceptibility to meropenem. PPMOs were designed to complement the regions around the AUG start codons of the mRNA of essential or KPC genes, conjugated to a cell penetrating peptide, and evaluated *in vitro* by measuring the minimal inhibitory concentration (MIC) and the reduction of viability. Twelve PPMOs that target essential genes such as *acpP* (acyl carrier protein), *rpmB* (ribosomal protein), and *ftsZ* (cell division ring) were analyzed for potency in a panel of 40 strains, including multidrug-resistant and KPC-expressing strains. The most potent PPMO targeted *acpP* and had an IC_{90} of 1 μM. This AcpP PPMO (at 2 x MIC) reduced viability of selected strains by > 10^3 cfu/ml within 8 h. Two PPMOs were designed to inhibit expression of bla_{KPC} carbapenemase. Using 2 μM (12 μg/ml) of either KPC PPMO in combination with meropenem reduced the MIC of meropenem from 32 μg/ml to 4 μg/ml. These data show that a PPMO targeted to a highly conserved, essential gene (*acpP*) has broad bactericidal efficacy across multiple *K. pneumoniae* strains. In addition, the data show that PPMOs can be used in a strategically different way, as an adjunct therapeutic acting as a highly specific beta-lactamase inhibitor, that restores susceptibility to approved antibiotics.

Author Disclosure Block:

E. Sully: None. **L. Li:** None. **S. Bailey:** D. Employee; Self; Sarepta Therapeutics. **M. Wong:** D. Employee; Self; Sarepta Therapeutics. **D.E. Greenberg:** H. Research Contractor; Self; Sarepta Therapeutics. **B.L. Geller:** C. Consultant; Self; Sarepta Therapeutics.

Poster Board Number:

SUNDAY-524

Publishing Title:

Carbapenem-inactivating Effects of L-Cysteine Derived from A549 Cells

Author Block:

H. Takemura, S. Terakubo, N. Okamura, J. Shimada, H. Nakashima; St. Marianna Univ., Kawasaki, Japan

Abstract Body:

Background: At the 52nd ICAAC in San Francisco, CA, in 2012, we reported that antimicrobial activities of several carbapenems (Cps) decreased in the supernatants of the human alveolar epithelial cell line A549. In the subsequent experiments metabolomics analysis of the culture supernatants revealed that they contained L-cysteine (L-cys), which was not among the original contents of the medium, RPMI1640 (RPMI). RPMI contains L-cystine (LC), one of the L-cys oxide, but does not L-cys. L-cys has been reported to hydrolyse imipenem (IPM) concentration dependently. In this study, we investigated how L-cys production by A549 cells causes Cp inactivation.**Methods:** Cells were incubated in RPMI, RPMI supplemented with 10% FCS (F-RPMI), Earle's balanced salt solution (EBSS), and EBSS supplemented with amino acids for 1, 3, 6, 8, and 12 hours at 37 °C in an atmosphere with 5% CO_2. L-cys in the collected culture supernatants were measured by a colorimetric assay using Ellman's reagent. For the assay for Cp inactivation, diluted supernatants were incubated with IPM for 3 hours, and the activities of IPM were detected by an agar diffusion microbiological bioassay with *Micrococcus luteus*. To reveal the contribution of LC in the medium, similar experiments were performed in RPMI without LC.**Results:** L-cys in the A549 RPMI culture supernatants increased time-dependently from 57.1±19.0 μM (n=3) at 1h to 280.0±23.7 μM (n=3) at 8 h. L-cys in the F-RPMI supernatants was about half of that in RPMI. The supernatants exhibited IPM-inactivating effects according to their L-cys contents. The supernatants cultured with EBSS and RPMI without LC did not contain detectable L-cys and did not inactivate IPM while those cultured with the medium containing LC inactivated IPM. The concentration adjusted L-cys control solutions exhibited Cp-inactivating effects consistent with the results.**Conclusions:** The A549 culture supernatants exhibited strong IPM-inactivating effects according to their L-cys contents. As LC in the medium were necessary for the effects, it is speculated that L-cys, the reductive products of LC with the A549, was a major potent contributor to the effects.

Author Disclosure Block:

H. Takemura: None. **S. Terakubo:** None. **N. Okamura:** None. **J. Shimada:** None. **H. Nakashima:** None.

Poster Board Number:

SUNDAY-525

Publishing Title:

Clinical Characteristics & Risk Factors for Acquisition of Less Commonly Isolated (Lci) Carbapenem-Resistant Enterobacteriaceae (Cre): A Case-Case-Control Study

Author Block:

Y. Cai, S-R. Chandra, C. Seah, J. Teo, T-P. Lim, W. Lee, T-H. Koh, T-T. Tan, A-L. Kwa; Singapore Gen. Hosp., SG, Singapore

Abstract Body:

Background: CRE infections are a major healthcare problem. While focus has been on common CRE [*K. pneumoniae* (KP), *E. coli* (EC) & *Enterobacter spp.* (ENT)], infections caused by LCI CRE (e.g. *Citrobacter spp., Serratia spp.*) are increasingly seen. It is unknown if unique risk factors are associated with LCI CRE acquisition. Our study aimed to elucidate factors associated with LCI CRE acquisition & describe outcomes of patients with LCI CRE. **Methods:** A matched case-case-control study was conducted at a tertiary Singapore hospital. Patients admitted from 2013 - 14 with positive LCI (defined as non-KP, EC or ENT) CRE cultures & those with LCI carbapenem-susceptible Enterobacteriaceae (CSE) were compared with a common control group with no Gram negative infections. Case & controls were matched for time at risk & location. Variables analyzed include demographics; comorbidities; healthcare exposure & antibiotic use. Two parallel multivariable logistic regression models were used to identify unique risk factors for LCI CRE. Outcomes of patients with clinically significant LCI CRE & LCI CSE infections were compared. **Results:** 42 patients with LCI CRE [31 (74%) surveillance; 11 (26%) clinical isolates], 42 patients with LCI CSE & 126 controls were included. *Citrobacter spp.* (95%) was the most common LCI CRE. Of the 11 clinical LCI CRE, the most common isolation site was urine (55%) & wound (27%). Half (52%) of LCI CRE patients had previous hospitalization. In the univariate analysis, old age, high SOFA score, previous hospitalization or instrumentation, & exposure to $3^{rd}/4^{th}$ generation cephalosporins & carbapenems were associated with LC CRE acquisition. In multivariable analysis, old age (OR: 1.06, 95% CI: 1.01-1.11) & high SOFA score (OR: 1.21, 95% CI: 1.00-1.46) independently predicted LCI-CRE acquisition. Patients with LCI CRE infe**c**tions had longer post-infection stay (median: 27 days vs. 13 days) & higher in-hospital mortality (18% vs. 7%) when compared to LCI CSE infections, but the differences were not statistically significant. **Conclusion:** Infections caused by LCI CRE are emerging, & may be associated with poorer outcomes. As age & SOFA score predicts LCI CRE acquisition, risk-based screening (i.e. in elderly or critically-ill) may be valuable to limit LCI CRE spread.

Author Disclosure Block:

Y. Cai: None. **S. Chandra:** None. **C. Seah:** None. **J. Teo:** None. **T. Lim:** None. **W. Lee:** None. **T. Koh:** None. **T. Tan:** None. **A. Kwa:** None.

Poster Board Number:

SUNDAY-526

Publishing Title:

OXA-48 and OXA-370 Detection Directly from Spiked Blood Culture Vials and Fecal Swabs

Author Block:

C. S. Nodari[1], A. C. Gales[2], A. L. Barth[1], C. M. Magagnin[1], A. P. Zavascki[1], C. G. Carvalhaes[2]; [1]Hosp. de Clínicas de Porto Alegre, Porto Alegre, Brazil, [2]Univ.e Federal de São Paulo, São Paulo, Brazil

Abstract Body:

Oxacillinases are carbapenemases with low hydrolytic activity against carbapenems. Although many variants have been described worldwide, OXA-48 group is the most relevant among *Enterobacteriaceae.* To date, only OXA-370-producing isolates were reported in Brazil. Recently, an immunochromatographic assay has become commercially available for the detection of OXA-48 and its variants. The aim of this study was to evaluate the performance of OXA-48 K-SeT® (CORIS BioConcept) assay for the detection of OXA-48- and OXA-370-producing isolates directly from spiked blood cultures and stool swabs. A serial ten-fold dilutions obtained from a 0.5 McFarland inoculum of two *K. pneumoniae* strains (one OXA-48 producer and one OXA-370 producer) was obtained from overnight cultured colonies. Each dilution was plated for counting of viable colonies. One mililiter of each dilution was inoculated in sterile blood culture vials (VersaTREK® system), as well as 1 mL of donated human blood. When blood culture vials signalled positive, bacterial pellet was obtained by centrifugation. One mililiter of each bacterial suspension was also inoculated into E-Swab® vials (Copan) containing AMIES media, TSB, and donated stool, and incubated for two hours. The bacterial pellet was obtained by centrifugation. A 10 µL loop of the bacterial pellet was added to 10 drops of LY-LA buffer (TRIS, NaN_3, detergent; pH 7.5). Then, 3 drops of the resulting suspension were placed on the designated spot of the OXA-48 K-SeT cassette and interpreted as recommended by the manufacturer. The inoculum obtained ranged from 10 to 10^5 UFC/mL for both, blood culture vials and E-swabs. We were able to detect OXA-48 and OXA-370 directly from blood culture vials, regardless of the starting inoculum but the differences remained in the time of positivity (from 5 to 10 hours). We were also able to detect the carbapenemases from swabs inoculated with stool, with a sensitivity of 10^4 UFC/mL for both OXA-48 and OXA-370. In summary, the OXA-48 K-SeT assay is a rapid and reliable test for detecting OXA-48 and OXA-370 directly from clinical specimens.

Author Disclosure Block:

C.S. Nodari: None. **A.C. Gales:** None. **A.L. Barth:** None. **C.M. Magagnin:** None. **A.P. Zavascki:** None. **C.G. Carvalhaes:** None.

Poster Board Number:

SUNDAY-527

Publishing Title:

Rapid Detection of Polymyxin Resistance in Enterobacteriaceae; The Missing Tool

Author Block:

L. Poirel, A. Jayol, P. Nordmann; Univ. of Fribourg, Fribourg, Switzerland

Abstract Body:

Background. Enterobacterial strains resistant to polymyxins are increasingly reported worldwide. Currently available polymyxins susceptibility methods are fastidious, time-consuming (24 h) and some methods are not reliable. They are poorly adapted to the clinical need and to the prevention of the dissemination of those multidrug resistant isolates. Therefore, we have developed the Rapid Polymyxin NP test that is rapid, reliable and cost-effective detect polymyxin resistant *Enterobacteriaceae.* **Materials/methods.** The Rapid Polymyxin NP test is based on the detection of bacterial growth in presence of a defined concentration of colistin (or polymyxin B) meaning colistin resistance in a well-defined medium. Growth is evidenced by acid formation related to glucose metabolism (aerobic and anaerobic) observed through a color change (orange to yellow) of a pH indicator (red phenol). A total of 196 enterobacterial strains from varied species were used to evaluate the performance of the Rapid Polymyxin NP test. Five strains were intrinsically resistant to colistin, 152 strains had an acquired mechanism of resistance to colistin, and 39 strains were susceptible to colistin. For 93 *Klebsiella* spp. isolates, resistance to colistin was associated to chromosomic alterations in genes modifying the LPS. For 10 *E. coli* isolates, resistance to colistin was mediated by the plasmid-borne *mcr-1* gene. The mechanism of colistin resistance was unknown for the remaining enterobacterial isolates. MICs of polymyxins were determined using the reference broth microdilution technique according to the CLSI guidelines and results were interpreted according to the breakpoints of the EUCAST.**Results.** The sensitivity and the specificity of the Polymyxin NP test were excellent, being 99.3 and 92.3 %, respectively, as compared to the broth microdilution taken as the gold standard. It was rapid (less than 2h) and reproducible.**Conclusion.** The Rapid Polymyxin NP test combines multiple advantages. It is easy to perform, rapid, reliable, cost-effective, sensitive, specific and implementable worldwide. It detects polymyxin-resistant enterobacterial strains from any species regardless the molecular mechanism of resistance to polymyxins (intrinsic, chromosomic and/or plasmid-mediated).

Author Disclosure Block:

L. Poirel: None. **A. Jayol:** None. **P. Nordmann:** None.

Poster Board Number:

SUNDAY-528

Publishing Title:

Modifying the Structure of a Membrane-targeting Small Molecule Antibiotic Alters Its Mechanism of Action

Author Block:

K. Hurley, V. Heinrich, T. Santos, D. Weibel; Univ. of Wisconsin, Madison, Madison, WI

Abstract Body:

The bacterial membrane is a clinically validated antibacterial target, however FDA-approved antibiotics that target the membrane are largely ineffective against gram-negative organisms. Developing chemotherapeutic agents that target the cellular membrane of both gram-positive and gram-negative bacteria can expand the clinical significance of this target. 2-((3-(3,6-dichloro-9*H*-carbazol-9-yl)-2-hydroxypropyl)amino)-2-(hydroxymethyl)propane-1,3-diol (DCAP) and its hydrophobic analog isopentyl-DCAP (i-DCAP) are broad-spectrum, membrane-targeting small molecules that increase the permeability of the membrane. Analogous to some membrane-active antimicrobial peptides, the antibacterial activity of DCAP and i-DCAP is antagonized by divalent cations (e.g., Mg^{2+} and Ca^{2+}). Using epifluorescence microscopy, we observed the temporal and local characteristics of membrane permeability after treatment of *E. coli* MG1655 cells with DCAP and i-DCAP. We discovered that cell lysis occurred after treatment with DCAP, however this phenotype was not observed after treatment with i-DCAP. Cells that became permeable after treatment with DCAP were predominantly dividing and contained a septum. After treatment with i-DCAP, cells from all phases of growth and division became permeable. Liposomal calcein release assays with liposomes constructed of various phospholipid compositions established that DCAP and i-DCAP disrupt artificial lipid bilayers of all bacterial phospholipids. We are currently investigating the effect of DCAP and i-DCAP on the membrane fluidity of the phospholipid bilayer in vivo using FRAP (fluorescence recovery after photobleaching). We are also pursuing computational modeling studies with stimulated phospholipid bilayers to determine the depth of insertion of DCAP and i-DCAP into the membrane. The DCAP-family of antibiotics may be an effective scaffold for developing chemotherapeutic agents that target the cellular membrane of both gram-negative and gram-positive bacteria.

Author Disclosure Block:

K. Hurley: None. **V. Heinrich:** None. **T. Santos:** None. **D. Weibel:** None.

Poster Board Number:

SUNDAY-529

Publishing Title:

Class A and D Carbapenemases Can be Differentiated by Indirect Tris/Edta (Te) Disk Testing with Boronic Acid

Author Block:

K. S. Thomson, J. W. Snyder, G. K. Thomson; Univ. of Louisville, Louisville, KY

Abstract Body:

Background: Convenient tests are needed to detect isolates producing OXA-48 and other class D carbapenemases. The lack of OXA-48-producing isolates available to US labs makes it difficult to evaluate OXA-48 detection tests, but a small 2015 US study showed that indirect tests with TE disks (BD Diagnostics) detected OXA-48 carbapenemases but did not differentiate them from class A carbapenemases (1). BA inhibits AmpCs and class A but not OXA (class D) carbapenemases. Therefore a study was designed to investigate whether the Indirect TE disk test could be modified by incorporating an additional TE disk containing BA (TEBA) to differentiate between AmpCs and class A vs class D carbapenemases.**Methods:** The isolates were 10 *K. pneumoniae* isolates producing OXA-48-like carbapenemases, 5 OXA-23-producing *A. baumannii*, 26 *Enterobacteriaceae* (EBE) producing the class A carbapenemases KPC-2, -3, -4, KPC-like, NMC-A, or SME-like, 8 EBE and 1 *P. aeruginosa* producing high level AmpC, 4 EBE and 1 *A. baumannii* MBL producers, and 2 EBE producers of a class A and B carbapenemase. The Indirect TE test was performed on a Mueller-Hinton agar plate inoculated with a lawn of *E. coli* ATCC 25922. A TE and a TEBA disk (containing 200 μg BA) were inoculated heavily with the test organism and positioned with the inoculated side in contact with the agar 1 mm to either side of an imipenem disk. After overnight incubation at 35^{o}C, an indentation of the inhibition zone near an inoculated TE disk indicated a positive test. No indentation indicated a negative test.**Results:** All 15 OXA carbapenemase producers were positive with both TE and TEBA disks. All 26 class A carbapenemase-producing EBE were positive with TE disks but negative with TEBA disks. One AmpC producer was positive in TE disk tests but all 9 AmpC producers were negative with TEBA disks. As expected, MBL producers yielded mixed results with one isolate positive with both disks and 4 isolates negative with both disks.**Conclusions:** Indirect TE tests differentiated between producers of class D carbapenemases vs AmpCs and Class A carbapenemases. Variable results with MBL producers indicated that this test is unsuitable for MBL-positive isolates. A limitation of the study was the small number of class D carbapenemase producers but the promising results suggested that further testing of a larger number of OXA carbapenemase producers is warranted.

Author Disclosure Block:

K.S. Thomson: C. Consultant; Self; BD Diagnostics. **J.W. Snyder:** None. **G.K. Thomson:** None.

Poster Board Number:

SUNDAY-530

Publishing Title:

Salicylate Induced Antibiotic Resistance Through the *Marrab*-Independent Pathway

Author Block:

T. Wang, I. El Meouche, M. J. Dunlop; The Univ. of Vermont, Burlington, VT

Abstract Body:

It has long been known that salicylate can induce antibiotic resistance by inhibiting MarR binding with the *marRAB* promoter and further activating MarA and its downstream genes related to resistance. Salicylate imposes a significant cost, slowing cell growth. This cost is traditionally attributed to expression of MarA-activated downstream resistance genes. Here, we show that salicylate can also induce equal levels of antibiotic resistance in *E.coli* through a *marRAB*-independent and *marRAB*/*rob*/*soxRS*-independent pathway. Unlike the findings from previous studies with salicylate, we find MarA activation to be a low-cost process. In order to study the cost of antibiotic resistance we established a genomic model which has MarR binding site mutations in *marRAB* promoter. We find that, unlike data obtained with salicylate treated cells, the genomic MarR binding inhibition model has a significantly higher minimum inhibitory concentration (MIC) for carbenicillin without a detectable cost of resistance. Our research indicates that the *marRAB*-independent pathway plays an important role in salicylate induced resistance. Furthermore, we demonstrate that the *marRAB* system is highly efficient, induce high levels of antibiotic resistance without detectable cost.

Author Disclosure Block:

T. Wang: None. **I. El Meouche:** None. **M.J. Dunlop:** None.

Poster Board Number:

SUNDAY-531

Publishing Title:

Mechanism Of Resistance In Carbapenem-Resistant Enterobacteriaceae Isolates At A Single Center In Northern California

Author Block:

C. Truong[1], N. Watz[2], I. Tickler[3], I. Budvytiene[2], N. Amiali[1], F. Tenover[3], N. Banaei[1]; [1]Stanford Univ. Sch. of Med., Stanford, CA, [2]Stanford Hlth.Care, Stanford, CA, [3]Cepheid, Sunnyvale, CA

Abstract Body:

The mechanism of carbapenem-Resistant Enterobacteriaceae (CRE) resistance is commonly due to carbapenemases and/or high-level of expression of ampC and ESBL coupled with altered outer membrane porins (omp). The underlying mechanism of resistance may have therapeutic implications for the newer β-lactam combination drugs. The aim of this study was to characterize the underlying mechanism of resistance in CRE isolates at our institution.From 2013-2015, all CRE isolates at Stanford Health Care meeting the pre-2015 CDC definition were included in this study. MICs for imipenem, meropenem, and ertapenem were established using MicroScan and confirmed by Etest. Real-time PCR and CheckPoint assay were used to detect carbapenemase (KPC, VIM, IMP, NDM-1, OXA-48 like) and cephalosporinase (ESBL and ampC) genes, respectively. Reverse transcriptase real-time PCR was used to quantify expression of *ompC* and *ompF* and their analogs. In total, 45 CRE infections were detected (11 in 2013, 14 in 2014, 20 in 2015). Carbapenemase genes could be detected in 38% (17/45) and included 4 KPC, 5 NDM, 1VIM, 2 IMP, and 5 OXA-48 like. In CREs characterized thus far, 69% (9/13) and 15% (2/13) of carbapenemase-positive isolates and 33% (9/27) and 15% (4/27) of carbapenemase-negative isolates were positive for ESBL and ampC, respectively. *ompC* and *ompF* expression were down regulated at least 2-fold in 0% (0/14) and 50% (7/14) of isolates with carbapenemase genes compared with 56% (10/18) and 56% (10/18) of isolates without a carbapenemase gene (78% [14/18] showed down regulation of either *ompC* or *ompF*), respectively.In summary**,** majority of CRE isolates at our institution do not encode a carbapenemase gene. Downregulation of omp is consistent with a porin loss in majority of carbapenemase-negative isolates.

Author Disclosure Block:

C. Truong: None. **N. Watz:** None. **I. Tickler:** None. **I. Budvytiene:** None. **N. Amiali:** None. **F. Tenover:** None. **N. Banaei:** None.

Poster Board Number:

SUNDAY-532

Publishing Title:

Avibactam (AVI) Inactivation of PER-2 β-Lactamase: Exploring the Landscape of Diazabicyclooctane (DBO) Inhibition

Author Block:

M. Ruggiero[1], K. M. Papp-Wallace[2], G. Gutkind[1], R. A. Bonomo[2], **P. Power**[1]; [1]U. Buenos Aires, Buenos Aires, Argentina, [2]Cleveland VAMC, CWRU, Cleveland, OH

Abstract Body:

Background: PER β-lactamases are prevalent in South America and the Middle East and readily hydrolyze oxyimino-cephalosporins. This enhanced hydrolytic activity may result from the presence of a unique fold in the Ω loop and in the β3 strand. These structural differences enlarge the active site entrance by 2-fold compared to other class A β-lactamases. PER-2 possesses higher catalytic efficiency towards ceftazidime (CAZ) compared to PER-1 (~22-fold higher). Examination of the apo-structure reveals that the H-bonding (HB) network between S70-Q69-HOH-T237-R220 may also play a role in their different catalytic profiles. AVI is a reversible DBO β-lactamase inhibitor (BLI) that inactivates Ambler class A and C β-lactamases. Our goal was to assess the ability of AVI to inhibit PER-2, restore susceptibility to clinical strains when combined with CAZ, ceftaroline (TAR), and aztreonam (ATM) and to probe the mechanism of inhibition. **Methods:** MIC testing was conducted using clinical isolates of *Enterobacteriaceae* (Ent) possessing PER-2 using ampicillin (AMP), AMP-AVI, CAZ, CAZ-AVI, TAR, TAR-AVI, ATM, and/or ATM-AVI. AVI inhibition constants (k_2/K) were determined using nitrocefin as a reporter substrate. Modeling of PER-2 with AVI was conducted with Autodock Vina and Yasara.**Results:** Ent possessing PER-2 demonstrated high MICs to CAZ (≥ 512 μg/ml), TAR (16-512 μg/ml), and ATM (≥ 128 μg/ml). When CAZ, TAR, and ATM were combined with AVI, only the TAR-AVI combination restored susceptibility (MICs ≤1 μg/ml) for all isolates. The k_2/K value for AVI was 2,000 $M^{-1}s^{-1}$, similar to AmpC inactivation constants (10^3 $M^{-1}s^{-1}$ range), but 100-fold less than that for other class A β-lactamases (e.g., TEM-1 and CTX-M-15), and 10-fold less than KPC-2. As observed in other class A β-lactamases, HB interactions between AVI and S130, E166, T235, and T237 are postulated. The Q69, H170, N132 and T104 residues are predicted to form hydrophobic interactions with AVI.**Conclusions:** Compared to CAZ and ATM, AVI combined with TAR effectively lowers MICs vs. Ent. The unique structural features of the PER active site and new interactions formed impact AVI inhibition of PER-2 and likely effect the deacylation of the AVI-PER-2 complex.

Author Disclosure Block:

M. Ruggiero: None. **K.M. Papp-Wallace:** E. Grant Investigator; Self; AstraZeneca, Actavis, Merck, Wockhardt. **G. Gutkind:** None. **R.A. Bonomo:** E. Grant Investigator; Self; AstraZeneca, Actavis, Merck, Wockhardt, GSK. **P. Power:** E. Grant Investigator; Self; Actavis.

Poster Board Number:

SUNDAY-533

Publishing Title:

Potentiating Fluoroquinolone Bactericidal Activity by Increasing Reactive Oxygen Species Production in Quinolone-Resistant *Escherichia coli*

Author Block:

J. Machuca[1], E. Recacha[1], P. Diaz de Alba[1], M. Ramos-Guelfo[1], Á. Pascual[1], J. Rodríguez-Martínez[2]; [1]Univ. Hosp. Virgen Macarena, Seville, Spain, [2]Univ. of Seville, Seville, Spain

Abstract Body:

Background: Bactericidal antibiotics, including fluoroquinolones (FQ), share a common mechanism of action involving the Reactive Oxygen Species (ROS) as part of its bactericidal power. The aim of this study was to evaluate the contribution of ROS overproduction in the bactericidal activity of microorganisms with acquired FQ resistance mechanisms.**Methods:** *E. coli* ATCC 25922 and three isogenic mutants harbouring chromosomal- and/or plasmid-mediated quinolone resistance mechanisms were used as background strains [EC04: S83L (*gyrA*) + S80R (*parC*); EC09: S83L-D87N (*gyrA*) + S80R (*parC*) + Δ*marR*; EC59: S83L-D87N (*gyrA*) + S80R (*parC*) + Δ*marR* + *qnrS1*]. Derived strains containing deletion in *sdhC* (Succinate dehydrogenase complex subunit C) or *cyoA* (Cytochrome oxidase subunit II) genes that increase ROS were obtained by chromosomal inactivation. MICs of FQs [ciprofloxacin (CIP), levofloxacin, moxifloxacin, norfloxacin, ofloxacin (OFX)] and nalidixic acid were determined by microdilution. Frequency of mutants was calculated at 4xMIC of CIP and OFX, 1 mg/L of CIP and 2 mg/L of OFX for selected strains. Time-kill curves were determined in Mueller-Hinton broth at CIP and OFX concentration of 2xMIC values. Growth curves of each strain in LB and M9 were drawn (with and without FQs).**Results:** Deletion in sdhC and cyoA genes did not affect the MICs of FQ in any E. coli genotype. No significant increase in the rate of mutation was observed in tested strains (ATCC 25922, EC04 and derived strains with deletion in sdhC or cyoA genes). For both quinolones evaluated, killing-curves assays showed a significant increased killing for strains with deletion in sdhC gene (sdhC inactivation reduced the survival in 1 - 2 log10 at 4 hour). cyoA inactivation effect on bacterial survival was lower than that of sdhC inactivation (0.3 - 1 log10 at 4 hour). The growth curve of strains with sdhC inactivation in M9 medium without antimicrobial showed a shorter lag phase compared to wild-type strains. This effect was not observed in LB medium.**Conclusions:** Inactivation of *sdhC* gene potentiates the killing of *E. coli* with acquired quinolone resistance mechanisms by FQ. Potentiation of ROS production might be used as adjuvant therapy of FQ use.

Author Disclosure Block:

J. Machuca: None. **E. Recacha:** None. **P. Diaz de Alba:** None. **M. Ramos-Guelfo:** None. **Á. Pascual:** None. **J. Rodríguez-Martínez:** None.

Poster Board Number:

SUNDAY-534

Publishing Title:

Characterisation Of A Tigecycline Specific Abc Transporter Isolated From A Human Saliva Metagenomic Library

Author Block:

L. J. Reynolds[1], M. F. Anjum[2], A. P. Roberts[1]; [1]UCL Eastman Dental Inst., London, United Kingdom, [2]Animal and Plant Hlth.Agency, London, United Kingdom

Abstract Body:

Background: Tigecycline is a novel glycylcycline that has been developed for the treatment of infections caused by antibiotic resistant pathogens including MRSA and *Acinetobacter baumannii*. Although the antibiotic is able to overcome most tetracycline resistance mechanisms, resistance to this antibiotic has been described.**Methods:** A human saliva metagenomic library was created in *E. coli* EPI300 using pCC1BAC. The library was screened for the presence of tetracycline resistant clones by growing them on 5 µg/ml tetracycline. Plasmid inserts from clones of interest were sequenced to determine any open reading frames (ORFs) were present. To determine the genes responsible for observed resistance phenotypes subcloning and site directed mutagenesis was employed.**Results:** 28,000 clones from the human saliva metagenomic library were screened to identify any tetracycline resistant members. A tetracycline resistant clone that also had reduced susceptibility to tigecycline was isolated. The plasmid isolated from the clone contained a 7,766 base pair insert with five ORFs, two of which encoded putative ABC transporters. These two genes were shown to confer resistance to tetracycline and tigecycline indicating their products created a heterodimeric ABC transporter. Mutants with deleted Walker A motifs were unable to confer resistance to these antibiotics.**Conclusions:** Screening of the metagenomic library revealed a clone exhibiting high levels of tetracycline and tigecycline resistance. Two genes encoding a putative heterodimeric ABC transporter were shown to be solely responsible for this phenotype. Walker A motifs are required for ABC transporter functionality and deletion of these regions resulted in a loss of resistance indicating that an ABC transporter is responsible for the observed phenotype.

Author Disclosure Block:

L.J. Reynolds: None. **M.F. Anjum:** None. **A.P. Roberts:** None.

Poster Board Number:

SUNDAY-535

Publishing Title:

Elucidating the Mechanisms of *Staphylococcus aureus* Pathogenesis During Urinary Tract Infections

Author Block:

J. Walker, A. Flores-Mireles, C Pinkner, HL. Schreiber, MG Caparon, and SJ Hultgren; Washington Univ. Sch. of Med., St. Louis, MO

Abstract Body:

Background: *Staphylococcus aureus* is a cause of urinary tract infections (UTI) in high-risk patient populations, with catheterized patients particularly susceptible. Importantly, methicillin-resistant *S. aureus* (MRSA) bacteriuria in high-risk populations has a high rate of dissemination to bacteremia and is a significant cause of shock following bacteremic UTI. *S. aureus* invasive infections, such as bacteremia, are associated with significant morbidity and mortality. Although invasive infections have been under intense research, few studies assess how MRSA enters the bloodstream to cause severe disease.**Results:** To address important gaps in understanding the mechanisms of MRSA dissemination, we adapted a mouse model of catheter-associated UTI (CAUTI). It is well established that catheterization leads to bladder tissue damage in patients and recent studies of mouse models of CAUTI indicate damage induced by the catheter triggers the release of fibrinogen (Fg) in the bladder. Further, we conducted a small clinical study assessing catheters from 50 patients undergoing catheterization as part of a standard of care and confirmed Fg was deposited on catheters, replicating what we see in the mouse model. Importantly, in our adapted MRSA CAUTI model **i)** MRSA required a catheter to establish persistent UTI; **ii)** Fg accumulated in the bladder and subsequently coated the catheter; **iii)** MRSA co-localized with Fg deposited on catheters; and **iv)** MRSA rapidly disseminated to bacteremia and subsequently colonized the spleen and heart. Further, *in vitro* data shows MRSA grown in human urine supplemented with Fg produces a thick, extracellular-like matrix via MRSA-Fg interactions, potentially capable of providing protection from the host immune system and antibiotics during CAUTI. Importantly, two patients enrolled in our clinical study had positive *S. aureus* urine cultures prior to their procedures. Surprisingly, even after appropriate antibiotic treatment, *S. aureus* was detected on these patient's catheters and the pathogen co-localized with Fg.**Conclusions:** These studies indicate the catheterized bladder is an ideal environment for MRSA infection and will provide a better understanding of MRSA dissemination in order to develop more effective treatment options to prevent progression of CAUTI to invasive disease.

Author Disclosure Block:

J. Walker: None.

Poster Board Number:

SUNDAY-536

Publishing Title:

Acute Injury to Internal Organs During Staphylococcal Infective Endocarditis

Author Block:

K. Kulhankova, J. M. King, K. Gibson-Corley, C. Stach, B. Vu, W. Salgado-Pabon; Univ. of Iowa, Iowa City, IA

Abstract Body:

Staphylococcal infective endocarditis (SIE) is a serious complication of hospital- and community-acquired staphylococcal infections. Clinical course of SIE is accompanied by acute damage to deep tissues and organ systems that manifests in about 40-70% of cases. These systemic complications are a primary cause of death in SIE patients. In humans, pathogens as *Staphylococcus aureus*, viridans streptococci, and enterococci induce different types of complications during IE. However, whether different *S. aureus* strains with varying repertoire of virulence factors differentially induce systemic complications is not known. Utilizing a rabbit model of IE and the four clinical *S. aureus* isolates with different superantigen (SAg) and hemolysin profiles, we investigated the gross pathology and histological characteristics of various organs during course of the disease. IA116 (α-toxin^{HI}) and IA209 (β-toxin^{HI}) are two current USA100 isolates that produce SAgs at low level. IA1471 (staphylococcal enterotoxin C, SEC^{HI}) and IA1871 (SEC negative) are two current USA600 isolates with low-level of hemolysins. Diffuse liver damage was significantly greater in rabbits infected with IA116 and IA209 compared to IA1471, consistent with their significantly greater production of hemolysins. On the other hand, IA1471, which expresses SEC, but low levels of hemolysins, resulted in a significantly greater acute lung damage and greater lethality. Acute kidney injury tended to be more severe in rabbits infected with IA116 - a high α-toxin and PSMs producer, compared to IA209. Altogether, these data provide evidence for the differential role of SEC and hemolysins in acute multi-organ injury during SIE. Elucidating the role of *S. aureus* genotypic and phenotypic characteristics in pathogenesis of clinical IE complications will further our understanding of the role of virulence factors in organ-tropism during complicated SIE.

Author Disclosure Block:

K. Kulhankova: None. **J.M. King:** None. **K. Gibson-Corley:** None. **C. Stach:** None. **B. Vu:** None. **W. Salgado-Pabon:** None.

Poster Board Number:

SUNDAY-537

Publishing Title:

Overexpression of Heat Shock Protein 70 Reduces *Chlamydia* Induced Pathology in Mice in a Tissue-Specific Fashion

Author Block:

W. Li, K. Vlcek, J. Do, A. Seetharaman, M. Ciancio, K. Ramsey, A. K. Murthy; Midwestern Univ., Downers Grove, IL

Abstract Body:

Chlamydia trachomatis genital infection leads to severe pathological consequences including hydrosalpinx (fluid-filled oviduct dilatation), pelvic inflammatory disease and infertility in a subset of untreated women. In the mouse model, a variety of host immunological mediators have been implicated in pathogenesis. Therefore, we hypothesized that over-expression of heat shock protein 70 (Hsp70), an evolutionarily conserved stress-induced protein with significant cytoprotective and immunoregulatory activities, would protect against *Chlamydia*-induced oviduct pathology in mice. C57BL/6 mice with Hsp70 transgene under the control of villin promoter were generated. The villin promoter is active only in the ciliated columnar epithelium of the mouse oviduct but not in other parts of the female mouse genital tract. HSP70 levels in transgenic (Tg) mice oviduct tissue were higher than that in non-transgenic mice. Six- to ten-week old female Hsp70 Tg and non-transgenic (NTG) littermates (n=12) were infected intravaginally with $5x10^4$ IFU of *C. muridarum*. Vaginal chlamydial shedding and chlamydial burden in the upper genital tract, and serum antibody levels were comparable between Hsp70 Tg and NTG mice. However, *Chlamydia*-specific cytokine production (IFN-γ, TNF-α, and IL-17) from medial iliac lymph nodes were significantly reduced in Hsp70 Tg mice. Hsp70 Tg mice displayed significant reduction in incidence and severity of oviduct dilation, not uterine horn pathology, at 80 days after bacterial inoculation when compared to NTG mice. In summary, these results suggest that Hsp70 over-expression in the oviduct epithelium leads to reduced antigen-specific cytokine production and to reduced oviduct pathology following chlamydial infection. These results support further evaluation of Hsp70 as a target to reduce clinically relevant *Chlamydia*-induced pathology.

Author Disclosure Block:

W. Li: None. **K. Vlcek:** None. **J. Do:** None. **A. Seetharaman:** None. **M. Ciancio:** None. **K. Ramsey:** None. **A.K. Murthy:** None.

Poster Board Number:

SUNDAY-538

Publishing Title:

Impact of Trauma on *Streptococcus pyogenes* Dissemination

Author Block:

L. Lamb[1], C. Scudamore[2], N. N. Lynskey[1], L. Tan[1], C. E. Turner[1], S. Sriskandan[1]; [1]Imperial Coll., London, United Kingdom, [2]MRC, Oxford, United Kingdom

Abstract Body:

Background: Historically, invasive infections caused by *S.pyogenes* or group A streptococcus (iGAS) were associated with penetrating trauma during combat until the introduction of penicillin. More recently, cases of severe iGAS and toxic shock have been reported in healthy individuals subjected to blunt trauma. We aimed to determine if contusion injury enhances the development or dissemination of iGAS systemically. **Methods:** A murine model of mild blunt contusion was developed and characterized. The effects of soft tissue contusion were assessed on a model of bacteremia and soft tissue infection with *S.pyogenes*. Bacterial phenotypic changes observed after contusion were investigated by sequencing. **Results:** Application of a 15.7mJ force resulted in histological changes in muscle consistent with mild contusion with no bony injury and minimal cytokine response. In a mouse model of bacteremia, blunt contusion did not lead to specific seeding of contused tissue and had no effect on bacterial burden or cytokine response. In a mouse model of soft tissue infection, blunt contusion was associated with increased dissemination of *S.pyogenes* to ipsilateral draining lymph node, and linked to an increase in a stable mucoid phenotype in both lymph node and spleen. Hyaluronan production wasenhanced in these colonies. Whole genome sequencing of mucoid colonies from spleen demonstrated a mutation in the global regulator covRS, and from lymph node an unexpected deletion in the promoter region of the *hasABC* capsule operon. *HasABC* promoter GAS mutants demonstrated a clearly enhanced ability to disseminate to local lymph node, consistent with a recently described interaction between hylauronan and the lymphatic endothelial receptor (LYVE-1). **Conclusions:** Contusion enhanced lymphatic dissemination of *S.pyogenes* to local lymph nodes. Potentially contusion leads to damaged lymphatic endothelium and enhanced exposure of GAS to LYVE-1. Whether lymphatic transit or local inflammation select for hyperencapsulated GAS mutants is uncertain. Although the findings do not rule out the possibility of seeding following more severe blunt trauma, mild contusion appears likely to impact on lymphatic spread. The study highlights a possible mechanism for interaction between contusion and *S. pyogenes* virulence.

Author Disclosure Block:

L. Lamb: None. **C. Scudamore:** None. **N.N. Lynskey:** None. **L. Tan:** None. **C.E. Turner:** None. **S. Sriskandan:** None.

Poster Board Number:

SUNDAY-539

Publishing Title:

Bacterial Presence and Histopathology in an Experimental Animal Model of Chronic *Propionibacterium acnes* Foreign Body Infectionhistopathology in a Prolonged Infection Model

Author Block:

H. Tyner[1], K. Greenwood Quaintance[2], R. Patel[2]; [1]Mayo Clinic Sch. of Graduate Med. Ed., Rochester, MN, [2]Mayo Clinic, Rochester, MN

Abstract Body:

Background: *Propionibacterium acnes* is a common contaminant in surgical specimens, but can also cause foreign body (especially shoulder arthroplasty) infection. *P. acnes* prosthetic joint infection is often not associated with elevated inflammatory markers or other classic features of infection.**Methods**: We developed a new rat model of chronic *P. acnes* foreign body infection wherein threaded stainless steel Kirschner wires were infected with *P. acnes* by allowing a *P. acnes* biofilm to form on the wires prior to surgical implantation into the tibia of rats. At 3 and 6 week time points, 9 rats were sacrificed, 2 uninfected rats and 7 infected rats per time point. After the surgically manipulated tibias were removed, bone within 5 mm of the implanted wire (one uninfected and 5 infected) was weighed, crushed, and quantitatively cultured. The remaining bones (one uninfected and 2 infected for each time point) were sent for histopathology to evaluate for evidence of inflammation and to undergo Brown and Brenn Gram staining.**Results**: At week 3, *P. acnes* was recovered from 5 of 5 infected bones (mean, range: 4.80, 1.64-6.15 $\log_{10}$cfu/g bone), and at week 6, *P. acnes* was recovered from 4 of 5 infected bones (mean, range: 2.81, 0-5.08 $\log_{10}$cfu/g bone). Histopathologically, infected 3 week bones showed no evidence of inflammation and bacterial stains were negative. One infected six week bone stained positive for many Gram-positive rods and the other showed no organisms; however, neither showed evidence of inflammation.**Conclusion**: In an experimental animal model of chronic foreign body infection, *P. acnes* can persist over time. A lack of inflammation was observed on histopathological examination.

Author Disclosure Block:

H. Tyner: None. **K. Greenwood Quaintance:** None. **R. Patel:** None.

Poster Board Number:

SUNDAY-540

Publishing Title:

Abnormal Intestinal Function in Kittens Experimentally Infected with Enteropathogenic *E. coli*

Author Block:

V. E. Watson, M. Jacob, J. Bruno-Barcena, J. L. Gookin; North Carolina State Univ., Raleigh, NC

Abstract Body:

Infectious diarrhea is a leading cause of death in children and kittens. In children, enteropathogenic *E.coli* (EPEC) is a common cause of diarrhea and is associated with an increased risk of mortality. We demonstrated that sick kittens dying in animal shelters have colonization of the intestinal epithelium by EPEC and PCR amplification of the intimin gene (*eae*) from fecal DNA is significantly associated with diarrhea. We hypothesize that EPEC is an important cause of intestinal dysfunction and diarrhea in kittens.Sixteen purpose-bred kittens were randomized to receive antibiotic-induced dysbiosis or no antibiotics prior to infection with 3 different serotypes of atypical EPEC (10^8 CFU each) obtained from kittens with diarrhea. To establish an infectious dose, the EPEC burden of naturally infected kittens with diarrhea was estimated by establishing a qPCR assay relating amplification of *eae* from fecal DNA to CFU of EPEC. Survival of EPEC following gavage was estimated by quantitative culture of each isolate in a simulated gastric environment. Following experimental infection, feces were cultured and isolated *E. coli* confirmed as EPEC by *eae* amplification. The effect of EPEC infection on intestinal absorption of D-xylose and permeability to iohexol were quantified and diarrhea established on the basis of fecal frequency, consistency score, and wet weight.Efficiency of *eae* qPCR for estimating fecal CFU of EPEC was 110 ± 4.7%. *Eae* qPCR of fecal DNA from 34 shelter kittens with diarrhea estimated carriage of 4×10^8 ($\pm 1.6 \times 10^8$) CFU of EPEC per 100 g of feces. No loss of EPEC was observed upon culture in a simulated gastric environment. After experimental infection, EPEC was cultured from feces of all kittens. EPEC infection led to significant malabsorption of D-xylose and a significant decrease in intestinal permeability to iohexol. In kittens with antibiotic induced dysbiosis prior to infection, EPEC significantly increased fecal wet weight at 3 days post infection ($p<0.01$;t-test).This study is the first to demonstrate that EPEC infection is sufficient to cause a significant decrease in intestinal absorption in kittens. Dysbiosis prior to infection increases the impact of EPEC on fecal water content. These results suggest a significant effect of EPEC on intestinal function in kittens and a role for intestinal bacterial dysbiosis in susceptibility to diarrhea.

Author Disclosure Block:

V.E. Watson: None. **M. Jacob:** None. **J. Bruno-Barcena:** None. **J.L. Gookin:** None.

Poster Board Number:

SUNDAY-541

Publishing Title:

Investigating the Relationships between Virulent *Dichelobacter nodoosus*, Interleuken-1-Beta (IL1β) and Histopathology Lesions in Footrot

Author Block:

M. Agbaje, G. Maboni, M. Bexon, C. S. Rutland, M. A. Jones, S. Tötemeyer; Univ. of Nottingham, Loughborough, United Kingdom

Abstract Body:

Background: Footrot is the major cause of lameness and a predominant welfare and economic concern for sheep farmers worldwide. Footrot is characterized by the separation of the hoof from the skin and this morphologic pathology has been attributed to the host immune response rather than the bacterial aetiology. In a study, IL1β (pro-inflammatory cytokine) was reported to have an increased expression in footrot ovine feet in comparison with healthy feet. However, the role of the innate immune system in footrot remains unclear. Virulent *Dichelobacter nodosus* is the causative agent of underrunning footrot. The aim of this study was to investigate the relationships between virulent *D. nodosus*, expression of the pro-inflammatory cytokine IL1β and histopathological lesions in footrot. **Methods:** From 55 healthy and 30 footrot ovine feet, two sets of parallel biopsies (one fixed for histology, one stored in RNA later for total DNA and RNA extraction) were collected post-slaughtered from an abattoir. H&E stained sections were analysed for histological lesions, virulent *D. nodosus* load quantified by qPCR and IL1β mRNA expression was determined by RT-qPCR. **Results:** We observed that significantly higher levels of IL1β corresponded with increased inflammatory cell infiltration score in footrot ($p<0.05$) but not in healthy biopsies. However, virulent *D. nodosus* load as well as histological lesions including cell ballooning, parakeratosis, epidermal micro-abscesses and inflammatory cell infiltration were similar in healthy and footrot conditions. **Conclusions:** This suggests, while virulent *D. nodosus* is the primary aetiology of footrot, other factors contribute to the exacerbation of the host immune response leading to footrot lesions. Also, the presence of virulent *D. nodosus* as well as histological lesions observed in the healthy ovine feet with no clinical symptoms of footrot may indicate on-going subclinical infections or early stages of infection.

Author Disclosure Block:

M. Agbaje: None. **G. Maboni:** None. **M. Bexon:** None. **C.S. Rutland:** None. **M.A. Jones:** None. **S. Tötemeyer:** None.

Poster Board Number:

SUNDAY-542

Publishing Title:

Development of an *In Vitro* Wound Model of Infection to Evaluate the Effect of Topical Application of Antimicrobials

Author Block:

B. L. Price, C. B. Dobson; Univ. of Manchester, Manchester, United Kingdom

Abstract Body:

Background: A relevant and reliable model for infected wounds colonised with biofilm has not yet been established. We have previously developed a collagen gel *in vitro* model for a chronic diabetic foot ulcer contaminated with a *P. aeruginosa* or *S. aureus* biofilm. In the present study we have further refined the model by introducing mammalian cells into the matrix, enabling release of cytokines and other inflammatory markers to be measured in response to microbial colonisation. **Methods:** Collagen gel matrices seeded with human neonatal foreskin fibroblasts were prepared containing central 'voids' bathed in media. Cells were incubated for five days, before models were inoculated with *Pseudomonas aeruginosa* PA01 and incubated for one day. The model was then incubated with calcium sulfate beads containing gentamicin for one day. Following incubation bacteria from the model were counted. We confirmed characteristics indicative of biofilm formation and the growth of mammalian cells was confirmed using fluorescent microscopy. Mammalian cell biochemical responses were investigated using Luminex targeting specific cytokines.**Results:** Histology confirmed the production of bacterial polysaccharide matrix while the presence of microcolonies within the matrix indicated growth of a biofilm. The biofilm was visualised within the collagen matrix by SEM. The biofilm showed decreased susceptibility to antimicrobials. Fluorescent microscopy identified fibroblasts growing within the collagen matrix and Luminex confirmed the presence of pro-inflammatory cytokines in the culture medium in response to infection. After one day of incubation with gentamicin loaded calcium sulfate beads, no bacterial colonies were recovered from the wound model.**Conclusions:** We have successfully developed an *in vitro* collagen wound model combined with fibroblasts, to assess biofilm colonisation of wounds confirmed using a range of techniques. We have demonstrated with this model that both biofilm colonisation of a wound and the resulting early inflammatory events can be modelled using this system. Addition of calcium sulfate beads combined with antibiotics directly to a novel biofilm wound model clears an established biofilm. This approach may offer an alternative clinical strategy for patients who are not responding to oral antibiotics.

Author Disclosure Block:

B.L. Price: None. **C.B. Dobson:** None.

Poster Board Number:

SUNDAY-543

Publishing Title:

Characterization of Pathogenesis Observed in BALB/c Mice Exposed to Aerosolized *Burkholderia pseudomallei* HBPUB10134a

Author Block:

K. A. Fritts, M. Hunter, C. P. Klimko, J. A. Bozue, R. G. Toothman, J. J. Bearss, J. L. Dankmeyer, P. L. Worsham, K. Amemiya, S. L. Welkos, C. K. Cote; USAMRIID, Frederick, MD

Abstract Body:

Burkholderia pseudomallei, the etiologic agent of melioidosis, is a gram negative bacterium designated a category B threat agent by the CDC. This bacterium is endemic in Southeast Asia and northern Australia and can infect humans and animals by several routes of infection. It has also been estimated to present a considerable risk as a potential biothreat agent. There are currently no effective vaccines for B. pseudomallei and antibiotic treatment can be hampered by nonspecific symptomology and also the high rate of naturally occurring antibiotic resistant strains. .Our goal is to develop well-characterized animal models to facilitate research on disease pathogenesis and evaluation of novel medical countermeasures. Female BALB/c were infected with a low dose of aerosolized B. pseudomallei HBPUB10134a (approximately 6 CFUs). Mice were euthanized on days 1, 2, 3, 4, 7, 10, and 14 post-infection. Some of these mice were used for pathological analyses and others were used to determine the bacterial burden on selected tissues (blood, lungs, and spleens). Additionally, spleen homogenate, lung homogenate, and sera samples were analyzed to better characterize the resulting host immune response after exposure to aerosolized bacteria. This work represents the next step in our efforts to developing standardized animal models for melioidosis. The data when compared to those collected using differentially virulent strains of B. pseudomallei (i.e. 1106a) demonstrate differences in pathogenesis amongst strains of bacteria in the mouse model and adds to the growing body of data concerning appropriate animals models for B. pseudomallei infections.

Author Disclosure Block:

K.A. Fritts: None. **M. Hunter:** None. **C.P. Klimko:** None. **J.A. Bozue:** None. **R.G. Toothman:** None. **J.J. Bearss:** None. **J.L. Dankmeyer:** None. **P.L. Worsham:** None. **K. Amemiya:** None. **S.L. Welkos:** None. **C.K. Cote:** None.

Poster Board Number:

SUNDAY-544

Publishing Title:

Serratia marcescens*: Blowing Corneal Epithelial Cell Bubbles with *gumB

Author Block:

R. M. Q. Shanks, K. M. Brothers, N. A. Stella; Univ. of Pittsburgh, Pittsburgh, PA

Abstract Body:

Background: *Serratia marcescens* causes bacterial keratitis and contact lens associated complications. Previous studies have shown that another bacteria, *Pseudomonas aeruginosa*, induces blebs in corneal cells using a type III secretion system. We observed that *S. marcescens* induced epithelial bleb formation in human corneal limbal epithelial cells (HCLE) and human keratinocytes in vitro, yet *S. marcescens* lacks a type III secretion system. The purpose of this study was to identify the novel mechanism by which *S. marcescens* causes epithelial cell bleb formation.**Methods:** A mariner transposon library of corneal keratitis isolate K904 was screened for mutations that inactivated the bacterial bleb induction in HCLE cells.**Results:** ~7,000 mutants were used to challenge HCLE layers and the challenged epithelial cells were examined microscopically. Of the tested mutants, 5 were defective in inducing bleb formation. Two of the bleb-induction deficient isolates had transposons that map to different loci in an uncharacterized gene in *S. marcescens* named *gumB*. A deletion mutation of the *gumB* gene was generated and the resulting mutant was defective in bleb formation. Complementation of the *gumB* open reading frame mutant confirmed the role of this gene in bleb induction. Other bleb-inducing defective bacteria had mutations in a type V secretion system. Expression of the type V system in *Escherichia coli* conferred the ability to induce blebs.**Conclusions:** This analysis identified a novel mechanism for inducing necrotic blebs in corneal cells. Future studies to inhibit this mechanism may help reduce contact lens associated complications and keratitis associated vision loss.

Author Disclosure Block:

R.M.Q. Shanks: None. **K.M. Brothers:** None. **N.A. Stella:** None.

Poster Board Number:

SUNDAY-545

Publishing Title:

In Susceptible Mice Infected with *M. pulmonis*, Host Lung Damage Is Associated with Recruitment of Il-17a$^+$ Lymphocytes and Neutrophils into the Lung

Author Block:

M. Mize, J. Simecka; Univ. of North Texas Hlth.Sci. Ctr., Fort Worth, TX

Abstract Body:

Background: Possessing the smallest genomes, *mycoplasma* induce pneumonia in humans and animals resulting in chronic airway inflammation. Exacerbating other respiratory conditions (i.e. asthma), *mycoplasma* have evolved to resist modern antibiotics and vaccines induce the same damage seen during actual infection[1]. A cytokine contributing to chronic pathology and neutrophil-mediated host protection, IL-17A is secreted during infection with *mycoplasma*. Here, we investigate whether IL-17A can promote damage characteristic of *mycoplasma* disease. Our results will help development of vaccines that confer protection and lack side-effects. **Methods:** Murine pneumonia, induced by *M. pulmonis*, resembles the pulmonary pathogenesis seen in human *mycoplasma* diseases. Furthermore, BALB/c models have been well established for studying chronic *mycoplasma* infection[2]. Briefly, *M. pulmonis* was administered intra-nasally. At select time points post-infection, mice were sacrificed and aspects of pathogenesis analyzed. **Results:** Injecting neutralizing antibodies against IL-17A into BALB/c mice reduced inflammation during infection without altering bacterial burden. Attenuating the effects of IL-17A reduced both airway cell numbers and total lung IL-17A$^+$ lymphocytes by Day (14). The increase in IL-17A$^+$ cells was associated with increased airway neutrophils, appearing as early as Day (1) post-infection. The early presence of neutrophils appears alongside CD4$^+$, CD8$^+$, and γδ T-cells that secrete IL-17A. By Day (9) post-infection, the described T-cell populations are replaced by CD4$^+$, SCA-1$^+$, and NK cells that contribute to IL-17A levels. While IL-17A$^+$CD4$^+$ T-cells reach their maximum response at Day (1) post-infection, this was the only T-cell population that persisted in their production of IL-17A by Day (14). The generation of IL-17A$^+$ lymphocytes, and subsequent recruitment of neutrophils was associated with disease pathogenesis. **Conclusions:** During infection with *M. pulmonis*, neutrophil recruitment into the lungs is associated with the presence of IL-17A$^+$ lymphocytes. Neutrophils and IL-17A$^+$ cells drive host damage; neutralizing IL-17A reduces airway neutrophils, total IL-17A$^+$ lung cells and host damage. Blocking IL-17A lowers lung lesion development, thus IL-17A and neutrophils promote respiratory damage.

Author Disclosure Block:

M. Mize: None. **J. Simecka:** None.

Poster Board Number:

SUNDAY-546

Publishing Title:

Characterization of Pathogenesis Observed in BALB/c Mice Exposed to Aerosolized *Burkholderia pseudomallei* 1106a

Author Block:

C. P. Klimko, M. Hunter, K. A. Frittspinnock, J. L. Shoe, C. W. Schellhase, J. J. Bearss, J. L. Dankmeyer, A. M. Woodson, R. G. Toothman, J. A. Bozue, P. L. Worsham, K. Amemiya, S. L. Welkos, C. K. Cote; USAMRIID, Frederick, MD

Abstract Body:

Burkholderia pseudomallei, the etiologic agent of melioidosis, is a gram negative bacterium designated a category B threat agent by the CDC. This bacterium is endemic in Southeast Asia and northern Australia and can infect humans and animals by several routes of infection. It has also been estimated to present a considerable risk as a potential biothreat agent. There are currently no effective vaccines for B. pseudomallei and antibiotic treatment can be hampered by non-descript symptomology and also the high rate of naturally occurring antibiotic resistant strains. Our goal is to develop well-characterized animal models to facilitate research on disease pathogenesis and evaluation of novel medical countermeasures. Female BALB/c were infected with a low dose of aerosolized B. pseudomallei HBPUB10134a (approximately 7,000 CFUs). Mice were euthanized on days 1, 2, 3 and 4 days post-infection. Some of these mice were used for pathological analyses and others were used to determine the bacterial burden on selected tissues (blood, lungs, and spleens). Additionally, spleen homogenate, lung homogenate, and sera samples were analyzed to better characterize the resulting host immune response after exposure to aerosolized bacteria. This work represents the next step in our efforts to developing standardized animal models for melioidosis. The data when compared to those collected using differentially virulent strains of B. pseudomallei (i.e. HBPUB10134a) demonstrate potential differences in pathogenesis amongst strains of bacteria in the mouse model and adds to the growing body of data concerning appropriate animals models for B. pseudomallei infections.

Author Disclosure Block:

C.P. Klimko: None. **M. Hunter:** None. **K.A. Frittspinnock:** None. **J.L. Shoe:** None. **C.W. Schellhase:** None. **J.J. Bearss:** None. **J.L. Dankmeyer:** None. **A.M. Woodson:** None. **R.G. Toothman:** None. **J.A. Bozue:** None. **P.L. Worsham:** None. **K. Amemiya:** None. **S.L. Welkos:** None. **C.K. Cote:** None.

Poster Board Number:

SUNDAY-547

Publishing Title:

Atypical Enteropathogenic *E. coli* Is Associated with Diarrhea and Diarrheal Death in Kittens

Author Block:

V. E. Watson[1], M. Jacob[1], J. Elfenbein[1], C. DebRoy[2], J. L. Gookin[1]; [1]North Carolina State Univ., Raleigh, NC, [2]Pennsylvania State Univ., University Park, PA

Abstract Body:

Infectious diarrhea is a leading cause of death in children and kittens. In children, enteropathogenic *E.coli* (EPEC) is a common cause of diarrhea and is associated with increased risk of mortality. We demonstrated that sick kittens dying in animal shelters have colonization of the intestinal epithelium by EPEC. We hypothesized that EPEC is significantly associated with diarrhea in kittens and that phenotypic or genotypic characteristics could be used to distinguish pathogenic from non-pathogenic EPEC in kittens.A prospective, case-controlled study of EPEC infection in shelter kittens with and without diarrhea was performed. DNA was extracted from feces for conventional and qPCR amplification of intimin (*eae*). Feces were cultured and isolated *E. coli* identified as EPEC by PCR (*eae+*, *stx1/2-*). EPEC were genotypically characterized using PFGE, serotyping, and PCR detection of *bfp* (atypical -vs- typical EPEC) and virulence genes on the Locus of Enterocyte Effacement (*EspA*, *EscN*, *Ler*). Phenotypic characteristics of EPEC were determined by quantifying motility on semi-solid media and adherence to HEp-2 cells examined using FITC-phalloidin.Conventional PCR detected *eae* in fecal DNA from 50% (31/62) of kittens with and 23% (12/53) of kittens without diarrhea (OR=3.4; 95% CI=1.5 to 7.7; $p<0.01$). Quantitative PCR demonstrated a greater copy number of *eae* in feces from kittens with diarrhea ($p<0.01$;t-test). Culture of live EPEC was achieved in 21% (6/29) of kittens with diarrhea-associated death and 16% (3/19) of control kittens. All culture isolates of EPEC were identified as atypical (*eae+*, *bfp-*). Methods to identify genotypic or phenotypic differences were not able to distinguish pathogenic from non-pathogenic EPEC.This study demonstrated that *eae* can be commonly amplified from the feces of kittens and is significantly associated with diarrhea and diarrheal death. Isolates of EPEC from kittens are phenotypically and genotypically diverse. Naturally occurring EPEC infection in kittens may represent a novel animal model for study of EPEC infection in children.

Author Disclosure Block:

V.E. Watson: None. **M. Jacob:** None. **J. Elfenbein:** None. **C. DebRoy:** None. **J.L. Gookin:** None.

Poster Board Number:

SUNDAY-548

Publishing Title:

Role of *msaabcr* Operon in the Staphylococcal Chronic Osteomyelitis Pathogenesis

Author Block:

G. S. Sahukhal[1], M. A. Tucci[2], G. A. Wilson[2], H. A. Benghuzzi[2], M. O. Elasri[1]; [1]The Univ. of Southern Mississippi, Hattiesburg, MS, [2]Univ. of Mississippi Med. Ctr., Jackson, MS

Abstract Body:

Background: *Staphylococcus aureus* is a major cause of both health care associated and community-associated infections. *S. aureus* is a primary agent of chronic bone infection also known as osteomyelitis. Treatment of osteomyelitis is very complicated and may require surgical debridement followed by prolonged antibiotic courses. Osteomyelitis patients often experience serious life threatening complications like septicemia, thrombosis and pathological fractures thus leading to high mortality and morbidity. In this study, we have investigated the role of the operon, *msaABCR* in pathogensis of osteomyelitis caused by *S. aureus*.**Methods:** In this study, we used the modified chronic osteomyelitis infection model using SD rats. Medical implants (K-wire pin) were coated with *S. aureus* biofilm (wild type, *msaABCR* deletion mutant and the *msaABCR* complement), and surgically transplanted transcortically through the metaphysis in the tibia. The infected tibias were harvested after 4, 8 and 15 days and were used for microbiological, X-ray and Microcomputed tomography (MicroCT) analysis.**Results:** X-ray and MicroCT images revealed that the wild type *S. aureus* strain colonized bone tissue and triggered significant bone damage of the infected tibia, whereas the *msaABCR* deletion mutant of *S. aureus* was attenuated and unable to cause chronic osteomyelitis. Bacterial count from infected tibias, blood and various organs (Liver, kidney, heart, spleen, testes) showed a significant difference between animals infected with the *msaABCR* mutant versus the wild type.**Conclusions:** This study shows that the *msaABCR* operon plays a role in the pathogenesis osteomyelitis cause by *S. aureus*. In particular, this model shows the importance of the operon in implant-associated infections which cause significant morbidity.

Author Disclosure Block:

G.S. Sahukhal: None. **M.A. Tucci:** None. **G.A. Wilson:** None. **H.A. Benghuzzi:** None. **M.O. Elasri:** None.

Poster Board Number:

SUNDAY-549

Publishing Title:

Trx1 Is Required For Candida Albicans Biofilm Formation

Author Block:

A. T. Fishburn, C. J. Nobile, M. Gulati; Univ. of California, Merced, Merced, CA

Abstract Body:

Background: *Candida albicans* is the predominant pathogenic fungus in humans, causing mucosal and life-threatening bloodstream infections. A major virulence factor of *C. albicans* is its ability to form biofilms, particularly on medical devices (e.g. catheters and heart valves). Biofilms are communities of adhered cells encased in a protective extracellular matrix. We previously discovered the entire transcriptional network controlling biofilm formation in *C. albicans*, which consists of six "master" transcriptional regulators (Bcr1, Tec1, Efg1, Ndt80, Rob1, and Brg1), and over 1000 downstream target genes. Here we report on the analysis of nine selected target genes of the biofilm network that are highly downregulated upon deletion of *NDT80*. **Methods:** We constructed deletion mutants for each of the nine target genes, and assessed biofilm formation using the following standard in vitro C. albicans biofilm assays: dry weight, optical density, and quantitative biofilm dispersion. In addition to these traditional biofilm assays, we also developed a new in vitro assay for biofilm formation using a customizable BioFlux microfluidic flow device. This device allows biofilm formation to be monitored over time by live microscopy under shear flow conditions, thereby allowing us to mimic physiological biofilm conditions found in catheters and blood vessels.**Results:** Our results indicate that deletion of TRX1 leads to defects in biofilm formation. Biofilms formed by the trx1Δ/Δ strain are thin and fragile, and show an optical density reduction of 68.5% relative to wild-type (p=0.0032). Real-time BioFlux videos of biofilm development over time revealed alterations in cell morphologies and overall biofilm architectures in the trx1Δ/Δ strain. The majority of cells in the trx1Δ/Δ strain biofilm are predominantly pseudohyphal, compared with the round yeast and true hyphal cells observed in the wild-type biofilm. Our results also indicate that the trx1Δ/Δ strain is defective in biofilm dispersion in a 24, 48, and 60 hour biofilm, the largest deficiency observed at the 60 hour time point (reduced six-fold relative to wild-type; p=5.1E-11).**Conclusions:** In summary, Trx1 plays a role in cell morphology and is required for normal biofilm development. Trx1 is particularly important for biofilm dispersion, which is the least understood stage of the biofilm life cycle, where cells leave the biofilm to seed new sites of infection.

Author Disclosure Block:

A.T. Fishburn: None. **C.J. Nobile:** None. **M. Gulati:** None.

Poster Board Number:

SUNDAY-550

Publishing Title:

A Single Amino Acid Change in *Burkholderia dolosa* FixI Alters Biofilm Formation and Pathogenicity

Author Block:

M. M. Schaefers, T. L. Liao, G. P. Priebe; Boston Children's Hosp., Boston, MA

Abstract Body:

Background: While investigating the genomic diversity of 112 isolates of the *Burkholderia cepacia* complex member *B. dolosa* collected from an outbreak in chronically infected cystic fibrosis patients over 16 years, we identified *fixL* (BDAG_01161) as a gene under strong positive selective pressure. This gene has homology to *fixL* of the rhizobial FixL/FixJ two-component system. FixL is a sensory histidine kinase that detects oxygen tension and phosphorylates the transcription factor FixJ under low oxygen conditions, leading to activation of the *fixK* gene. Our previous work identified that the *fixLJ* system is involved in biofilm formation, motility, and persistence in a murine model. Interestingly the reference strain, *B. dolosa* AU0158, which was isolated from the index patient after several years of colonization, contains a mutation in the *fixL* gene compared to the ancestral isolate. In this study we sought to understand the implications of this mutation in *fix*-mediated phenotypes. **Methods:** A *fixLJ* deletion mutant was constructed in *B. dolosa* strain AU0158 using allelic replacement. We constructed isogenic strains by complementing the deletion mutant with either S439 *fixL* (reference sequence) or W439 *fixL* (ancestral sequence) and *fixJ*. We measured swimming motility as well as the ability of these constructs to form biofilm on PVC plates. In a murine model of pneumonia after intranasal inoculation, we assessed the ability of *B. dolosa* to persist in the lung and disseminate to the spleen.**Results:** *B. dolosa* carrying the ancestral FixL sequence (W439) produced significantly (*p* value <0.001) more biofilm than isogenic bacteria carrying the reference *fixL* sequence (S439). Interestingly, *B. dolosa* carrying the ancestral *fixL* were non-motile while *B. dolosa* carrying the reference sequence were motile. In the murine pneumonia model, *B. dolosa* carrying the ancestral FixL (W439) was cleared faster in the lungs and spleen than *B. dolosa* carrying the reference FixL (S439) at 7 days post infection (*p* value <0.05).**Conclusion:** *B. dolosa* carrying a FixL sequence variant arising later during human infection is less able to form biofilms, more motile, and better able to persist in vivo in the murine lung and spleen compared to *B. dolosa* carrying the ancestral genotype.

Author Disclosure Block:

M.M. Schaefers: None. **T.L. Liao:** None. **G.P. Priebe:** None.

Poster Board Number:

SUNDAY-551

Publishing Title:

Implications of an *Frha* snp Affording Enhanced Attachment on Increased Virulence Capabilities of *Vibrio cholerae* Clinical Isolates

Author Block:

K. D. Brumfield, B. M. Carignan, M. S. Son; Plymouth State Univ., Plymouth, NH

Abstract Body:

Background: Cholera is a severe diarrheal disease caused by the consumption of food or water contaminated with the aquatic Gram-negative bacterium *Vibrio cholerae*. Infected hosts will experience vomiting and severe watery diarrhea, and if not treated properly, will ultimately succumb to death by dehydration. Recently, *V. cholerae* clinical isolates have demonstrated increase virulence, with patients experiencing more severe symptoms and the disease progressing at a faster rate than previously observed. In the environment, *V. cholerae* is commonly found attached to the chitinous exoskeleton of planktonic crustacean copepods, allowing for increased survivability of the bacteria. The *f*lagellar *r*egulated *h*emagglutinin A, coded by *frhA*, is known to mediate chitin attachment by *V. cholerae*. Through deep sequencing data analysis, a single nucleotide polymorphism (SNP) in *frhA*, as well as four regulatory genes (*hns*, *hapR*, *luxO*, and *vieA*), has been identified in clinical isolates of *V. cholerae* (compared to the wild-type (WT) El Tor N16961). The purpose of this study is to assess the effects of the *frhA* SNP in concert with the other regulatory SNPs while developing a *V. cholerae* attachment model. We hypothesize strains containing the *frhA* SNP will have increased attachment capabilities and promotes increased virulence capabilities of *V. cholerae*. **Methods:** The *frhA* SNP was introduced into the WT N16961 using allelic exchange, and enhanced attachment determined using a newly developed copepod model. Briefly, *V. cholerae* cultures were incubated with planktonic marine copepods for 18 to 24 hours, and attachment levels determined by PCR. **Results:** The clinical isolate harboring the *frhA* SNP demonstrated earlier attachment to the copepod compared to the WT N16961. In addition, a working model using marine copepods has been developed allowing qualitative measurement of attachment by *V. cholerae*. **Conclusion:** The clinically relevant *frhA* SNP demonstrated enhanced attachment compared to the WT N16961 strain, and the copepod model demonstrated proof-of-principle. Understanding the role of this *frhA* SNP alone and in conjunction with the other identified SNPs, and its effects on attachment will provide insight into regulatory mechanisms linking attachment and virulence gene expression during *V. cholerae* pathogenesis.

Author Disclosure Block:

K.D. Brumfield: None. **B.M. Carignan:** None. **M.S. Son:** None.

Poster Board Number:

SUNDAY-552

Publishing Title:

Esta Controls Virulence Gene Expression in *Enterohemorrhagic e. coli* O157:H7

Author Block:

E. S. McKenney, M. M. Kendall; Univ. of Virginia, Charlottesville, VA

Abstract Body:

Enterohemorrhagic *Escherichia coli* O157:H7 (EHEC) is a foodborne, intestinal pathogen that causes hemorrhagic colitis and hemolytic uremic syndrome. Each year, more than 70,000 people in the United States become ill due to EHEC infections, resulting in approximately 50 deaths and an estimated $1 billion per year in economic costs. EHEC establishes infection in the human colon by intimately attaching to epithelial cells through the formation of attaching and effacing (AE) lesions. Additionally, EHEC produces Shiga toxin, which causes hemolytic uremic syndrome and the mortality associated with EHEC infections. In order to establish infection, EHEC must sense molecules within the host to control the expression of genes that encode for AE lesion formation and Shiga toxin production. For example, the metabolite ethanolamine is abundant in the intestinal tract and is an important signaling molecule for EHEC.Ethanolamine controls the expression of several EHEC virulence factors through the transcription factor EutR. However, ethanolamine induces the expression of some virulence factors independent of EutR, suggesting a second ethanolamine-signaling pathway. We identified an ethanolamine responsive gene that influences the expression of virulence factors in a Δ*eutR* background, and we renamed this gene ethanolamine signal transduction gene A, or *estA*. To determine a global role for EstA, we generated an *estA* deletion strain and performed microarray analysis, which demonstrated that EstA influences the expression of genes with diverse functions in EHEC, including virulence genes. These data suggest that EstA is involved in a complex regulatory network. For example, genes encoding Shiga toxin are upregulated at both the transcript (measured by qRT-PCR) and protein level (measured by western blot) in Δ*estA*, indicating that EstA negatively regulates Shiga toxin expression. Additionally, EstA positively influences the formation of AE lesions, as Δ*estA* was deficient in forming AE lesions on HeLa cells in a fluorescein actin staining assay. Elucidating how EstA influences gene expression is ongoing, and will contribute to the understanding of a novel ethanolamine-dependent signaling pathway that modulates virulence in EHEC.

Author Disclosure Block:

E.S. McKenney: None. **M.M. Kendall:** None.

Poster Board Number:

SUNDAY-553

Publishing Title:

Lysr-Type Transcriptional Regulators are Required for Biofilm and Virulence of *Acinetobacter baumannii*

Author Block:

A. C. Jacobs, S. D. Tyner, **D. V. Zurawski**; Walter Reed Army Inst. of Res., Silver Spring, MD

Abstract Body:

Acinetobacter baumannii is a Gram-negative bacterial species that in the last several decades has emerged as an important nosocomial, opportunistic pathogen. In the last 10 years this pathogen has gained more attention as Wounded Warriors returning to the U.S. from Iraq and Afghanistan have been infected. While *A. baumannii* infections were reduced with the end of these conflicts, XDR and pandrug resistant strains are still being isolated from Wounded Warriors and civilians, and still represent a significant percentage of all bacterial wound infections at Walter Reed National Military Medical Center. While a handful of virulence factors have been described for *A. baumannii*, none of these elements provide a comprehensive understanding of pathogenesis. One way to address this issue is to study global gene regulators in order to gain insight into the signaling networks required for survival in environments such as the host. With this idea in mind, our laboratory began studying LysR-Type Transcriptional Regulators (LTTR), a helix-turn-helix superfamily of DNA binding proteins that regulate a diverse set of genes, including genes related to biofilm and virulence. In *P. aeruginosa* the LTTR MvfR was found to regulate quorum-sensing, was required for virulence, and interruption of MvfR activity with novel compounds remediated *P. aeruginosa* infection in mouse models. Based on these data, our laboratory hypothesized that LTTRs may also play an important role in *A. baumannii* pathogenesis. As part of our preliminary work, 25 Tn*5*-insertion mutants with LTTR homology were assessed in various assays. First, biofilm formation was assessed using a standard crystal violet method. Ratios of biofilm formation to culture growth were determined and compared to wild type. Five mutants were found to have aberrant biofilm formation, and one of these interrupted genes had homology to the *P. aeruginosa* MvfR. When compared in the wax worm model of *A. baumannii* infection, this homologue also had reduced fitness compared to wild type. Taken together, these data demonstrate that some LTTRs in *A. baumannii* play a role in biofilm formation, and suggest at least one regulator is required for virulence. Further characterizing these genes will provide a better understanding of *A. baumannii* pathogenesis and possible novel targets for therapeutic intervention.

Author Disclosure Block:

A.C. Jacobs: None. **S.D. Tyner:** None. **D.V. Zurawski:** None.

Poster Board Number:

SUNDAY-554

Publishing Title:

Elucidating the Function of *Enterococcus faecalis* Eep Protease in Virulence-related Peptide Signaling and Biofilm Formation

Author Block:

K. L. Frank[1], A. M. T. Barnes[2], G. M. Dunny[2]; [1]Uniformed Services Univ. of the Hlth.Sci., Bethesda, MD, [2]Univ. of Minnesota, Minneapolis, MN

Abstract Body:

The *E. faecalis* Eep site 2 membrane metalloprotease contributes to the spread of antibiotic resistance by processing signal peptides of certain lipoproteins into peptide pheromones that induce conjugative transfer of certain plasmids (e.g., pCF10). Eep also plays a role in *E. faecalis* evasion of the innate immune response by conferring resistance to lysozyme. We previously established that a Δ*eep* mutant has an aberrant cell and matrix distribution phenotype in biofilms and is attenuated in animal models of biofilm-associated infection involving both a foreign body (catheter-associated urinary tract infection) and a host surface (infective endocarditis). In wild-type cells with pCF10, endocarditis virulence is primarily mediated by pheromone-induced expression of aggregation substance (PrgB), which mediates cell clumping. One mechanism preventing autocrine induction of conjugation machinery by endogenous pheromone involves a plasmid-encoded inhibitory pheromone (e.g., iCF10) that is also processed by Eep. The tight ratio between inducer and inhibitor peptides that usually prevents autocrine activation is perturbed *in vivo*, allowing self-induction that leads to enhanced expression of PrgB. Our previous data revealed that the Δ*eep* endocarditis attenuation phenotype is rescued by pCF10 in a PrgB-independent manner. In contrast, our recent data show that exogenous induction of pCF10 does not restore lysozyme resistance in Δ*eep* cells, suggesting that Eep contributes to multiple steps in *E. faecalis* endocarditis pathogenesis. Our data show that Δ*eep*(pCF10) cells clump in response to a lower concentration of exogenous activator peptide compared to the wild-type strain. This is correlated with a 5-fold increase in the transfer frequency of pCF10 in Δ*eep* relative to wild-type cells in solid-surface conjugation assays. This effect is a result of Eep proteolytic activity, as evidenced by the fact that a strain with a point mutation in the catalytic site phenocopies the Δ*eep* strain in conjugation assays. Exogenous induction of pCF10 during biofilm formation also results in differences between wild-type and Δ*eep* biofilm biomass accumulation and biofilm architecture. Our data suggest that Eep has a fundamental role in virulence related peptide signaling and biofilm formation.

Author Disclosure Block:

K.L. Frank: None. **A.M.T. Barnes:** None. **G.M. Dunny:** None.

Poster Board Number:

SUNDAY-555

Publishing Title:

Role of Acetyltransferase *pg1842* in Activation and Maturation of Gingipains in *Porphyromonas gingivalis*

Author Block:

A. Mishra, F. Roy, Y. Dou, H. M. Fletcher; Loma Linda Univ., Loma Linda, CA

Abstract Body:

Porphyromonas gingivalis, the major etiologic agent in adult periodontitis, produces large amounts of proteases that are important for its survival and pathogenesis. Activation/maturation of gingipains, a major protease, in *P. gingivalis* involves complex networks of processes which are not fully understood. VimA, a putative acetyltransferase, is a virulence modulating multifunctional protein in *P. gingivalis* that is known to be involved in gingipains biogenesis. Deletion of the *vimA* gene resulted in an isogenic mutant (Δ*vimA*) that showed late onset gingipain activity at stationary phase indicating that there could be a functional VimA homolog that is most likely upregulated in stationary phase to overcome the *vimA* mutation. The aim of this study is to identify any functional homolog which may play a role in activating the gingipains in absence of VimA at stationary phase. Bioinformatics analysis showed five putative acetyltransferase genes in *P. gingivalis* genome structurally similar to *vimA*. Allelic exchange mutagenesis was used to make deletion mutants of all five acetyltransferases in *P. gingivalis* Δ*vimA* background. One of the mutants, Δ*PG1842*, did not show any late onset of gingipain activity at stationary phase as compared to parent strain Δ*vimA*. Comparison of western blot analysis of stationary phase extracellular fractions from the Δ*vimA* and Δ*PG1842* isogenic mutants showed the active catalytic domain band in Δ*vimA*, whereas only inactive proenzyme species were seen in Δ*PG1842*. This suggested that in absence of *PG1842*, the inactive proenzyme in Δ*vimA* was not processed into active catalytic form. Real time PCR showed similar expression level of *PG1842* during both exponential and stationary phase in Δ*vimA*. Collectively, these findings indicate that *PG1842* may play a significant role in activation/maturation of gingipains in *P. gingivalis*.

Author Disclosure Block:

A. Mishra: None. **F. Roy:** None. **Y. Dou:** None. **H.M. Fletcher:** None.

Poster Board Number:

SUNDAY-556

Publishing Title:

Characterizing the Mechanism of Bile Resistance and Related Biofilm Formation in S*higella flexneri*

Author Block:

K. P. Nickerson[1], R. B. Chanin[1], J. R. Sistrunk[2], P. Fink[1], D. K. V. Kumar[1], D. A. Rasko[2], **C. S. Faherty**[1]; [1]Massachusetts Gen. Hosp., Charlestown, MA, [2]Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract Body:

Shigella flexneri is a Gram-negative, facultative intracellular pathogen that causes millions of cases of diarrhea each year, predominantly in children in developing countries. While many aspects of colonic cell invasion are known, a crucial gap in knowledge remains regarding how the bacteria survive, transit, and regulate virulence gene expression prior to infection. *S. flexneri* is exposed to bile, a bactericidal host factor essential for digestion, as it enters the small intestine. Previous research has demonstrated that bile salts induce both *S. flexneri* adherence to and invasion of epithelial cells. In this study, we further characterized the effects of bile salts exposure on *S. flexneri* 2a strain 2457T. Growth curve analysis in the presence of bile salts revealed that 2457T grew normally within the physiological range of 0.2% to 2% w/v, but growth was slowed or inhibited at 5% and 10% w/v. Using RNA-sequencing as a comprehensive analysis of gene expression, we identified 172 genes induced in 0.4% w/v bile salts that are involved with central metabolism, gene expression, membrane structure, drug resistance, and virulence. Interestingly, to mimic small intestine transit, extended periods of bile salts exposure led to biofilm formation, which was confirmed by crystal violet staining, analysis of exopolysaccharide matrix production, and electron microscopy. Analysis of additional *Shigella*, *Salmonella*, and *Escherichia coli* strains demonstrated a common theme of bile salt-induced biofilm formation among enteric pathogens. Finally, analysis of a transposon mutant library has identified several 2457T mutants unable to survive bile salts exposure or form a biofilm. Our data demonstrate that *S. flexneri* has several mechanisms to survive bile exposure, and we hypothesize that biofilm formation is crucial in that process. Moreover, bile serves as an *in vivo* signal to activate *S. flexneri* virulence prior to entry in the colon. This work has led to a greater understanding of how *Shigella* transits the host to establish infection in the colon.

Author Disclosure Block:

K.P. Nickerson: None. **R.B. Chanin:** None. **J.R. Sistrunk:** None. **P. Fink:** None. **D.K.V. Kumar:** None. **D.A. Rasko:** None. **C.S. Faherty:** None.

Poster Board Number:

SUNDAY-558

Publishing Title:

Non-pathogenic *Escherichia coli* Enhance Stx2a Production of *E. coli* O157:H7 by Multiple Mechanisms

Author Block:

L. Xiaoli, K. Goswami, E. Dudley; Penn State Univ., University Park, PA

Abstract Body:

Inestinal colonization by the foodborne pathogen *E. coli* O157:H7 causes serious disease symptoms, including bloody diarrhea and severe abdominal cramps. The disease can further develop into hemolytic uremic syndrome (HUS) and hemorrhagic colitis (HC). Synthesis of one or more Shiga toxins (Stx) is essential for HUS and HC development. The genes encoding Stx, including Stx2a, are encoded by a lambdoid prophage integrated into the *E. coli* O157:H7 chromosome. Enhanced Stx2a expression was reported when specific non-pathogenic *E. coli* strains were co-cultured with O157:H7, and it was hypothesized that this phenotype required the former to be sensitive to infection by the Shiga toxin-converting phage. We tested this hypothesis by using a previously published method to replace *bam*A (an essential gene and phage receptor) in non-pathogenic *E. coli* strains with the ortholog from *Salmonella enterica*. Such heterologous gene replacement abolished the ability of *E. coli* strain C600 to enhance toxin production when co-cultured with *E. coli* O157:H7 strain PA2, which belongs to the hypervirulent clade 8 cluster. Two extracellular loops of BamA (loops 4 and 10) were further shown to be important for infection by Stx2a-converting phage. However, gene replacement in other non-pathogenic *E. coli* strains revealed a *bamA*-independent mechanism for toxin amplification. Collectively, these data suggest that multiple mechanisms exist for commensal *E. coli* to increase Stx production when they co-exist with *E. coli* O157:H7.

Author Disclosure Block:

L. Xiaoli: None. **K. Goswami:** None. **E. Dudley:** None.

Poster Board Number:

SUNDAY-559

Publishing Title:

The Role of H. *Pylori baba* Gene in Persistent Infection and Chronic Inflammation

Author Block:

S. A. Ansari[1], S. U. Kazmi[2]; [1]Dow Univ. of Hlth.Sci., Karachi, Pakistan, [2]Immunology and Infectious Disease Res. Lab. - Dadabhoy Inst. of Higher Ed. - Karachi - Pakistan, Karachi, Pakistan

Abstract Body:

Pathogenesis of Helicobacter pylori in severe gastric disorders is multifactorial and it involves intricate microbial communications with gastric mucosa. Several adhesion proteins *of H.pylori* which also have tendency to bind with Lewis a-1, 3/4 difucosylated blood group antigens found on gastric mucosa play an important role in the establishment of persistent infection and chronic inflammation whereas fucosylated host secretions inhibit adherence of *H. pylori* and other pathogens to the mucosal cell surfaces. The histo-blood group antigen binding adhesin (BabA) is one of the best-characterized *H. pylori* adhesins which binds in the gastric mucosa specially to the Lewis b antigen (Leb). We investigated the binding properties of *H.pylori* to gastric epithelium in humans, mediated by blood group antigens in control and test group to establish a correlation of *H. pylori* infection with gastritis and (BabA) adhesin. Gastric biopsies , saliva and blood samples of 574 patients who underwent gastro duodenal endoscopy during 2011 to 2014 were subjected to Helicourease (IIDRL- Rapid Urease) test, culture, histological examination and PCR for the detection of *H. pylori* and *babA* gene. . *H. pylori* infection was detected in biopsies of 138(24%) patients by rapid urease 263 (55.4%) by culture . Histologic observation confirmed infection in biopsy specimens (154) from patients who had inflamed and ulcerated gastric mucosa were positive for *H. pylori* by histology , PCR, cultures and rapid urease. Patients who had intact mucosa were excluded. A total of 343 (60%) biopsy samples we were positive for *H. pylori by PCR* using *16SrRNA (*HP1 andHP2) whereas 250 (43.55 when we used second *16SrRNA* (JW22, JW23). Among PCR positive *H. pylori* subjects 91(36%) had BabA gene . We observed a high frequency of *babA* gene in patients with gastritis , highly inflamed and ulcerated mucosa , which is strongly associated with colonization of *H. pylori* that contributes to virulence and development of *H. pylori* infection. Our study supports a strong association between gastritis, (BabA) adhesin and *H. pylori* infection.

Author Disclosure Block:

S.A. Ansari: None. **S.U. Kazmi:** None.

Poster Board Number:

SUNDAY-560

Publishing Title:

Mechanics of Gram-Positive Bacterial Adhesion: Covalent Attachment and Energy Dissipation

Author Block:

D. J. Echelman[1], J. Alegre-Cebollada[2], J. M. Fernandez[1]; [1]Columbia Univ., New York, NY, [2]Natl. Inst. of Cardiovascular Res. (CNIC), Madrid, Spain

Abstract Body:

Background: The challenges of microbial adhesion are as ancient as life. Bacteria, whether sticking to rocky surfaces, congregating in biofilms, or colonizing hosts, must withstand forces that are sufficient to cleave covalent bonds. Extracellular protein appendages, including bacterial pili, are necessary for adherence under mechanical stress. In Gram-positive bacteria, these extracellular structures harbor an abundance of unique *intra*molecular isopeptide and thioester bonds. However, it remains unknown how these structures function under force and manage adherence in such mechanically chaotic environs.**Methods:** Using AFM-based single molecule force spectroscopy, we measured the mechanical behavior of purified pili and of engineered polyproteins of pilus shaft and adhesin subunits.**Results:** We find that the pilus shaft subunits SpaA from *Corynebacterium diphtheriae*, and FimA from *Actinomyces oris*, exhibit partial unfolding under force that is delimited by internal isopeptide bonds. The forces of unfolding are unprecedented in strength, 525 ± 65 pN for SpaA (n = 25) and 690 ± 70 pN for FimA (n = 96), and weaken with loss of the isopeptide bond. Moreover, refolding after mechanical extension occurs rapidly at rates of 14 s^{-1} or greater. Meanwhile, the pilus adhesin Spy0125 from *Streptococcus pyogenes* exhibits partial unfolding under force that is delimited by an internal thioester bond. This thioester bond has previously been reported to mediate covalent attachment to host (1). Uniquely, we find that the thioester-containing domain is buried within the fold of a more mechanically stable domain that serves to protect the bond from mechanical exposure. Finally, we integrate our mechanical measurements to estimate the energy dissipation from pilus extension, which is many-fold greater than rigid “rod-like” or yielding “helix-like” models of pilus mechanics.**Conclusions:** We propose that Gram-positive pili are optimized to sustain the covalent attachment to host through mechanical protection of the thioester bond and mechanical energy dissipation.

Author Disclosure Block:

D.J. Echelman: None. **J. Alegre-Cebollada:** None. **J.M. Fernandez:** None.

Poster Board Number:

SUNDAY-561

Publishing Title:

Increased Expression of Fibrinogen-Binding Protein (*sdrg*) By Late Phase *Staphylococcus epidermidis* May Contribute to Increased Stiffness of Infected Fibrin Clots

Author Block:

R. A. Sturtevant, T. Ma, M. J. Solomon, J. VanEpps; Univ. of Michigan, Ann Arbor, MI

Abstract Body:

Background: Biofilm colonization of medical devices is known to be facilitated by a number of host factors, including the presence of a fibrin clot. We previously demonstrated that the mechanical properties of an infected fibrin clot depend on the growth phase of *Staphylococcus epidermidis*. That is, clots infected with late growth phase cells are stiffer than those infected with early growth phase cells. We hypothesize that these differences may be due to differential expression of adhesion-related proteins affecting cellular binding to the fibrin. Here we examine the expression of *sdrG*, a cell-surface receptor protein with a high affinity for fibrinogen binding, and *icaA*, which is involved in the synthesis of a key biofilm exopolymer, polysaccharide intercellular adhesion (PIA), as a function of growth phase.**Methods:** RNA was isolated from both early (mid-log) and late (stationary) phase planktonic cultures of *S. epidermidis*. One-step Taqman® RT-PCR was performed using primers and dual-labeled probes against *sdrG* and *icaA*. 16S rRNA was used as the endogenous control, and early phase as the reference sample. A fold-change in gene expression relative to the reference was expressed as $2^{-\Delta\Delta CT}$.**Results:** Compared to early phase, late phase cells exhibited a 45% increase in *sdrG* expression. On the other hand, *icaA* expression decreased by 93% in late vs early phase.**Conclusions:** An increase in *sdrG* expression supports our hypothesis that cellular adhesion proteins may play a role in the enhanced mechanical properties of clots infected with late phase *S. epidermidis.* Our future work will further investigate how these changes in *sdrG* expression specifically affect fibrinogen binding and will also examine the role of other adhesion related proteins, including accumulation associated protein (*Aap*) and a major autolysin (*atlE*).

Author Disclosure Block:

R.A. Sturtevant: None. **T. Ma:** None. **M.J. Solomon:** None. **J. VanEpps:** None.

Poster Board Number:

SUNDAY-562

Publishing Title:

Unveiling the Soil Metazoan Microbiome and Its Contribution to Nutrient Cycling

Author Block:

J. A. Ceja-Navarro, A. Arellano, M. Kvietok, E. L. Brodie; Lawrence Berkeley Natl. Lab., Berkeley, CA

Abstract Body:

Arthropods represent more than 60% of all living species on Earth, with an estimated global mass of 1 to 14 billion tons[1]. During their evolution, arthropods incorporated microbes and their genes into their living machinery where commensal microbes cooperate by conveying processes such as N fixation, transformation of complex plant polymers and the degradation of toxic compounds.In this study we have characterized the prokaryotic microbial communities associated with soil arthropods and tested their potential for nitrogen fixation and plant polymer transformation. Soil arthropods were separated from soil samples using Berlese funnels. Each individual was surface sterilized prior to DNA extraction. The DNA was then used for the amplification and high throughput sequencing of 16S and 18S rRNA markers. Most abundant arthropod populations were separated in groups and used for the isolation of nitrogen fixing bacteria, and cellulose and lignin degrading microorganisms. Selected arthropod groups were incubated under an atmosphere of 98% $^{15}N_2$ and 2% Oxygen while feeding on ^{13}C-labelled cellulose. Carbon and Nitrogen enrichment was measured with Gas Chromatography Isotope Ratio Mass Spectrometry (GC-IRMS) and Nanoscale Secondary Ion Mass Spectrometry (nanoSIMS).One hundred arthropods were extracted from soil samples and categorized based on morphology and 18S rRNA sequencing/phylogenetics. In broad terms the isolated arthropods were identified as collembolans, mites, pseudoscorpions, beetles, millipedes, and centipedes. The analysis of the 16S rRNA sequences confirmed that each group contains a distinct makeup of microbial communities that are possibly related to their feeding habits/position in the food web. Nine different bacterial species among fifty microbial isolates were obtained from selected groups of arthropods using N deficient media and media containing cellulose or lignin as a sole source of C. Our incubation experiments and analysis of enrichment are still ongoing.The study of soil arthropods and other members of the soil fauna as vessels of biological transformation will allow us to improve the current models for the study of soil nutrient cycling and may help to improve soil management practices while also extending the frontiers for bioprospecting.

Author Disclosure Block:

J.A. Ceja-Navarro: None. **A. Arellano:** None. **M. Kvietok:** None. **E.L. Brodie:** None.

Poster Board Number:

SUNDAY-563

Publishing Title:

Endophyte Populations And Induced Disease Resistance Following Treatment Of Plants With A Mixture Of Branched-Chain Alkanes

Author Block:

P. Goodwin, W. Gao; Univ. of Guelph, Guelph, ON, Canada

Abstract Body:

Eight bacterial endophyte types matching six species of *Bacillus* and two species of *Pseudomonas* were cultured from roots, stem+petioles and/or leaves of *Nicotiana benthamaiana* growing in an organic soil. Treatment of the soil with a branched-chain alkane mixture resulted in both induced resistance against foliar infection by *Colletotrichum orbiculare* and changes in the populations of several of the endophytes. Increased populations compared to the controls were observed for the *Pseudomonas* sp. LW3, *Bacillus simplex* LW4 and *Bacillus pumilis* LW5 colony types in roots and the *B. simplex* strain LW4 colony type in stem+petioles. The LW3 and LW4 colony types also had higher populations in pure cultures grown in minimal media containing the branched-chain alkane mixture compared to controls. All eight colony types were able to induce resistance against *C. orbiculare* when inoculated into roots, but differed in their effectiveness. All the endophyte colony types, except *B. simplex* LW4 and *B. marisflavi* LY1, could inhibit *C. orbiculare* growth *in vitro*, but the level of inhibition was not correlated with the level of induced resistance *in planta*. It appears that branched-chain alkanes can result in selective endophyte growth promotion *in planta*, affecting populations of endophytes that can possibly both catabolise alkanes and induce systemic resistance in plants.

Author Disclosure Block:

P. Goodwin: None. **W. Gao:** None.

Poster Board Number:

SUNDAY-564

Publishing Title:

Endophytic Microbiome Of Jatropha Curcas

Author Block:

K. Mighell[1], S. Van Bael, 70118[1], K. Saltonstall[2]; [1]Tulane Univ., New Orleans, LA, [2]Smithsonian Tropical Res. Inst., Panama City, Panama

Abstract Body:

Background: The soil is the principle source of bacterial symbionts of plants. Select bacteria colonize the rhizosphere and endophytic bacteria colonize root tissue and shoot tissue through the vascular system. Soil chemistry, edaphic factors, biogeography, and host biology all influence the rhizosphere and endophytic community of bacteria. *Jatropha curcas* (Euphorbiaceae) is a drought-tolerant biodiesel crop plant native to Central America. How do soil edaphic factors and biogeography affect the recruited endophytic and rhizosphere community?**Methods:** We transplanted sterile jatropha seedlings into three sites along a 125km transect in Panama. Each site had different soil chemical properties, experienced a range of precipitation levels, and had different use histories. Leaves, roots and rhizosphere samples were collected from the seedlings after growing in the soil at each site for ten weeks. We extracted the DNA for all samples. For a selection of roots and rhizosphere, we also extracted RNA and created cDNA libraries of the 16S gene. We sequenced the 16S community on a MiSeq and analyzed the results using Qiime.**Results:** Bacterial communities were all distinct by organ. Leaf and root communities were dominated by Proteobacteria, with leaves containing mostly Alphaproteobacteria and roots containing even mixtures of Alphaproteobacteria and Betaproteobacteria. The rhizosphere was dominated by Proteobacteria and Acidobacteria. The cDNA library showed that many different groups, principally Acidobacteria, were present in lower abundances in the cDNA library as compared to the DNA library. Ordination revealed that site was a significant factor driving community clustering, with sites experiencing similar rainfall being more similar, despite edaphic differences.**Conclusions:** We conclude that climatic factors have an effect on the symbiotic community of *J. curcas*. Bacterial 16S presence and activity differed, demonstrating that the select groups are more metabolically important than previously thought. Plant associated communities can strongly affect plant performance. As *J. curcas* is a drought tolerant plant, the symbiotic community may play a profound role in mitigating its stress tolerance. By comparing this work to plant performance data and bacterial community function, we can predict how different bacterial communities may act within the host.

Author Disclosure Block:

K. Mighell: None. **S. Van Bael:** None. **K. Saltonstall:** None.

Poster Board Number:

SUNDAY-565

Publishing Title:

Analysis of Volatile Organic Compounds Emitted by Biological Control Trichoderma and Volatile-Mediated Plant Growth

Author Block:

S. Lee, J. W. Bennett; Rutgers, The State Univ. of New Jersey, New Brunswick, NJ

Abstract Body:

Volatile organic compounds (VOCs) are a small portion of the total metabolites produced by organisms; however, their unique properties enable them to mediate important biological functions, especially in aerial and terrestrial environments. In agriculture, the potential uses of VOCs include volatile-mediated inhibition of pathogen growth and increased plant systemic resistance. Filamentous fungi in the genus *Trichoderma* are robust biological control agents as they utilize several modes of action including resistance, antibiosis, competition and myco-parasitism. Earlier work by our laboratory demonstrated the ability of *Trichoderma*-derived VOCs to stimulate *Arabidopsis* growth. In this study, *Trichoderma* emission profiles, concentrations, quantities, and VOC-mediated effects on plants were measured in order to develop a mechanistic understanding of the volatile-mediated *Trichoderma*-to-plant interactions. We identified several volatile-mediated plant growth promoting *Trichoderma* isolates. Volatile-mediated plant growth promotion was dependent on the age of the fungal culture, developmental stage of the plant, duration of the exposure, and was isolate-specific. GC-MS analysis of VOCs from *Trichoderma* isolates led to identification of more than hundred unique compounds and several unknown sesquiterpenes, diterpenes, and tetraterpenes. A large number of compounds were selected and tested individually on *Arabidopsis* in order to determine if individual compounds could mimic the effects of *Trichoderma* volatile mixtures on plant growth. Exposing plants to single compounds yielded significant increases in seed germination, plant fresh shoot weight, and chlorophyll. RNA sequencing analysis of *Arabidopsis* shoots treated with VOC for 72 hours identified differentially expressed genes involved in volatile response. Up-regulated genes were related to growth, responses to hormone and cell wall modifications inducible by auxin. We provide a list of candidate genes to screen in future research on the biological activities of fungal VOCs.

Author Disclosure Block:

S. Lee: None. **J.W. Bennett:** None.

Poster Board Number:

SUNDAY-566

Publishing Title:

Flavonoids and Auxin are Part of the Chemical Talk Between Plant and Cyanobacteria in the Rhizosphere

Author Block:

A. Hussain; Absul Wali Khan Univ., Mardan, Pakistan

Abstract Body:

Rhizospheric region of the roots is richly supplied with the secondary metabolites that orchestrate all sorts of rhizospheric interactions and hence communicate plant roots with their belowground competitors. A chemical dialogue is established between the microbe and plant roots, creating an atmosphere of complex and dynamic interactions flourishing either symbiosis or pathogenicity. A wide range of secondary metabolites are found there in rhizospheric region but particularly flavonoids and auxins are documented to be the most important signalling elements in plant-microbe interactions. Role of synthetic flavonoids and auxins as signalling compounds have largely documented in case of legume-rhizobia symbiosis but the field is lacking regarding the symbiosis of cyanobacteria with higher plants. Focusing on the establishment of artificial symbiosis between rice plant and the cyanobacteria, in the present study 21 different strains were isolated from the rice roots, rhizospheric region and the surface soil in rice fields of different areas of District Mardan. Under hydroponic condition, the strains were able to colonize on rice roots thereby enhancing seedling growth significantly. Root exudates showed increased flavonoid and auxin content in case of all the tested strains. Application of exogenous flavonoids and auxins increase the growth of the seedlings while the flavonoid and auxin content was significantly reduced in the root exudates. While opposite trend was observed in case of axenic strains where the both metabolites were significantly increased as compared to non-treated strains. Regarding co-culturing treatments exogenous flavonoids and auxins also show positive differences with the fact that the flavonoids and auxins in combination improved the colonization of roots by cyanosymbiont as well as the exogenous flavonoid and auxin content. Also, IAA and flavonoids producing strains of cyanobacteria efficiently colonized on rice root and enhanced growth of the seedlings.

Author Disclosure Block:

A. Hussain: None.

Poster Board Number:

SUNDAY-567

Publishing Title:

Metagenomic Analysis of the Root Nodule Endophyte Community of the Actinorhizal Plant Casuarina Glauca

Author Block:

T. S. D'Angelo[1], F. Ghodhbane-Gtari[2], A. Ktari[2], K. Hezbri[2], A. Gueddou[2], M. Gtari[2], L. S. Tisa[1]; [1]Univ. of New Hampshire, Durham, NH, [2]Univ. of Tunis El Manar, Tunis, Tunisia

Abstract Body:

Background: Actinorhizal plants host mutualistic symbionts of the nitrogen-fixing actinobacterial genus *Frankia* within nodule structures formed on their roots. Several plant-growth-promoting bacteria have also been isolated from actinorhizal root nodules, but little is known about them. We were interested investigating the *in planta* microbial community composition of actinorhizal root nodules using culture-independent techniques. To addresses this knowledge gap, 16s rRNA amplicon and shotgun metagenomic sequencing was performed on DNA from the nodules of the *Casuarina glauca.* **Methods:** DNA was extracted from *C. glauca* nodules collected in three different sampling sites in Tunisia, along a gradient of aridity ranging from humid to arid. Sequencing libraries were prepared using Illumina Nextera technology and 150 BP paired-end reads were sequenced using the Illumina HiSeq 2500 platform. After quality filtering, the three data sets, totaling 83 million reads, were combined *in silico* and assembled using the SPAdes assembler. Reads from each sampling location were aligned to the assembly using the Bowtie2 alignment tool. Unique coverage values, tetranucleotide frequency and GC content was used to cluster contigs into genome bins using the mmgenome R package. Genome bins extracted from the metagenome were taxonomically and functionally profiled. Community structure based off of preliminary 16s rRNA amplicon data was analyzed via the QIIME pipeline.**Results:** Reconstructed genomes were comprised of members of *Frankia*, *Micromonosopora*, *Bacillus*, *Paenibacillus*, *Phyllobacterium* and *Afipia*. *Frankia* dominated the nodule community at the humid sampling site, while the absolute and relative prevalence of *Frankia* decreased at the semi-arid and arid sampling locations. The genera of non-*Frankia* members in this community have been isolated from legumes and other plants.**Conclusions:** Actinorhizal plants harbor similar non-*Frankia* plant-growth-promoting-bacteria as legumes and other plants. The data suggests that the prevalence of *Frankia* in the nodule community is influenced by environmental factors, with *Frankia* being less abundant under more arid environments.

Author Disclosure Block:

T.S. D'Angelo: None. **F. Ghodhbane-Gtari:** None. **A. Ktari:** None. **K. Hezbri:** None. **A. Gueddou:** None. **M. Gtari:** None. **L.S. Tisa:** None.

Poster Board Number:

SUNDAY-568

Publishing Title:

Phyllosphere Microbial Ecology of Succulent Plants in a Greenhouse Environment

Author Block:

J. E. Einson, X. You, C. Randell, A. Rodriguez, M. Bartlett, D. Sela; Univ. of Massachusetts, Amherst, MA

Abstract Body:

It is established that the plant phyllosphere is a readily habitable environment for a wide range of microorganisms. The phyllosphere microbiome varies profoundly between plant species, suggesting that specific host traits may enhance or restrict cognate microbial populations. Here we have characterized the microbial communities associated with several succulent plants that are co-housed in the same environment. Succulents have experienced convergent evolution over the past 250 million years, where plant lineages have converged on similar phenotypes to thrive in arid environments. Although succulents exhibit physical similarities, their associated microbial communities may vary to reflect the underlying biology of plant-microbial interactions. Using culture-independent techniques, microbial community structure is characterized with much higher resolution, and in the absence of selective bias of culturing. Thus we tested the hypothesis that there is no significant difference in phylogenetic diversity between phyllospheres of succulent plants that are located in the same greenhouse. We collected samples (n≈30) from members of *Liliaceae, Cactaceae, Aizoaceae,* and *Crassulaceae* by swabbing various surfaces of these plants. Extracted community DNA was subjected to PCR amplification of the 16S rRNA phylogenetic marker gene using broad-range primers. Libraries were constructed and sequenced to characterize phylogenetic diversity. Various non-parametric statistical methods were employed to verify non-random diversity measurements. This study builds on our knowledge of how the eukaryotic world interacts with the bacterial world. If bacterial communities remain constant regardless of succulent host, the greenhouse environment would likely contribute the largest role in microbiome composition. However, if each plant fosters a distinct microbial community, this will require further investigation to determine the factors underlying these host-microbial relationships. One possibility is that the phyllosphere composition of succulent plants helped conserve water in their relatively harsh environment.

Author Disclosure Block:

J.E. Einson: None. **X. You:** None. **M. Bartlett:** None. **D. Sela:** None.

Poster Board Number:

SUNDAY-569

Publishing Title:

Formate, a Driver of Anaerobic Processes in the Rhizosphere of a Methane-Emitting Fen

Author Block:

H. L. Drake, S. Hunger, O. Schmidt, A. S. Goessner; Univ. Bayreuth, Bayreuth, Germany

Abstract Body:

Wetlands are important sources of globally-emitted methane. Plants mediate much of that emission by releasing root-derived organic carbon, including the root leakage of direct precursors of methane such as formate. Thus, the objective of this study was to identify potential formate-driven anaerobic processes linked to methanogenesis in the root-zone of fen-derived plants. Although formate was anticipated to trigger methanogenesis, the rapid anaerobic consumption of formate by *Carex* roots unexpectedly yielded H_2 and CO_2, an activity indicative of formate-H_2-lyase (FHL). Formate-derived H_2 augmented methanogenesis and acetogenesis, and molecular and cultivation approaches identified hydrogenotrophic methanogenic (*Methanoregula*, *Methanobacterium*, *Methanocella*) and acetogenic (*Acetonema*, *Clostridum*, *Sporomusa*) genera. Fifty-seven hydrogenase-containing family-level phylotypes were detected, and *Proteobacteria* appeared to contribute to the production of H_2. Root-derived FHL-containing fermentative *Citrobacter* and *Hafnia* isolates produced H_2 in response to formate, and co-substrate utilization of formate enhanced the utilization of glucose by the *Citrobacter*-affiliated species, an activity indicative of 'priming' observed at the system-level. The findings (a) indicate that root-associated FHL-containing fermenters produce formate-derived H_2 that can subsequently drive secondary anaerobic processes proximal to fen roots, (b) provides novel insights on remarkably diverse hydrogenase-containing taxa potentially linked to this trophic interaction, and (c) raise questions regarding the fate of formate-derived H_2 when it diffuses out of the root-zone.

Author Disclosure Block:

H.L. Drake: None. **S. Hunger:** None. **O. Schmidt:** None. **A.S. Goessner:** None.

Poster Board Number:

SUNDAY-570

Publishing Title:

Influence of Seed Microbiome on Fitness of *Epichloë* Infected Tall Fescue Seedlings

Author Block:

E. Roberts, **B. Mormile**; Southern Connecticut State Univ., New Haven, CT

Abstract Body:

Increased fitness in tall fescue (*Schedonorus arundinaceaus)* is attributed to infection by *Epichloë coenophiala*. However, the mechanisms of some host benefits associated with *Epichloë* infection are poorly understood. Similarly, plant-growth promoting (PGP) bacteria increase fitness of many types of plants, and PGP bacteria have been shown to dominate the rhizosphere microbiome of *E. coenophiala* infected (E+) tall fescue. While it is likely that some PGP bacteria are recruited from the soil, we hypothesized that such bacteria are dominant members of tall fescue seed microbiomes. Furthermore, we expect that PGP seed colonizers influence the fitness of developing tall fescue seedlings.Genetically identical clone pairs of endophyte-infected (E+) and endophyte-free (E-) tall fescue seeds were surface sterilized to remove epiphytic bacterial colonizers. Twenty surface sterilized and 20 control plants of each type were grown for six weeks prior to dry weight measurements of the above ground tissues. Forty individuals from certified E- (K32) and certified E+ (K31) tall fescue varieties were treated as mentioned above. Additionally, total 16S amplified DNA washed from the surfaces of each seed type was analyzed with Illumina sequencing to assess the microbial communities.Control E+ plants averaged 35% higher biomass than surface sterilized E+ plants. Conversely, biomass comparisons between the surface sterilized and non-sterilized E- varieties showed no statistical difference. E+ plant seeds are also dominated by plant-growth promoting bacteria (41% of the total community) *Rhizobium* sp., *Pedobacter* sp., *P. agglomerans, Agrobacterium* sp., and *Enterobacter cowaanii* which are either absent or in very minor quantities (6% of the total community) on E- fescue varieties. Our findings indicate that *Epichloë* infection influences the bacterial community composition of tall fescue seed surfaces, and that those bacteria play an important role in fitness of host seedlings.

Author Disclosure Block:

E. Roberts: None. **B. Mormile:** None.

Poster Board Number:

SUNDAY-571

Publishing Title:

Microscale Imaging of Microbial Communities on *Arabidopsis thaliana* L. Leaf Surfaces Using Confocal Laser Scanning Microscopy and Fluorescence *In Situ* Hybridization

Author Block:

E. L. Peredo, S. L. Simmons; Marine Biological Lab., Woods Hole, MA

Abstract Body:

The plant phyllosphere, formed by leaves and other aerial organs, harbors diverse microbial communities composed of yeast, fungi, archaea, bacteria and viruses. The leaf habitat is characterized by extreme environmental conditions with high UV and low water and nutrient availability. Leaf-associated taxa often display specialized adaptive traits such as aggregation, chemotaxis, and production of phytohormones, antibiotics or pigments. Microbes can also overcome harsh conditions in the phyllosphere by preferentially colonizing specific leaf microhabitats such as cuticle cracks, stomata, hydathodes, veins, trichomes, and glands. Variables at the microscale, not detectable by bulk methods, can play a key role on the assembly of microbial communities and affect the establishment of human pathogens on leaves, an important issue for food safety.We developed a microbial visualization protocol adapted to leaf surfaces, with specific fixation steps to minimize autofluorescence. We used CLSM (Zeiss LSM 780, lambda mode) to generate crosstalk-free multi-fluorescence images by parallel or sequential acquisition of images with spectral information of each pixel, followed by linear unmixing processing. Autofluorescence, greatly reduced after tissue pretreatments, was incorporated in the images as another spectral character.We demonstrate that our method is suitable for visualization of occurring bacteria on leaves of *in vitro* or glasshouse-grown *Arabidopsis*. Using CLASI-FISH (Valm *et al*., 2012) approaches we report simultaneous detection of at the genus level of three phylogenetically diverse microbes *in situ*. Finally, we demonstrate the difficulty of predicting bacterial distributions in the phyllosphere based solely on phylogenetic and physiological information. We investigated the spatial distributions of a reference and a newly isolated strain of the common pink-pigmented phyllosphere genus *Methylobacterium.* We initially hypothesized similar distributions on leaves due to their shared metabolic niche (facultative methylotrophy) and ability to produce active phytohormones. Our results demonstrate, however, that even closely-related bacterial species sharing common physiological traits can consistently occupy extremely different leaf microniches.

Author Disclosure Block:

E.L. Peredo: None. **S.L. Simmons:** None.

Poster Board Number:

SUNDAY-572

Publishing Title:

***Bacillus* sp. Inhibits Fungi and Enhances Resistance of Cotton Seedlings against Damping-off Disease Caused by *Fusarium* sp.**

Author Block:

I. Irizarry, Q. Chen, M. Bergen, J. White; Rutgers Univ., New Brunswick, NJ

Abstract Body:

Wild relatives of crops are sources of beneficial microbes that can improve health and promote growth of economically important crops. *Bacillus* sp. was isolated from the tropical coastal tree *Thespesia populnea* (Malvaceae), evaluated for its antifungal properties, and its ability to promote growth of cotton seedlings (*Gossypium hirsutum*; Malvaceae). *Bacillus* sp. was co-cultured with various seed-transmitted fungi on potato dextrose agar to observe its effects on fungal growth. The colony diameters and growth rates of fungal cultures were measured along with the width of 20 hyphal cells. Genes for lipopeptide synthesis in *Bacillus* sp. were amplified by PCR. Lipopeptides produced by *Bacillus* sp. in 1L of potato dextrose broth were extracted, identified by MALDI-TOF molecular weight estimation analysis, and tested for antifungal activity by disk diffusion assays. *Bacillus* sp. was also tested for its ability to enhance cotton seedling resistance against *Fusarium* sp. damping-off disease. Cotton seedlings were grown for 21 days on inoculated soil inside magenta vessels. The average total dry weight and the root/shoot ratio of four 21 day old cotton seedlings were calculated. T-tests were used to show significant difference between means. *Bacillus* sp. significantly reduced the colony diameter, growth rates, and hyphal width of *L. theobromae, Phomopsis* sp., *B. ochroleuca, Rhizopus* sp., *Curvularia lunata,* and *Fusarium* sp. ($p=<0.01$). The genes for surfactin and iturin synthesis, *srfC* and *ituD,* were amplified and a mixture of lipopeptides was detected in the extract including fengycin, iturin A, surfactin, and kannurin. The extract inhibited growth and induced chlamydospore production in *L. theobromae, B. ochroleuca, Phomopsis* sp., *C. lunata, Fusarium* sp., *Cladosporium cladosporioides, Neofusicoccum australe, Fusarium brachygibbosum,* and *Setosphaeria rostrata* ($p=<0.01$). Cotton seedlings inoculated with *Bacillus* sp. along with *Fusarium* sp. had increased biomass, root to shoot development, and enhanced resistance to *Fusarium* sp. damping-off disease ($p=<0.01$) after 21 days of growth. Our findings show that bioprospecting for beneficial bacteria in wild relatives of crops is a useful strategy. Bacteria like *Bacillus* sp. could be commercialized as biofertilizers to protect vulnerable cotton seedlings against fungal pathogens.

Author Disclosure Block:

I. Irizarry: None. **Q. Chen:** None. **M. Bergen:** None. **J. White:** None.

Poster Board Number:

SUNDAY-573

Publishing Title:

Estimation of Generation Times in Planta for the Non-cultivable Citrus Pathogen '*Candidatus* Liberibacter Asiaticus'

Author Block:

M. E. Hilf; USDA-ARS, Fort Pierce, FL

Abstract Body:

'*Candidatus* Liberibacter asiaticus' is an insect-transmitted bacterial pathogen of citrus. Infection of citrus trees by '*Ca.* Liberibacter asiaticus' results in a chronic, degenerative decline disease called huanglongbing or citrus greening. Its presence in the US was confirmed in Florida in August 2005. The bacterium can infect a range of economically important citrus varieties and since 2005 crop losses in Florida have approached 50% from tree and fruit loss due to infection. In citrus '*Ca.* Liberibacter asiaticus' colonizes phloem sieve cells, which are specialized vascular cells which transport carbohydrates synthesized in leaves to local and distal portions of the tree. A sustained laboratory culture of '*Ca.* Liberibacter asiaticus' has not been established, so population growth characteristics from *in vitro* culture are unknown. To characterize the growth of bacterial populations in planta trees with established infections were pruned to force the growth of new branches which would be of equivalent physiological and temporal age and which would have an equivalent susceptibility to colonization. Real time PCR assays were performed on nucleic acid extracts from leaves collected from the new growth at 30, 60 and 90 days post-pruning. Assuming binary fission as the primary mode of cell division, bacterial populations calculated from mean cell numbers indicated that population doubling times between 30 and 60 days post-pruning were 10.2, 5.3 and 7.2 days for the citrus varieties 'Carrizo' citrange, sour orange and *Citrus macrophylla* respectively. The calculated mean cell numbers suggested that populations were in an exponential phase of growth between 30 and 60 days, but by 90 days populations had transitioned to a slower-growing, possible stationary phase. For the 60 to 90 day period, the calculated doubling time for '*Ca.* Liberibacter asiaticus' in sour orange was 17.1 days, which was more than triple the calculated doubling time for the 30-60 day period. Data from this study indicates that forcing new growth on infected trees can help synchronize colonization of tissues by '*Ca.* Liberibacter asiaticus' and that populations of bacteria display host-dependent growth characteristics which may influence disease development in infected trees.

Author Disclosure Block:

M.E. Hilf: None.

Poster Board Number:

SUNDAY-574

Publishing Title:

The Genome and Genetics of a High Oxidative Stress Tolerant *Serratia* sp. LCN16 Isolated from the Plant Parasitic Nematode *Bursaphelenchus xylophilus*

Author Block:

C. Vicente[1], F. Nascimento[2], Y. Ikuyo[1], M. Mota[3], K. Hasegawa[1]; [1]Chubu Univ., Kasugai, Japan, [2]Univ.e Federal de Santa Catarina, Florianópolis, Brazil, [3]Univ.e de Évora, Évora, Portugal

Abstract Body:

Pine wilt disease (PWD) is an important disease of pine forests in East Asia and Europa forests and is caused by the pine wood nematode (PWN) *Bursaphelenchus xylophilus*. Bacteria are known to be associated with PWN in the host tree and may have an importante role in PWD. *Serratia* sp. LCN16 is an PWN associated bacterium that is highly resistant to oxidative stress *in vitro*, and which beneficially contributes to the PWN survival under these conditions. Oxidative stress is generated as a part of the basal defense mechanism used by plants to combat pathogenic invasion. Here, we investigated the interaction of the *Serratia* sp. LCN16 and PWN interaction in the disease development by exploring the LCN16 genome and functionally characterized some of the genes behind its impressive oxidative stress resistance. *Serratia* sp. LCN16 is phylogenetically most closely related to the phytosphere group of *Serratia*, which includes *S. proteamaculans*, *S. grimessi* and *S. liquefaciens*, and which have the ability to survive and colonize a plant-environment. Under conditions of oxidative stress (with H_2O_2, as the main stressor), LCN16 expresses *katG* (hydroperoxide I, HPI) under the positive regulation of OxyR. LCN16 *kat* (HPII) is OxyR-independent. LCN16 Δ*oxyR* was more sensitive to H_2O_2 than LCN16 Δ*kat*, and both deletion mutants failed protect the PWN from H_2O_2-stress exposure. *In planta*, co-inoculation of LCN16 Δ*oxyR* with PWN resulted in a slower development of disease symptoms. In contrast, the co-inoculation of wild-type LCN16 and PWN led to a faster PWD development than only PWN inoculation. This study provides new insights into the bacteria-nematode interaction in the natural host of PWD and provides further evidence of the potential role of bacteria in the first stages of PWN infection.

Author Disclosure Block:

C. Vicente: None. **F. Nascimento:** None. **Y. Ikuyo:** None. **M. Mota:** None. **K. Hasegawa:** None.

Poster Board Number:

SUNDAY-575

Publishing Title:

Study of Bacterial Diversity in Rhizosphere Soil of Two Caesalpinioideae (*Piliostigma reticulatum* and *Erythrophleum guineense*) Associated with Dry Cereal Crops in West Africa

Author Block:

N. NIANG[1], S. DEMANECHE[2], K. ASSIGBETSE[3], M. GUEYE[3], I. NDOYE[1], P. SIMONET[2], I. NAVARRO[2], **S. FALL**[4]; [1]Université Cheikh Anta DIOP (UCAD), LCM,LMI LAPSE, Dakar, Senegal, [2]Équipe "Génomique Microbienne Environnementale" (Environmental Microbial Genomics Group), UMR CNRS 5005, Ecully, France, [3]Inst. de Recherche pour le Développement (IRD), Dakar, Senegal, [4]Inst. Sénégalais de Recherches Agricoles, LNRPV, LCM, LAPSE, Dakar, Senegal

Abstract Body:

In Africa, 65% of agricultural lands are severely or moderately degraded. The association of cereal-legume crops is proposed as an alternative to improve both their organic status and productivity. In woodlands in Senegal, shrub and tree belonging to subfamily Caesalpinoideae (*Piliostigma reticulatum* and *Erythrophleum guineense*) are deliberately left in the fields by farmers for their roles in the water supply in the intercropping system and traditional medicines. However, their involvement in the soil nutrient cycles including nitrogen nutrition are very few documented. Therefore, the high-throughput sequencing technique (HTS) using Illumina Miseq platform combined with a profiling method (RISA) were used to assess the structural differences of bacterial communities, taxonomic groups and alpha and beta diversity between treatments (rhizosphere versus bulk soils). RISA analyses have shown a slight heterogeneity of bacterial community structure between the different treatments while HTS analysis showed high heterogeneity of bacterial community structures of soil rhizosphere associated with great diversity. Significant variations in relative abundances of dominant taxonomic groups were observed between the two types of soil, with the rhizosphere soils being dominated by *Proteobacteria* (35.8%) while *Actinobacteria* (26%) were dominant in the bulk soils. Bacterial communities associated with the rhizosphere of *P. reticulatum* were different from those of *E. guineense* and were dominated respectively by *Acidobacteria* (24.6%) and *Verrucomicrobia* (8.4%).Briefly, the high-throughput sequencing methods had increased our knowledge of the bacterial communities diversity in the rhizosphere of P. reticulatum and E. guineense associated with cereal crops in the savanna area.

Author Disclosure Block:

N. Niang: None. **S. Demaneche:** None. **K. Assigbetse:** None. **M. Gueye:** None. **I. Ndoye:** None. **P. Simonet:** None. **I. Navarro:** None. **S. Fall:** None.

Poster Board Number:

SUNDAY-576

Publishing Title:

Relation Between Pathogen Resistance and Rhizosphere Microbiome in Common Bean

Author Block:

L. W. Mendes[1], R. Mendes[2], M. de Hollander[1], J. M. Raaijmakers[1], S. M. Tsai[3]; [1]Netherlands Inst. of Ecology, Wageningen, Netherlands, [2]Embrapa Environment, Jaguariuna, Brazil, [3]Ctr. for Nuclear Energy in Agriculture, Piracicaba, Brazil

Abstract Body:

The microbial community in the rhizosphere plays a key role in the functioning of the host plant, influencing its physiology, growth & development. It has been postulated that plants modulate the microbiome composition to fend off pathogens. In this context, we investigated the rhizosphere microbiome of common bean cultivars that differ in resistance to the soil-borne pathogen *Fusarium oxysporum* (Fox). Using 16S rRNA sequencing and metagenomics we assessed the taxonomic composition and functional potential of the rhizosphere microbiome of common bean. The results show that bacterial abundance increased with an increase in resistance to Fox. The Fox-resistant bean cultivar presents a higher abundance Pseudomonadaceae, Bacillaceae, Solibacteraceae and Cytophagaceae as compared to the Fox-susceptible cultivar. At a finer taxonomic level, *Bacillus* and *Pseudomonas* were highly abundant in the rhizosphere of the Fox-resistant cultivar. The network analysis, with taxonomic, functional and environmental parameters, presented 3-4 fold more complexity in the Fox-resistant cultivar and showed a non-modular topology, indicating a more complex and highly connected community. Although the Fox-resistance of the common bean is cultivar linked to plant genetic traits our findings suggest that Fox-resistance also impacts the microbiome selected in the rhizosphere. Whether these changes in the rhizosphere microbiome contribute to the Fox-resistance phenotype, by means of specific microbial groups, functional traits, diversity and abundance, and network structure, is subject of ongoing experiments.

Author Disclosure Block:

L.W. Mendes: None. **R. Mendes:** None. **M. de Hollander:** None. **J.M. Raaijmakers:** None. **S.M. Tsai:** None.

Poster Board Number:

SUNDAY-577

Publishing Title:

Variation in the Microbiome of Wine Grapes from the Willamette Valley, Oregon

Author Block:

M. VandenBerg, D. Grenier, J. Weisz, A. Kruchten; Linfield Coll., McMinnville, OR

Abstract Body:

Numerous abiotic environmental factors influence the maturation of grapes and subsequently the wine made from them. It has recently by hypothesized that, in addition to these factors, the microbiome of the wine grapes may also have a significant effect on the maturation of grapes and contributions to wine that are not fully understood. To determine if small-scale environmental differences influence the composition of the microbiome, samples were taken across several blocks in a single vineyard in Amity, Oregon. Full clusters of grapes were sampled from the vineyard using sterile technique and DNA was extracted from four grapes of each cluster. DNA was sequenced via high throughput sequencing with a primer set for the 16S gene. Results show significant differences in microbiome composition between grapes of different varieties in neighboring blocks. Further, microbiomes also varied within a grape variety in blocks across the vineyard. Within the same vineyard, where macroenvironmental conditions are largely uniform, it is significant that the microbiome is highly variable. These data suggest that "microbial terroir" may be influenced by a variety of factors, including the nature of the grapes and microenvironmental variables.

Author Disclosure Block:

M. VandenBerg: None. **D. Grenier:** None. **J. Weisz:** None. **A. Kruchten:** None.

Poster Board Number:

SUNDAY-578

Publishing Title:

Genomic Evidence Of A Recent Host Restriction In An Emerging Plant Pathogen

Author Block:

L. R. Shapiro[1], O. Zhaxybayeva[2], J. Paulson[3], E. Scully[4], N. Pierce, 02138[1], G. Beattie[5], M. Gleason[5], R. Kolter[3]; [1]Harvard Univ., Cambridge, MA, [2]Dartmouth Univ., Hanover, NH, [3]Harvard Univ., Boston, MA, [4]USDA, Manhattan, KS, [5]Iowa State, Ames, IA

Abstract Body:

Background: Extensive landscape modification has accompanied human transition from nomadic to agricultural societies. This has resulted in the conversion of intact ecological habitats to simplified agro-ecosystems, containing large and genetically homogeneous populations of domesticated plants and animals with reduced genetic diversity. Some novel microbial variants are able to emerge into these populations and cause diseases that threaten agricultural production. Here, we present evidence that *Erwinia tracheiphila* (Smith) is one such recently emerged pathogen. *E. tracheiphila* infects two genera of wild and cultivated cucurbits, native *Cucurbita* spp. (squashes, pumpkins, and gourds), and introduced *Cucumis* spp. (cucumbers and melons). Despite the worldwide distribution of susceptible host plants, *E. tracheiphila* epidemics are geographically restricted to the Eastern United States and Southern Canada. **Results**: To investigate the evolutionary history of *E. tracheiphila*, we used long read PacBio sequencing to generate a reference sequence of an isolate from a *Cucurbita* plant and an isolate from a *Cucumis* plant, and then sequenced a 100 strain collection of isolates from across the geographic range where *E. tracheiphila* occurs. We find that the *E. tracheiphila* genome shows signs of recent, rapid change, including active phage infection, dramatic invasion and proliferation of transposable elements, horizontal acquisition of genes putatively important for host interactions, and extensive pseudogenization. Despite evidence of rapid change, sequences from the strain collection display negligible SNP diversity, and coalescent estimates suggest a recent emergence into its current niche. Further, *E. tracheiphila* appears to be undergoing an incipient lineage split associated with host adaptation to the two plant genera (*Cucumis* spp. and *Cucurbita* spp) it infects. This system provides a model to better understand the genetic and ecological factors affecting the evolution and rapid emergence of novel pathogens.

Author Disclosure Block:

L.R. Shapiro: None. **O. Zhaxybayeva:** None. **J. Paulson:** None. **E. Scully:** None. **N. Pierce:** None. **G. Beattie:** None. **M. Gleason:** None. **R. Kolter:** None.

Poster Board Number:

SUNDAY-579

Publishing Title:

Cellulose-targeting Sus-like Systems within the Gut Metagenomes of Herbivores

Author Block:

C. M. Bahr[1], P. B. Pope[2], A. E. Naas[2], N. M. Koropatkin[1]; [1]Univ. of Michigan Med. Sch., Ann Arbor, MI, [2]Norwegian Univ. of the Life Sci., Aas, Norway

Abstract Body:

Human gut Bacteriodetes target dietary glycans via the expression of several outer membrane complexes termed starch utilization (Sus)-like systems. Each Sus-like system targets a distinct carbohydrate and is comprised of proteins that sequentially bind, degrade and import carbohydrates. The proteins within individual Sus-like systems are functionally conserved, although they target different glycans, and it is the specificity of these proteins that dictate which glycans the cell can utilize. The focus of my research is to understand the molecular basis of how glycans are recognized by different Sus-like systems. Because Sus-like systems for nearly every plant cell wall fiber except cellulose have been identified in human gut isolates, it was thought that cellulose degradation in all mammalian guts, including herbivores, was likely regulated by Gram positive bacteria. Here we have characterized the extracellular glycan binding lipoproteins of a predicted Sus-like system within an uncultured Bacteroidales, found in the cow rumen metagenome, that is likely to target cellulose. Using non-denaturing affinity electrophoresis and isothermal titration calorimetry we demonstrate that these proteins target longer cello-oligosaccharides, comprised of five or more glucose residues, with high affinity. Interestingly, these Sus-like protiens from the cow rumen Bacteroidales systems can target a wide variety of β-glucan structures, in contrast to many human gut Sus-like systems that are highly specific for a discrete carbohydrate structure. We determined the crystal structure of the SusD-like protein in this system, and by comparison to other SusD-like protein structures we predicted and confirmed, via site directed mutagenesis, the cellulose-binding residues of the protein. The glycan-binding residues within the structure are arranged as a planar platform of tryptophans, giving it the ability to recognize the linear structure of cellulose, which reveals how and why specific Sus-like proteins can target one or more plant cell wall glycan. This important structural information may facilitate the development of novel probiotic strains that can target cellulose in the human diet.

Author Disclosure Block:

C.M. Bahr: None. **P.B. Pope:** None. **A.E. Naas:** None. **N.M. Koropatkin:** None.

Poster Board Number:

SUNDAY-581

Publishing Title:

Influence of Dietary and Ontogenetic Factors on the Microbiome of Blacklegged Ticks (*Ixodes scapularis*)

Author Block:

C. P. Zolnik[1], R. C. Falco[2], T. J. Daniels[1], S. O. Kolokotronis[1]; [1]Fordham Univ., Bronx, NY, [2]New York State Dept. of Hlth., Armonk, NY

Abstract Body:

Blacklegged ticks (*Ixodes scapularis*) are important disease vectors in the United States, capable of maintaining and transmitting a number of human pathogens. Although over 98% of their life is spent off-host in forested settings, they require three bloodmeals throughout the course of their life cycle. Blood feeding usually lasts for several days and involves the transfer of host blood to the tick. This close interaction between host and tick likely influences the overall bacterial community within the tick. The purpose of this study was to explore the influence of host bloodmeal, engorgement, digestion, and sex on the *I. scapularis* microbiome over the nymph-to-adult transition period. Nymphal blacklegged ticks were collected in June 2014 from a field site in southern NY, an area with high tick populations and associated tick-borne pathogen prevalence. Ticks were fed on white-footed mice (*Peromyscus leucopus*) and were sacrificed at intervals (immediately post-feeding, two weeks post feeding, immediately post-molt). Additionally, host-seeking nymphs (before feeding) were also used to provide a baseline. DNA was extracted from each tick and 16S rRNA gene amplicon sequencing was carried out on the MiSeq (300bp PE) Illumina platform. OTUs were clustered *de novo* using a 97% identity threshold and identified with the Greengenes database. Our results demonstrate bacterial community structuring between host bloodmeal sources and engorged ticks. Although 82 taxa were shared between blood and tick samples, an additional 90 were found exclusively in ticks. A decrease in bacterial diversity was noted from field-collected nymphs through the various levels of digestion and after molting into adults, suggesting that bacterial OTUs are lost after feeding and throughout digestion. These results are in contrast to our previous study where bacterial diversity across all developmental stages was highest in field-collected adult males. It is likely that ticks lose bacterial OTUs after feeding but continual exposure to the bacteria from the field environment counters this loss in field-collected ticks. Maintaining the ticks from engorgement, through digestion and molting in sterilized vials and incubators allowed us to observe the decrease in bacterial diversity.

Author Disclosure Block:

C.P. Zolnik: None. **R.C. Falco:** None. **T.J. Daniels:** None. **S.O. Kolokotronis:** None.

Poster Board Number:

SUNDAY-582

Publishing Title:

Cockroach Gut Microbiome Considering the Presence of a Gut Parasitic Nematode

Author Block:

C. Vicente, S. Ozawa, K. Hasegawa; Chubu Univ., Kasugai, Japan

Abstract Body:

Since the ancestor of the Blattodea spread from Mantodea in about 200 million years ago, cockroaches have evolved to cope with harsh climate changes and survival competition during long evolutional history and now being considered as one of the most successful insect groups on earth. From the fact that thelastomatid parasitic nematodes have been reported from many Blattodea species (Ozawa et al., 2014), it is very interesting to examine the evolutionary history of the cockroach and gut parasitic nematodes. In addition, cockroach microbiome, specifically, gut microbiota is extremely specified and adapted to the gut environment, their mutualistic balance might be highly sophisticated and indispensable for the host high environmental adaptability. The present study aims to understand the effect of the parasitic nematode *Leidynema appendiculatum* on the microbiome of the Smokybrown cockroach *Periplaneta fuliginosa* using a metagenomic approach. A total of 18 lab-reared cockroach individuals (9 nematode-infected and 9 nematode-free), fed with the same diet, were dissected, the gut extracted and divided into foregut, midgut and hindgut. Six independent libraries of the V3-V4 region of the 16S rRNA gene were prepared and sequenced using Illumina MiSeq System. Intraspecific variation between individuals is not considered in our analysis. Our results contribute to shed light into the ecological role of the universal symbiotic relationship between host animals, parasitic nematodes and microbiome.

Author Disclosure Block:

C. Vicente: None. **S. Ozawa:** None. **K. Hasegawa:** None.

Poster Board Number:

SUNDAY-583

Publishing Title:

Restriction of Dietary Zinc and Its Impact on the Structure and Function of the Gut Microbiome of Adult Zebrafish

Author Block:

C. A. Gaulke, L. M. Beaver, C. L. Barton, R. L. Tanguay, E. Ho, T. J. Sharpton; Oregon State Univ., Corvallis, OR

Abstract Body:

Growing evidence suggests that the microbiome plays an important role in establishing and maintaining gastrointestinal homeostasis. Changes in diet are known to have rapid and profound impacts on microbiome structure. However, less is known about how micronutrients contribute to the structure and function of the gut microbiome and how this in turn can influence host health. Zinc is an essential micronutrient that plays a vital role in normal growth and development, protein synthesis and immune function. Zinc is also essential for the growth of most microorganisms and the host microbiota competes for zinc in the gastrointestinal tract. We examined the effects of limiting dietary zinc concentrations on the host microbiome structure and function using whole-genome shotgun and 16S amplicon sequencing for adult zebrafish fed a zinc deficient defined diet (n=15), zinc adequate defined diet (n =15), and a zinc adequate commercially available zebrafish chow (n=15). While the animals receiving the zinc adequate diets harbored microbiomes similar in taxonomic composition and abundance, significant differences in taxonomic abundance and composition were observed between the microbiome of fish receiving the zinc adequate and zinc deficient diets. The abundance of the phyla Fusobacteria and Tenericutes were significantly increased and Proteobacteria decreased in the animals receiving the zinc deficient diet when compared to those receiving zinc adequate diets ($p < 0.05$). Similar observations were made at lower taxonomic and OTU level analysis. The functional capacity of the gut microbiomes of fish receiving zinc deficient and zinc adequate diets was then quantified using shotgun metagenomics. Comparisons of functional differences between fish receiving zinc deficient and zinc adequate diets were correlated to host transcriptional profiles to examine potential relationships between altered microbiome function and host physiology. These experiments provide novel insights into the effects of nutrient limitation on gut microbiome structure and function and expand our understanding of how these shifts may be involved in altered host physiology.

Author Disclosure Block:

C.A. Gaulke: None. **L.M. Beaver:** None. **C.L. Barton:** None. **R.L. Tanguay:** None. **E. Ho:** None. **T.J. Sharpton:** None.

Poster Board Number:

SUNDAY-584

Publishing Title:

Evaluation of 16s Rrna Amplicon Sequencing Using Two Next-Generation Sequencing Technologies for Phylogenetic Analysis of the Rumen Bacterial Community in Steers

Author Block:

P. R. Myer[1], M. Kim[2], H. C. Freetly[2], T. P. L. Smith[2]; [1]Univ. of Tennessee, Univ. of Tennessee Inst. of Agriculture, Knoxville, TN, [2]USDA-ARS, U.S. Meat Animal Res. Ctr., Clay Center, NE

Abstract Body:

Next generation sequencing technologies have vastly changed the approach of sequencing of the 16S rRNA gene for studies in microbial ecology. Three distinct technologies are available for large-scale 16S sequencing. All three are subject to biases introduced by sequencing error rates, amplification primer selection, and read length, which can affect the apparent microbial community. The objective of this study was to compare and analyze short read 16S rRNA variable regions, V1-V3, with that of near-full length 16S regions,V1-V8, using highly diverse microbial communities from the rumen contents of steers (n=32), in order to examine the impact of technology selection on phyogenetic profiles. Short paired end reads from the Illumina MiSeq platform were used to generate V1-V3 sequence, while long "circular consensus" reads from the Pacific Biosciences RSII instrument were used to generate V1-V8 data. The two platforms predicted similar microbial operational taxonomic units (OTUs) as well as species richness, Good's coverage, and Shannon diversity. However, the V1-V8 amplified ruminal community resulted in significant increases in several orders of taxa, such as phyla Proteobacteria and Verrucomicrobia ($P < 0.05$). Taxonomic classification accuracy was also greater for the near full-length read ($P < 0.05$). UniFrac distance matrices using jackknifed UPGMA clustering also noted differences between the communities. These data support the general consensus that longer reads result in a finer phylogenetic resolution that may not be achieved by shorter 16S rRNA gene fragments. Our work on the cattle rumen bacterial community demonstrates that utilizing near full-length 16S reads may be useful in conducting a more thorough study, or for developing a niche-specific database to use in analyzing data from shorter read technologies when budgetary constraints preclude use of near-full length 16S sequencing.

Author Disclosure Block:

P.R. Myer: None. **M. Kim:** None. **H.C. Freetly:** None. **T.P.L. Smith:** None.

Poster Board Number:

SUNDAY-585

Publishing Title:

In-Depth Analysis Of Broiler Chicken Gut Microbiome Responses Over Time Fed A Commercial Yeast-Based Prebiotic

Author Block:

S. Park, S. Lee, S. C. Ricke; Univ. of Arkansas, Fayetteville, AR

Abstract Body:

Prebiotics are considered fermentable food additive that selectively utilized by beneficial bacteria in lower gastrointestinal tracts of host. Several prebiotics based on yeast cell walls have been developed and evaluated for the effects on host for pathogenic bacteria reduction since usage of antibiotics in poultry industry has been identified with the rise of multidrug resistant pathogens. The aims of this study were to assess commercial broiler cecal microbiome response over time when supplemented by an antibiotic (BMD50) or a commercial yeast-based prebiotic (Biolex® MB40) into feeds. For microbiome analysis, DNA from individual birds was extracted to construct libraries and sequenced using an Illumina MiSeq platform. Acquired sequencing data sets were applied to QIIME pipeline to generate operational taxonomic units (OTUs) tables and identify relative abundance of microorganisms from phyla to genus levels. Although there were no significant increases or reductions of microorganisms in relative abundance among groups, the maturity of birds exhibited specific microbial population shifts in the prebiotic supplemented group. By considering and comparing cecal microbiomes of birds that are supplemented with feed additives, a better understanding of the relationship between specific microbial abundance and bird growth performance as well as health status can be achieved for developing a rationale on prebiotic selection strategies.

Author Disclosure Block:

S. Park: None. **S. Lee:** None. **S.C. Ricke:** None.

Poster Board Number:

SUNDAY-586

Publishing Title:

Effect of Diet on Enterobacterial Community Composition of the Wood-eating Catfish *Panaque nigrolineatus*

Author Block:

R. C. McDonald[1], J. E. M. Watts[2], H. J. Schreier[1]; [1]Univ. of Maryland Baltimore County, Baltimore, MD, [2]Univ. of Portsmouth, Portsmouth, United Kingdom

Abstract Body:

The Amazonian catfish *Panaque nigrolineatus* consumes large quantities of wood as part of its diet and may rely heavily on the metabolic activities of its enteric microbial community for nutrient acquisition. However, unlike exclusively wood-feeding organisms, *P. nigrolineatus* is capable of transitioning between wood-only and mixed wood/detritus diets depending on environment. This strategy provides a unique opportunity to examine the impact of a changing diet in a wood-feeding organism and enables the description of the core microbiome composition essential for fish nutrition. In the present study, the MiSeq platform was used to compare 16S rRNA gene sequences of the enteric microbial communities from fish fed either a wood/algae (mixed) diet or wood alone. A shift in microbiome composition and structure was observed across feeding regimens and within different GI tract regions. The wood-fed fish microbiome included predominantly Alphaproteobacteria, Sphingobacteriia, Gammaproteobacteria, Verrucomicrobiae and Bacilli, while mixed diet comprised predominantly Alphaproteobacteria, Gammaproteobacteria, Clostridium, Bacteroidetes, and Flavobacteria. Unlike mixed diet-fed fish, the microbial communities of wood-fed fish were remarkably consistent across tissue regions, suggesting wood consumption selects for a specific community across the entire GI tract length. This contrasts with the highly compartmentalized microbial communities observed in other wood-feeding organisms. Phylogenetic analysis identified two predominant OTUs with high sequence similarity to *Candidatus* Amoebophilus asiaticus (7-10%) and *Candidatus* Cardinium hertigii (5-8%) found exclusively in wood-fed fish. Both organisms are obligate intracellular symbionts of eukaryotes, but their role in fish nutrition is unclear. In addition to the symbionts, several phylotypes with high sequence similarity to nitrogen-fixing Alphaproteobacteria were identified in both diets in agreement with our previous findings. This study demonstrates that diet has a major impact on the microbiome of *P. nigrolineatus;* the ability to shift microbiomes may provide a selective advantage allowing the fish to assimilate less refractory substrates when available and has implications for microbial recruitment.

Author Disclosure Block:

R.C. McDonald: None. **J.E.M. Watts:** None. **H.J. Schreier:** None.

Poster Board Number:

SUNDAY-587

Publishing Title:

Bat Microbiota as Biocontrols for White-Nose Syndrome

Author Block:

D. E. Northup[1], A. S. Kooser[1], N. A. Caimi[1], P. S. Hamm[2], A. Porras-Alfaro[2], J. C. Kimble[1], J. M. Young[1], D. C. Buecher[3]; [1]Univ. of New Mexico, Albuquerque, NM, [2]Western Illinois Univ., Macomb, IL, [3]Buecher Biological Consulting, Tucson, AZ

Abstract Body:

Background: Recent studies have shown that mammals, such as humans, have a natural external microbiota that acts as a filter against pathogens. We know little about this potential defense in bats and its effectiveness against *Pseudogymnoascus destructans*, the causative agent of white-nose syndrome (WNS). **Methods:** One hundred-eighty-six bats from 14 species from pre-WNS New Mexico (NM) and Arizona (AZ) were swabbed and sequenced using 454 next gen sequencing.**Results:** Our Qiime and R Studio analysis of bat bacterial microbiota revealed that microbiota present on bats caught in caves are substantially different from those netted on the surface. Our results also reveal that the ecoregion from which the bat was caught, helps to differentiate different sampled microbiota. One major group present is the microbiota is the Actinobacteria, which are one of the most prolific producers of secondary metabolites, such as antifungals. These Actinobacteria may represent an effective biocontrol agent against *P. destructans*. To test this, we cultured external Actinobacteria from NM and AZ cave-caught and surface netted bats, subcultured 2100 isolates using media targeting Actinobacteria, and tested for the production of antifungals that are effective against *P. destructans*. Tests were conducted using a bi-layer method in the laboratory. Thirty-five isolates inhibited *P. destructans*, most of which were *Streptomyces* spp. One *Rhodococcus rhodochrous* also produced significant inhibition.**Conclusions:** Understanding the antifungal potential of the external microbiota on bats will help to identify potential WNS biocontrol agents. Also, investigations of microbiota, and Actinobacteria in particular, give us insight into what management strategies are likely to be successful.

Author Disclosure Block:

D.E. Northup: None. **A.S. Kooser:** None. **N.A. Caimi:** None. **P.S. Hamm:** None. **A. Porras-Alfaro:** None. **J.C. Kimble:** None. **J.M. Young:** None. **D.C. Buecher:** None.

Poster Board Number:

SUNDAY-589

Publishing Title:

Different Influence of *Mycobacterium tuberculosis* H37Rv and *Mycobacterium bovis* BCG Infections in the Expression of Toll-Like Receptor Signaling and Pro-Inflammatory Cytokines of A549 Alveolar Epithelial Type II Cells

Author Block:

Y. Li[1], D-x. Liu[2], L. Cheng[2], X-p. Liu[3], Q-j. Ren[2], X-m. Liu[1]; [1]Key Lab. of Ministry of Ed. for Conservation and Utilization of Special Biological Resources in the Western China, Yinchuan, Yinchuan, China, [2]Coll. of Life Sci., Ningxia Univ., Yinchuan, China, [3]The Fourth People's Hosp. of Ningxia Hui Autonomous Region, Yinchuan, China

Abstract Body:

Background: Alveolar epithelial type II cells (AEC II) are the first target cells infected by pathogens of pulmonary tuberculosis inhaled through respiratory tracts. **Methods:** The objective of present study was to explore the molecular mechanisms of immune response and persistent infection of *M.tuberculosis* (Mtb) in A549 AEC II cells by analyzing the differential expression of TLRs signaling and pro-inflammatory cytokines in A549 cells infected with H37Rv and BCG at different time points. The A549 cells were infected by H37Rv or BCG bacilli for different times (6, 12 and 24 h), and the expression of key molecules and proinflammatory cytokines in TLR signaling cascade were ascertained in terms of qRT-PCR and Western-blotting assays.**Results:** The result showed that, the expression of *TLR2, TLR4, NF-κB* and *MyD88* were up-regulated significantly in cells infected with H37Rv as compared with BCG-infected group at 6 h time after the infection. However, *TLR2/4* was also increased in both H37Rv and BCG infected groups, there was no statistically significant difference of increase between cells infected with these two strains at 24 h time point. In addition, the expression of IRAK4 and TRAF3 were significantly higher in the infected A549 cells with BCG than that of H37Rv-infected cells, in which the expression of IRAK4 in BCG-infected cells was up-regulated at the time of 6 and 12 h but was down-regulated at 24 h post-infection. Furthermore, the expression of pro-inflammatory cytokines *IL-1a, IL-6, IL-12a, IL- 8, TNF-α* and *CSF2* were significantly increased in both H37Rv- and BCG-infected A549 cells in a time-dependent manner, and the expression of *IL-1a, IL-6 IL-8, TNF-α* and *CSF2* was more pronounced in H37Rv-infected relative to the BCG infection.**Conclusions:** These results indicated that the TLRs signaling pathway was apparently suppressed in AECII cells infected with the virulent *Mycobacterium* strain H37Rv, but it induced more robust inflammation responses in A549 cells as compared with the attenuated strain BCG.

Author Disclosure Block:

Y. Li: None. **D. Liu:** None. **L. Cheng:** None. **X. Liu:** None. **Q. Ren:** None. **X. Liu:** None.

Poster Board Number:

SUNDAY-590

Publishing Title:

The Voltage-Dependent Anion Channels in the Membrane of *Mycobacterium avium* Phagosome is Required for the Transport of Lipids into the Macrophage Cytosol and for Pathogen Survival

Author Block:

L. Danelishvili, L. E. Bermudez; Oregon State Univ., Corvallis, OR

Abstract Body:

Background: *Mycobacterium avium* subsp. *hominissuis* is an environmental mycobacteria associated with infection of immunocompromised individuals as well as patients with chronic lung disease. *M. avium* infects macrophages and prevents the maturation of the phagosome. The bacterium also interferes with trafficking and many pathways inside of the macrophage, indicating that bacterial effector molecules perform functions in the cytoplasm, after being secreted and transported across the vacuole membrane. Since intracellular mycobacterium is found juxtaposed to the phagosome membrane, our goal of this study was to identify possible phagosomal proteins that are employed by *M. avium* to export virulence factors into the cytosol of host cells. **Methods:** To investigate whether *M. avium* structures to attach to proteins on the internal surface of the vacuole before releasing secreted molecules, intravascuolar *M. avium* and vacuole membrane proteins were purified using biotin labeling and magnetic purification technique, and interactions among both were evaluated with the yeast two-hybrid system. **Results:** The voltage dependent anion channels (VDAC) were identified as components of *M. avium* vacuoles in macrophages. *M. avium* mmpL proteins were found to bind to VDAC-1 protein. The inactivation of VDAC-1 function by pharmacological means or siRNA lead to significant decrease of intracellular *M. avium* survival. Although we could not establish a role of VDAC channel in the transport of known secreted *M. avium* proteins, our data suggests that at least in some cases, the secretion of effectors begins with the recognition of a transport system in the vacuole membrane by a *M. avium* protein. We demonstrated that the VDAC porin channels are associated with the transport of bacterial cell wall lipids outside of vacuole. **Conclusions:** We identified a transport mechanism for *M. avium* secreted lipids. Suppression of the transport of pathogeneffectors may serve as an approach for therapy of infectious diseases.

Author Disclosure Block:

L. Danelishvili: None. **L.E. Bermudez:** None.

Poster Board Number:

SUNDAY-591

Publishing Title:

***Mycobacterium tuberculosis* Dissemination and Extrapulmonary Spread**

Author Block:

M. G. Moule, Preeti Sule, Hee-jeong Yang, Katri Anttonen, Denise K. Zinniel, Raul G. Barletta, Jeffrey D. Cirillo; Texas A&M Hlth.Sci. Ctr., Bryan, TX

Abstract Body:

Mycobacterium tuberculosis is the causative agent of tuberculosis, a disease that remains one of the leading causes of death worldwide despite major advances in diagnostics and therapeutics. An estimated one third of the global population is currently infected with *M. tuberculosis,* with 8.6 million new cases and 1.3 million deaths each year. The majority of these infections are latent, capable of reactivation at any time, and cannot be completely cleared even with antibiotic treatment. In addition, up to 20% of infections in healthy adults and over 50% of infections in children and immunocompromised patients result in extrapulmonary infections that are particularly difficult to detect and treat and have high mortality rates. While most clinical interventions against *M. tuberculosis* have focused on pulmonary infections, developing approaches that target dissemination could lead to diagnostics capable of detecting extrapulmonary infection and therapeutics that prevent the disease from progressing to a more lethal stage. In addition, *M. tuberculosis* latency is highly associated with secondary lesions in the lungs that are established following bacterial dissemination out of primary lesions, and thus targeting dissemination could also potentially address the establishment of latent infections. We have developed a genetic approach to investigate the mechanisms of *M. tuberculosis* dissemination using Tn-seq to identify genes required for *M. tuberculosis* dissemination using a library of 50,000 *M. tuberculosis* CDC1551 mutants. By combining this screen with a real-time live imaging technique known as reporter enzyme fluorescence (REF), we can characterize the role of each gene identified in our screen in the context of an infection. REF imaging will allow us to follow the progress of dissemination in individual animals over time, determining the nature and kinetics of each attenuated dissemination mutant to establish extrapulmonary infections. Our initial pilot screen has identified multiple candidate dissemination mutants, the first of which has been tested and confirmed by REF imaging, validating the effectiveness of our screening method. We believe these experiments will lead to a greater understanding of this critical disease process and open the door for the development of novel therapeutics designed to prevent bacterial dissemination.

Author Disclosure Block:

M.G. Moule: None.

Poster Board Number:

SUNDAY-592

Publishing Title:

***Mycobacterium tuberculosis* Interact with Stem Cell Self-Renewal Mechanism Through Hypoxia-Inducible Factor 1 Alpha Signaling Pathway**

Author Block:

J. Garhyan[1], S. Gayan[2], R. Bhatnagar[1], B. Das[3]; [1]Jawaharlal Nehru Univ., New Delhi, India, [2]Kavikkrisna Lab., Guwahati, India, [3]The Forsyth Inst., Cambridge, MA

Abstract Body:

Background: Adult stem cells employ self-renewal mechanisms to remain quiescent for a prolonged period of time. We speculated that Mycobacterium tuberculosis (Mtb) might interact with the self-renewal mechanisms to hide intracellular to adult stem cells in a non-replicating state for a prolonged period of time. Indeed, our work in human and mice with Mtb infection identified CD271+/CD133+ bone marrow mesenchymal stem cells (CD271+ BM-MSCs) as a candidate stem cell type, where Mtb reside intracellular for months. Importantly, Mtb infected CD271+ BM-MSCs exhibited high expression of hypoxia inducible factor-1α (HIF-1α), a transcription factor involved in the self-renewal of both hematopoietic and mesenchymal stem cells. Here we investigated the host/pathogen interaction between Mtb and HIF-1alhpa mediated metabolism in CD271+ BM-MSCs. **Method:** GFP-H37Rv infected and healthy human CD271+BM-MSCs were cultured under aerobic condition and qRT-PCR was carried out to compare the H1F1 α transcript levels. MRC-5 cell derived CD271+ cells were also used to study Mtb-H37Rv modulation of HIF-1alpha signaling. **Result:** Here we show that under aerobic condition, HIF-1α and related genes including genes involved in aerobic glycolysis and LD formation were induced in Mtb infected CD271+BM-MSCs by 3-5-fold as compared to non-infected cells. Increased LD formation were confirmed by 3-fold increase in Nile red RFU and a 2 fold increment in TG content in infected CD271+BM-MSCs compared to healthy cells. Silencing of HIF-1α with siRNA lead to differentiation of loss of Mtb viability, increased ROS production, and reversal of aerobic glycolysis, as well as LD formation. Importantly, siRNA HIF-1alpha led to loss of self-renewal capacity of CD271+ BM-MSCs. Next, in a mouse model of Mtb-m18b infection, a streptomycin dependent strain of 18b (2), we show that inhibition of HIF-1alpha by FM19G11 led to the differentiation of CD271+ BM-MSCs and the loss of Mtb viability. **Conclusion and significance:** Our findings suggest that Mtb evolved to interact with the self-renewal mechanism of stem cells.

Author Disclosure Block:

J. Garhyan: None. **S. Gayan:** None. **R. Bhatnagar:** None. **B. Das:** None.

Poster Board Number:

SUNDAY-593

Publishing Title:

Identification of Genes Involved with Bicarbonate Sensing and Extracellular Dna Export in Nontuberculous Mycobacterial Biofilms

Author Block:

S. J. Rose, L. E. Bermudez; Oregon State Univ., Corvallis, OR

Abstract Body:

We recently reported the initial finding and characterization of extracellular DNA (eDNA) in mycobacterial biofilms. When studied further, we have determined that bicarbonate triggers the export of eDNA in *Mycobacterium avium* and other nontuberculous mycobacteria (NTM) including *M. abscessus*. Various pathogens use elevated carbon dioxide or bicarbonate in the host to upregulate virulence gene expression, but a link to biofilms or eDNA has not been described. The current study was carried out to identify genes associated with eDNA export in NTM. An *M. avium* strain A5 bank of mutants was screened for eDNA production by co-incubating inoculums with 3µM cell-impermeable propidium iodide and fluorescently measuring undisturbed biofilms over a time course. eDNA-deficient mutants were complemented with wild-type gene and native promoter using an integrative pMV306Apr plasmid. Out of 4,048 mutant clones screened in *M. avium* for eDNA export, we identified and sequenced 145 mutants that were severely deficient. Mutants included a unique DNA transporting FtsK-like pore, a carbonic anhydrase, 8 mutants located in a novel genomic region, various transcriptional regulators, many genes encoding hypothetical proteins, and genes associated with metabolism and energy production. Interestingly, most of the eDNA deficient mutants tested were attenuated during *in vitro* macrophage infections. Additionally, we sequenced the surface proteome of the bicarbonate-exposed biofilm and found many proteins that match sequenced attenuated mutants, or are located in operons with mutants. Ten mutants of interest were complemented and upon biofilm formation exhibited restoration of eDNA to wild-type levels. To corroberate the findings in *M. avium*, 1,080 *M. abscessus* mutants were also screened for eDNA export and 69 of them were classified as eDNA deficient. Thirty of the most deficient mutants were sequenced and several of the genes inactivated are located in operons with genes identified in the *M. avium* screen. Multiple laboratories, including ours, have suggested active export of eDNA in bacterial biofilms, but the mechanisms are unknown. Our findings identify genes involved with bicarbonate sensing and eDNA export, which could be potential virulence factors for mycobacterial infection.

Author Disclosure Block:

S.J. Rose: None. **L.E. Bermudez:** None.

Poster Board Number:

SUNDAY-594

Publishing Title:

Nontuberculous Mycobacteria from Hawaii Demonstrate Varying Degrees of Virulence

Author Block:

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Abstract Body:

Hawai'i has the highest rate of NTM lung disease in the U.S. Our previous work indicates that *Mycobacterium chimaera* predominates in clinical samples and households in Hawai'i. It's plausible that clinical and environmental isolates vary in virulence. We previously show a unique virulence mechanism of pathogenic NTM; *i.e.*, resistance to and inactivation of cathelicidin (LL-37). Intracellular growth in macrophages and evasion to LL-37 were used to assess virulence differences between genetically similar clinical and environmental *M. chimaera* from Hawai'i. *rpoB* sequence analysis was used to speciate NTM and phylogenomic methods were used to assess genetic relatedness between identified *M. chimaera* strains. Two pairs of clinical (CL) and environmental (ENV) *M. chimaera* were shown to have a high degree of genetic similarity within each pair. Virulence differences were determined by the number of intracellular *M. chimaera* recovered from THP-1 cells and whether *M. chimaera* resist and inactivate LL-37. The antimicrobial activity of LL-37 following exposure to NTM was assessed by *Escherichia coli* viability. *M. chimaera* pairs CL10 and ENV56 and CL8 and ENV16 were shown to be genetically similar within each pair. Among the pairs, no difference in the number of *M. chimaera* were observed between the clinical and environmental isolates at any timepoint tested; but, compared to the CL10 and ENV56 pair, significantly more intracellular CL8 and ENV16 were recovered from THP-1 at the 96-hour timepoint ($p=0.03$). Incubation with 25 and 125 μg/ml of LL-37 did not affect the viability of CL8 or ENV16 and the LL-37 exposed to this pair lost its potent antimicrobial activity against *E. coli*. In contrast, CL10 and ENV56 showed susceptibility to 125 μg/ml of LL-37. Whereas CL8 and ENV16 inactivated all LL-37 concentrations tested, 125 μg/ml of LL-37 incubated with either CL10 or ENV56 retained activity. Our data indicate that virulence does not significantly differ between clinical and environmental *M. chimaera*, but genetically dissimilar *M. chimaera* do differ in virulence and evasion capacity to LL-37. The genetic differences between *M. chimaera* isolate pairs likely determine the observed variation in virulence.

Author Disclosure Block:

J.R. Honda: None. **N.A. Hasan:** None. **Y. Zhou:** None. **E.D. Chan:** None.

Poster Board Number:

SUNDAY-595

Publishing Title:

Cross Talk Between T-Cells and Antigen Presenting Cells During Mice Infection with *Mycobacterium avium* subspecies

Author Block:

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Abstract Body:

Background: *Mycobacterium avium* subspecies (ssp.) represent a group of genetically closely related bacteria with different, specific phenotypical and genotypical features. *M. avium* ssp. *paratuberculosis* (MAP) is the causative agent of paratuberculosis (Johne's disease), a chronic intestinal disease, in cattle and other ruminants. MAP is also suggested as the possible cause of Crohn's disease, a persistent inflammation of the bowel in humans. In contrast to its closest relatives *M. avium* ssp. *avium* (MAA) and *M. avium* ssp. *hominissuis* (MAH) MAP exhibits a strong intestinal tropism. There is strong evidence that MAP escapes local immune surveillance to establish long term infection leading to clinical disease. However, only little is known about the underlying immunological mechanism.**Objectives:** The presented study was performed to analyze the functional capacity and the phenotypes of antigen presenting cells and T-cell response after MAP infection of mice in comparison to infection with the closely related MAA. **Methods:** We established *ex vivo* and *in vivo* antigen specific T-cell proliferation assays. Female C57BL/6 mice were infected with MAP or MAA. At different time points after intraperitoneal infection, splenic dendritic cells (DCs) were sorted and co-cultured with CD4 T-cells from OT II TCR transgenic mice in the presence ovalbumin protein or peptide. T-cells proliferation was measured in terms of CFSE dilution. Similarly, antigen specific proliferation was measured *in vivo* after adoptive transfer of OT-II CD4 T-cells.**Result:** Our *ex vivo* and *in vivo* experiments showed that MAA but not MAP infection of mice affected the antigen presenting capacity of splenic DCs resulting in the inhibition of antigen specific CD4 T-cell proliferation. Antigen presenting cells co-stimulatory and co-inhibitory molecules were differently regulated. In contrast to MAP infection MAA infection affected the myeloid cell compartment by inducing B-cells and T-cells loss. TLR2 dependent interleukin 10 production was common to all subspecies *in vitro*.**Conclusion:** Overall, these data show that MAP and MAA differ in their immune escape mechanism in mice. They suggest that MAP escapes the host immune response while MAA seems to actively subvert it.

Author Disclosure Block:

K. Abdissa: None. **A. Nerlich:** None. **S. Weiss:** None. **R. Goethe:** None.

Poster Board Number:

SUNDAY-597

Publishing Title:

Human Endosomal Tlr Response Upon Recognition of *Mycobacterium tuberculosis* Nucleic Acids

Author Block:

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Abstract Body:

Mtb, the causative agent of TB, is a facultative intracellular pathogen that infects macrophages where it avoids elimination by interfering with host defense mechanisms. Toll-like receptors (TLRs) expressed on macrophages can recognize Pathogen Associated Molecular Patterns (PAMPs) on Mtb and mediate the production of immune-regulatory cytokines such as tumor necrosis factor (TNF) and type I Interferons (IFNs). The latter are produced by activation of endosomal TLRs after recognition of nucleic acids in the phagosome. TLR importance is underlined by the fact that the TLR-adaptor molecule MyD88 pathway in macrophages is important in confining Mtb within phagolysosomes, and it is required for mycobacterial clearance and an adequate innate and adaptive immune response against mycobacterial infection. We herein aimed to characterize the endosomal TLR-signaling of human macrophages, as they are appear crucial in determining the fate of the disease. To explore if variations in Type I IFN responses correlates with differences in Mtb nucleic acids derived from different Mtb genotypes, human monocytic cell line THP-1 derived macrophages, expressing two inducible reporter constructs for interferons (IFNs) and for NF-kB, were stimulated with DNA obtained from Mtb strains of different lineages. We found that DNA from different *Mtb* phylogenetic lineages elicits differential NF-kB and IRF inflammatory responses in human macrophages. We also studied the effect of Vitamin D (VD) on these responses, as it is known to activate macrophages and enhance their antimycobacterial activity. Pretreatment of cells with VD increased the response to DNA from HN878 and virulent strain H37Rv, and increased phagocytosis of live *M.smegmatis*. It also enhanced the response to RNA from *M.smegmatis* and attenuated *M.bovis* strain BCG. Although correlation between different Mtb strains and lung epithelial invasiveness and inflammatory response has been reported, none of these studies evaluated Type I or Type II IFNs. The significance of the herein reported differences between different Mtb strains is of great importance as type I IFNs have been demonstrated to strongly inhibit monocytes and macrophages responsiveness to IFN-γ, and are associated with active TB disease.

Author Disclosure Block:

J. Cervantes: None. **E. Oak:** None. **J. Garcia:** None. **J.C. Salazar:** None.

Poster Board Number:

SUNDAY-599

Publishing Title:

Profiling the Mouse Genome to Find Correlates of Vaccine-Induced Protection against *Mycobacterium tuberculosis*

Author Block:

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Abstract Body:

Despite extensive world-wide use of BCG, tuberculosis (TB) remains a serious global public health threat. New vaccines are difficult to develop due to incomplete understanding of optimal protective mechanisms of immunity against *M. tuberculosis* (*M. tb*). Using mouse models, our laboratory adapted an *in vitro* co-culture system to identify immune mediators whose relative gene expression is significantly associated with the degree of protection. In this strategy, *Mycobacteria*-infected macrophage monolayers are co-cultured with TB-immune lymphocytes to evaluate control of intramacrophage bacterial replication. Previously, we used a panel of BCG-related vaccines to establish a hierarchy of vaccine-induced protection against *M. tuberculosis* challenge. Splenocytes or peripheral blood lymphocytes (PBL) from vaccinated mice controlled intracellular *Mycobacteria* replication in patterns that represented the relative protection afforded against *in vivo* MTB challenge. We further applied this approach to study protective mechanisms. We hypothesized that genes specifically upregulated in protective versus less or non-protective vaccines represent potential immune correlates. We then screened a panel of 90 chemokine and cytokine genes, and found several molecules whose expression levels in splenocytes and PBLs corresponded with protection. Given this success, here we expanded screening to include the whole mouse genome as measured by microarray. We used RNA derived from splenocytes co-cultured with *M.tb*.-infected macrophages. In addition to mediators found in our original screen, such as IFN-gamma, Hmox, and Nos-2, we found an additional ~830 genes whose expression was at least two-fold different between the “best” vaccine and the naïve samples. Of these, the 190 strongest candidates have subsequently been validated with qRT-PCR. We are now also evaluating this subset in samples derived from PBL. To date, the strongest associations include Cxcl9, Ubd, Mgl, Lpl, and Plin2. Further, pathway analysis of the most upregulated gene targets show involvement of pathways related to cell maintenance, cell death, and cell trafficking. These findings will expand the knowledge of immune mechanisms that underlie successful vaccination, and may ultimately provide a panel of relevant correlates.

Author Disclosure Block:

S.L. Kurtz: None. **K.L. Elkins:** None.

Poster Board Number:

SUNDAY-600

Publishing Title:

Meta-analysis of the Sputum Microbiome in Pulmonary Tuberculosis

Author Block:

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Abstract Body:

Background: The lung has received less attention compared to other body sites in terms of microbiome characterization, and its study carries special technological difficulties related to obtaining reliable samples as compared to other body niches. Tuberculosis (TB) is a worldwide health problem with high morbidity and mortality. It is still unclear if changes in the lung microbiome composition are associated with pulmonary TB. Up to date, five studies reported the sputum microbiome on TB patients and controls, with somewhat contradictory results [1-5].**Methods:** A systematic NCBI PubMed searched identified 5 studies on sputum microbiome and TB through December 2015. Available sequence data from three of these studies plus a recen analysis of the lung microbiota in healthy individuals [6] were analyzed by phylotype approach using microbiome analysis platforms mothur and qiime. We compared data from lower respiratory tract samples, i.e. sputum and/or bronchoalveolar lavage (BAL), in both TB cases vs. healthy controls.**Results:** Overall, a signature of TB did not appear consistent between studies, even when the data were analyzed with consistent methods. A decreased in diversity was found in the sputum bacterial composition of TB patients compared to healthy controls. A predominance of *Prevotella* and *Streptoccocus* was observed in the composition of TB sputum at the genus level, while *Clostridia* and *Bacteroides* were the most predominant genera in healthy controls.**Conclusions:** Although our findings are narrowed by a limited number of studies, they underline the fact that the lung microbiome in TB patients differs from normal individuals. Future larger studies are needed to validate a consistent presence of a few genera across the studies analyzed. This could potentially allow for discrimination from healthy controls with significant accuracy statistically. Lung microbiome dysbiosis observed in TB patients may play a role in disease onset, progression, recurrence, and outcome after treatment. Understanding the composition of the lung microbiome in health and comparing it to that of pulmonary TB cases would elicit clues into the pathogenesis of *Mycobacterium tuberculosis* infection at the pulmonary alveolus and would help the design of promising treatment options for TB with potential direct beneficial consequences for patients and public health.

Author Disclosure Block:

A. Adami: None. **B. Hong:** None. **J. Cervantes:** None.

Poster Board Number:

SUNDAY-601

Publishing Title:

Identification of *Borrelia burgdorferi* Oxidative Stress Response Genes and Characterization of Their Role *In Vivo* Using Transposon Insertion Sequencing

Author Block:

M. E. Ramsey[1], J. A. Hyde[2], T. Lin[3], L. Gao[3], M. E. Lundt[1], S. J. Norris[3], J. T. Skare[2], L. T. Hu[1]; [1]Tufts Univ., Boston, MA, [2]Coll. of Med., Texas A&M Hlth.Sci. Ctr., Bryan, TX, [3]UTHlth.McGovern Med. Sch., Houston, TX

Abstract Body:

The Lyme disease spirochete *B. burgdorferi* lacks homologs of several canonical oxidative stress response genes, and the cellular targets of oxidative damage differ compared to model organisms such as *E. coli*. We conducted an unbiased search for genes involved in the *B. burgdorferi* oxidative stress response using transposon insertion sequencing (Tn-seq). Tn-seq relies on high-throughput sequencing to quantify the frequency of transposon (Tn) mutants in a population before and after a selective pressure. A *B. burgdorferi* Tn library was exposed to DEA/NO (a nitric oxide donor) and to two ROS reagents: hydrogen peroxide (H_2O_2) and t-butyl hydroperoxide (TBHP). Tn mutants with insertions in DNA repair genes exhibited the largest decreases in frequency following DEA/NO exposure, consistent with previous reports that DNA is a target of RNS in *B. burgdorferi*. We confirmed the NO-sensitivity of several Tn mutants, including those with insertions in genes encoding the excinuclease subunit UvrC (previously shown to be important for RNS resistance), the helicase UvrD, and the ribonuclease RnhA. In contrast, after exposure to H_2O_2 and TBHP, Tn mutants with insertions in genes encoding transmembrane proteins exhibited the largest decreases in frequency. We confirmed the ROS-sensitivity of Tn mutants with insertions in four of these genes: *bb0202*, encoding a hemolysin-like protein, *bb0017*, encoding a GlnB-like protein, *bb0164*, encoding a putative sodium/calcium exchanger, and *bb0412*, encoding a putative membrane protein. We then investigated whether the genes identified in the *in vitro* screens played a role *in vivo* by infecting C57BL/6 mice with a mini-library of 39 Tn mutants and found that all of the Tn mutants exhibited fitness defects *in vivo*. Collectively, these studies identify new gene functions involved in ROS and RNS resistance in *B. burgdorferi* and demonstrate a role for these genes *in vivo*. Ongoing studies aim to test the infectivity of the Tn mutants in mice deficient in ROS and RNS production.

Author Disclosure Block:

M.E. Ramsey: None. **J.A. Hyde:** None. **T. Lin:** None. **L. Gao:** None. **M.E. Lundt:** None. **S.J. Norris:** None. **J.T. Skare:** None. **L.T. Hu:** None.

Poster Board Number:

SUNDAY-602

Publishing Title:

Effects of *Moraxella Catarrhalis*-Derived Nitric Oxide on Different Human Cell-Lines in Co-Cultures

Author Block:

B. MOCCA, C. Johnson, W. Wang; FDA, Silver Spring, MD

Abstract Body:

Moraxella catarrhalis is an important cause of the most common childhood infectious diseases, such as acute otitis media (AOM) and OM with effusion (OME) and forms biofilms on the mucosal surface of the mid-ear of children with OME. In adults with chronic obstructive pulmonary disease (COPD), *M. catarrhalis* infection can cause exacerbations of COPD. Recent study reported that the expression of a nitrite reductase (AniA) is up-regulated in *M. catarrhalis* biofilms formed on human bronchial epithelial cells (HBEC) in co-cultures, which, in the presence of pathological levels of nitrite, produces higher levels of nitric oxide (NO) that stimulated host cell expression of proinflammatory cytokines, including IL-1α and TNF-α, and induces HBEC to undergo apoptotic death. In addition, bacteria-derived NO is toxic to biofilm-grown bacteria but not to planktonically-grown *M. catarrhalis*. These study results indicated that bacteria-derived nitric oxide might paly pathogenic roles in *M. catarrhalis*-associated otitis media. Because *M. catarrhalis* caused childhood disease is very different from *M. catarrhalis* associated exacerbation of COPD, it is important to examine the effects of *M. catarrhalis*-derived NO on different human cells. When the A549 cells (adenocarcinomic human alveolar basal epithelial cells) were used as host cells in co-cultures, preliminary data showed that bacterial cells can grow either attached or unattached to A549, and the growth of A549 was only slightly reduced by bacteria-derived NO.

Author Disclosure Block:

B. Mocca: None. **C. Johnson:** None. **W. Wang:** None.

Poster Board Number:

SUNDAY-603

Publishing Title:

Bacterial Production of Glutathione Protects Group B *Streptococcus* from Killing by Healthy and Nadph Oxidase - Deficient Murine Macrophages

Author Block:

T. Vongsurbchart, S. Rowse, B. Branneky, A. Riffle, T. Khurana, C. Kranz, K. Vyas, A. Modi, **B. E. Janowiak**; Saint Louis Univ., St. Louis, MO

Abstract Body:

Background: Group B Streptococcus (GBS) is a pathogen that can cause sepsis in immune compromised patients. Previously, we have found that GBS produces and uses large amounts of a potent antioxidant, glutathione (GSH), to protect itself against immunologically relevant reactive oxygen species (ROS). We hypothesize that a glutathione-deficient strain of GBS (in which we genetically inactivated the gene *gshAB*) would be cleared more readily by weakened macrophages than either the parental (wild type) strain or a genetically complemented strain of GBS.**Methods**: In order to test our hypothesis, we infected J774A.1 macrophage-like cells, as well as freshly isolated murine intraperitoneal macrophages from either NADPH oxidase-deficient (NADPH oxidase is the rate-limiting enzyme in the ROS cascade) or wild type mice, with GSH-deficient, GSH-complemented, or wild type GBS. We then measured how efficient the macrophages were at clearing the GBS strains, and we also measured the effect of GBS strains on the viability of the macrophages over time.**Results**: We observed significantly higher killing of GSH-deficient GBS than wild type or GSH-complemented GBS for all three infections (J774A.1 cells, healthy macrophages, and NADPH-oxidase deficient macrophages). Likewise, we observed less macrophage cytotoxicity post infection with GSH-deficient GBS than wild type or GSH-complemented GBS for all three infections. Supporting our hypothesis, we observed that GSH-deficient GBS were efficiently cleared from both healthy and NADPH-oxidase deficient macrophages.**Conclusion**: Our studies suggest that GSH plays an important role in helping GBS to survive in both healthy and immune compromised immune cells. Additionally, since GSH seems to be critical for both the survival of GBS as well as the cytotoxic activity in macrophages, GSH synthesis may be a viable drug target. Future studies will explore that possibility.

Author Disclosure Block:

T. Vongsurbchart: None. **S. Rowse:** None. **B. Branneky:** None. **A. Riffle:** None. **T. Khurana:** None. **C. Kranz:** None. **K. Vyas:** None. **A. Modi:** None. **B.E. Janowiak:** None.

Poster Board Number:

SUNDAY-604

Publishing Title:

Catabolite and Oxygen Regulation of Enterohemorrhagic *Escherichia coli* Virulence

Author Block:

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Abstract Body:

Nutritional competition among human gastrointestinal (GI) bacteria drives differential gene expression, including expression of virulence factors. The host mucus layer provides sugars for GI commensal bacteria while preventing enteric pathogens from directly accessing intestinal cells. However, the enteric pathogen enterohemorrhagic *Escherichia coli* (EHEC) uses these mucus derived sugars (i.e. glucose and fucose) to regulate virulence gene expression. EHEC express the locus of enterocyte effacement (LEE) pathogenicity island, which encodes a type three secretion system, effectors, and an adhesin protein necessary to form attaching and effacing lesions on enterocytes. LEE activity is modulated by many signaling molecules. Under gluconeogenic conditions, the transcription factors, KdpE and Cra, activate expression of the LEE; however, under glycolytic conditions, expression of the LEE is decreased. In addition to glycolytic environments, high fucose concentrations also repress expression of the LEE. EHEC senses fucose through a histidine sensor kinase, FusK, which constitutes a two-component system with its cognate response regulator FusR. FusR represses expression of the LEE genes and prevents premature LEE expression before EHEC finds a target enterocyte. Once EHEC reaches the enterocyte and senses low concentrations of carbon sources, LEE expression is activated. Therefore, EHEC regulates expression of the LEE through the spatial and temporal sensing of sugars. In addition to sensing sugars, EHEC also uses oxygen to control expression of virulence factors. We have measured expression of the LEE under different oxygen concentrations under gluconeogenic conditions using qPCR and Western blots for secreted virulence proteins. These data enhance our understanding of how the host environment is sensed by EHEC to coordinate expression of virulence factors required for infection.

Author Disclosure Block:

K. Carlson-Banning: None. **V. Sperandio:** None.

Poster Board Number:

SUNDAY-605

Publishing Title:

Identification of the Cellular Targets of Nitric Oxide in the Lyme Disease Spirochete Borrelia Burgdorferi

Author Block:

T. J. Bourret[1], F. C. Gherardini[2]; [1]Creighton Univ., Omaha, NE, [2]Rocky Mountain Lab., Hamilton, MT

Abstract Body:

Borrelia burgdorferi is the etiologic agent of Lyme disease and is transmitted to humans by ticks of the genus *Ixodes*. During its lifecycle in *Ixodes* ticks, *B. burgdorferi* encounters various environmental challenges including shifts in pH, temperature, and nutrient availability, as well as innate host defenses, including reactive oxygen species (ROS) and reactive nitrogen species (RNS). We show here that *I. scapularis* ticks produce RNS both in the salivary glands and midguts during feeding. Therefore, *B. burgdorferi* likely encounters a significant degree of nitrosative stress during its lifecycle in its arthropod host. Previously, we have shown that RNS produced *in vitro* are cytotoxic for *B. burgdorferi* due to the *S*-nitrosylation of cysteine thiols of both zinc-dependent and zinc-independent proteins. The aim of this study was to identify *B. burgdorferi* proteins that are subject to *S*-nitrosylation following the exposure of cells to sublethal concentrations of the NO donor diethylamine NONOate. In addition to our previously identified targets, the *Borrelia* oxidative stress regulator (BosR) and the neutrophil activating protein A (NapA), we have identified 29 additional proteins subject to *S*-nitrosylation at 1 or more cysteine residues using the biotin-switch technique combined with LC-MS sequencing analysis. Targets identified include enzymes involved in central metabolism, transcription/translation, DNA replication/repair, protein turnover, chaperones, transport, gene regulation, antioxidant defenses and cell division. Collectively, these data suggest that RNS may have a significant impact on the physiology, gene expression and overall virulence of *B. burgdorferi*.

Author Disclosure Block:

T.J. Bourret: None. **F.C. Gherardini:** None.

Poster Board Number:

SUNDAY-606

Publishing Title:

Immunomoduratory Roles of Metformin in Murine *Legionella* Infection

Author Block:

C. Kajiwara, Y. Kusaka, S. Kimura, K. Tateda; Toho Univ., Tokyo, Japan

Abstract Body:

Background: *Legionella pneumophila* (*Lp*) is a facultative intracellular pathogen which replicates within macrophages and monocytes and finally causes a severe pneumonia known as Legionnaires' disease. Reactive oxygen species (ROS) production, autophagy network, shift to Th1 phenotype immune response were required for the effective control of intracellular pathogens. Metformin (MET: dimethylbiguanide) a prescribed drug for type 2 diabetes, has been reported to have anti-tuberculosis effect caused by increasing the ROS production via AMPK signaling. However, the all other pathogens were incompletely understood. Here we showed that MET inhibited the intracellular growth of *Lp*, restricted the progression of disease. **Methods:** *In vivo* studies were performed to determine the effect of MET on survival, bacterial counts, and expression of Th1 cytokines in the lungs after administration of *Lp*. A/J mice were received 5mg/ml MET or not dissolved in the drinking water 7 day before the infection, and then mice were i.t. administered with *Lp* (10^5 CFU). *In vitro* studies, bone marrow-derived macrophages (BMMs) and RAW264.7 cells treated with different doses of MET were infected with *Lp* (MOI: 0.1). ROS production was determined using a flow cytometer. Phosphorylated AMPK was detected by western blotting. **Results:** Significantly higher survival rates were observed in mice treated MET. No difference in bacterial burden was observed on day 2 and day 3 post challenge between control and MET groups, however, significantly lower bacterial number was observed in MET-treated mice on day5 (p=0.035). In MET-treated mice, sustained productions of Th1 cytokines, such as IFN-gamma and IL-12, were observed in the lungs during the first 3 days, whereas early reductions of these cytokines were noted in control mice (p=0.03 and p=0.0026, respectively). MET treatment inhibited the intracellular growth of *Lp* in a dose-dependent manner in BMMs and RAW264.7 cells. Interestingly, MET treatment reversed *Lp* -induced suppression of ROS synthesis within just 6 hours of drug exposure. **Conclusion:** The present data demonstrated that MET pretreatment significantly enhanced host defense systems against *Lp* infection, probably through Th1-directed responses and sustained ROS productions. Potential of MET as immunomodulators is warranted for future' investigation.

Author Disclosure Block:

C. Kajiwara: None. **Y. Kusaka:** None. **S. Kimura:** None. **K. Tateda:** None.

Poster Board Number:

SUNDAY-607

Publishing Title:

Effects of Neutrophil Depletion on M1/M2 Macrophage Polarization and Lethality of Mice with *Legionella pneumophila* Pneumonia

Author Block:

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Abstract Body:

Background: *Legionella pneumophila* is an intracellular pathogen, which replicate mainly in macrophages, and cause severe pneumonia. Lately, the role of macrophage subtypes and polarization in M1/M2 are becoming a topic in host immunity. Previously, we have reported that neutrophil depletion by anti-Gr-1 antibody induced immunological shift from T1 to T2 lymphocyte subset, characterized by T1/T2 cytokine valances. In the present study, we analyzed effects of neutrophil depletion on M1/M2 macrophage polarization and lethality of mice with *L. pneumophila* pneumonia.**Methods**: We induced *L. pneumophila* pneumonia by intratracheal administration of bacteria. To deplete neutrophils, anti-Gr-1 antibody was administered intra-muscularly 1 day to 7 days before the infection. The infected lungs and blood were harvested and analyzed by FACS and real time PCR 2 and 3 days after the infection. M1 (CD86) and M2 surface marker (CD206) were used for FACS. M1 (iNOS) and M2 marker (Arg-1 or YM-1) were applied for real time PCR. The effect of neutrophil depletion on the survival of mice and bacterial number in the lungs was determined in the designated time points.**Results**: Anti-Gr1-treatment (1 day before the infection) induced the shift of macrophages subtypes from M1 to M2 in the lung and of peripheral blood, which was well associated with increase of mortality. On 3 days after the infection, CD 206 (M2) expression was 2-4 times higher, whereas CD86 (M1) expression decreased in neutrophil-depleted mice. Similarly, anti-Gr1-treatment induced an increase of Arg-1 (M2) and decrease of iNOS (M1). Interestingly, when anti-Gr1 treatment was performed 7 days before the infection, higher neutrophil number, lower bacterial burden and higher survival were observed in anti-Gr1-treated mice, which were well correlate with M1-directed immune responses in the lungs.**Conclusion**: The present data demonstrated that neutrophils accumulation and depletion affected M1/M2 macrophage polarization, which were associated with disease severity and survival of mice with *L. pneumophila* pneumonia.

Author Disclosure Block:

Y. Kusaka: None. **C. kajiwara:** None. **K. Tateda:** None.

Poster Board Number:

SUNDAY-608

Publishing Title:

Role of Airsr Two-component System in Survival of *Staphylococcus aureus* in Human Blood

Author Block:

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Abstract Body:

Staphylococcus aureus is a major pathogen and a common cause of hospital- and community-acquired infections. The pathogenicity of *S. aureus* partially relies on the coordinately-regulated expression of virulence factors that allow the bacterium to evade the host immune system or promote survival during infection. It has been demonstrated that AirSR(YhcSR) two-component system senses oxygen and modulates the expression of pathways responsible for dissimilatory nitrate reduction, cellular homeostasis and alternative sugar catabolism pathways. The role of AirSR in pathogenesis of *S. aureus* is still unclear. We conducted experiments to determine if AirSR contributes to the pathogenesis of *S. aureus* using an antisense RNA interference technology, an inducible overexpression system, and gene deletions. Depletion of AirSR by antisense RNA or deletion of the genes resulted in significant decrease in bacterial survival in human blood. Conversely, overexpression of AirR significantly promoted survival of *S. aureus* in blood. AirR promoted the secretion of virulence factors that inhibits opsonin-based phagocytosis. Move rover, we revealed that AirSR mediates the production of the cysteine endopeptidase and golden pigment. Our data demonstrated that the enhanced bacterial survival by AirR is attributable to the direct regulation of the biosynthesis of golden pigment, staphyloxanthin. Taken together, our data are the first to indicate that AirSR(YhcSR) is an important virulence regulator for modulating expression of antioxidants and survival within phagosomes. Our studies provide new insights into the molecular pathogenesis of *S. aureus.*

Author Disclosure Block:

J. Hall: None. **J. Yang:** None. **H. Guo:** None. **Y. Ji:** None.

Poster Board Number:

SUNDAY-609

Publishing Title:

Characterization of the Putative Oxygen Sensor Dosp in *Listeria monocytogenes*

Author Block:

D. McClung[1], **S. White**[1], H. N. Jenkins[1], O. Paul[1], H. Abdelhamed[1], K. Pendarvis[2], J. R. Donaldson[1]; [1]Mississippi State Univ., Mississippi State, MS, [2]Univ. of Arizona, Tuscon, AZ

Abstract Body:

Background: The food-borne pathogen *Listeria monocytogenes* is the causative agent of the disease listeriosis and has a mortality rate of 20-30%. The ability of this pathogen to survive following exposure to stressors encountered throughout the gastrointestinal tract, such as acidic conditions, hypoxic conditions, and bile, is critical for the establishment of listeriosis. Recent work from our laboratory has indicated that certain strains of *L. monocytogenes* have an enhanced ability to resist stressors when exposure occurs under anaerobic conditions. It is hypothesized that the ability to sense oxygen availability can influence the stress response of *L. monocytogenes.* In this study, the involvement of the putative oxygen sensor DosP in intracellular replication was tested in relation to oxygen availability. **Methods:** The *dosP* gene was removed from the *L. monocytogenes* 4b strain F2365 and confirmed through gene sequencing. The impact of *dosP* on intracellular invasion was determined using human colon cell line C2BBe1, which is a clonal cell line of Caco-2. F2365 and the isogenic *dosP* mutant were cultured overnight in tryptic soy broth in either aerobic or anaerobic conditions. C2BBe1 cells were then infected with F2365 and the *dosP* mutant at a MOI of 1:100 for 1, 3, and 5 h. Cells were lysed and intracellular bacteria were plated to determine the intracellular survival. Proteome expression differences were also determined at 1 h using an LTQ mass spectrometer.**Results:** The data indicate that F2365 invaded uniformly at 0.03% between both aerobic and anaerobic conditions. However, the *dosP* mutant displayed variation between aerobic and anaerobic conditions, with an increased invasion potential of 0.1% under aerobic conditions compared to 0.03% under anaerobic conditions ($P < 0.007$). The intracellular proteomic expression of *dosP* in comparison to the wild type strain indicated that this protein is involved in regulation of various metabolic processes.**Conclusions:** These data indicate that DosP is involved in regulating intracellular invasion, but only in the presence of oxygen. Further research is needed to determine how the function of this protein in the progression of listeriosis.

Author Disclosure Block:

D. McClung: None. **S. White:** None. **H.N. Jenkins:** None. **O. Paul:** None. **H. Abdelhamed:** None. **K. Pendarvis:** None. **J.R. Donaldson:** None.

Poster Board Number:

SUNDAY-610

Publishing Title:

***In Vivo* Fitness Adaptation of Colistin-resistant *Acinetobacter baumannii* Isolates to Oxidative Stress**

Author Block:

C. Jones[1], S. Singh[1], Y. Alamneh[1], L. Casella[2], R. Ernst[2], E. Lesho[1], P. Waterman[1], D. Zurawski[1]; [1]Walter Reed Army Inst. of Res., Silver Spring, MD, [2]Univ. of Maryland, Baltimore, MD

Abstract Body:

Acinetobacter baumannii is a Gram negative pathogen that acquires resistance to multiple antibiotics. Resistance of *A. baumannii* to colistin, an antibiotic of last resort, has been associated with decreased bacterial fitness. Considering the ubiquitous nature of *Acinetobacter*, we hypothesized that host-related stress placed selective pressure on *A. baumannii* to compensate for the fitness loss associated with acquisition of colistin resistance *in vivo*. For this study, we evaluated *A. baumannii* isolates serially obtained from the same patient. We show here that colistin-resistant (CR) isolates obtained shortly after *in vivo* exposure to colistin were outcompeted for growth in nutrient broth by the parental colistin-susceptible (CS) isolate. In contrast, CR isolates obtained during the latter stages of infection were able to compete for growth at a level similar to the CS isolate. This same trend was observed when colistin resistant isolates were competed for growth in the lungs of neutropenic mice after 48 hours. Decreased fitness was not associated with defects in membrane integrity or loss of LPS. Interestingly, these early CR strains exhibited increased susceptibility to hydrogen peroxide killing and decreased catalase production when compared to late CR and CS strains. Taken together, this data shows that early fitness loss of *A. baumannii* is associated with increased susceptibly to oxidative stress as a result of decreased catalase activity, and bacteria compensate for this loss during the course of infection by modulating catalase activity.

Author Disclosure Block:

C. Jones: None. **S. Singh:** None. **Y. Alamneh:** None. **L. Casella:** None. **R. Ernst:** None. **E. Lesho:** None. **P. Waterman:** None. **D. Zurawski:** None.

Poster Board Number:

SUNDAY-611

Publishing Title:

Pirin-like Proteins are Regulated by Oxidative Stress and Iron in *Bacteroides fragilis* and Involved in the Modulation of Central Energy Metabolism and Metronidazole Susceptibility

Author Block:

E. R. ROCHA[1], A. M. Gough[1], P. J. O'Bryan[2], T. R. Whitehead[2], C. J. Smith[1]; [1]East Carolina Univ. Brody Sch. of Med., Greenville, NC, [2]USDA Agricultural Res. Service, Peoria, IL

Abstract Body:

Bacteroides fragilis is the most frequent anaerobe isolated from human infections. Clinical isolates of *B. fragilis* are among the highest aerotolerant anaerobic bacteria. The oxidative stress response (OSR) in *B. fragilis* induces an array of genes enabling them to survive prolonged oxygen exposure in the aerobic extra-intestinal tissues until abscess formation provides a favourable anaerobic infectious environment. Among the OSR genes, two pirin-like homologues, Pirin 1 (Pir1) and Pirin 2 (Pir2) were found to be induced by oxygen exposure and iron availability determined by whole genome transcriptional analysis and real-time RT-PCR. Pirin-like proteins are iron-containing proteins of the cupin superfamily present in all kingdoms ranging from archae to humans. Real time RT-PCR also confirmed the up-regulation of the genes for *pir1* (7.2-fold) and *pir2* (9.5-fold) following exposure to oxygen compared to anaerobic culture controls. Under anaerobic low-iron conditions, *pir1* and *pir2* genes were induced approximately 7.6-fold and 9.3-fold respectively compared to the levels under iron-replete conditions in a Fur-independent manner. To investigate the role of Pir1 and Pir2 in *B. fragilis*, we have used bacterial two-hybrid system to show that Pir1 and Pir2 interacts with pyruvate:ferridoxin oxidoreductase (PFOR) and alcohol dehydrogenase (ADH). This suggests that pirin-like proteins possibly play a role in response to oxidative stress by interacting with enzymes involved in *B. fragilis* central energy metabolism. The *B. fragilis* strains constitutively expressing Pir1 and Pir2 had a strong reduction in lactate levels and increase in acetate and ethanol levels compared to parent strain under anaerobic conditions. Moreover, constitutively expressing Pir1 and Pir2 strains were more sensitive to the antibiotic metronidazole compared to parent strain possibly due to the protein-protein interactions between the PFOR and Pir-like proteins. Taken together, these findings show that there is a coordinate regulation of pirin proteins during OSR and iron limitation in *B. fragili*s that affects central energy metabolic fermentative pathways and antibiotic resistance.

Author Disclosure Block:

E.R. Rocha: None. **A.M. Gough:** None. **P.J. O'Bryan:** None. **T.R. Whitehead:** None. **C.J. Smith:** None.

Poster Board Number:

SUNDAY-612

Publishing Title:

A Novel Rtx Toxin Encoded on a Colv-type Plasmid Contributes to *Escherichia coli* Urinary Tract Infection

Author Block:

J. Saoud[1], S. Houle[1], A. Garénaux[1], F. J. Veyrier[1], F. Daigle[2], **C. M. Dozois**[1]; [1]INRS-Inst. Armand-Frappier, Laval, QC, Canada, [2]Université de Montréal, Montréal, QC, Canada

Abstract Body:

Extra-intestinal pathogenic *E. coli* (ExPEC) cause a variety of diseases in humans and animals. Some ExPEC strains contain large virulence plasmids such as Colicin V (ColV) or similar plasmids. The objective was to investigate ColV-type virulence plasmids for potential novel virulence factors. Plasmid extracts from ExPEC strains containing sequences associated with ColV plasmids were analyzed by pulsed field gel electrophoresis. Two strains found to contain very large plasmids were sent for whole genome sequencing. Sequencing results identified a large ColV-type plasmid (201 kb) in ExPEC strain QT598 containing a 7 kb gene cluster predicted to encode a RTX toxin distinct from previously characterized RTX toxins. Based on the plasmid origin of the system, it was named ***prtCABD*** (for **p**lasmid-encoded **R**TX **t**oxin). The ***prt*** genes were cloned in the pUCm-T vector in *E. coli* K-12 DH5-[[Unsupported Character - Symbol Font ]]. SDS-Page demonstrated a 91 kD protein corresponding to the ***prtA*** product secreted in the culture supernatant. Secretion was dependent on the ***prtCD*** genes as well as ***tolC*** which is required for secretion of other known RTX toxins. Tests were done to determine if the ***prt*** system conferred cytolytic and hemolytic activity. *E. coli* K-12 that secreted **PrtA** demonstrated cytolytic activity during interaction with human macrophage, human bladder epithelial, and chicken fibroblast cell lines. Further, colonies demonstrated hemolysis when grown on blood agar plates. However, wild-type strain QT598 did not demonstrate cytolytic or hemolytic activity in these assays. A ***prtCABD*** deletion mutation was constructed by allelic exchange in ExPEC strain QT598 and tested in a urinary tract model in CBA/J mice. In co-infection experiments, the WT strain outcompeted the mutant a mean of two-fold ($p=0.002$) in the kidneys and 1.5 times in the bladder ($p=0.008$). In single strain infections, the mutant demonstrated lower numbers in the kidneys ($p=0.04$), but there was no significant difference in colonization of the bladder. Taken together, results demonstrate that PrtCABD represents a new RTX toxin demonstrating hemolytic and cytolytic activity that may contribute to urinary tract colonization and virulence of certain ExPEC strains.

Author Disclosure Block:

J. Saoud: None. **S. Houle:** None. **A. Garénaux:** None. **F.J. Veyrier:** None. **F. Daigle:** None. **C.M. Dozois:** None.

Poster Board Number:

SUNDAY-613

Publishing Title:

Isolation and Structural Characterization of Several Candidate Precolibactins Produced by the *pks* Island Provides Insights into Colibactin's Biosynthesis and Biological Activity

Author Block:

M. R. Wilson, C. A. Brotherton, L. Zha, E. P. Balskus; Harvard Univ., Cambridge, MA

Abstract Body:

Growing evidence suggests that the human gut microbiota influences the development and progression of several human cancers including colorectal cancer (CRC). One microbial metabolite strongly associated with cancer development is colibactin, a small-molecule genotoxin produced by commensal *E. coli* inhabiting the human gut. Colibactin-producing *E. coli* causes DNA double strand breaks *in vitro* and chromosomal instability *in vivo*. The increased abundance of *pks*$^+$ *E. coli* found in CRC patients and its ability to potentiate tumorigenesis in mice suggests that colibactin may promote the progression of CRC in humans. However, the mechanism by which colibactin exerts genotoxicity remains uncharacterized because it has not been isolated and its chemical structure is therefore unknown. Our previous characterization of the genotoxin's self-resistance mechanism revealed that colibactin is initially biosynthesized as a larger, inactive prodrug called precolibactin that is later cleaved by the periplasmic peptidase ClbP to release the active genotoxin, colibactin. Herein, we report the isolation and structural characterization of several candidate precolibactins from an *E. coli* mutant missing the peptidase ClbP. These isolation efforts revealed structural features likely present in colibactin such as a spirocyclopropane warhead and DNA intercalating elements, which suggests colibactin's mode of action may involve DNA alkylation.

Author Disclosure Block:

M.R. Wilson: None. **C.A. Brotherton:** None. **L. Zha:** None. **E.P. Balskus:** None.

Poster Board Number:

SUNDAY-615

Publishing Title:

The Carboxy Terminal Region Mediates Differential Cell Binding and Endocytosis between Variants of *Clostridium difficile* Toxin B

Author Block:

J. J. Hunt, J. L. Larabee, J. D. Ballard; Univ. of Oklahoma Hlth.Sci. Ctr., Oklahoma City, OK

Abstract Body:

The carboxy-terminal region of TcdB from ribotypes 012 and 027 share 88% amino acid identity. However, ribotype 027 exhibits comparatively enhanced toxicity and antibodies raised to the carboxy-terminal region of TcdB do not effectively cross neutralize toxin from dissimilar ribotypes. Several studies have indicated that there may be two distinct regions of TcdB which contribute to the binding and uptake of the toxin; residues 1500-1851 and 1851-2366 (CROP region), respectively. This suggested enhanced toxicity of $TcdB_{027}$ may be a result of structural changes in this carboxy-terminal region that affect binding of the toxin to its receptor. We sought to determine if the sequence differences proximal to the CROP region affect the clearance of toxin from the cell surface, or the localization of toxin to acidified endosomes. To this end, we tracked the binding, uptake and acidification of labeled toxin, or toxin fragments using flow cytometry and fluorescence microscopy. Our findings suggest the region proximal to the CROP domain, encompassed by residues 1653-1851, is responsible for the enhanced uptake of $TcdB_{027}$ and for proper localization of toxin to acidified endosomes.

Author Disclosure Block:

J.J. Hunt: None. **J.L. Larabee:** None. **J.D. Ballard:** None.

Poster Board Number:

SUNDAY-616

Publishing Title:

A *Clostridium difficile* Tcdb-Derived Peptide That Blocks the Actions of Tcdb

Author Block:

J. L. Larabee, J. J. Hunt, J. D. Ballard; Univ. of Oklahoma Hlth.Sci. Ctr., Oklahoma city, OK

Abstract Body:

Clostridium difficile causes debilitating antibiotic associated diarrhea in hospital patients and is a pathogen in which alternatives to antibiotic therapies are necessitated. *C. difficile* infections (CDI) are dependent on the activities TcdB, which is an intracellular toxin whose sequence and toxicity varies among different strains of *C. difficile*. A therapy that targets TcdB along with variant forms of this toxin would be an ideal approach to counter CDI and could serve as an alternative to antibiotics. Here, we have discovered peptides derived from the carboxyl-terminal region of TcdB2 that inhibits the cytotoxicity of both TcdB1 (previously termed $TcdB_{012}$ and $TcdB_{Hist}$) and TcdB2 (previously termed $TcdB_{027}$ and $TcdB_{HV}$). This discovery built on our previous TcdB2 work that identified a region proximal to the carboxyl-terminal CROPS that mediates intramolecular interactions and protects neutralizing epitopes. In this region (1753-1851), specific sets of amino acids in TcdB2 (1773-1780 and 1791-1798) were identified that are necessary for supporting protein-protein interactions in TcdB. These studies also revealed that these carboxyl-terminal amino acids (1773-1780 and 1791-1798) influence the amino-terminal domains of TcdB. These data led to the prediction that this region proximal to the CROPS may be necessary for maintaining the overall tertiary structure of TcdB. To test this prediction, a series of peptides were designed spanning this region (1753-1851), and these peptides were screened to determine if any could disrupt inter-domain interactions and alter the activity of TcdB. As shown herein, four peptides with TcdB-inhibitory activity were uncovered in this screen and a detailed analysis of one peptide demonstrated a novel inhibitory mechanism. These studies demonstrate the principle of designing peptides that disrupt inter-domain interactions that are critical for the function of multidomain protein toxins such as TcdB.

Author Disclosure Block:

J.L. Larabee: None. **J.J. Hunt:** None. **J.D. Ballard:** None.

Poster Board Number:

SUNDAY-617

Publishing Title:

TPL-2 Is a Key Regulator of Inflammation in *C. difficile* Infection

Author Block:

Y. Wang[1], S. Tzipori1[2], H. Feng[3], X. Sun[1]; [1]Morsani Sch. of Med., Univ. of South Florida, Tampa, FL, [2]Tufts Univ. Cummings Sch. of Vet. Med., North Grafton, MA, [3]Sch. of Med., Univ. of Maryland, Baltimore, Baltimore, MD

Abstract Body:

Background: Tumor progression locus 2 (TPL-2) has a critical role in the response to inflammatory signals as it functions as a serine/threonine kinase in the MAPK signal transduction cascade known to regulate both innate and adaptive immunity. The pro-inflammatory actions of TPL-2 are mediated by the activation of MAPKs, including ERK, c-Jun NH_2-termianl kinase (JNK) and p38 MAPK. Both *Clostridium difficile* TcdA and TcdB are capable of inducing pro-inflammatory cytokines including TNF-α and IL-1β, which are implicated in the development and progression of *C. difficile* infection (CDI). Previously, we showed that both TcdA and TcdB could activate p38 MAPK and ERK in both Raw264.7 macrophages and bone marrow-derived dendritic cells (BMDCs). We also found that TcdA-mediated TNF-α production in RAW264.7 cells was mediated through p38 MAP kinase and MEK/ERK signaling pathways. **Results:** In this study, we investigated whether TPL-2 has a central role in CDI severity by mediating the production of pro-inflammatory cytokines including TNF-α, IL-1β and IL-6. We report here that in BMDCs, a TPL2 specific inhibitor abolished TcdB-induced production of TNF- α, IL-1β and IL-6. We further demonstrated that TPL-2 inhibitor dramatically blocked TcdB-induced activation of ERK and p38 MAP kinase, but not of JNK in BMDCs. We confirmed these results using BMDCs extracted from TPL2-KO mice. Finally, we demonstrated TPL2-KO mice were significantly more resistant than wild-type mice to *C. difficile* infection in mice. **Conclusions:** Our data suggest TPL-2 represents a potential therapeutic target for CDI treatment.

Author Disclosure Block:

Y. Wang: None. **S. Tzipori1:** None. **H. Feng:** None. **X. Sun:** None.

Poster Board Number:

SUNDAY-618

Publishing Title:

Identification of Genetic Factors Required for *Serratia marcescens* Extracellular Phospholipase Activity

Author Block:

L. A. Bradford[1], M. T. Anderson[1], A. M. Machnacki[2], H. L. Mobley[1]; [1]Univ. of Michigan Med. Sch., Ann Arbor, MI, [2]Univ. of Michigan, Ann Arbor, MI

Abstract Body:

Serratia marcescens, a widespread opportunistic pathogen, causes multiple types of infections, including urinary tract infections and bacteremia. *S. marcescens* infections are frequently healthcare-associated and are often difficult to treat due to a high incidence of antibiotic resistance. Although the mechanisms by which *S. marcescens* survives and replicates within the host remain poorly characterized, this organism is noted for secreting multiple candidate virulence factors. PhlA, an extracellular phospholipase A, is secreted by *S. marcescens* primarily during stationary phase and is predicted to have a role in pathogenesis. To identify *S. marcescens* factors that are involved in the production and secretion of PhlA, a genetic screen was conducted using a *mariner* transposon insertion library. Mutant isolates (>15,000) were screened for the loss of PhlA activity on agar plates containing phosphatidylcholine. In total, mutations were identified in six genes that resulted in the complete loss of extracellular phospholipase activity. Four genes (*cyaA, crp, fliJ and fliP)* encode products that are known to be involved in the transcriptional regulation and export of flagellar components, suggesting that a functional flagellar assembly apparatus is necessary for extracellular phospholipase activity. These results are consistent with previous studies demonstrating that *Serratia* PhlA is secreted through the flagellar transport apparatus when expressed exogenously in *Escherichia coli*. The two remaining genes identified in this screen, *helD and cysE,* are predicted to encode a DNA helicase and serine acetyltransferase, respectively, and have not been previously associated with phospholipase activity or flagellar assembly. The roles of the *helD* and *cysE* gene products in extracellular PhlA activity are currently under investigation. Together these results suggest a putative export pathway for the *S. marcescens* phospholipase exoenzyme and provide further insight into the genetic requirements for production and secretion of this putative virulence factor.

Author Disclosure Block:

L.A. Bradford: None. **M.T. Anderson:** None. **A.M. Machnacki:** None. **H.L. Mobley:** None.

Poster Board Number:

SUNDAY-619

Publishing Title:

Serum Elicits a Massive Increase in Production, Secretion, and Release of *Bordetella* Adenylate Cyclase Toxin

Author Block:

L. Gonyar[1], M. Gray[1], C. Hoffman[1], F. Damron[2], E. Hewlett[1]; [1]Univ. of Virginia, Charlottesville, VA, [2]West Virginia Univ., Morgantown, WV

Abstract Body:

The number of cases of whooping cough, caused by *Bordetella pertussis*, is increasing in the United States and internationally despite high vaccine coverage. Adenylate cyclase toxin (ACT), a critical factor in establishing respiratory tract infection, intoxicates target cells by increasing levels of intracellular cyclic AMP, thereby inhibiting the immunologic response. ACT is almost exclusively surface-bound during *in vitro* growth. However, we have shown that newly secreted, not surface-associated, ACT is responsible for intoxication. Free ACT is present in nasal washes of infected baboons and humans, suggesting that it is secreted and released *in vivo.* Recently, we found that serum (fetal bovine or human) shifts the relative distribution of ACT to the supernate versus the bacterial cell surface and increases the amount up to 30-fold. This serum effect occurs in all nine *B. pertussis* and *B. bronchiseptica* strains tested, including a recent *B. pertussis* clinical isolate.Western blot analysis and J774A.1 cell cytotoxicity indicate that serum increases the amount of ACT protein, rather than just increasing enzyme activity; this is supported by inhibition of the serum effect by blocking protein synthesis with chloramphenicol. Transcript levels (by qRT-PCR) of genes that encode and secrete ACT as well as the BvgA/S two-component system, the master regulator of virulence in *Bordetella*, are unchanged +/- serum, suggesting regulation at the level of protein synthesis or stability. Other virulence determinants, specifically pertussis toxin and filamentous hemagglutinin, are also altered in response to serum, suggesting a global response resulting in coordinate expression of virulence factors. We are currently employing unbiased transcriptomic and proteomic methods to understand the regulatory mechanism/s of this striking serum effect and are working to identify the responsible component/s in serum. Our hypothesis is that these host-derived signaling molecules are present during establishment of infection in the respiratory tract and play a critical role in coordinating expression of essential virulence factors.

Author Disclosure Block:

L. Gonyar: None. **M. Gray:** None. **C. Hoffman:** None. **F. Damron:** None. **E. Hewlett:** None.

Poster Board Number:

SUNDAY-621

Publishing Title:

Pro-Inflammatory Peptidoglycan Fragment Release and Recycling in Pathogenic and Non-Pathogenic *Neisseria*

Author Block:

J. Chan, J. P. Dillard; Univ. of Wisconsin-Madison, Madison, WI

Abstract Body:

Neisseria gonorrhoeae (GC) and *Neisseria meningitidis* (MC) are human pathogens that cause gonorrhea and meningococcal meningitis respectively. Both GC and MC release a number of small peptidoglycan (PG) fragments, although GC releases higher levels of pro-inflammatory PG monomers compared to MC. Since MC colonizes the human nasopharynx as asymptomatic colonizers in 8-40% of the population, we hypothesize that MC limits the release of PG monomers to evade immune clearance in the nasopharynx. When applied to Fallopian tube (FT) explants, PG monomers induce the production of pro-inflammatory cytokines and cause the death and sloughing of ciliated FT cells. These PG fragments are generated in the periplasm during normal PG turnover to allow for cell growth and cell separation. Most of the PG fragments generated are efficiently taken back into the cytoplasm for recycling by AmpG, an inner membrane permease that specifically transports PG fragments from the periplasm to the cytoplasm. Substitutions of GC AmpG residues 273 and 385 from glycine to aspartate inactivate AmpG and result in a large increase in PG monomers released. Since MC is more efficient at recycling compared to GC, we hypothesize that more efficient recycling leads to lower levels of PG monomers released. When MC *ampG* is expressed in GC, we see a ~50% decrease in the amount of PG monomers released. Thus, we seek to determine the factors that modulate AmpG recycling efficiency. We found that AmpG residues 391, 398 and 402 modulate AmpG recycling efficiency through mechanisms we do not yet understand. Substitutions of these residues from the GC to the MC variants cause a ~50% decrease in the amount of PG monomers released. We also investigated PG fragment release and recycling in non-pathogenic *Neisseria*. *N. sicca* and *N. mucosa* are asymptomatic colonizers of the human nasopharynx that release a number of small PG fragments, although they release no PG dimers and lower amounts of PG monomers compared to GC. Both *N. sicca* and *N. mucosa* are more efficient at AmpG-mediated recycling compared to GC. However, expression of *ampG* from *N. sicca* and *N. mucosa* could not complement an *ampG* deletion in GC. We hypothesize that the AmpG sequence differences between *N. sicca*, *N. mucosa* and GC cause the non-pathogenic AmpG to be non- or less functional when expressed in GC, perhaps due to a loss of interaction with potential interaction partner(s).

Author Disclosure Block:

J. Chan: None. **J.P. Dillard:** None.

Poster Board Number:

SUNDAY-622

Publishing Title:

Interaction between the Type III Effector VopO and GEF-H1 Activates the RhoA-ROCK Pathway

Author Block:

Hirotaka Hiyoshi, **T. Kodama**[1], S. Matsuda[2], K. Gotoh[2], Y. Akeda[2]; [1]RIMD, Osaka Univ., Suita, Osaka, Japan, [2]Osaka Univ., Suita, Osaka, Japan

Abstract Body:

Vibrio parahaemolyticus is an important pathogen that causes food-borne gastroenteritis in humans. The type III secretion system encoded on chromosome 2 (T3SS2) plays a critical role in the enterotoxic activity of *V. parahaemolyticus*. Previous studies have demonstrated that T3SS2 induces actin stress fibers in various epithelial cell lines during infection. This stress fiber formation is strongly related to pathogenicity, but the mechanisms that underlie T3SS2-dependent actin stress fiber formation and the main effector have not been elucidated. In this study, we identified VopO as a critical T3SS2 effector protein that activates the RhoA-ROCK pathway, which is essential pathway for the induction of the T3SS2-dependent stress fiber formation. We also determined that GEF-H1, a RhoA guanine nucleotide exchange factor (GEF), directly binds VopO and is necessary for T3SS2-dependent stress fiber formation. The GEF-H1-binding activity of VopO via an alpha helix region correlated well with its stress fiber-inducing capacity. Furthermore, we showed that VopO is involved in the T3SS2-dependent disruption of the epithelial barrier. Thus, VopO hijacks the RhoA-ROCK pathway in a different manner compared with previously reported bacterial toxins and effectors that modulate the Rho GTPase signaling pathway.

Author Disclosure Block:

T. Kodama: None.

Poster Board Number:

SUNDAY-623

Publishing Title:

The *Legionella pneumophila M*ajor Metalloprotease is Translocated out of the Pathogen Vacuole in a Novel, Type II Secretion-Dependent Manner

Author Block:

H. K. Truchan, N. P. Cianciotto; Northwestern Univ., Chicago, IL

Abstract Body:

Legionella pneumophila is a Gram negative, facultative intracellular bacterium that is increasingly recognized as the cause of significant outbreaks of severe pneumonia, known as Legionnaires' disease. Reported cases of the disease have increased 279% since 2000. *L. pneumophila* is ubiquitous in the environment and exists in fresh waters primarily as a parasite of amoeboid protists. Human infection commonly occurs when aerosolized water droplets from contaminated water systems are inhaled. Within the infected mammalian host, the bacteria replicate in alveolar macrophages in a host-derived phagosome, termed the *Legionella*-containing vacuole (LCV). *L. pneumophila* protein secretion systems promote virulence and intracellular infection. Our lab has determined that the Lsp type II secretion (T2S) system secretes > 25 substrates including degradative enzymes as well as novel and eukaryotic-like proteins. The location of these T2S substrates during intracellular infection is unknown. Here, we show that the T2S substrate ProA, a major metalloprotease, is translocated outside the LCV and into the macrophage cytosol where it associates with the LCV membrane (LCVM). This translocation can be observed as early as 10 h post-infection (PI), approximately the midpoint of the intracellular life cycle. However, it can occur as early as 6 h PI if the protein is constitutively expressed from a plasmid. This indicates that translocation is dependent on the timing and level of ProA expression and that any other necessary factors for translocation are in place as early as 6 h PI. Our data also indicate that translocation and localization to the LCVM occur with different *L. pneumophila* strains and in both macrophages and amoebae. Moreover, we determined that ProA translocation is dependent on a functional T2S system and is not mediated by outer membrane vesicles. Thus, the protein is first secreted into the vacuolar lumen by the T2S system and is then subsequently trafficked into the macrophage cytosol via a novel mechanism. Taken together, these data represent the first evidence of a *L. pneumophila* T2S substrate in the infected host cell cytosol. We hypothesize that this localization allows ProA to target cytosolic host cell proteins and perform unique, unidentified functions.

Author Disclosure Block:

H.K. Truchan: None. **N.P. Cianciotto:** None.

Poster Board Number:

SUNDAY-624

Publishing Title:

***In Vitro* and *In Vivo* Characterization of *Stenotrophomonas maltophilia* Type II Secretion and Its Proteases**

Author Block:

A. L. DuMont, S. M. Karaba, M. Y. Nas, N. P. Cianciotto; Northwestern Univ., Chicago, IL

Abstract Body:

Respiratory and blood stream infections caused by the multi-drug resistant, opportunistic pathogen *Stenotrophomonas maltophilia* continue to rise in both the hospital and community setting. The mechanisms utilized by *S. maltophilia* to promote antibiotic resistance have been well characterized, but our understanding of *S. maltophilia* virulence remains limited. *S. maltophilia* pathogenicity in the murine lung is characterized by inflammation and tissue damage, but the virulence factors mediating these effects are not known. Our laboratory has attributed the Xps type II secretion (T2S) system, and primarily two Xps-secreted serine proteases, StmPr1 and StmPr2, to *S. maltophilia*-mediated rounding, detachment, and death of A549 human lung epithelial cells. StmPr1 and StmPr2 are also primarily responsible for the degradation of extracellular matrix (ECM) proteins and the pro-inflammatory cytokine interleukin 8 (IL-8). We now show that an additional secreted serine protease, StmPr3, contributes to Xps-mediated protease activities, by constructing a mutant lacking *stmPr1*, *stmPr2*, and *stmPr3* in the *S. maltophilia* strain K279a background. The triple protease mutant phenocopies an *xps* mutant in both A549 cell detachment and ECM protein degradation assays, indicating that StmPr1, StmPr2, and StmPr3 entirely account for these activities. StmPr1 contributes the most to strain K279a protease activities due to its high abundance in culture supernatant. Therefore, StmPr1 was overexpressed in the triple protease mutant and purified from culture supernatant using benzamidine sepharose. We found that purified StmPr1 was sufficient to cause rounding, detachment, and death of A549 cells. Lastly, to examine the role of Xps T2S *in vivo*, mice were infected intranasally with the *xps* mutant or the K279a wild type (WT) strain, and bacterial burden and histopathology was evaluated in the lungs at various time points. We found that mice infected with the *xps* mutant had lower bacterial burden in the lung at 8-12 hours post-infection compared to WT-infected mice, and that the *xps* mutant caused less lung tissue damage than the WT strain. Taken together, these data attribute the previously described Xps-dependent protease activities entirely to StmPr1, StmPr2, and StmPr3, and indicate that Xps T2S is important for *S. maltophilia* virulence.

Author Disclosure Block:

A.L. DuMont: None. **S.M. Karaba:** None. **M.Y. Nas:** None. **N.P. Cianciotto:** None.

Poster Board Number:

SUNDAY-625

Publishing Title:

***in Vitro* characterization Of The *pseudomonas Aeruginosa* cytotoxin Exou**

Author Block:

A. Zhang, J. L. Veesenmeyer, A. R. Hauser; Northwestern Univ., Chicago, IL

Abstract Body:

Background: *Pseudomonas aeruginosa* is a Gram-negative bacterium and an important cause of acute infections in immunocompromised patients. Its type III secretion system is a major virulence factor and allows effector proteins to directly translocate into targeted host cells. One such effector protein, ExoU, harbors a patatin-like phospholipase domain known to require two host cell factors for activation. Previous work from our lab and others has also shown that ExoU exhibits alpha-complementation. While certain truncation mutants of ExoU were non-cytotoxic inside mammalian cells, co-expression of these mutant proteins partially restored cell killing. However, the mechanism of this phenomenon is currently unknown. We hypothesize that oligomerization of ExoU mediates alpha-complementation and plays an important role in ExoU cytotoxicity. **Methods/Results:** Using lactate dehydrogenase release assays as a measure of cell death, we confirmed and extended previous results showing that transfection of HeLa cells with N- and C-terminal truncations of ExoU individually resulted in no cytotoxicity but that co-transfection of these variants resulted in partial cell death relative to wildtype ExoU. We also observed that differentially epitope-tagged ExoU could be co-immunoprecipiated when expressed in HeLa cells. Using size-exclusion chromatography and blue-native polyacrylamide gel electrophoresis, we showed that purified recombinant ExoU does not form oligomers. Co-incubation of ExoU with one of its host cofactors, ubiquitin, also did not result in oligomerization of ExoU. However, co-incubation with phosphatidylinositol 4,5-bisphosphate (PIP2), the lipid co-activator of ExoU, was sufficient to induce oligomerization *in vitro*. **Conclusion:** Our results suggest that ExoU oligomerizes *in vitro*. This oligomerization is mediated by one of the mammalian cofactors of ExoU required for phospholipase activation. In combination with the previous observation that regions of ExoU exhibit alpha-complementation, we believe this data indicates ExoU oligomerization may play a role in mediating cytotoxicity.

Author Disclosure Block:

A. Zhang: None. **J.L. Veesenmeyer:** None. **A.R. Hauser:** None.

Poster Board Number:

SUNDAY-626

Publishing Title:

Bacterial Social Interactions and Their Impact on Virulence Evolution in *Pseudomonas aeruginosa*

Author Block:

E. T. Granato, R. Kümmerli; Univ. of Zurich, Zurich, Switzerland

Abstract Body:

Understanding the evolution of virulence in human pathogens is of great interdisciplinary interest. This is particularly true for opportunistic pathogens, such as *Pseudomonas aeruginosa*, that often cause chronic infections and undergo within-host evolution during disease progression. Here, we use experimental evolution to elucidate the interplay between virulence factor secretion, spatial structure and the evolution of virulence in the model host *Caenorhabditis elegans*. Virulence in bacterial infections is typically mediated through secreted metabolites that can be cooperatively shared across the bacterial collective within the host. Although many of these metabolites are essential to establish infections, populations of metabolite producers can potentially be invaded by "cheating" mutants, which no longer contribute but still benefit from the shareable metabolites produced by others. Consequently, conditions selecting for cheats should ultimately reduce virulence. We predict that the reduction of virulence due to cheat invasion should be particularly strong in spatially unstructured environments, where cheating mutants have been shown to enjoy highest selective advantage because secreted metabolites and cells mix readily. We tested this prediction by experimentally evolving *P. aeruginosa* in liquid (unstructured) and agar-based (structured) medium in the presence or absence of its host *C. elegans*. After the evolution, populations of bacteria that had evolved in a liquid environment displayed strongly decreased virulence towards *C. elegans*. Phenotypic screening revealed that many bacteria had lost the ability to produce pyoverdine, a secreted metabolite used to scavenge iron from the environment and the host tissue. This was exclusively observed in clones that evolved in liquid medium. Additionally, the production of pyocyanin, a secreted toxin important in killing *C. elegans*, dropped dramatically during evolution, and production levels were lowest among bacteria that evolved in liquid medium with the host. Altogether, our results support the idea that non-virulence factor producing cheats can invade populations of virulence-factor producing pathogens, thereby steering pathogen populations towards lower virulence. This opens possibilities for therapeutic interventions that aim at exploiting cooperator-cheat dynamics.

Author Disclosure Block:

E.T. Granato: None. **R. Kümmerli:** None.

Poster Board Number:

SUNDAY-627

Publishing Title:

Prevalence of Exfoliative Toxin Gene Carriage in *Staphylococcus pseudintermedius* Isolates from Humans and Dogs

Author Block:

L. K. Bryan[1], M. Höök[2], C. A. Burnham[3], N. D. Cohen[1], S. D. Lawhon[1]; [1]Texas A&M Univ., College Station, TX, [2]Texas A&M Hlth.Sci. Ctr., Houston, TX, [3]Washington Univ. Sch. of Med., St. Louis, MO

Abstract Body:

Staphylococcus pseudintermedius is an emerging human pathogen and is the leading cause of canine pyoderma. Staphylococcal exfoliative exotoxins (ET) are thought to exacerbate skin infections in humans and dogs by cleaving cell-cell junction proteins in the superficial epidermis. Three ET genes have been identified in *S. pseudintermedius*: *siet*, *exi* and *expB*. An additional gene, *speta*, may encode an exotoxin. *S. pseudintermedius* isolates collected from healthy (n = 142) and diseased (n = 373) dogs and diseased humans (n = 45) were analyzed via polymerase chain reaction (PCR) to determine prevalence of ET gene carriage and to compare host factors and antimicrobial drug susceptibility in isolates with differing ET carriage profiles. Five ET genotypes occurred: only *speta; speta+siet; speta+siet+exi; speta+siet+expB;* and, *speta+siet+exi+expB*. The *exi* and *expB* genes always occurred in conjunction with *speta* and *siet*. The *speta+siet+exi+expB* and only *speta* genotypes were not observed in human-derived isolates. The majority of isolates from humans and dogs were *speta+siet* (84 and 71%, respectively), followed by *speta+siet+expB* (11 and 15%, respectively) and *speta+siet+exi* (5 and 12%, respectively). The proportions of ET genotypes were compared via Pearson's chi-squared and Fisher's exact tests. The *exi* gene was more prevalent (P = 0.0038) among canine pyoderma isolates than among dogs with other diseases. The odds of surgical site infection were also 3-fold greater (P = 0.0316) for isolates that were *speta+siet* than for other genotypes. The *speta+siet+expB* genotype had a higher probability of being methicillin-sensitive (P = 0.003) than resistant in dogs. There was no significant difference between ET gene carriage and overall health status in dogs or between ET genotype and methicillin resistance in the human-derived isolates.

Author Disclosure Block:

L.K. Bryan: None. **M. Höök:** None. **C.A. Burnham:** None. **N.D. Cohen:** None. **S.D. Lawhon:** None.

Poster Board Number:

SUNDAY-628

Publishing Title:

Loss of the MFS Transporters *fptg* and *fptb* Delays Host Exit of *Francisella tularensis*

Author Block:

P. Balzano, E. Barry; Univ. of Maryland, Baltimore, Baltimore, MD

Abstract Body:

Background: *Francisella tularensis (Ft*) is a Gram negative facultative intracellular coccobacillus, and is the causative agent of the human disease tularemia. Due to past weaponization, a small inoculum through the aerosol route, a mortality rate approaching 50%, and no licensed vaccine, *Ft* is a major bioterror concern. After the bioterror attacks of 9/11, there are renewed vaccine development efforts for this select agent pathogen.**Methods:** The targeted deletion of selected genes in *Ft* to produce candidate live attenuated vaccine strains is a strategy we have pursued. We produced 2 vaccine candidates containing single deletions of major facilitator superfamily (MFS) transporters, *fptG,* and *fptB,* an isoleucine transporter, in the Type A strain Schu S4. Based on the attenuating capacity of these deletions in the LVS background, we hypothesized that deletion of either transporter would alter the intracellular replication rate of the virulent Type A strain. Gentamicin protection assays that eliminate or allow cell-to-cell spread in human THP-1 macrophage-like cells were utilized to identify intracellular replication patterns for both mutants.**Results:** Gentamicin protection assays that eliminate cell-to-cell spread yielded intracellular bacterial counts of up to ten times more bacteria for both mutants than wild type at 24 hours post infection. Further invasion assays allowing cell-to-cell spread indicated that SchuS4Δ*fptG* replicated at the same rate as wildtype, but appeared delayed in escaping the host cell compared to the wildtype. SchuS4Δ*fptB* similarly appeared delayed in exiting the host cell, but also exhibited a significant growth defect, doubling 1 to 1.5 fewer times in the first 24 hours. SchuS4Δ*fptG* and SchuS4Δ*fptB* are the first MFS transporter knockouts to be created in the virulent Schu S4 strain.**Conclusions:** The mutants' phenotype of delayed cellular escape is novel in *Ft* and suggests a more active role in host escape played by the bacteria beyond simply overwhelming the host with high levels of replication. These strains are valuable tools to study the intracellular lifecycle of *Ft* and may be candidates for a live attenuated vaccine.

Author Disclosure Block:

P. Balzano: None. **E. Barry:** None.

Poster Board Number:

SUNDAY-629

Publishing Title:

Cold Shock Domain Family Proteins Promote Survival in Human Macrophages and Expression of Key Virulence Genes in Listeria Monocytogenes

Author Block:

Athmanya Eshwar, Roger Stephan, **T. Tasara**; Univ. of Zurich, Zurich, Switzerland

Abstract Body:

Background: The intracellular foodborne pathogen *Listeria monocytogenes* causes rare but serious disease (listeriosis) and high rates of mortality among those with diminished immunity. Cold shock domain protein family (Csps) are small global gene expression regulating proteins with diverse cellular functions that promote virulence and stress responses in bacteria. In this study the contribution of Csps to the intracellular survival and growth of *L. monocytogenes* within human macrophages and in modulating the expression of key virulence factors was investigated. **Methods:** A series of in-frame *csp* deletion mutants of *L. monocytogenes* EGDe that lack all (Δ*cspABD*) or retain one (Δ*cspBD*, Δ*cspAD* and Δ*cspAB*) of the three Csp encoding genes (*cspA*, *cspB* and *cspD*) in this bacterium and their parental wild type strain were compared. The strains were evaluated with respect to human macrophage infection and expression of genes encoding key virulence factors using qRT-PCR and WB analysis.**Results:** The capacity of *L. monocytogenes* to infect human macrophages and express key virulence genes was significantly compromised without Csp functions and in presence of individual Csp variants. A mutant incapable of producing Csps (Δ*cspABD*) was most severely impaired compared to the parental wild type EGDe strain in its ability to survive and grow in THP-1 human macrophages, as well as in the expression of genes encoding the PrfA, LLO, Mpl, ActA, PlcA and PlcB virulence factors. Analysis of mutants harboring individual Csp variants revealed that there is both functional redundancy and hierachy (CspB [Δ*cspAD*] >CspD [Δ*cspAB*] >CspA [Δ*cspBD*]) in the role of the individual Csps in promoting intramacrophage survival and growth, and modulating the expression of key virulence genes in this bacterium.**Conclusion:** These studies have shown that Csp-dependent gene expression regulation is crucial for the full expression of *L. monocytogenes* virulence in human host macrophages. Csp functional contribution to *L. monocytogenes* virulence responses is associated with their role in promoting the gene expression of key virulence factors in this bacterium.

Author Disclosure Block:

T. Tasara: None.

Poster Board Number:

SUNDAY-630

Publishing Title:

Comparative Analysis of Genes Involved in Intracellular Survival of *Salmonella enterica* serovar Typhimurium in Murine Macrophages and *Dictyostelium discoideum*

Author Block:

A. Sabag[1], S. Riquelme[1], C. Valenzuela[1], C. Bravo[1], V. Barra[1], J. Ugalde[2], R. Canals[3], P. Desai[3], V. Martinez[1], F. Chavez[1], M. McClelland[3], S. A. Alvarez[1], C. A. Santiviago[1]; [1]Univ. de Chile, Santiago, Chile, [2]Univ. del Desarrollo, Santiago, Chile, [3]Univ. of California Irvine, Irvine, CA

Abstract Body:

Pathogenicity of *Salmonella* is associated to its ability to evade the innate immune system of the host. For instance, *S.* Typhimurium can replicate within phagocytic cells such as macrophages, neutrophils and dendritic cells of many hosts, causing illnesses ranging from gastrointestinal infections to severe systemic diseases. In the environment, *Salmonella* can interact with other types of professional phagocytic cells, such as free-living amoebae. Amoebas like *Dictyostelium discoideum* ingest bacteria as a source of nutrients and may provide a niche for bacterial replication similar to the intra-macrophage environment. Therefore, we hypothesized that *S.* Typhimurium survival in macrophages and amoebas involves a common set of genes. To identify these genes, we infected RAW264.7 murine macrophages and *D. discoideum* AX4 with a single-gene deletion mutant collection of *S.* Typhimurium 14028s (SGD-K) using a MOI of 10 and 100 bacteria/cell, respectively. After co-incubation, extracellular bacteria were killed by gentamicin treatment. Intracellular bacteria were recovered at different times of infection (0 and 6 h), grown in LB broth for 12 h, collected by centrifugation and treated for genomic DNA extraction, amplification and massive parallel sequencing. A preliminary analysis of the sequencing data allowed us to identify 591 and 963 mutants under negative selection in RAW264.7 murine macrophages and *D. discoideum*, respectively. The comparison of both datasets showed that 281 mutants undergo negative selection in both models. Most of these mutants are associated to genes involved in carbohydrate and amino acid metabolism and transport, energy production, cell motility, virulence and signal transduction. Altogether, our data indicate that *S.* Typhimurium requires a common set of genes to survive within murine macrophages and *D. discoideum.*

Author Disclosure Block:

A. Sabag: None. **S. Riquelme:** None. **C. Valenzuela:** None. **C. Bravo:** None. **V. Barra:** None. **J. Ugalde:** None. **R. Canals:** None. **P. Desai:** None. **V. Martinez:** None. **F. Chavez:** None. **M. McClelland:** None. **S.A. Alvarez:** None. **C.A. Santiviago:** None.

Poster Board Number:

SUNDAY-631

Publishing Title:

Phenotypic and Genomic Characterisation of Atypical *Salmonella enterica* Serovars Linked to Multistate Foodborne Outbreaks in the United States

Author Block:

D. Hurley[1], M. Hoffmann[2], M. Martins[1], E. W. Brown[2], S. Fanning[1]; [1]Univ. Coll. Dublin, Dublin, Ireland, [2]U.S. Food and Drug Admin., College Park, MD

Abstract Body:

Salmonella Typhimurium pathogenicity has traditionally been studied using *in vivo* models and murine cell lines. Few studies using human models for other non-typhoidal serovars have been described, despite their epidemiological importance in several outbreaks. In this study, 10 atypical serovars cultured from multistate U.S. outbreaks were characterised by infection biology techniques and comparative sequence analyses.**Objective:** To study the genomes and virulence of 10 foodborne outbreak isolates.Intracellular survival was assessed *ex vivo* in murine RAW 264.7 and human THP-1 macrophages. Release of 17 proinflammatory cytokine and chemokine proteins from infected macrophages was quantified. *S*. Typhimurium ST4/74 was used as a reference strain. Whole genome sequencing was performed using the Illumina MiSeq platform. Complete genome sequences for isolates of particular interest were determined on the Pacific Biosciences RS II.In THP-1 macrophages, *S*. Tennessee CFSAN001387 exhibited a 1-Log_{10} reduction between 2 and 168 hours post infection, demonstrating an increased ability to persist within these cells. Similarly, *S*. Weltevreden CFSAN001415 exhibited a >2-Log_{10} reduction, surviving for 7 days compared to the reference strain which was unrecoverable. Many isolates stimulated increased cytokine (IL1B, TNF) and chemokine (CCL2, CCL3/4) release compared to the reference strain.*S*. Anatum CFSAN003959 has an altered SPI-2 SsaB protein being associated with an attenuated infection phenotype thereby rendering the isolate unrecoverable early during infection. SptP, a T3SS effector, was altered in *S*. Heidelberg CFSAN002063 contributing to consistently higher levels of TNF production compared to other isolates. The SseBCD translocon is integral to the intracellular survival of *Salmonella* and in *S*. Tennessee CFSAN001387 this was highly altered when compared to *S*. Typhimurium ST4/74. This may explain the ability of the former isolate to survive within RAW 264.7 and THP-1 macrophages.These phenotypic and genomic analyses extend our understanding of the bacterial host-cell response and have identified virulence factors that further explain the ability of these isolates to cause outbreaks.

Author Disclosure Block:

D. Hurley: None. **M. Hoffmann:** None. **M. Martins:** None. **E.W. Brown:** None. **S. Fanning:** None.

Poster Board Number:

SUNDAY-632

Publishing Title:

Characterization of a Temperature Sensitive Aminoacyl-tRNA Synthetase C*hlamydia trachomatis* Mutant

Author Block:

J. A. Brothwell[1], B. D. Stein[2], D. E. Nelson[1]; [1]Indiana Univ. Sch. of Med., Indianapolis, IN, [2]Indiana Univ., Bloomington, IN

Abstract Body:

Chlamydia spp. are obligate intracellular pathogens that are causative agents of blinding trachoma, sexually transmitted infections, and pneumonia in humans. These pathogens exhibit a characteristic biphasic developmental cycle, which is initiated by endocytosis of the infectious, non-metabolically active elementary body (EB). The EB then differentiates into a non-infectious, metabolically active reticulate body (RB) inside a parasitophorous vacuole, termed the inclusion. What signals EB to RB differentiation, and whether this program is dependent on activation of specific biosynthetic pathways, is unknown. Genes that control chlamydial differentiation would be predicted to be essential, so we screened a library of ethyl methanesulfonate (EMS)-mutagenized *C. trachomatis* isolates for temperature sensitive (TS) mutants that could survive in HeLa cells at the permissive temperature of 37°C, but which could not survive at the non-permissive temperature of 40°C. We identified a TS mutant that had a nonsense mutation in *gltX* (*gltX*^{Q487*}). GltX is required for aminoacylating both tRNA^{Glu} and tRNA^{Gln}, which suggested that protein synthesis of this mutant was impeded at 40°C. Previous studies have shown that *de novo C. trachomatis* protein synthesis initiates within 15 minutes of infection. However, *gltX*^{Q487*} growth could be rescued as late as 18 hours post infection, suggesting that this mutant might survive in a persistent state. *gltX*^{Q487*} exhibited normal growth kinetics at 37°C, but viable progeny and genome copy number of this strain were severely reduced at 40°C. Examination of inclusion ultrastructure at 40°C showed that *gltX*^{Q487*} was able to differentiate into RB and undergo at least 1 cellular division. These results contrast those from another persistence model where tryptophan is depleted via interferon-γ treatment. Our results suggests that *C. trachomatis* responds differently to the loss of aminoacylated tRNAs than it does to the absence of individual amino acids and that EB may contain a complete set of aminoacylated tRNAs that facilitate their early protein synthesis and differentiation.

Author Disclosure Block:

J.A. Brothwell: None. **B.D. Stein:** None. **D.E. Nelson:** None.

Poster Board Number:

SUNDAY-633

Publishing Title:

Characterization of Virulence-Factor Deletion Strains of *Burkholderia mallei* That Are Attenuated in Inhalational Glanders Challenge in Mice

Author Block:

J. A. Bozue[1], **R. Toothman**[1], S. Chaudhury[2], K. Amemiya[1], J. Chua[1], C. Cote[1], J. Dankmeyer[1], C. Klimco[1], C. Wilhelmsen[1], J. Raymond[1], N. Zavaljevski[3], J. Reifman[2], A. Wallqvist[3]; [1]USAMRIID, Fort Detrick, MD, [2]BHSAI, Frederick, MD, [3]BHSAI, Fort Detrick, MD

Abstract Body:

Background: *Burkholderia mallei* is a highly infectious intracellular pathogen and the causative agent of glanders. It is a zoonotic disease primarily of horses, donkeys and mules and endemic to Africa, Asia, and Middle East. Human infection, although rare, is almost always fatal without antibiotic intervention. *B. mallei* has been observed to have a high level of antibiotic resistance, and there is currently no approved vaccine. **Methods:** Previously, we identified novel virulence factor genes *BMAA0553* (encoding a putative serine/threonine phosphatase) and *BMAA0728* (encoding for a hypothetical protein in gene cluster of components of a type 6 secretion system). In the present study, we used recombinant allelic exchange to successfully construct deletion mutants of *BMAA0553* and *BMAA0728*. **Results:** We showed that both deletions completely abrogated virulence at doses of >100 times the LD_{50} of the wild-type *B. mallei* strain. Subsequent *in vitro* work confirmed significantly decreased intracellular growth for both mutant strains and delayed multinuclear giant cell formation in macrophages. The Δ*BMAA0553* strain also demonstrated a defect in actin active-based motility during macrophage infection. In addition, we explored the use of Δ*BMAA0728* as a candidate live-attenuated vaccine. We vaccinated mice with two aerosolized immunizations of Δ*BMAA0728* and observed a 21-day survival rate of 67% after a high-dose aerosolized challenge with the wild-type ATCC 23344 strain, compared to a 0% survival rate for unvaccinated mice. However, the bacteria were still able to establish a chronic infection in the surviving mice. Measurement of antibody, cellular, and cytokine responses of vaccinated mice showed a modest IgG response and prolonged elevation of pro-inflammatory cytokines. **Conclusions:** These data do demonstrate the essential role of these proteins as virulence factors in both intracellular replication in macrophages and for aerosolized glanders of mice.

Author Disclosure Block:

J.A. Bozue: None. **R. Toothman:** None. **S. Chaudhury:** None. **K. Amemiya:** None. **J. Chua:** None. **C. Cote:** None. **J. Dankmeyer:** None. **C. Klimco:** None. **C. Wilhelmsen:** None. **J. Raymond:** None. **N. Zavaljevski:** None. **J. Reifman:** None. **A. Wallqvist:** None.

Poster Board Number:

SUNDAY-634

Publishing Title:

Two Stable Variants of *Burkholderia pseudomallei* Strain MSHR5848 Express Broadly Divergent *In Vitro* Phenotypes Associated with Their Virulence Differences

Author Block:

A. Shea, R. Bernhards, D. Rozak, M. Wolcott, D. Fetterer, J. Ladner, G. Palacios, J. Bozue, P. Worsham, C. Cote, **S. Welkos**; U S A M R I I D, Frederick, MD

Abstract Body:

Burkholderia pseudomallei (*Bp*) is the HHS Tier 1 cause of melioidosis, a disease with manifestations ranging from acute and rapidly fatal to protracted and chronic. *Bp* is highly infectious by aerosol, can cause disease with nonspecific symptoms, and is resistant to many antibiotics. Effective treatment is long and difficult, and no vaccine exists. The development of more effective countermeasures requires a better understanding of disease pathogenesis. Unlike many *Bp* strains which exhibit random variability in traits such as colony morphology, DOD Unified Culture Collection stock of BURK178 (derived from *Bp* strain MSHR5848) exhibited two distinct and fairly stable colony morphologies on sheep blood agar plates: a smooth pale yellow colony (type 1) and a flat dry white colony (type 2). Passage of these variant colonies under standard lab conditions produced cultures composed nearly exclusively of the single corresponding type 1 or type 2 morphotype. However both types could switch to the other one at frequencies which varied with the growth condition. The two MSHR5848 variants were extensively characterized to demonstrate the broadly divergent phenotypes associated with them, including differences in microscopic and colony morphologies, metabolic and antimicrobial sensitivities, and macrophage infectivity and mouse virulence. Here we present whole genome sequence comparisons which show that the variants are genetically relatively conserved. However, Biolog Phenotype Microarray^{TM} (PM) profiles revealed distinct differences between the variants. Type 1 was more metabolically active than type 2 and used a greater number of diverse carbon sources. Nevertheless, type 2 was more active in the presence of sulfur sources. The type 2 variant also had a higher response when the peptide nitrogen sources included aromatic amino acids. Also, type 1 grew more rapidly *in vitro* than type 2, and whereas type 1 cells stained with the fluorescent DNA-binding dye propidium iodide, type 2 failed to stain. These distinct phenotypic and *in vivo* differences shown by the BURK178 morphotypes may provide a model for identifying *in vitro* markers associated with different infection stages and help guide vaccine and therapeutic development.

Author Disclosure Block:

Shea: None. **R. Bernhards:** None. **D. Rozak:** None. **M. Wolcott:** None. **D. Fetterer:** None. **J. Ladner:** None. **G. Palacios:** None. **J. Bozue:** None. **P. Worsham:** None. **C. Cote:** None. **S. Welkos:** None.

Poster Board Number:

SUNDAY-635

Publishing Title:

Metabolic Evidence That Biotin Acts as a Nutritional Virulence Factor in the Intracellular Pathogen *Francisella novicida*

Author Block:

Y. Feng; Zhejiang Univ., Hangzhou, China

Abstract Body:

Background: Biotin (Vitamin H), the sulfur-containing enzyme cofactor, is an essential micronutrient for three domains of life. The physiological function of biotin requires biotin protein ligase activity in order to attach the coenzyme to its cognate proteins, which are enzymes involved in central metabolism. The model intracellular pathogen *Francisella novicida* is unusual in that it encodes two putative biotin protein ligases rather than the usual single enzyme. *F. novicida* BirA has a ligase domain as well as an N-terminal DNA-binding regulatory domain, similar to the prototypical BirA protein in *E. coli*. However, the second ligase, which we name BplA, lacks the N-terminal DNA binding motif. It has been unclear why a bacterium would encode these two disparate biotin protein ligases, since *F. novicida* contains only a single biotinylated protein. **Methods:** Integrative approaches were employed that included *in vivo* complementation, enzyme assays, biochemical & biophysical experiments, and cell lines/mice-based infection trials.**Results:** BirA and BplA both were found to be functional biotin protein ligases, but BplA is a much more efficient enzyme. BirA (but not BplA) regulated transcription of the biotin synthetic operon. Expression of *bplA* (but not *birA*) increased significantly during *F. novicida* infection of macrophages. BplA (but not BirA) was required for bacterial replication within macrophages as well as in mice. During infection, BplA seems primarily employed to maximizethe efficiency of biotin utilization without limiting the expression of biotin biosynthetic genes, representing a novel adaptation strategy that may also be used by other intracellular pathogens. We also determined a new gene *bioJ* (*FTN_0818*) with the involvement of biotin metabolism and required for *F. novicida* virulence. Genetic and biochemical analyses suggested that BioJ is functionally equivalent to the paradigm pimeloyl-ACP methyl ester carboxyl-esterase, BioH. Structure-guided mapping combined with site-directed mutagenesis revealed that the BioJ catalytic triad consists of Ser151, Asp248 and His278, all of which are essential for activity and virulence. **Conclusion:** To the best of our knowledge, the data above represents further evidence linking biotin synthesis to bacterial virulence in *F. novicida*.

Author Disclosure Block:

Y. Feng: None.

Poster Board Number:

SUNDAY-636

Publishing Title:

Pyomelanin Production Contributes to Virulence in the Human Pathogen *Burkholderia cenocepacia* j2315

Author Block:

S. C. Fankhauser[1], M. L. Ivey[2], J. B. Goldberg[2]; [1]Oxford Coll. of Emory Univ., Oxford, GA, [2]Emory Univ. Sch. of Med., Atlanta, GA

Abstract Body:

Those living with chronic granulomatous disease (CGD) or cystic fibrosis (CF) are extremely susceptible to lung infections. Patients with CGD are particularly prone to repeat bacterial infections, and prophylactic antibiotic therapy is necessary to limit bacterial infection and the severity of bacterial infection. For CF patients, about 80-90% of these patients eventually succumb to respiratory failure due to chronic bacterial infection. Of the select pathogens that infect both these patient populations, *Burkholderia cenocepacia* is especially troublesome due to its inherent antibiotic resistance, capacity to survive in supposedly sterile hospital solutions, and ability to spread between patients. Effective prevention and treatment measures are critical to reducing the devastating impact that *B. cenocepacia* has on patients. Yet, developing these measures requires a clear understanding of the mechanisms that contribute to *B. cenocepacia* infection. We investigated whether the conspicuous phenotype of pigment production, which has been observed in several different *B. cenocepacia* strains, could impact virulence as it has been shown to in other bacterial pathogens. Specifically we investigated the role of the pigment pyomelanin, which is produced by the human pathogen *B. cenocepacia* J2315. Compared to a non-pyomelanin producing isogenic mutant, J2315 is significantly more virulent in an intratrachael mouse infection model. We have determined that pyomelanin produced by J2315 protects against the reactive nitrogen species nitric oxide, and that this increased protection may contribute to the increased virulence we observed in the mouse infections. Together our studies have identified and begun to characterize a novel virulence factor in *B. cenocepacia* and these findings may be applicable to other bacterial pathogens that produce pyomelanin.

Author Disclosure Block:

S.C. Fankhauser: None. **M.L. Ivey:** None. **J.B. Goldberg:** None.

Poster Board Number:

SUNDAY-637

Publishing Title:

***Legionella pneumophila* Philadelphia-1 p45 IntegrativeGenomic Element Plays a Role in Virulence**

Author Block:

L. M. Christensen, S. L. G. Cirillo, M. Strain, S. Parikh, J. D. Cirillo; Texas A&M Hlth.Sci. Ctr., Bryan, TX

Abstract Body:

As ubiquitous environmental organisms, *Legionella spp.* are capable of living in a vast array of environmental conditions. This adaptability is made possible, in part, due to the notorious plasticity of their genomes, composed of several mobile elements capable of horizontal transfer. One such site-specific integrative element, p45, is found more commonly in pathogenic strains of *Legionella,* which are capable of causing a potentially fatal form of pneumonia called Legionnaires' disease. In order to examine the role of the p45 element in virulence, we utilized *Legionella pneumophila* Philadelphia-1 (Phil-1), a clinical isolate containing p45. In addition to Lp01, a laboratory strain derived from Phil-1, which displays attenuated virulence-related phenotypes and is lacking p45. We complemented Lp01 with p45 using natural conjugation from Phil-1. The resulting p45-containing Lp01 strain ($Lp01^{p45}$), Phil-1, and Lp01 were compared for their ability to survive in the presence of sodium, infect macrophages, and cause disease in guinea pigs. Results from these experiments suggest that p45 plays a key role in *Legionella* virulence, as the $Lp01^{p45}$ strain phenotypes mimicked those of Phil-1 throughout the above assays. These exciting results warrant further study of the p45 element in order to identify the specific components and mechanisms involved.

Author Disclosure Block:

L.M. Christensen: None. **S.L.G. Cirillo:** None. **M. Strain:** None. **S. Parikh:** None. **J.D. Cirillo:** None.

Poster Board Number:

SUNDAY-638

Publishing Title:

Lipid Differentiation of *Burkholderia pseudomallei* Following *In Vitro* Infection

Author Block:

R. Dermody, D. Fletcher, T. Eckstein; Colorado State Univ., Fort Collins, CO

Abstract Body:

This experiment was conducted in order to assess the lipid makeup of *Burkholderia pseudomallei* following an *in vitro* infection. *B. pseudomallei* is a bacterium associated with the human disease melioidosis. It is observed to be similar to *Mycobacterium avium* subspecies *paratuberculosis* (MAP), which causes Johne's disease in ruminants. My work aims to further existing research that associates species similar to MAP with receptor-mediated lipid transformation. Such research has been limited in the past due to the highly infective quality of subject bacteria. The method I used began with the infection of live macrophages with a culture of *B. pseudomallei* ΔpurM (Bp82), an adenine and thiamine auxotrophic form of *B. pseudomallei* . After infection, the macrophages were lysed in order to expel the bacteria. Lipid was extracted from the bacteria post-infection by a process of lyophilization and Folsch washing. Bp82 from broth that did not undergo infection was submitted to the same lipid extraction protocol. The lipid produced from both samples was run through a series of thin layer chromatography systems of varying polarity and also submitted for LC/TOF-MS, using the Sartain Method in both positive and negative modes. My analysis has suggested that infection increases the abundance and intensity of lipids possessing greater polarity. These results may be used to improve knowledge of the composition of *B. pseudomallei* for use in vaccination and treatment.

Author Disclosure Block:

R. Dermody: None. **D. Fletcher:** None. **T. Eckstein:** None.

Poster Board Number:

SUNDAY-639

Publishing Title:

***In vivo* Clearance and Tissue Distribution of Purified *Burkholderia pseudomallei* Capsular Polysaccharide in a Murine Model**

Author Block:

A. Kirosingh[1], T. Nualnoi[1], S. G. Pandit[1], P. Thorkildson[1], D. E. Reed[1], P. J. Brett[2], M. N. Burtnick[2], D. P. AuCoin[1]; [1]Univ. of Nevada Sch. of Med., Reno, NV, [2]Univ. of South Alabama, Mobile, AL

Abstract Body:

A Gram-negative bacillus, *Burkholderia pseudomallei,* is the causative agent of melioidosis. Melioidosis has been a significant source of morbidity and mortality in northern Australia and Southeast Asia. The "gold standard" for melioidosis diagnosis is bacterial isolation, which takes several days to complete. The delay in diagnosis can lead to administration of inappropriate antibiotics, which could result in death. *B. pseudomallei* produces a capsular polysaccharide (CPS), an outer membrane component important for the virulence of the bacterium, that has been identified as a biomarker during infection. Rapid lateral flow immunoassays using monoclonal antibodies specific to CPS have been developed and used in endemic areas for clinical testing. However, the tissue distribution and clearance of CPS is not well understood. In this study, mice were injected with purified CPS intravenously and concentrations of CPS found in the serum, urine, and major organs (kidneys, lungs, liver and spleen) were monitored using antigen capture ELISAs at different time intervals. The tissue distribution study indicates no accumulation of CPS in the major organs. Our results also show that CPS is predominantly eliminated in urine. Western blot analysis revealed that CPS was not degraded during excretion; how a large molecule like CPS was eliminated from the kidneys is still being investigated. Interestingly, 3D computer modeling reveals that CPS has a rod-like shape with a small diameter that could allow it to flow through the glomerulus. To calculate CPS clearance from serum, the data were fit to four different exponential decay models and corrected Akaike Information Criterion was used for the model selection. The results show that CPS was eliminated with a relatively short half-life of 3 to 4.5 hours. The rapid clearance of CPS through urine indicates that the presence of CPS in the serum and urine correlates with active melioidosis infection and provides a marker to monitor the effectiveness of treatment of melioidosis.

Author Disclosure Block:

A. Kirosingh: None. **T. Nualnoi:** None. **S.G. Pandit:** None. **P. Thorkildson:** None. **D.E. Reed:** None. **P.J. Brett:** None. **M.N. Burtnick:** None. **D.P. AuCoin:** None.

Poster Board Number:

SUNDAY-640

Publishing Title:

***ehrlichia Chaffeensis* Survival Is Linked To Trp120 Dependent Activation Of The Notch Pathway**

Author Block:

T. Lina; Univ. of Texas Med. Branch, Galveston, TX

Abstract Body:

Background: *Ehrlichia chaffeensis* is an obligately intracellular bacterium responsible for the life-threatening tick transmitted zoonosis, human monocytotropic ehrlichiosis (HME). *E. chaffeensis* exhibits tropism for mononuclear phagocytes and survives through a strategy of subverting innate immune defenses, but the mechanisms are unknown. We have shown *E. chaffeensis* type 1 secreted tandem repeat protein (TRP) effectors such as TRP120 promote intracellular survival through diverse pathogen-host protein interactions, including Notch signaling pathway components such as the metalloprotease, ADAM17. In our current study, we demonstrate *E. chaffeensis*, via the TRP120 effector, activates canonical Notch signaling pathway to promote intracellular survival. **Methods:** Human Notch PCR array, RT-PCR, western blot and immunofluorescence microscope (IFA) was used to study the expression of different Notch components and target genes during *E. chaffeensis* infection and TRP120 stimulation. Pharmacological inhibitors and siRNA transfection was used to inhibit different Notch signaling components and ehrlichial infection was determined by either Diff-Quick staining, or by determining the *dsb* copy number using qPCR.**Results:** Nuclear translocation of the transcriptionally active Notch intracellular domain (NICD) occurs in response to *E. chaffeensis*, resulting in upregulation of Notch signaling pathway components and target genes *notch1*, *adam17*, *hes* and *hey*. Significant differences in canonical Notch signaling gene expression levels (>40%) were observed during early and late phase of infection, indicating activation of the Notch pathway. We linked Notch pathway activation specifically to the TRP120 effector, which interacts with ADAM17 of the Notch receptor complex, and results in Notch signaling activation. Pharmacological inhibitors and siRNAs against γ-secretase enzyme, RBPjκ, Notch1 and ADAM17, confirmed that Notch signaling is required for ehrlichial survival.**Conclusions:** This investigation reveals a novel mechanism whereby *E. chaffeensis* exploits Notch pathway to promote intracellular survival. Detailed studies to identify the role of Notch signaling in inhibition of innate immune responses during ehrlichial infections are currently in progress.

Author Disclosure Block:

T. Lina: None.

Poster Board Number:

SUNDAY-641

Publishing Title:

***Salmonella enterica* Serovar Typhi Fails to Elicit Apoptosis and T_h1/M1 Polarization in Infected Human Macrophages and Humanized Mice**

Author Block:

L. A. Singletary, J. E. Karlinsey, S. J. Libby, T. A. Stepien, F. C. Fang; Univ. of Washington, Seattle, WA

Abstract Body:

The human pathogen *Salmonella enterica* serovar Typhi (*S.* Typhi) causes more than 20 million infections and 200,000 deaths each year. Typhoid fever is characterized by a prolonged incubation period, invasive disease and a high rate of asymptomatic carriage following disease resolution. Survival within macrophages is an essential feature of *Salmonella* pathogenesis. Accordingly, a T_H1 helper T cell response and M1 macrophage activation in response to T_H1 cytokines are important for host resistance to intracellular pathogens such as *Salmonella*. Individuals with congenital or acquired immunodeficiencies resulting in impaired T_H1 immunity, such as HIV/AIDS, are more susceptible to invasive nontyphoidal salmonellosis but not typhoid fever, suggesting that nontyphoidal serovars and *S.* Typhi may differ in their propensity to initiate a T_H1 immune response in infected hosts. We have found that *S.* Typhi persists within cultured human THP-1 macrophages, in contrast to nontyphoidal serovar *S.* Typhimurium, by avoiding the induction of apoptosis. *S.* Typhimurium induces apoptosis via caspase-3 cleavage, in a process dependent upon the *Salmonella* pathogenicity island 2 (SPI2) type III secretion system and the ADP-ribosylating toxin SpvB encoded on the *Salmonella* virulence plasmid pSLT. Furthermore, *S.* Typhi-infected macrophages exhibit reduced activation of the M1 transcription factor STAT1 and the T_H1 cytokine IL-12 in comparison to *S.* Typhimurium-infected macrophages. A hu-SRC-SCID humanized mouse model mirrors these results, with *S.* Typhi-infected mice displaying significantly lower levels of serum IL-12 and IFN-γ in comparison to *S.* Typhimurium-infected mice. The T_H1 polarization of human macrophages by *S.* Typhimurium is also SPI2-dependent. However, in contrast to apoptosis, T_H1 polarization is not dependent on SpvB, and the specific effector(s) required for STAT1 activation have not been identified. Collectively these observations suggest that the ability of *S.* Typhi to cause persistent infection may result from avoidance of a host T_H1/M1 response.

Author Disclosure Block:

L.A. Singletary: None. **J.E. Karlinsey:** None. **S.J. Libby:** None. **T.A. Stepien:** None. **F.C. Fang:** None.

Poster Board Number:

SUNDAY-642

Publishing Title:

Stimulation of Large Vesicle Formation for Increased Secretion of *Salmonella* Typhimurium Effector Proteins by Overproduction of MdsABC Efflux Pump

Author Block:

Y-H. Kim, G. Kim; Catholic Univ. of Daegu Sch. of Med., Daegu, Korea, Republic of

Abstract Body:

Background: *Salmonella* Typhimurium is a primary pathogen which causes gastrointestinal disease worldwide in both humans and animals.**Methods:** In this study, we showed that overproduction of *Salmonella*-specific MdsABC efflux pump promoted the early secretion of some virulence factors and an inner membrane phospholipid, 1-palmitoyl-2-stearoyl-phosphatidylserine (PSPS), by a non-classical pathway.**Results:** This activity was related with reduced growth rate of the cells in exponential phase, but not in stationary phase. MdsABC overproduction stimulated formation of large vesicles containing DNA by changing the bacterial cell shape from confocal images. Exogenous overexpression of MdsABC and external addition of PSPS had a similar effect on the expression of high levels of *sipABCD* genes in intracellular bacteria. The two treatments on infection of mouse macrophage RAW 264.7 cells by *S.* Typhimurium influenced a complex regulatory network of the master transcription factors, HilACD. Transmission electron microscopy images showed some evidence of the pathological role of PSPS in infected cells with the loss of pseudopods.**Conclusions:** We suggest that an apoptotic mechanism of bacterial death induced by overexpression of MdsABC and hypersecretion of PSPS may contribute to pathogenesis of *S.* Typhimurium infection which stimulates cytoskeleton remodeling by the delivery of bacterial effector proteins into the host cell cytosol.

Author Disclosure Block:

Y. Kim: None. **G. Kim:** None.

Poster Board Number:

SUNDAY-644

Publishing Title:

Characterizing Human Norovirus Binding to Bacterial Ligands

Author Block:

E. Almand, M. Moore, L-A. Jaykus; North Carolina State Univ., Raleigh, NC

Abstract Body:

Human norovirus is a leading cause of gastroenteritis. Recent studies suggest bacteria may influence infectivity, persistence and replication of this virus, yet bacteria-virus interactions are poorly understood. It is possible that gastrointestinal bacteria interact with human norovirus because they possess histo-blood group-like antigens (HBGAs; putative norovirus receptor), but these glycoproteins remain uncharacterized. This study sought to elucidate the bacterial moieties mediating norovirus binding. The binding of seven bacterial species (2 ATCC strains; 5 natural fecal isolates) to multiple norovirus strains was investigated. Isolates were grown in minimal media and lysed. Whole bacterial lysates were examined via Western blot for three characteristics (1) HBGA activity (AB, B, H, Le^a, Le^b, and Le^y); (2) lectin binding; and (3) human norovirus binding (GI.6, GII.1, GII.4, and GII. 17). Bacterial moieties were isolated and further assessed using LC-MS. Bacteria-virus binding was visualized via TEM to determine localization. Bacteria-norovirus interactions varied by bacteria type and virus strain. The TEM results showed that GII.4 norovirus bound select Gram-positive and Gram-negative bacteria on outer membrane and/or protruding pili structures. The seven bacterial species screened possessed varying HBGA residues with differences in (1) overall activity (Western blot relative density 0-0.79); (2) HBGA banding patterns (1 vs 7 reactive glycoproteins); and (3) glycoprotein size (13-140 kDa). To probe for specific sugars rather than the entire HBGA (i.e. N-acetyl-galactosamine, L-fucose), lectin-binding assays were done. This analysis yielded a relative density range of 0-10.68, 3-5 glycoproteins per lectin, and sizes from 17-140kDa. A virus overlay demonstrated that common human norovirus binding residues were located in association with glycoproteins of 140, 35, and 17 kDa size. A 35 kDa glycoprotein isolated from a fecally-derived *Bacillus* strain bound all four of the human norovirus strains tested, including GII.1, which currently lacks a known cellular receptor. This is one of the first studies investigating bacteria-norovirus interactions and delving into the bacterial residues responsible for those interactions. Future studies will focus on molecular binding mechanisms and how interactions with bacteria impact norovirus infection and/or environmental behavior.

Author Disclosure Block:

E. Almand: None. **M. Moore:** None. **L. Jaykus:** None.

Poster Board Number:

SUNDAY-645

Publishing Title:

Hepatitis B Spliced Protein (HBSP) Promotes Hepatoma Cell Migration and Invasion by Triggering Epithelial-Mesenchymal Transition (EMT) via Interaction with Transforming Growth Factor β-1-Induced Transcript 1 Protein (TGFβ1|1)

Author Block:

W. Chen, J-g. Huang, F-f. Liang, X-j. Lin, X. Lin; Fujian Med. Univ., Fuzhou, China

Abstract Body:

Background: The hepatitis B spliced protein (HBSP) encoded by a 2.2 kb singly spliced defective HBV genome (spliced between positions 2447 nt and 489 nt) was a ubiquitously detectable protein in the liver tissues and the serum from patients with hepatitis B (Günther *et al.*, 1997; Soussan *et al.*, 2000). In our previous study, the HBSP protein was shown to be an oncogenic protein enhancing the invasion and migration of hepatoma cells via interaction with a lysosomal cysteine protease Cathepsin B (CTSB) through activation of MMP9 and uPA and activation of MAPKs and Akt signaling pathway(Chen *et al.*, 2012). **Methods:** The cytoplasmic yeast two-hybrid screening was applied to identify the intracellular proteins interacting with HBSP, followed with protein interaction identification by in *vitro* GST pull-down and *in vivo* co-immunoprecipitation. TGFβ1-induced EMT and cell invasion and migration were explored by knockdown of TGFβ1I1 by siRNA in hepatoma cell lines expressing HBSP. **Results:** TGFβ1I1 was identified as an intracellular interacting partner of HBSP by the cytoplasmic yeast two-hybrid screening, and the interaction of TGFβ1I1-HBSP was further confirmed by in *vitro* GST pull-down and *in vivo* co-immunoprecipitation. The influences as well as the mechanisms of HBSP-TGFβ1I1 interaction on the TGFβ1-induced EMT and cell invasion and migration were also explored by knockdown of TGFβ1I1 by siRNA in hepatoma cell lines expressing HBSP. It was demonstrated that interaction of HBSP and TGFβ1I1 promote the EMT by activating TGFβ1/Smad signaling pathway, and ultimately resulted in the enhanced invasion and migration of hepatoma cells. **Conclusions:** Our findings highlight new molecular mechanisms for HBSP-induced HCC progression.

Author Disclosure Block:

W. Chen: None. **J. Huang:** None. **F. Liang:** None. **X. Lin:** None. **X. Lin:** None.

Poster Board Number:

SUNDAY-647

Publishing Title:

Identifying an Astrovirus Receptor

Author Block:

C. Johnson[1], S. Marvin[2], S. Schultz-Cherry[2]; [1]Rhodes Coll., Memphis, TN, [2]St. Jude Children's Res. Hosp., Memphis, TN

Abstract Body:

Infectious diarrhea is a leading cause of morbidity and mortality worldwide. Astroviruses (HAstV), small, non-enveloped positive RNA viruses, are a leading cause of diarrhea in children, the elderly, and the immunocompromised, making it a major public health problem. HAstV are extremely prevalent; around 90% of the population 9 years and older have antibodies against HAstV. However, little is known about HAstV pathogenesis including the cellular receptor(s) required for infections of enterocytes. For any intracellular pathogen, bacterial or viral, the first step in causing disease is to establish an infection by binding to cellular receptor(s) on the host cell. However, to date, no receptor for any astrovirus species has been identified. Our goal is to identify candidate astrovirus receptor(s). Crystal structure of the putative receptor binding domain of the HAstV capsid (outer coat) protein suggests the use of carbohydrates as receptors. Yet treatment of the human colorectal adenocarcinoma cell line (Caco-2), one of the few cells that productively support HAstV replication, with chondroitinase, heparinase, and neuraminidase does not impact viral replication or binding. We also explored the role of carbohydrate moieties through binding to an extensive panel of distinct carbohydrates in collaboration with the Carbohydrate consortium. This also failed to identify a candidate receptor. As an alternative approach, we found that baby hamster kidney (BHK), while able to produce progeny virions upon transfection of HAstV mRNA, do not support infection suggesting they lack surface molecule(s) important for HAstV binding and/or entry. This was confirmed by flow cytometry. Thus, BHK cells were transfected with a Caco-2 cDNA library and cells supporting HAstV infection are being sorted. The transfected gene will then be identified by PCR, followed by single cell suspension, which will allow us to sequence the plasmid containing the cDNA. Preliminary results suggest that the top 0.5% fluorescent cells to be sequenced must be captured and grown in bacteria three times in order to amplify the amount of potential receptor DNA. If successful, our studies will identify astrovirus receptor(s), important information for understanding viral pathogenesis and targeted drug design.

Author Disclosure Block:

C. Johnson: None. **S. Marvin:** None. **S. Schultz-Cherry:** None.

Poster Board Number:

SUNDAY-648

Publishing Title:

The Surface Protein (MPER) of HIV on the Q-Beta Coliphage as a Vaccine Candidate

Author Block:

T. Egbo[1], **C. Sanders**[1], B. K. Robertson[1], G. W. Nchinda[2], A. Bopda Waffo[1]; [1]Alabama State Univ., Montgomery, AL, [2]The Chantal Biya Intl. Reference Ctr. for Res. onn the Prevention and Maanagement of HIV/AIDS, Yaounde-Messa, Cameroon

Abstract Body:

Background: HIV infection has been of great concern to public health with the recent estimate of 1,201,100 infected with the virus in the United States, according to the Center for Disease Control (CDC). There is a critical need for a safe and effective vaccine to protect the uninfected. Studies have shown that the membrane proximal external region (MPER) represents one of the most neutralizing targets for HIV vaccine research. The tryptophan-rich proximal region of its external membrane mediates binding entry of the virion.**Methods:** We anticipate that the MPER, which is also a neutralizing site of the transmembrane protein, will serve as an antigen to stimulate antibodies once presented on the Q-Beta phage. The novel Q-Beta phage was preferred because it can be easily separated, purified with genotype and phenotype linkages. We designed the PCR template using oligos that represent a specific region of the 50 amino acid MPER. We performed the cloning by fusing the MPER motif in frame at the end of the A1 gene.**Results:** The recombinant plasmid was used to transform *E. coli* HB101. The binding of the phage exposing MPER motif with antibody was achieved using Western blot and ELISA, and the analysis of antibodies from HIV patients with Q-BetaMPER using ELISA.**Conclusions:** Further analysis will be done using dot blot, and EM to further reveal the fusion of the MPER-A1 on the surface of Q-Beta, and also, animal immunization with Q-BetaMPER. In all, we anticipate that the Q-BetaMPER will serve as a vaccine candidate and as a standard for vaccine efficacy.

Author Disclosure Block:

T. Egbo: None. **C. Sanders:** None. **B.K. Robertson:** None. **G.W. Nchinda:** None. **A. Bopda Waffo:** None.

Poster Board Number:

SUNDAY-649

Publishing Title:

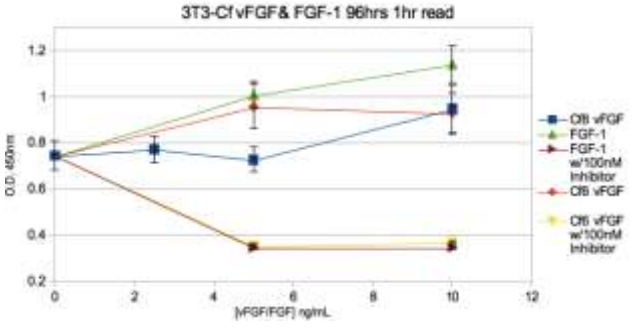
Baculovirus Fibroblast Growth Factors are Mitogens for Both Insect and Mammalian Cells

Author Block:

C. M. Finnerty, D. R. Meier; Winona State Univ., Winona, MN

Abstract Body:

Nearly all baculoviruses encode homologues of fibroblast growth factor (FGF). Previous studies on baculovirus FGFs have demonstrated that these cytokines have chemotactic activity and may be involved in tissue remodeling during pathogenesis.The purpose of this study was to investigate baculovirus FGFs for mitogenic activity in both insect and mammalian cells. Our data demonstrate that recombinant baculovirus FGF induces cell proliferation in both the Sf9 and NIH/3T3 cell lines.The FGF genes from *Autographa californica* MNPV and *Choristoneura fumiferana* MNPV were separately cloned and expressed in *E. coli* to generate maltose-binding protein-FGF fusion proteins denoted as Ac6 and Cf6. A recombinant MBP-LacZ protein served as a negative control. All the MBP fusion proteins were purified via affinity chromatography using an amylose resin. For some experiments, an untagged recombinant FGF from *Cf*MNPV(denoted as "Cf8") was tested as well. The purified proteins were applied to monolayers of Sf9 and NIH/3T3 cells in 96-well plates. Human FGF-1 and FGF-2 served as positive controls. In addition, the effect of the FGF receptor inhibitor PD173074 in combination with these FGFs was also tested. Cell densities were measured using Dojindo CCK-8 reagent, and the absorbance was determined using a plate reader.At concentrations of 5 and 10 ng/ml, the Ac6 and Cf6 MBP-FGFs produced cell densities that were significantly elevated compared to the negative control protein in both the Sf9 and NIH/3T3 cell lines. Cf8 also showed mitogenic activity at the 10 ng/ml concentration. FGFR inhibitor had an inhibitory effect on cell proliferation, though this may have been a result of cell mortality due to high inhibitor concentrations. Representative data are shown. These data demonstrate that baculovirus FGF functions as a mitogen *in vitro*.



Author Disclosure Block:

C.M. Finnerty: None. **D.R. Meier:** None.

Poster Board Number:

SUNDAY-650

Publishing Title:

Ebola Virus Makona Infection Induces Early Transcriptome Changes *In Vivo* within T Cells and Monocytes

Author Block:

A. Rivera[1], K. Versteeg[2], T. Geisbert[2], I. Messaoudi[1]; [1]Univ. of California, Riverside, Riverside, CA, [2]Univ. of Texas Med. Branch, Galveston, TX

Abstract Body:

The high virulence of Ebola virus (EBOV) is largely attributed to the ability of this virus to: 1) subvert host innate immune pathways thereby preventing priming of an adaptive immune response; and 2) induce an uncontrolled inflammatory response that results in increased vascular permeability and lymphocyte death. However, mechanisms underlying filovirus-induced immune dysregulation are not well understood. Most studies investigating the interactions between filoviruses and the innate immune system have been mostly carried out in vitro. Genomic studies in nonhuman primates have provided us with insight into the global host response networks, but have not examined gene expression within specific immune cell subsets. Therefore, the impact of filovirus infection within specific immune cell subsets *in vivo* remains poorly understood. To address this knowledge gap, we used RNA-seq to uncover EBOV-induced changes in gene expression profiles of T cells and monocytes. Magnetic microbeads were used to purify CD2+ T cells and CD14+ monocytes from PBMCs isolated at 0, 1 and 2 days post infection (dpi) from 2 cynomolgus macaques infected with EBOV Makona strain. Purity was assessed by flow cytometry. RNA transcripts were mapped to both host as well as viral genome to determine viral replication and we detected differentially expressed genes (DEGs) at 2 dpi. Most DEGs were downregulated in T cells and functional enrichment of downregulated genes mapped to gene ontology processes related to transcriptional regulation and innate immune response. A larger number of DEGs was detected in monocytes at 2 dpi. Upregulated genes enriched to monocyte effector functions, notably regulation of tumor necrosis factor and nitric oxide biosynthesis and inflammatory response, suggesting that EBOV infection does stimulate inflammatory changes in monocytes in vivo. Moreover down-regulated genes within monocytes include genes associated with Type I IFN signaling, which is consistent with previous reports of EBOV-induced IFN suppression. Our data sheds light on the role of T cells and monocytes in EBOV pathogenesis.

Author Disclosure Block:

A. Rivera: None. **K. Versteeg:** None. **T. Geisbert:** None. **I. Messaoudi:** None.

Poster Board Number:

SUNDAY-651

Publishing Title:

Characterization of Ebola Virus Makona Pathogenesis in Cynomolgus Macaques

Author Block:

K. Versteeg[1], A. Rivera[2], I. Messaoudi[3], T. Geisbert[1]; [1]Univ. of Texas Med. Branch, Galveston, TX, [2]Univ. of California, Riverside, Riverside, CA, [3]Univ. of California, Riverside, Riverside, CT

Abstract Body:

Ebola virus (EBOV) continues to cause significant morbidity and mortality as highlighted by the recent outbreak in West Africa. With no FDA approved vaccines or therapeutics, understanding the mechanisms that contribute to EBOV pathogenesis and identifying therapeutic strategies are of utmost importance. However, much of what is known about the immune response to EBOV infection has been acquired through in vitro observations or studies focused on host response in the final stages of disease. To better understand the development of an immune response to EBOV, we used flow cytometry and RNA-seq to uncover infection-induced changes in phenotype and gene expression profiles of whole blood (WB) and peripheral blood mononuclear cells (PBMCs) isolated from cynomolgus macaques infected with wild type EBOV-Makona. A total of 10 macaques were inoculated with a 1000 PFU Makona. Blood was collected on days 0, 1, 2, 3, 4 and 6 post infection. Following infection, viral titers were detected 3 days post infection (dpi) and continued to increase throughout infection. Hematological analysis and serum biochemistry showed less dramatic clinical changes previously reported in macaques infected with previous strains. Flow cytometry revealed an increase of monocytes at 2-3 dpi and an increase in frequency of nonclassical monocytes at 4 and 6 dpi. RNA transcripts were mapped to both host as well as viral genome and we detected differentially expressed genes (DEGs) and viral transcripts at 3dpi, which steadily increased as infection progressed. There were distinct changes in gene expression in WB compared to PBMC, suggesting EBOV infections results in significant changes in RNA transcripts in cells present only in WB. Functional enrichment analysis showed that upregulated genes in WB and PBMC at 3-6dpi, which consisted largely of interferon stimulated genes, mapped to Defense Response and Immune System Process, suggesting that IFN response may correlate with disease progression. Downregulated genes in WB at 6 dpi enriched to Regulation of Vascular Permeability and Lymphocyte activation. Overall, our data give insight into the pathogenesis of the new EBOV strain.

Author Disclosure Block:

K. Versteeg: None. **A. Rivera:** None. **I. Messaoudi:** None. **T. Geisbert:** None.

Poster Board Number:

SUNDAY-652

Publishing Title:

Transcriptional Signatures Associated with Influenza A/H1n1-Specific Igg Memory-Like B Cell Response in Older Individuals

Author Block:

I. Haralambieva, I. Ovsyannikova, R. Kennedy, M. Zimmermann, K. Goergen, D. Grill, A. Oberg, G. Poland; Mayo Clinic, Rochester, MN

Abstract Body:

Background. Studies suggest that the predominantly recall-based humoral immune response to influenza A/H1N1 originates from activated memory B cells. The aim of this study was to identify blood transcriptional signatures associated with memory B cell immune response measures following influenza vaccination.**Methods**. We used pre- and post-vaccination (Day 3, early and Day 28, late) mRNA-Seq transcriptional profiling on PBMC samples from 159 subjects (50-74 years old) following the receipt of trivalent influenza vaccine containing the A/California/7/2009/H1N1-like virus, penalized-regression methods, and network modeling to identify associations of genes and pathways with peak (Day 28 - Day 0) memory B cell ELISPOT response after vaccination.**Results**. We observed significant genes (p-value range 7.92E-08 to 0.00018, q-value range 0.00019 to 0.039) with baseline, early or late gene expression associated with peak memory B cell response after influenza vaccination. Genes and genesets exhibiting significant associations with memory B cell response included metabolic (cholesterol and lipid metabolism-related), cell migration/adhesion, MAP kinase and NF-kB cell signaling (chemokine/cytokine signaling), and transcriptional regulation signatures correlated with the development of memory B cell response after influenza vaccination.**Conclusion**. Through an unbiased transcriptome-wide profiling approach, our study identified gene signatures of memory B cell response following influenza vaccination, highlighting the underappreciated role of metabolic changes (among the other immune function-related events) in the regulation of vaccine-induced immune memory.

Author Disclosure Block:

I. Haralambieva: None. **I. Ovsyannikova:** None. **R. Kennedy:** None. **M. Zimmermann:** None. **K. Goergen:** None. **D. Grill:** None. **A. Oberg:** None. **G. Poland:** A. Board Member; Self; Chair of a Safety Evaluation Committee for novel investigational vaccine trials being conducted by Merck Research Laboratories. C. Consultant; Self; Consultative advice on vaccine development to Merck & Co. Inc., CSL Biotherapies, Avianax, Dynavax, Novartis Vaccines and Therapeutics, Emergent Biosolutions, Adjuvance, and Microdermis..

Poster Board Number:

SUNDAY-653

Publishing Title:

Micro-RNAs in Regulation of Early Host Responses to Hepatitis E Virus Infection

Author Block:

S. K. Panda, S. P. k. Varma, P. Joshi, D. Paliwal; All India Inst. of Med. Sci., New Delhi, India

Abstract Body:

Hepatitis E virus (HEV) is a 7.2kb plus stranded RNA virus and a globally prevalent cause of acute hepatitis. Micro-RNAs (miRNA) are known to regulate host responses in various viral infections. The role of host miRNAs in Hepatitis E virus (HEV) infection is unexplored.We performed mRNA and miRNA profiling of HEV replicon transfected Huh7 cells in biological duplicates at two time points (24hours and 72hours) post transfection and compared with time matched non-transfected controls on next generation sequencing platform (Ion Proton). Data analysis was done using Partek Flow and Partek Genomics Suite and genes with ≥ 2 fold change and p-value <0.05 were considered differentially expressed (DE). Correlations within DE mRNA-miRNA datasets were determined by Pearson's correlation coefficient using 7 databases for *in silico* miRNA target prediction (TargetScan v7.0, miRDB, miRANDA, PITA, microcosm, miRTarbase and miRecords). Biologically relevant 7 miRNAs and 8 mRNAs were validated by real time PCR.A total of 148 and 121 mRNAs were differentially expressed in 24hr and 72hr samples respectively with 15 common genes. A high representation of interferon and immune related genes was observed. While 27 miRNAs were found to be DE at 24 hrs, there were only 3 altered miRNAs in the 72hr samples. *In-silico* correlation analysis in the 24hr samples predicted 46 miRNA–mRNA pairs ($-0.95 > \rho > +0.95$, p-value < 0.05) formed by 10 miRNAs, of which 2 pairs exhibited inverse fold changes and the rest 44 showed a positive correlation. The miRNAs identified were hsa-miR-556-3p, hsa-miR-921, hsa-miR-142-3p, hsa-miR-520c-3p, hsa-miR-202-5p, hsa-miR-6844, hsa-miR-1302, hsa-miR-4798-3p, hsa-miR-548ap-3p and hsa-miR-190a-5p, all of which were upregulated (fold-change range +2 to +4.12) while the upregulated target mRNAs represented diverse pathways such as interferon (IFI6, IFIT5, IRF9), chemokines (CCL5, CXCL10), apoptosis (BIRC3, XAF1) and anti-viral responses (TRIM22, RSAD2, OASL). Downregulated target mRNAs represented cell surface molecules (HS6ST3, DENND1C).We report that micro-RNAs may regulate early host responses in HEV infection. However, with time this miRNA mediated regulation appears to be curtailed.

Author Disclosure Block:

S.K. Panda: None. **S.P.K. Varma:** None. **P. Joshi:** None. **D. Paliwal:** None.

Poster Board Number:

SUNDAY-654

Publishing Title:

Ebp1 Negatively Regulates Hepatitis C Virus Replication and Translation

Author Block:

P. Mishra, U. Dixit, A. Pandey, V. N. Pandey; Rutgers Sch. of BioMed. Sci., Newark, NJ

Abstract Body:

HCV infection is the leading cause of chronic liver diseases, which, with time, progress to liver cirrhosis, hepatocellular carcinoma, and liver failure. The majority of liver failure patients requiring liver transplant have a history of CHC. The HCV genome is a positive-stranded RNA with conserved and highly structured untranslated 5' and 3’ terminal regions having multiple regulatory elements that are essential for viral replication and translation. Recently, using a novel affinity probe, we have captured the replicating HCV RNA *in situ* and identified 83 cell factors that are associated with the viral RNA. ErbB3binding protein (Ebp1) was one of them. Here we show that the shorter isoform that lacks the N terminal 54 amino acid sequences is capable of inhibiting HCV replication. We found that overexpression of p42 could inhibit viral protein expression level whereas p48 had no significant effect. By transient expression of each isoform in Ebp1 kd cells, we found that p42 could restore the effect of knock down whereas p48 could not. We confirmed these observations through exogenously added which p42 inhibits in vitro endogenous HCV replication in cell free replicative lysate. We saw inverse correlation between p42 and HCV replication. Ebp1 immunoprecipitate also co-precipitated HCV non structural proteins, NS5A and NS5B. Thus through knock down and overexpression studies we have identified that the shorter isoform negatively regulates HCV replication. p42 functions as host defense molecule (protein) by suppressing viral protein expression. Although the clear mechanism through which the shorter isoform mediates its effect is still to be deciphered, our findings suggest that p42 may have therapeutic role in HCV infection.

Author Disclosure Block:

P. Mishra: None. **U. Dixit:** None. **A. Pandey:** None. **V.N. Pandey:** None.

Poster Board Number:

SUNDAY-656

Publishing Title:

Endocrine-disrupting Chemicals Affect Mosquito Development and Mosquito-borne Virus Replication

Author Block:

J. R. Anderson, A. M. Tingle; Radford Univ., Radford, VA

Abstract Body:

Endocrine-disrupting chemicals (EDCs) are found in numerous manmade products and can interfere with normal metabolic processes regulated by hormones and other steroids. Numerous studies have described these detrimental effects in both vertebrate and invertebrate organisms. Our goal was to determine whether EDCs can affect the likelihood of mosquito-borne virus transmission. Toward that end, we exposed developing mosquito larvae to varying concentrations of three EDCs and assessed larval mortality and emergent adult body size and fecundity. All EDCs resulted in significant increases in mortality, while the effects on body size varied by EDC, sex, and species. Females exposed to EDCs deposited more eggs than those reared in water only. We also investigated the effects of EDC exposure on virus replication in cultured cells. EDC exposure tended to result in higher levels of virus replication during the first cell passage, while replication was hindered early in the second passage before again producing more virus. Mosquitoes breeding in plastic or other containers made with EDCs may alter the dynamics of mosquito-borne virus transmission. Important factors in such transmission include the number of mosquitoes in a population, the size of the mosquito, and the ability of the virus to replicate in the mosquito. Our results indicate that all three of these factors can be affected, although how they affect transmission vary based on the EDC and the mosquito studied. Future studies will aim to investigate whether EDCs can directly affect the likelihood of virus transmission by mosquitoes.

Author Disclosure Block:

J.R. Anderson: None. **A.M. Tingle:** None.

Poster Board Number:

SUNDAY-657

Publishing Title:

Roles of Catecholamines of Enterovirus 71 Infection in Mice Models

Author Block:

S-M. Wang; Natl. Cheng Kung Univ. and Hosp., Tainan, Taiwan

Abstract Body:

Background: Enterovirus 71 (EV71) has been recognized as highly neurotropic and associated with a diverse range of neurological complications. Brainstem encephalitis (BE) was the cardinal feature of EV71 CNS involvement. The plasma concentration of norepinephrine (NE) in EV71-infected patients with autonomic nervous system (ANS) dysregulation and pulmonary edema (PE) were significantly higher than those with BE.**Methods:**Seven-day-old ICR mice were infected with LD_{50} of MP4 by intraperitoneal injection. Norepinephrine, epinephrine, and dopamine were detected by a commercial immunoassay kit (Labor Diagnostika Nord GmbH & Co. KG, Germany). 6-hydroxydopamine (6-OHDA) (Sigma, St. Louis, Mo., USA) was dissolved in sterile saline containing 0.01% (w/v) ascorbic acid (vehicle) and was injected intraperitoneally (i.p.) at a concentration of 25-100 μg/g. Control mice received i.p. injections of an equal volume of vehicle alone.**Results:** Norepinephrine and epinephrin increased at day 2 post infection by EV71. The survival rate of infected mice treated with 6-OHDA (78%) was higher than vehicle-treated mice (50%). The clinical scores of EV71-infected and treated with 6-OHDA group were markedly lower than EV71-infected and vehicle group at day 8 to 12 post infection. The plasma levels of norepinephrine and epinephrine in EV71-infected and treated with 6-OHDA group were significantly lower than EV71-infected and vehicle group.**Conclusions:** EV71 infection induced sympathetic over activity plays an important role in the pathogenesis of neurological complications.

Author Disclosure Block:

S. Wang: None.

Poster Board Number:

SUNDAY-658

Publishing Title:

Sybr Green I Real-Time Pcr versus Taqman Probe Techniques in Detection of Hepatitis C Virus RNA in Infected Patients

Author Block:

D. E. Metwally[1], A. N. Amer[2], H. M. Mostafa[1], G. E. A. El Sawaf[1], O. A. Kader[1]; [1]Med. Res. Inst. - Alexandria Univ., Alexandria, Egypt, [2]Faculty of Pharmacy - Univ. of Pharous, Alexandria, Egypt

Abstract Body:

Background: The prevalence of hepatitis C virus (HCV) is highest in Egypt compared to other countries. Nucleic acid amplification test (NAT) may provide an accurate and sensitive method to quantify HCV- RNA . The routine use of NAT as a screening tool for blood products or in clinical practice is quite limited due to its cost. The aim of this study was to compare two common RT-PCR methods, TaqMan probe technique and SYBR Green method in quantitative detection of HCV RNA for diagnosis and follow up of HCV patients. **Methods:** Blood samples were collected from 220 HCV patients and subjected to RNA extraction using a QIAamp viral RNA kit (QIAGEN, Valencia, CA), determination of viral load by TaqMan technique using HCV Qiagen kit (Artus) and detection of HCV RNA by SYBR Green technique using Sigma-Fermentas one step RT PCR kit. Real-time PCR was performed by using the Mx3000P Stratagene real-time PCR system. The PCR products were identified by melting curve analysis. Standard sera with known concentrations of HCV RNA were used to validate our assays.**Results:** Among the recruited 220 HCV patients, 154 (70%) were HCV-RNA positive by both the techniques, while 24 (10.9%) were negative by both techniques. On the other hand, 40 (18.2%) cases were HCV RNA positive only by SYBR Green technique, and 2 (0.9%) only by TaqMan probe technique. Forty (20.4%) of the 196 chronic HCV cases were HCV-RNA positive by SYBR Green but negative by TaqMan probe technique.**Conclusions:** SYBR Green assay is perfectly suitable for both routine clinical use and large-scale screening of donated blood due to its good level of sensitivity and specificity, ease of handling, relatively low cost, and low contamination rate.

Author Disclosure Block:

D.E. Metwally: None. **A.N. Amer:** None. **H.M. Mostafa:** None. **G.E.A. El Sawaf:** None. **O.A. Kader:** None.

Poster Board Number:

SUNDAY-659

Publishing Title:

Persistent Nontypeable *H. influenzae* Isolates from the Airways of Adults with Copd: Novel Vaccine Candidates and Mechanisms of Diversity

Author Block:

C. P. Ahearn[1], C. Kirkham[1], A. Brauer[1], A. Johnson[1], Y. Kong[2], M. M. Pettigrew[3], T. F. Murphy[1]; [1]Univ. at Buffalo, Buffalo, NY, [2]Yale Sch. of Med., New Haven, CT, [3]Yale Sch. of Publ. Hlth., New Haven, CT

Abstract Body:

Nontypeable *Haemophilus influenzae* (NTHi) is a human restricted pathogen. NTHi colonizes the airways of adults with chronic obstructive pulmonary diseases (COPD) inducing airway inflammation and causes acute otitis media in children. There is no vaccine against NTHi licensed in the US. A 15 year prospective study collected 101 persistent NTHi strains from sputum samples of 78 adults with COPD. The genome sequences of the first and final isolates of persistent strains have been determined to evaluate the genomic changes that occurred during persistence.We hypothesize that NTHi expresses conserved antigens that are surface exposed and immunogenic. Using Reverse Vaccinology methods we identified 30 potential vaccine antigens based on predicted surface exposure and antigenicity. Reverse transcriptase PCR showed that two novel uncharacterized hypothetical genes are expressed. Sequence analyses showed that the genes are conserved and stable during persistent COPD infection. We will determine surface exposure and protective ability of recombinant forms of the antigens *in vivo*.We hypothesize that surface antigens change during persistence due to varying abilities to repair DNA damage. We determined that 4 previously described candidate vaccine antigens in select strains show marked sequence variations during persistence. To test the capacity of strains that change with persistence to resist DNA damage, we subjected selected strains to sublethal DNA damaging treatments and assessed growth characteristics. The results showed differential resistance among strains, with some strains being resistant and some strains susceptible. Genomic comparisons of resistant versus susceptible strains will divulge the sequence variations in repair mechanisms responsible for sequence diversity and antigenic variation.We have identified NTHi antigens to investigate their ability as protective vaccine antigens. We have defined NTHi strains that are differentially resistant to sublethal DNA damage treatment, which provides the foundation to investigate the mechanisms of antigenic variation during persistent NTHi infection.

Author Disclosure Block:

C.P. Ahearn: None. **C. Kirkham:** None. **A. Brauer:** None. **A. Johnson:** None. **Y. Kong:** None. **M.M. Pettigrew:** None. **T.F. Murphy:** None.

Poster Board Number:

SUNDAY-660

Publishing Title:

Comparative Genomics of Foot and Mouth Disease Virus Type O Circulating in Bangladesh

Author Block:

H. Ullah, M. Siddique, R. Chakrabarty, M. Ali, M. Sultana, M. Hossain; Univ. of Dhaka, Dhaka, Bangladesh

Abstract Body:

Background: The causative agent of Foot-and-mouth disease (FMD), FMD virus (FMDV), is a positive sense RNA virus and belongs to the genus *Aphthovirus* within the family *Picornaviridae*. There are seven immunogenically distinct serotypes, i.e. O, A, C, Asia-1 and South African Territories (SAT) 1-3 and vaccine protect against one serotype to another are limited. Here we report isolation and genome wide analysis of FMDV type O from Bangladesh. **Methods:** Virus from infected blister tissue was cultured in BHK-21 cell line followed by nucleic acid extraction. Serotype O [BAN/NA/Ha-156/2013] virus was selected for complete genome analysis. **Results:** The genome was found 8131 nt. in length, which includes a 1020 nt. 5´ un-translated region (UTR), a 6999 nt. length open reading frame (ORF) encoding a polyprotein of 2332 (excluding stop codon) amino acids or 2304 amino acid residues due to two alternative initiation sites and 91nt 3´-UTR plus 21nt poly(A) tail. 5'-UTR contains a short S-fragment, poly C tract, multiple pseudoknots of unknown function, *cis* acting replication element and internal ribosome entry sites (IRES) which directs the initiation of poly protein synthesis. The genome sequence has been deposited in to the NCBI Genbank database with Accession Number KF985189. Regarding to coding region, the sequence of non-structural proteins is much more conserved than that of the structural proteins in the FMDV genome. Among all the compared regions, 5´-UTR, VP1 and 3A were found lowest conservative. Comparative genome wide analysis with reference sequence (RefSeq) revealed that an 82 nt. deletion in S-fragment and 43 nt. consecutive insertion in 5´untranslated region (UTR) was evident introducing an extra pseudoknot (PK) structure. Comparison of structural protein VP1 indicated variation in B-C loop (40~60), G-H loop (133~160) and C-terminal linear epitope amino acid segment (200~211) whereas non-structural protein 3A showed that a 10 amino acid insertion (position 92~101) in the 3A protein.**Conclusions:** The genomic structure of serotype O [BAN/NA/Ha-156/2013] differed from Refseq of the virus. The functional implication of this altered genomic structure needed to be further elucidation.

Author Disclosure Block:

H. Ullah: None. **M. Siddique:** None. **R. Chakrabarty:** None. **M. Ali:** None. **M. Sultana:** None. **M. Hossain:** None.

Poster Board Number:

SUNDAY-662

Publishing Title:

Quantification Of Rotavirus Particles In Ht29.F8 Cells Treated With T-A3

Author Block:

C. Witcher[1], H. Lockwood[1], M. Mattila[1], R. Havner[1], J. Taylor[1], F. Medina-Bolivar[2], J. Ball[3], R. Parr[1]; [1]Stephen F Austin State Univ., Nacogdoches, TX, [2]Arkansas State Univ., Jonesboro, AR, [3]Texas A&M Univ., College Station, TX

Abstract Body:

Rotaviruses (RV) causes severe life threatening diarrhea in young children. Several effective vaccines have been developed that are protective against specific strains of RV but not all known strains of RV. To discovery new strategies to use against RV infections, it is important to understand the mechanisms of RV pathogenesis and how the host responds to RV infections. Previously, our laboratory has shown that a natural product from peanuts, trans-arachidin- 3 (t-A3), significantly inhibits simian RV replication. Although the molecular mechanism(s) of action for t-A3 on RV infections are not known, the decrease in infectious virus particles suggests that one effect is on RV maturation. The objective of this study is to characterize a human RV strain virions during an infection with/without t-A3. Three methods were used; 1) Virus plaque assays to quantify infectious virus particles, 2) Tunable resistive pulse sensing technology using the qNano instrument to quantify and determine the size distribution of all the virus particles, and 3) Transmission electron microscopy (TEM) to observe the gross distribution of the virus particles and the effects on cell structures. The results with a human RV strain and t-A3 obtained from this study indicates: 1) at least a 50 fold decrease of infectious virus particles with t-A3; 2) the qNano system data shows a distribution of virus particles size between 80-115nm for both treatments; and 3) TEM data displays both mature and immature virus particles. This data confirms that the human RV strain replication is effected by t-A3. Furthermore, the size distribution of virus particles was consistent when observed with both the qNano system and TEM. Also, the TEM micrographs indicate that the structures of the cells with RV alone showed signs of apoptosis at 18 hours post infection, whereas the cells with RV and t-A3 appeared to have more normal ultrastructural appearance. This suggests t-A3 may have a protective effect on the host cells. Thus, these natural products are promising anti-RV therapeutic agents.

Author Disclosure Block:

C. Witcher: None. **H. Lockwood:** None. **M. Mattila:** None. **R. Havner:** None. **J. Taylor:** None. **F. Medina-Bolivar:** None. **J. Ball:** None. **R. Parr:** None.

Poster Board Number:

SUNDAY-665

Publishing Title:

Host-Pathogen Interaction Network Among *Sulfolobus islandicus* And Its Viruses

Author Block:

J. A. Black, M. A. Bautista, R. J. Whitaker; Univ. of Illinois at Urbana-Champaign, Urbana, IL

Abstract Body:

Viruses that infect hyperthermophilic archaea of the genus *Sulfolobus* and other organisms from the order Sulfolobales often show distinct, novel morphologies as compared to viruses that infect eukaryotes or bacteria [1]. The co-evolution and pathogenic interactions between these strains and their Archaeal hosts will provide great insight into the diversity and ancient origins of viral defense, and are prime targets for further exploration. To further our understanding of evolutionary ecology and local adaptation between viruses and their microbial hosts in this system, we have focused our research on two geographically isolated populations of *Sulfolobus islandicus* from Yellowstone National Park, USA, and the Mutnovsky Volcano region of the Kamchatka peninsula, Russia [2]. Using 31 strains of *S. islandicus* and viruses isolated from multiple hot springs in both YNP and Kamchatka, we have performed cross-infections of panels of 12 host and 19 virus strains. The patterns of host-virus interactions are determined experimentally by crossing standardized concentrations of both cells and viruses for each host-virus pair using traditional spot-on-lawn assays. The challenged lawns are monitored for plaque formation, size, as well as an increase in plaque turbidity over time that may indicate recovery of an immune host population, or presence of a resistant host minority. To date, a network of host-pathogen interactions between genetically and geographically distinct *S. islandicus* hosts has not been described. Our data shows that some virus strains produce different infective patterns and plaque morphologies across different host strains. These patterns of host-virus interactions may be influenced by the varying sequences of CRISPR-Cas repeat-spacer arrays [3] across individual host strains on the panel, and these experimentally derived patterns can be compared to expected patterns of immunity as predicted by CRISPR-Cas sequence data. By assembling networks of host-pathogen interactions both experimentally and through sequence data, we can resolve a clearer picture of the co-evolutionary process in this extreme environment and uncover novel mechanisms of virus defense.

Author Disclosure Block:

J.A. Black: None. **M.A. Bautista:** None. **R.J. Whitaker:** None.

Poster Board Number:

SUNDAY-666

Publishing Title:

Endothelial Dysfunction in Human Immunodeficiency Virus (Hiv)- Infected Subjects

Author Block:

F. Arnaiz de las Revillas[1], E. Palacio Portilla[2], C. González Rico[1], V. González Quintanilla[2], A. Oterino Duran[2], **M. Fariñas Álvarez**[1], C. Armiñanzas Castillo[1]; [1]Infectious Disease Unit. Hosp. Univ.rio Marqués de Valdecilla, Santander, Spain, [2]Neurology Dept.. Hosp. Univ.rio Marqués de Valdecilla, Santander, Spain

Abstract Body:

Background: The aim of this study was to determine whether patients with HIV infection have higher prevalence of endothelial dysfunction than healthy individuals **Methods:** Case-control study, which included 23 non-smoker patients with HIV infection, with intermediate or low cardiovascular risk calculated according to Framingham risk scale and with more of 5 years after diagnosis, compared with a control group of 23 healthy subjects matched by age and sex. Analytical and ultrasound parameters were analyzed.**Results:** Eleven women and 12 men with HIV infection were enrolled; the mean age was 48.9 years [37-62].All patients had a viral load <20 copies/mL and the median CD4+ T-cell was 733 / mm3 [235-2055]. Patient´s average body mass index (BMI) and waist circumference were respectively 26.18 kg / m^2 [20-32] and 95.33 cm [79-111] without differences in BMI or waist circumference when patients were compared to controls. Cases have mean total cholesterol of 175 mg/dL [120-245], mean LDL cholesterol of 92 mg / dl [91-151], while in the control group mean total cholesterol was 187 mg/dL and mean LDL cholesterol was 114,6 mg/dL, the differences were not statistically significant (p >0.05). The mean intima media thickness (IMT) of the control group was 0.86 cm in both of the common carotid arteries, higher than IMT observed in patients, which had an average IMT of 0.66 cm in the right common carotid and 0.68 cm in the left common carotid (p>0.05). Two patients presented atherosclerotic plaques in the carotid versus none in the control group (p <0.01). Breath holding index (BHI) was higher in the patients with HIV infection than in the control group, 1.1% and 1.05% respectively (p>0.05). The endothelium-dependent vasodilation was significantly higher in the control group (11%) than in the group of HIV patients (9.5%) (p= 0.01).**Conclusions:** Patients with HIV infection present impaired endothelial function, as measured by the rate of endothelium-dependent vasodilation and BHI, as a marker of early vascular damage, compared to normal subjects. These findings suggest that the HIV infection itself acts as risk factor for the development of atherosclerosis.

Author Disclosure Block:

F. Arnaiz de las Revillas: None. **E. Palacio Portilla:** None. **C. González Rico:** None. **V. González Quintanilla:** None. **A. Oterino Duran:** None. **M. Fariñas Álvarez:** None. **C. Armiñanzas Castillo:** None.

Poster Board Number:

SUNDAY-667

Publishing Title:

Regulation of the Transcriptome of Rotavirus-Infected HT29.F8 Cells by Trans-Arachidin-3

Author Block:

H. Lockwood[1], C. Witcher[1], R. Havner[1], F. Medina-Bolivar[2], B. Clack[1], J. Taylor[1], J. Ball[3], R. Parr[1]; [1]Stephen F. Austin State Univ., Nacogdoches, TX, [2]Arkansas State Univ., Jonesboro, AR, [3]Texas A&M Univ., College Station, TX

Abstract Body:

The leading cause of severe diarrhea in infants and young children worldwide is rotavirus (RV) infections that can lead to dehydration and death. Although vaccines are available, some are cost prohibitive in emerging countries, and the efficacies are dependent on timing of vaccination and RV strain specificity. Continuing surveillance for newly emerging RV strains, assessment of vaccine efficacies, and development of cost effective antiviral drugs remain important strategies for the prevention of RV pathology. Previously, our laboratory has determined the antiviral activity of a stilbenoid, trans-arachidin-3 (t-A3), on inhibiting RV replication. The objective of this study was to determine host genes that are regulated in a RV-infected human intestinal cell line (HT29.f8) treated with t-A3. Microarray analyses provided insights into the regulation of the transcriptome of HT29.f8 cells at eight hours post RV/RV+t-A3 infections, and quantitative real-time PCR (qRT-PCR) validated the microarray experiments. One of the findings of this study demonstrates that the apoptotic pathway that leads to cell death is activated with a RV infection. Quantitative RT-PCR confirmed that the transcripts for caspase 7 and 9 are significantly upregulated while caspase 3, 8 and 10 remain relatively constant. This suggests that the intrinsic pathway involving mitochondria dysfunction leading to apoptosis is involved during a RV infection. Additionally, transmission electron microscopy (TEM) was used to observe the ultrastructure of cells with the addition of RV alone or RV+t-A3. The data showed an increase in number of mitochondria around the viroplasms with RV alone than with RV+t-A3. Taken together the data presented in this study implies a mechanism of action by t-A3 that interferes with viral replication, and supports the development of t-A3 as an antiviral agent.

Author Disclosure Block:

H. Lockwood: None. **C. Witcher:** None. **R. Havner:** None. **F. Medina-Bolivar:** None. **B. Clack:** None. **J. Taylor:** None. **J. Ball:** None. **R. Parr:** None.

Poster Board Number:

SUNDAY-668

Publishing Title:

Repression of Aflatoxin Biosynthesis and Elicitation of Conidiation Regulatory Genes in *Aspergillus flavus* by Molasses

Author Block:

S. T. Hua[1], P-K. Chang[2], S. B. L Sarreal and C. H. Tam; [1]USDA-ARS, Western Regional Res. Ctr., Albany, CA, [2]USDA-ARS, Southern Regional Res. Ctr., New Orleans, CA

Abstract Body:

Background: Aflatoxin (AF) B_1 is very toxic to humans and animals and the most potent natural carcinogens known. The toxin is often detected in agricultural crops, dried fruits and spices. AF contamination is a serious and recurrent problem and causes substantial economic losses. We observed that when toxigenic strains of *A. flavus* grown in 8% molasses medium, aflatoxin production was non-detectable by HPLC analysis, and the amount of fungal sclerotia was significantly reduced with concurrent increase of conidiophores and spores. The goal of this project was to examine the mechanism of molasses inhibition. **Methods:** Molasses liquid medium (MB) and agar (MA), potato dextrose agar (PDA) and broth (PDB) were used as growth media. Total RNA from fungal hyphae were extracted and used for cDNAs synthesis followed by Real Time PCR. Dual vitality fluorescent stains were also applied to probe the physiological conditions of fungal hyphae grown in molasses. **Results:** Eight genes, *aflR, aflJ, norA, omtA, omtB, ordB, pksA, vbs* and *ver-1*in the aflatoxin biosynthetic pathway were all suppressed ranging from several to 10,000 fold in fungal cultures grown in MB compared to PDB. The expression levels of conidiation regulatory genes, *brlA, abaA* and *wetA* were significantly increased in MB compared to those in PDB. Consistent with the observed difference in the expression levels of *brlA, abaA* and *wetA* in MB and PDB, microscopic observation showed more conidiophores were produced on MA than on PDA at 48 h. The conidiophores on the former also showed much more conidia, yielding larger heads as evidenced by yellowish green conidial chains. **Conclusion:** Although the underlying mechanism(s) of molasses on the inhibition of AF production and effect on fungal development remains to be elucidated, an early-scheduled increase in conidiation activities may result in a delay or even switching off of secondary metabolism of aflatoxin biosynthesis. Molasses may provide a tool for new findings in aflatoxin control.

Author Disclosure Block:

S.T. Hua: None. **P. Chang:** None.

Poster Board Number:

SUNDAY-669

Publishing Title:

Characterization of the Cpx-Dependent Regulation in *Klebsiella pneumoniae*

Author Block:

C-J. Liu[1], T-Y. Liu[1], Z-C. Wang[2], W-F. Lin[3], H-L. Peng[1]; [1]Natl. Chiao Tung Univ., HsinChu, Taiwan, [2]Academia Sinica, Taipei, Taiwan, [3]Linkou Chang Gung Mem. Hosp., Taoyuan, Taiwan

Abstract Body:

The envelope stress two component system Cpx is composed of a sensor kinase CpxA and the cognate response regulator CpxR. We have previously found that deleting *cpxAR* genes from *Klebsiella pneumponiae* CG43S3 significantly enhanced the expression of both type 1 and type 3 fimbriae. The mutation also affected the production of outermembrane proteins, cellulose and capsular polysaccharides. When compared to the parental strain CG43S3, the Δ*cpxAR* mutant was more sensitive to paraquat, SDS and deoxycholate. To investigate how the Cpx-dependent regulation works, a comparative transcriptomic analysis of CG43S3, CG43S3Δ*cpxAR*, CG43S3Δ*cpxR* using RNA sequencing and qRT-PCR was employed and analyzed. Specifically, the transcript levels of *fimA* and *mrkA*, respectively coding for the major subunit of type 1 and type 3 fimbriae, *mrkH*, coding for the c-di-GMP binding protein were significantly increased, while that of RcsA, the major regulator for the capsular polysaccharides synthesis and Fur were reduced by the *cpxAR* or *cpxR* deletion. In addition, expression of the outermembrane protein, iron homeostasis system, oxidative stress responsive enzymes SodA and SodB, and the c-di-GMP synthesis enzyme YfiN are all differentially affected. If CpxAR participates in regulating the intracellular c-di-GMP levels or the biosynthesis of the envelope components thereby affects the expression of type 1 and type 3 fimbriae is being investigated and the results will be reported.

Author Disclosure Block:

C. Liu: None. **T. Liu:** None. **Z. Wang:** None. **W. Lin:** None. **H. Peng:** None.

Poster Board Number:

SUNDAY-670

Publishing Title:

Identification of Virulence Genes in the Regulon of a New Lysr-Type Transcriptional Regulator in the Bacterium *Burkholderia thailandensis*

Author Block:

S. Le Guillouzer, M-C. Groleau, F. Mauffrey, R. Villemur, E. Déziel; INRS - Inst. Armand-Frappier, Laval, QC, Canada

Abstract Body:

Quorum sensing (QS) is a global regulatory mechanism of gene expression depending on bacterial density. QS involves intercellular signaling molecules that are synthesized and secreted by all bacteria in a population. These signals modulate, synchronously throughout the bacterial population, the expression of specific genes resulting in the coordination of microbial activities. Burkholderia pseudomallei is a human pathogenic bacterium responsible for melioidosis, a potentially fatal septicemic infection, and is considered as a probable bioterrorism weapon. Its pathogenicity is attributed to multiple virulence factors which are mostly under QS control through N-acyl-L-homoserine lactones (AHLs) molecular signaling. This pathogen also produces putative signaling molecules belonging to the 4-hydroxy-3-methyl-2-alkylquinolines (HMAQs) family. These molecules, likewise AHLs, are possibly involved in virulence gene regulation. Our investigations on the regulatory mechanism of HMAQs biosynthesis revealed a novel LysR-type transcriptional regulator called HmqR, which is ubiquitous in species belonging to the Burkholderia genus. In order to identify targets of HmqR, and in particular those associated with virulence, we conducted a comparative transcriptomic analysis by RNA sequencing of the wild-type and its hmqR- mutant using the non-pathogenic bacterium Burkholderia thailandensis strain E264, which constitutes a model system for the study of B. pseudomallei. We determined that HmqR acts as a global activator of gene expression in B. thailandensis and that numerous genes controlled by HmqR are potentially involved in host-pathogen interactions. These genes, including several encoding multiple B. thailandensis secretion systems, could play a role in bacterial virulence. Accordingly, infection assays in the model host Drosophila melanogaster showed that HmqR affects the pathogenicity of B. thailandensis. HmqR might represent a potential target for the development of new therapeutic strategies.

Author Disclosure Block:

S. Le Guillouzer: None. **M. Groleau:** None. **F. Mauffrey:** None. **R. Villemur:** None. **E. Déziel:** None.

Poster Board Number:

SUNDAY-671

Publishing Title:

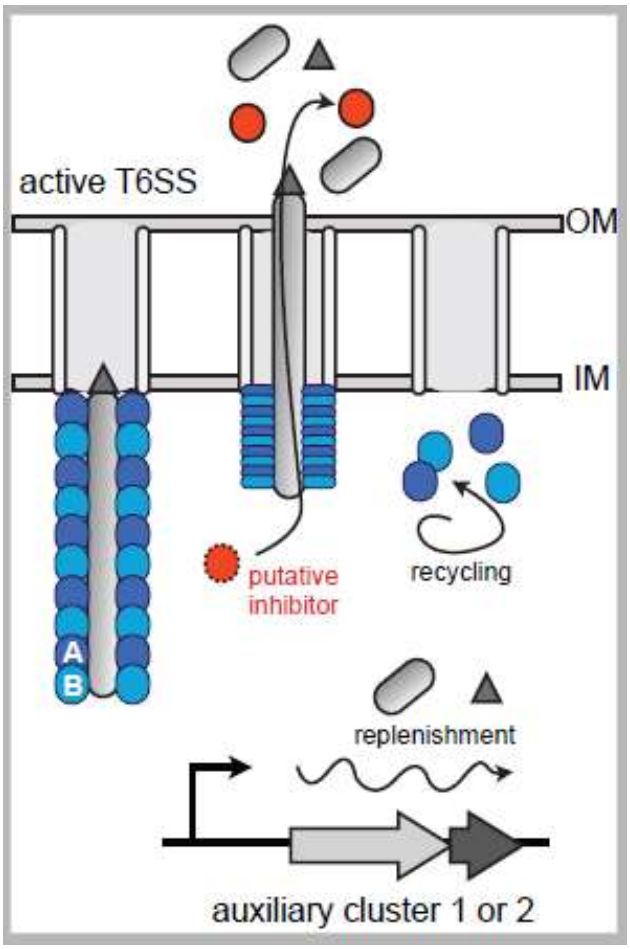
Activity-linked Supply and Demand of the Type Vi Secretion System Components

Author Block:

F. Caro, J. J. Mekalanos; Harvard Med. Sch., Boston, MA

Abstract Body:

Background: The type VI secretion system (T6SS) is a molecular puncturing device that transports toxic protein effectors from the bacteria cytoplasm to the extracellular environment or directly into target cells. How bacteria control recycling and replenishment of the T6SS components remains poorly understood. This work details experiments aimed at describing the regulatory network that allows *Vibrio cholerae* to link the firing of the T6SS apparatus to the transcription of its components. **Materials**: The *V. cholerae* isolate 2740-80 with a constitutively active T6SS, a ΔvipA mutant of this strain with an inactive T6SS, and a complemented strain, were grown *in vitro* to extract RNA for transcriptome sequencing. Genetic suppressor screens were used to pinpoint candidates that act as inhibitors of T6SS gene expression in these strains. **Results**: A comparison of the transcriptomes of the three strains showed that T6SS activity positively influences the expression of its own components. The genetic suppressor screen yielded three candidate genes encoding for transcriptional regulators. **Conclusion**: The *V. cholerae* type VI secretion system (T6SS) functions like a weapon; to restore the T6SS following a round of firing, the T6SS substrates have to be re-charged while the structural components can be recycled. The data obtained shows that T6SS firing of the system dictates the generation of more substrates, which could be mediated by a transcriptional feedback mechanism, linking T6SS activity to T6SS gene expression. A deeper understanding of T6SS gene control will reveal the mechanism by which *V. cholerae* strains maintain homeostasis of a ubiquitous virulence factor in diverse environments. It will also enable therapeutic approaches for disrupting T6SS homeostasis during pathogenesis in the human host.



Author Disclosure Block:

F. Caro: None. **J.J. Mekalanos:** None.

Poster Board Number:

SUNDAY-672

Publishing Title:

The Landscape Of Rnap Polymerase-Associated Proteins

Author Block:

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Abstract Body:

A plethora of transcription factors interact with RNA polymerase (RNAP) and regulate its activities during all stages of the transcription cycle, including initiation, elongation and termination. Although many transcription factors are sequence-specific DNA-binding proteins that recruit RNAP to specific promoters via a direct protein-protein interaction with RNAP, others bind to RNAP without binding the DNA and influence its behavior at steps other than promoter recognition. In this study we have used a bacterial two-hybrid assay to discover and characterize the precise protein-protein interactions that occur between E. coli RNAP and its associated proteins. Specifically, we have assembled a library of 30+ surface-exposed independently folded RNAP fragments, and tested these individually for interaction with 40 previously identified candidate RNAP-associated proteins. Using this systematic approach we have uncovered and characterized the RNAP-binding determinants for numerous transcription factors and have begun to assemble a landscape interaction map for all RNAP-associated proteins.

Author Disclosure Block:

C.P. Garcia: None. **N. Verdini:** None. **K. Barrasso:** None. **A. Hochschild:** None. **P. Deighan:** None.

Poster Board Number:

SUNDAY-673

Publishing Title:

Proteomic Analysis of a *Candida albicans Pga1* Null Strain

Author Block:

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Abstract Body:

Background: The opportunistic fungal pathogen *Candida albicans* is one of the leading agents causing death in immunocompromised individuals. In a pathogen the cell wall in general and cell wall proteins in particular are antigenic determinants as they are the first elements to contact the host. Cell wall proteins have been shown to be involved in adhesion, virulence, response to host oxidative stress among other functions. We have previously characterized Pga1, a cell wall protein that we found to be involved in virulence, adhesion, biofilm formation, chitin deposition, and resistance to oxidative stress. In this study we investigated differential proteomic expression of a *pga1 null* mutant strain compared to the wild-type strain to determine protein expression patterns that explain the above mentioned phenotypes **Methods:** This was accomplished through an advanced protein sequencing technique that combines liquid chromatography and MS/MS analysis. First, cell wall proteins from mutant and wild type parental strains were extracted and subjected to tryptic digestion. The peptides were separated by NanoLC and analysed using MALDI TOF mass spectrometry. The resulting MS/MS data was searched using Protein Pilot Software and MASCOT Server to identify the proteins. In MS/MS Ion Search, Swissprot and NCBI databases were searched and unidentified peptide sequences were blasted on NCBI and Candidagenome.org. **Results:** Under filamentous conditions several proteins such as Dor14, Ssr1, Als7, Skn1 were exclusively expressed in the wild type parental strain, whilst Pga60, pir1, and Bmt6 were present only in the wild type under non filamentous conditions. ALs7 and Pga60 are adhesins and their absence in the mutant might explain the decrease in adhesion, and biofilm formation. Dor14 has been found to be strongly antigenic in mice and its absence in the mutant is reflected as a decrease in virulence. The decrease in resistance to oxidative stress, and decrease in chitin deposition might be due to the absence of Ssr1, Skn1, Pir1, and Bmt6 that are involved in cell wall structure, 1,6-β glucan synthesis, 1-3-β glucan linkage, and β-1,2 mannosylation of cell wall phospholipomannans resulting in a less rigid less resistant cell surface **Conclusions:** MALDI TOF analysis provides a precise and rapid method to analyze cell surface proteome differences in yeast

Author Disclosure Block:

R.A. Khalaf: None. **A. Awad:** None. **P. El Khoury:** None. **I. El Khatib:** None.

Poster Board Number:

SUNDAY-674

Publishing Title:

The Expression Of Several Genes In *salmonella* Is Similarly Regulated By The Transcription Factor Slya In Response To Ros And In Bacteria Residing In Immune Cells

Author Block:

A. BRIONES[1], C. CABEZAS[1], C. PARDO-ESTÉ[1], J. CASTRO-SEVERYN[1], C. SALINAS[1], C. AGUIRRE[1], S. BAQUEDANO[1], E. H. MORALES[2], E. CASTRO-NALLAR[1], **C. P. SAAVEDRA**[1]; [1]Univ. Andrés Bello, Santiago, Chile, [2]Univ. de Santiago de Chile, Santiago, Chile

Abstract Body:

Salmonella Typhimurium is an intracellular pathogen capable of generating systemic fever in the murine model. During infection of the host, *Salmonella* faces different kinds of stresses, among which Reactive Oxygen Species (ROS) are the most harmful ones. However, *Salmonella* is able to survive this stress and complete a successful infective process, given that it is equipped with different defense mechanisms whose expression is finely tuned by different transcription factors, including OxyR and SoxRS. Another transcription factor involved in the response to oxidative stress and invasion of murine macrophages is SlyA. In our laboratory we evaluated the role of SlyA in the response to H_2O_2 and HOCl, finding that the expression of SlyA increases when it is exposed to these toxics. However, the target genes and the molecular mechanisms by which SlyA is involved in the infective process are unknown. We analyzed changes at the transcriptional level (RNA-seq) of a wild type and Δ*slyA* strain exposed to these toxics, and determined the genes regulated by SlyA in response to the toxics. Among the genes that change their expression, we found some related with protection against ROS (*spy, katG*), virulence (*sopD*), central metabolism (*kgtP, fruK, glpA*), outer membrane permeability (*ompW*), among others. Experiments with transcriptional fusions (GFP) further confirmed that SlyA regulates the expression of these genes, and a direct interaction of SlyA with the promoter regions of several of these genes was confirmed by EMSA. Furthermore, *in-vitro* assays showed that the same genes are also regulated by SlyA in *Salmonella* when infects macrophages and neutrophils. Therefore, the expression of several genes in *Salmonella* is similarly regulated by the transcription factor SlyA in response to ROS and in bacteria residing in immune cells capable of generating ROS, namely macrophages and neutrophils.

Author Disclosure Block:

A. Briones: None. **C. Cabezas:** None. **C. Pardo-esté:** None. **J. Castro-severyn:** None. **C. Salinas:** None. **C. Aguirre:** None. **S. Baquedano:** None. **E.H. Morales:** None. **E. Castro-nallar:** None. **C.P. Saavedra:** None.

Poster Board Number:

SUNDAY-675

Publishing Title:

Understanding the Regulation and Mechanisms Involved in Multiple Antibiotic Resistance

Author Block:

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Abstract Body:

Antibiotic resistance in bacteria is a serious problem. Such pathogens cause difficult to treat diseases world-wide. Multiple antibiotic resistance in bacteria can be driven by the transcriptional regulators in AraC-XylS family. The *Escherichia coli* '*mar'* regulon is considered a paradigm for such systems. The mar locus consists of 3 genes; *marRAB*. MarA, a transcriptional activator that activates drug efflux pump expression. Transcriptional activation is mediated by binding of MarA to "marbox" sequences at target promoters. The marbox is conserved in many gram negative pathogens. We identified 64 new targets by ChIP-Seq analysis in Enterotoxigenic *E.coli* (ETEC). All targets were enriched for marbox motifs. Few of the 64 new targets of marA were characterised previously. This research provides new insights into understanding of antibiotic resistance at molecular level.

Author Disclosure Block:

P. Sharma: None. **D.C. Grainger:** None.

Poster Board Number:

SUNDAY-676

Publishing Title:

Mining Transcriptional Expression Data to Identify Genes Responsible for Enhanced Alkane and Lipid Droplet Production in Cyanobacteria

Author Block:

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Abstract Body:

Cyanobacteria have the ability to produce lipid droplets (LDs). These LDs are enriched with alpha-tocopherol and hydrophobic energy-dense compounds such as triacylglycerides and alkanes with biotechnological potential. Previous work from our lab has shown that introducing extrachromosomal copies of two genes (*aar*, *adc)* into *N. punctiforme* caused enhanced production of heptadecane. LDs also increase in number, likely as an adaptation to detoxify excess alkane production. A putative lipase that when over-expressed alone increased alkane production was identified to be associated with purified LDs by Western blotting. Over-expression of this lipase in conjunction with *aar* and *adc* further increased LDs and heptadecanes compared to the over-expression of *aar*/*adc*. The addition of extra copies of the putative lipase also repaired a growth defect observed in the *aar/adc* over-expressor strain. To identify transcriptional changes associated with increased heptadecane and LD production, and complementation of the growth phenotype in the 2-gene over-production strain, RNA from wild-type and over-expresser strains were extracted and subjected to RNA Seq analysis. Genes that showed a >1.5-fold statistically significant change in the 2 and 3-gene over-expressor strains relative to a plasmid-only control were hypothesized to enhance or cope with over-production of LDs and alkanes. Up and down regulated genes encode different sets of proteins including sigma factors, transcriptional regulators, sugar modifying proteins, and stress proteins. A different subset of genes were found to change expression only in the 2-gene samples and returned to wild-type levels in the 3-gene overexpression samples. These included down-regulated genes in the 2-gene over-expressor strain associated with growth, and up-regulated genes including general stress proteins, indicating the lipase may alleviate stress when over-expressed. Genes showing up-regulation have been transformed back into the wild-type strain on a multi-copy plasmid to test the hypothesis that increased gene copy number can mimic the increased LD phenotype. This work is important for using LDs in biotechnological applications.

Author Disclosure Block:

D.B. Arias: None. **A. Peramuna:** None. **A. Solomon:** None. **M.L. Summers:** None.

Poster Board Number:

SUNDAY-677

Publishing Title:

Gatase1-Like Arac-Family Transcriptional Regulators (Gatrs) in *Burkholderia thailandensi*s

Author Block:

A. M. NOCK, M. J. Wargo; Univ. of Vermont, Burlington, VT

Abstract Body:

GATase1-like AraC-family Transcriptional Regulators (GATRs) are a subgroup of AraCs that are widely distributed among Proteobacteria with an N-terminal class-1 glutamine amidotrasferase-like domain. Based on functions of other GATase1 superfamily members and inferences from our previous studies, we hypothesized that this domain is likely to be used to identify various amine-containing small molecules, particularly those present when microbes are in close association with eukaryotic organisms. The few characterized GATRs are involved with metabolism of eukaryotic-derived amines, and two have links to bacterial virulence.*Burkholderia thailandensis* encodes thirteen GATRs, more than most other sequenced Proteobacteria. The genetic tractability of *B. thailandensis* combined with the high number of GATRs makes it an excellent model organism in which to investigate this family of transcriptional regulators. We generated knockouts of each GATR and are screening each mutant against custom set of amine-containing compounds to assess the metabolic pathways within which these GATRs may play regulatory roles. Based on homology to known regulators in *Pseudomonas aeruginosa*, here we describe the roles of *gbdR1*, *gbdR1*, and *souR* in choline catabolism; *argR* in arginine metabolism; and *cdhR* in carnitine catabolism. These validated orthologs provide valuable comparators for bioinformatic analysis to identify resides involved in ligand specificity and to characterize those GATRs without predicted orthologs. Intriguingly, amongst the eight uncharacterized GATRs is one that appears to be associated with one of the capsule operons shared by *B. thailandensis* and *Burkholderia pseudomallei*. Our goal is to use this data to enable us to infer GATR functions in other Proteobacteria and extend our knowledge of this regulatory family.

Author Disclosure Block:

A.M. Nock: None. **M.J. Wargo:** None.

Poster Board Number:

SUNDAY-678

Publishing Title:

A Spectrum of Cody Activities Drives Metabolic Reorganization and Virulence Gene Expression in *Staphylococcus aureus*

Author Block:

N. R. Waters[1], D. J. Samuels[1], J. Livny[2], K. Y. Rhee[3], **S. R. Brinsmade**[1]; [1]Georgetown Univ., Washington, DC, [2]Broad Inst. of MIT and Harvard, Cambridge, MA, [3]Weill Cornell Med. Coll., New York, NY

Abstract Body:

CodY is a global regulator that controls the expression of dozens of genes in many Firmicutes and is known to link nutrient availability and virulence in pathogenic species like *Staphylococcus aureus* by sensing branched-chain amino acids (BCAAs) and GTP. We previously reported that CodY-dependent genes in *Bacillus subtilis* are controlled in a hierarchical manner. While there is overlap between the *B. subtilis* and *S. aureus* CodY regulons (i.e., metabolic genes), *S. aureus'* regulon includes nearly all of the known virulence genes and nothing is known regarding the threshold activities of CodY required to regulate these genes. To test the hypothesis that *S. aureus* uses CodY to grade metabolic and virulence gene expression as a function of BCAA availability, we constructed *S. aureus* strains that produce mutant CodY proteins with varying regulatory activity due to reduced sensitivity to intracellular pools of BCAAs. Using RNA-Seq and LC-MS analysis, we profiled the transcriptome and metabolome to determine the response of *S. aureus* to nutrient depletion. We measured > 1.5-fold increases in steady-state pools of amino acids as CodY activity was reduced ($p < 0.05$, Cuzick's test for trend). Cells lacking CodY activity produce a PIA-dependent biofilm, but development is restricted in strains with moderate CodY activity ($p < 0.05$, Games-Howell test). Relative to *codY* cells, *codY*$^+$ cells repress the production of secreted nuclease >10-fold in an *sae*- and nutrient-dependent manner, revealing cascading virulence regulation. Using *k*-means clustering, genes encoding proteins that mediate the host-pathogen interaction and subvert the immune response are shut off at intermediate levels of CodY activity, while genes coding for proteins that extract nutrients from tissue, that kill host cells, and that synthesize amino acids are the last genes to be turned on. We conclude that *S. aureus* uses CodY to limit host damage to the most severe starvation conditions, providing insight into one potential mechanism by which *S. aureus* transitions from a commensal bacterium to an invasive pathogen.

Author Disclosure Block:

N.R. Waters: None. **D.J. Samuels:** None. **J. Livny:** None. **K.Y. Rhee:** None. **S.R. Brinsmade:** None.

Poster Board Number:

SUNDAY-679

Publishing Title:

Crosstalk Between Fimbrial and Flagellar Gene Expression in Uropathogenic *Escherichia coli* is Linked Through Regulation of *papx*

Author Block:

C. Luterbach, H. L. T. Mobley; Univ. of Michigan, Ann Arbor, MI

Abstract Body:

Uropathogenic *Escherichia coli* (UPEC) are the main cause of uncomplicated urinary tract infections (UTIs). During a UTI, expression of adherence structures, called fimbriae, promote binding of UPEC to host cell receptors and can thereby aid in colonization of the urinary tract. P fimbriae, encoded by the *pap* operon, bind to the P-blood group antigen found on kidney epithelium, and *E. coli* strains that carry the *pap* operon are more likely to colonize the kidneys during infection, resulting in pyelonephritis. P fimbriae have been confirmed by Molecular Koch's postulates as a virulence factor for CFT073, a pyelonephritis isolate, in the murine model of UTI. Unlike the majority of other fimbrial operons, the 3' end of the *pap* operon encodes a MarR-like transcription factor, PapX. Using SELEX and high-throughput sequencing, our lab has shown that PapX binds to a 29-bp sequence upstream of *flhDC*, the master regulator of flagellar gene expression, and represses motility. We hypothesize that PapX assists in the coordinated regulation of fimbrial and flagellar gene expression observed during UTI. Our lab has previously shown by quantitative PCR that *papX* is transcribed with the *pap* operon. However, using 5' RACE we identified an additional promoter for *papX*. Also, our lab has shown by RNAseq on a clinical UTI *E. coli* isolate, HM69, that *papX* transcription compared to *papA*, which encodes the major fimbrial subunit, is elevated during growth in urine and during UTI compared to growth in LB, suggesting that regulation of *papX* differs from the *pap* operon. To test the regulation of *papX* we constructed transcriptional *lacZ* fusions of the *papX* and *papA* promoters and plan to investigate the impact on regulation of *papX* in strains lacking known regulators of *pap* expression and under different environmental conditions. *E. coli* CFT073 also carries the *foc* operon, which encodes F1C fimbriae and the transcription factor, FocX, which shares 96.7% amino acid sequence identity to PapX. We have tested that FocX and PapX are both capable of repressing motility by soft agar motility assay, and we have constructed transcriptional *lacZ* fusions with the *focX* and *focA* promoters to evaluate regulation of *focX* and cooperation between FocX and PapX. This approach will allow us to determine the impact of encoding multiple "X" proteins on the crosstalk between adherence and motility during UPEC pathogenesis.

Author Disclosure Block:

C. Luterbach: None. **H.L.T. Mobley:** None.

Poster Board Number:

SUNDAY-680

Publishing Title:

The Expression of Virulence Genes of *H. pylori* in the Presence of Reuterin

Author Block:

V. H. Urrutia Baca, M. A. De la Garza Ramos, R. A. Gomez Flores, E. Escamilla Garcia; Univ. Autonoma de Nuevo León, Monterrey, Mexico

Abstract Body:

Background: *Helicobacter pylori* is the pathogen most often associated with diseases of the gastrointestinal tract, about 50% of the world population is infected. *H. pylori* has many virulence factors that allow establishment, colonization and damage in the gastric epithelium Actually, is widely described the use of probiotics and their benefits for the treatment against this infection, however, the antimicrobial activity that may have its metabolites has not been fully tested. In addition, these compounds have shown the ability to inhibit expression of virulence factors in other pathogens. In this study, a metabolite called reuterin produced by *Lactobacillus reuteri* was evaluated. The objective of this investigation was to determine whether reuterin is capable of inhibit the growth or reduce the expression of virulence genes in *H. pylori*.**Methods:** The minimum inhibitory concentration (MIC) was assessed using microdilution method in 96-well plate. The relative expression of virulence genes of *H. pylori* in the presence of 100 µM reuterin was carry out by RT-qPCR.**Results:** The MIC for reuterin against *H. pylori* was 50 mM and a dose-dependent effect was observed. Otherwise, A significant decrease of expression was observed mainly on *flaA*, *vacA* and *luxS* genes at 3 h post-treatment (Figure 1). **Conclusions:** Reuterin has a moderate antimicrobial activity against *H. pylori* in *in vitro* assays and low doses can inhibit the expression of virulence genes. The modulation of virulence genes shown in this study opens the possibility for new approaches about of the mechanisms of interaction of probiotics with gastric pathogens.

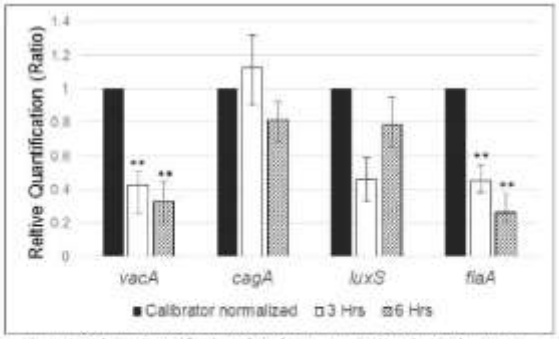

Figure 1. Relative Quantification of virulence genes in *H. pylori* in the presence of reuterin, calculated by the ΔΔCT method. Mean Ratio ± Standard Error. *p<0.05, ** p<0.01

Author Disclosure Block:

V.H. Urrutia Baca: None. **M.A. De la Garza Ramos:** None. **R.A. Gomez Flores:** None. **E. Escamilla Garcia:** None.

Poster Board Number:

SUNDAY-681

Publishing Title:

Transcriptomic Analysis of *Pichia anomala* WRL-076 in Dual Culture with *Aspergillus flavus*

Author Block:

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Abstract Body:

Background: *Pichia anomala* (*Wickerhamomyces anomalus*) WRL-076 is a biocontrol yeast which has been shown to inhibit growth and aflatoxin production of *Aspergillus flavus*. The molecular mechanism of biological control was further characterized by the temporal transcriptome response of *P. anomala* to *A. flavus* in a liquid growth medium at ratio of 1:1 during 72h experimental period. **Methods:** Total RNA was extracted and processed using an Illumina TruSeq RNA Sample Prep kit. RNA-seq reads were mapped to the *W. anomalus* genome using tophat2 with default settings. Differential expression analysis was performed using edgeR. Gene ontology (GO) annotation of *P. anomala* was retrieved from http://genome.jgi.doe.gov/. Enrichment of GO categories in differentially regulated genes was determined using Fisher's exact test in the R environment. **Results:** Comparison of yeast gene expression with and without *A. flavus*, a large number of genes were differentially expressed. At 24 h, 662 genes and 679 genes out of a total of 6,423 genes were up- and down-regulated respectively. Specifically, genes involved in protein phosphorylation, protein kinase, DNA-templated regulation of transcription, and microtubule-based movement. They were enriched in the down-regulated genes at 24 h, but in up-regulation at later time points. This suggests that *P. anomala* was recuperating from the competition of *A. flavus*. Transport was enriched in up-regulated genes at 48 h, which implies that *P. anomala* was actively utilizing nutrients from the environment to build its biocontrol activities. **Conclusion:** *A. flavus* has a significant influence on *P. anomala* gene expression. We applied GO annotations to identify major categories of genes that were involved in biological control processes. A group of genes in defense function, such as signal transduction were up-regulated. The lytic enzymes, exo-beta-1,3-glucanase 1 and 2 were slightly higher at 48 and 72 h. Further analysis is warranted for a better understanding on biocontrol efficacy.

Author Disclosure Block:

S.T. Hua: None. **G. Wu:** None. **S.L. Sarreal:** None. **P. Chang:** None. **J. Yu:** None. **R.W. Li:** None.

Poster Board Number:

SUNDAY-682

Publishing Title:

Molecular and Structural Considerations of the TF-DNA Binding for the Generation of Biologically Meaningful and Accurate Phylogenetic Footprinting Analysis in Proteobacteria. The LysR-Type Transcription Regulatory Family as a Study Model

Author Block:

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Abstract Body:

The in silico identification of Transcription Factor Binding Sites (TFBSs) is a key issue for many Molecular Biology studies aimed to characterize the regulatory elements in sequence genomes. The goal of many algorithms developed to find TFBSs has been the identification of discrete sequence motifs that are overrepresented in a statistically significant manner of a given set of sequences where a TF is expected to bind. Despite their extensive use, the accuracies reached with these methods are still low. In many cases, the true TFBSs are excluded from the identification process or imprecisely identified, especially in those cases when the TFBS correspond to a low affinity. Here we present a new computational protocol named PProCoM (Phylogenetic Profile of Consensus Motifs) to identify in a very precise way, all of the TFBSs in a regulatory sequence, including those with a low conserved sequence or degenerated TFBSs. Our protocol is based on the construction of non-conventional Phylogenetic Profiles consistent of increasing sequence lengths of a set of consensus motifs, obtained by canonical phylogenetic footprinting technique. In this sense, we analyzed the regulatory regions of six members of the LysR-Type Transcriptional Regulator (LTTR) family in Proteobacteria. We have divided these members in three groups according to their regulatory activity, considering predicted TFBSs positions regarding to the promoter sequences of regulated genes. The first group is composed by MetR and GcvA regulatory systems, both of them showed two conserved TFBSs in the intergenic regulatory region shared between the TF and its target gene, while the second group composed by OxyR, IlvY and CynR, have three conserved TFBSs, two of them are spliced. The last is composed by the single regulatory system LysR. We were able to infer the evolutionary change of TFBSs between different organisms and have elucidated possible activation-repression TFBSs roles in accordance of the presence/absence of their corresponding inducers. The in all these information, we have proposed new Molecular Regulatory Models for each one of the LTTR considered in our study.

Author Disclosure Block:

M. Peralta-Gil: None. **P. Oliver:** None. **M.L. Tabche:** None. **T. Romero:** None. **V.H. Perez-España:** B. Collaborator; Self; Victor H. Perez-Espana. **E. Merino-Pérez:** None.

Poster Board Number:

SUNDAY-683

Publishing Title:

Improving Crispr Interference (Crispri) to Achieve Finely-tuned Transcriptional Repression of *Escherichia coli* Genes

Author Block:

N. J. Backes, G. J. Phillips; Iowa State Univ., Ames, IA

Abstract Body:

Background: The application of Clustered Regularly Interspaced Short Palindromic Repeats Interference (CRISPRi) has been shown to enable repression of selected genes in *E. coli* (Qi, et al. 2013. Cell. 52:1173-83). This approach has significant potential to allow interrogation of gene function in multiple bacterial species. To more fully utilize the potential of this technology, we have developed a CRISPRi system that allows for sufficiently tight regulation of targeted genes to allow essential genes to be conditionally expressed. We have further modified the nuclease-defective Cas9 protein from *Steptococcus pyogenes* (dCas9) to allow for visualization of specific regions of the bacterial chromosome and episomes. **Methods:** To maximize efficiency of dCas9 expression in *Enterobacteriaceae*, we synthesized a codon-optimized variant of the *S. pyogenes* gene and placed it under regulation of multiple promoters, including *tetR, araB* and *lac*. These constructs were integrated into the *E. coli* chromosome for expression in single copy by either site-specific recombination, or by Tn7 transposition. A dCas-GFP variant was also constructed and expressed in single copy. Guide RNAs were expressed from ColE1-derivative plasmids. **Results:** We achieved sufficient regulation of the dCas9 to allow reversible genetic switching of expression of multiple essential genes, including those encoding components of the *E. coli* signal recognition particle, as evidenced by conditional growth. Furthermore, we were able to use the CRISPRi system to detect the cellular location of the *lac* operon on both the bacterial chromosome as well as on F' *lac*. **Conclusions:** The CRISPRi system described here represents an easy, efficient system to repress essentially any selected gene for transcriptional repression. In addition, use of dCas9-GFP can allow interrogation of chromosome structure and subcellular location of DNA elements. This system should also be amenable to a broad range of bacterial hosts within the *Enterobacteriaceae* family to better understand gene function.

Author Disclosure Block:

N.J. Backes: None. **G.J. Phillips:** None.

Poster Board Number:

SUNDAY-684

Publishing Title:

An RNA-Guided System for Analyzing Genetic Interactions Involving Essential Genes in *Escherichia coli* K-12

Author Block:

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Abstract Body:

Escherichia coli K-12 has served as an outstanding model organism for several decades from the development of traditional forward genetics to the modern era of high-throughput systems approaches. Although *E. coli* K-12 is one of the best-studied organisms, the physiological function of a large number of its genes has yet to be characterized experimentally. Furthermore, physiological network structure, such as genetic interaction, has only recently been started and there is a long way to its complete understanding at a systems level. Defining gene essentiality is not simple because it depends on a variety of physiological properties, such as growth media, temperature, other environmental factors, and the genetic background. Elucidating the physiological functions of essential genes is crucial to full understanding of a cell. Furthermore, essential genes often serve as hubs of functional networks in a cell. The identification of epistatic relationships provides a powerful approach to connect genes to their functional partner genes. For this purpose, we applied the CRISPR-Cas system (clusters of regularly interspaced short palindromic repeats and associate protein), to isolate conditional knockdown mutants of target essential genes. The CRISPR-Cas system, first discovered in *E. coli* nearly 30 years ago (Nakata et al., 1989; PMID2656660), contains small guide RNAs to specify the target DNA fragments and nuclease domain to cleave the DNA. Here, we introduce a system based on the CRISPR-Cas9 platform for controlling the expression of target essential genes in *Escherichia coli*, in which we employed a mutant Cas9 (dCas9) protein lacking endonuclease cleavage activity. To facilitate systematic analysis of genetic interactions between essential gene knockdown and non-essential single-gene knockouts, we developed a self-transferable CRISPR system. With this system, quantification of growth rates of the essential gene knockdown alone and in combination with a non-essential knockout reveals genetic interactions between essential and non-essential genes.

Author Disclosure Block:

X. Yang: None. **A. Muto:** None. **K.M. Esvelt:** None. **B.L. Wanner:** None. **H. Mori:** None.

Poster Board Number:

SUNDAY-685

Publishing Title:

Development of a Crispr-Cas9 Based Gene Knockout System for Bifidobacteria

Author Block:

y. yang, L. Yu, D. J. O'Sullivan; Univ. of Minnesota, Saint Paul, MN

Abstract Body:

Bifidobacteria are recognized as beneficial microbes in the human gastrointestinal tract, and are frequently used as probiotic cultures in foods. However, our understanding of how they interact with the host and its microbiome is still quite limited. One of the main research limitations is the lack of molecular tools for analyzing these bacteria, particularly targeted genome editing. Therefore, it was necessary to develop an efficient and reproducible genome editing system for bifidobacteria. CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)-Cas systems, which have evolved in bacteria and archaea to impart an acquired immunity to invasive DNA, have been used for genome editing in a wide variety of organisms. In this study we adapted the type II bacterial CRISPR-Cas9 system from Streptococcus pyogenes for site specific gene knockouts in Bifidobacterium longum DJO10A using an efficient conjugation-based gene transfer approach. The CRISPR/Cas9 genome editing plasmid pDOJHR-WD6-Y was designed for site specific mutagenesis of the lanR1 gene and comprises a target-specific guide RNA, a codon-optimized cas9 gene, and an RP4 specific oriT. In order to enhance the gene expression, a modified 16s RNA promoter was constructed upstream of the cas9 gene, as well as for the guide RNA. To increase the efficiency of transcription, a transcriptional terminator was inserted downstream of these unique regions. This novel CRISPR-Cas9 based gene knockout system has now been used for the introduction of a frame-shift mutation into the lanR1 gene of B. longum DJO10A and can be redesigned to target any gene of interest in bifidobacteria.

Author Disclosure Block:

Y. yang: None. **L. Yu:** None. **D.J. O'Sullivan:** None.

Poster Board Number:

SUNDAY-686

Publishing Title:

Cas9 Nickase-Assisted Rna Repression Enables Stable Manipulation Of Essential Genes And Combined Metabolic Engineering In *clostridium Cellulolyticum*

Author Block:

T. Xu, J. Zhou; Univ. of Oklahoma, Norman, OK

Abstract Body:

Clostridium cellulolyticum is an anaerobe with potential use in consolidated bioprocessing; however, increasing its industrial value by diminishing acetate production and conducting combined metabolic engineering in this strain is challenging due to the essentiality of acetate-producing genes. Here, we developed new genetic engineering strategies to overcome these difficulties. Plasmid-based expression of antisense RNA (asRNA) molecules targeting the phosphotransacetylase (*pta*) gene successfully reduced PTA activity by 35% in cellobiose-grown *C. cellulolyticum*, metabolically decreased acetate titer by 15% and 52% in wildtype transformants on cellulose and xylan, respectively. Transformants of the double mutant (LM) of lactate dehydrogenase and malate dehydrogenase reduced acetate titer by more than 33%, concomitant with negligible lactate formation. However, further testing showed that chromosomal integrants created by double-crossover recombination exhibited only very weak repression because DNA integration lessened gene dosage. With the design of a tandem repetitive promoter-driven asRNA module and the use of a new Cas9 nickase genome editing tool, a powerful integrant (LM3P) was generated in a single step and successfully enhanced RNA repression, which reduced acetate titer to 73% of LM on 10 g/L cellulose. From batch fermentation, the LM3P integrant exhibited additional changes in cell growth, cellulose utilization, and other fermentation products especially on 50 g/L cellulose. AsRNA-mediated gene repression performed very well in reducing acetate and enabled co-manipulation of lactate and acetate production in *C. cellulolyticum*. This combinatorial method using the Cas9 nickase genome editing tool to integrate the gene repression module demonstrates easy-to-use and high-efficiency advantages, paving the way for stably manipulating genes, even essential ones, for functional characterization and microbial engineering.

Author Disclosure Block:

T. Xu: None. **J. Zhou:** None.

Poster Board Number:

SUNDAY-687

Publishing Title:

Identification of Virulence Genes of *V. vulnificus* by Using Signature-Tagged Mutagenesis

Author Block:

T. Kashimoto[1], K. Yamazaki[1], T. Kado[1], M. Yamamoto[2], K. Yamamoto[2], S. Ueno[1]; [1]Kitasato univ., Towada, Aomori, Japan, [2]Okayama Prefectural Univ., Soja, Okayama, Japan

Abstract Body:

Background: *Vibrio vulnificus* causes rapid disseminating septicemia by oral infection in infected individuals who have an underlying disease, especially chronic liver diseases. There have been reported a number of studies about several putative virulence factors, which include a capsular polysaccharide, RTX toxin, cytolytic hemolysin VvhA, metalloprotease VvpE, and iron-sequestering system. Although several virulence factors have been found in this organism, the pathogenic mechanisms of *Vibrio vulnificus* infection still unknown. Because in vitro conditions may not always reflect the complicated in vivo host environments, pathogenic genes expressed only in the infection process could be overlooked. Several screening methods for bacterial virulence genes that were expressed in vivo have been developed. In this study, we attempted to apply signature-tagged mutagenesis (STM) to investigate *Vibrio vulnificus* virulence genes that are active in vivo. **Methods:** *Vibrio vulnificus*CMCP6 was mated with *E. coli* BW19795 harboring pUT miniTn5Km2 labeled with one of the 63 unique signature tags. Each set group was used for as a pooled INPUT culture for mouse injection and for template DNA to prepare an INPUT probe. To estimate attenuated mutants, dot hybridization were performed, and the intensity of hybridization signals with INPUT and OUTPUT probes was compared. If tags were detected with INPUT but not with the corresponding OUTPUT, the transposon inserted gens of these attenuated mutants were amplified by AP-PCR and then sequenced. The genes with the highest identification with the database were predicted as transposon-disrupted genes of the attenuated mutants.**Results:** The genes with the highest identification with the database were predicted as transposon-disrupted genes of the attenuated mutants. Ferric vulnibactin receptor VuuA, polysaccharide export protein Wza, and lipid A core-O-antigen ligase were identified as the disrupted genes.**Conclusions:** Some genes which have already known as the putative virulence factor coding gene were identified in the transposon disrupted genes. Thus, we concluded that novel virulence genes could be identified by our STM system.

Author Disclosure Block:

T. Kashimoto: None. **K. Yamazaki:** None. **T. Kado:** None. **M. Yamamoto:** None. **K. Yamamoto:** None. **S. Ueno:** None.

Poster Board Number:

SUNDAY-688

Publishing Title:

The *Streptococcus agalactiae* Essential Genome as Determined by Tn-Seq

Author Block:

T. A. Hooven[1], A. J. Catomeris[2], L. J. Tallon[3], S. Ott[3], T. M. Randis[2], H. Tettelin[3], A. J. Ratner[2]; [1]Columbia Univ., New York, NY, [2]New York Univ., New York, NY, [3]Univ. of Maryland, Baltimore, MD

Abstract Body:

Background: *Streptococcus agalactiae* (group B *Streptococcus*; GBS) is the most common cause of early-onset sepsis and a major contributor to neonatal morbidity and mortality. Genome-wide determination of essential GBS genes may help identify new targets for treatment or prevention of GBS. **Methods:** We used next-generation sequencing of transposon-genome junctions (Tn-seq) to define the essential genome of GBS A909 (serotype Ia). A mini-*Himar1* transposon, which inserts without bias at any genomic TA dinucleotide site, was modified to contain MmeI restriction enzyme sites in the termini of its flanking inverted repeat sequences (necessary for Tn-seq) and delivered to GBS on a temperature-sensitive plasmid.Three independent mutant libraries were grown in rich media, and serially sampled. Illumina sequencing of PCR amplified transposon-genome junctions was followed by statistical analysis of insertion frequency using Tn-seq Explorer and ESSENTIALS software. We validated our results by comparison with the essential genome of *Streptococcus pyogenes*—a closely related Gram-positive pathogen—and mapped essential and critical genes onto predicted GBS signal transduction and metabolic pathways.**Results:** Gene fitness data from our mutant libraries were highly correlated ($r > 0.72$, $p < 0.0001$ Pearson correlation), indicating reproducibility between biological and technical replicates. After pooling of sequencing reads, library saturation was 39-45%, with 99.5% of nonessential genes interrupted at three or more TA sites. Our method identified 14.7% of A909 genes as either essential or critical. 93% of GBS gene fitness assignments were concordant with published *S. pyogenes* results. Essential genes mapped to key metabolic pathways such as aminoacyl-tRNA biosynthesis, nucleotide metabolism, and glycolysis. Our fitness data correctly predicted essentiality of the transcriptional regulator CcpA, which is nonessential in close relatives.**Conclusions:** We have developed and tested a reliable system for performing Tn-seq in GBS, and have determined the essential genome of strain A909. This Tn-seq system is flexible and should permit assessment of conditionally essential genes from biological challenge experiments (including *in vivo* systems).

Author Disclosure Block:

T.A. Hooven: None. **A.J. Catomeris:** None. **L.J. Tallon:** None. **S. Ott:** None. **T.M. Randis:** None. **H. Tettelin:** None. **A.J. Ratner:** None.

Poster Board Number:

SUNDAY-689

Publishing Title:

Elucidating Metabolic Networks in Archaeal Species Using Dynamic Control Valves

Author Block:

E. A. Moreb, A. Schmid, M. D. Lynch; Duke Univ., Durham, NC

Abstract Body:

Archaea are some of the least understood but most metabolically and physiologically diverse organisms on earth. *Haloferax volcanii*, a moderate halophile, is relatively simple to culture and has become a model organism for halophilicity and general archaeal biology. Although better characterized than other species, essential and nonessential genes involved in many aspects of metabolism, including carbon utilization and CO_2 fixation, remain unknown in *H. volcanii*. Identifying the metabolic function of uncharacterized enzymes can be difficult, particularly for essential enzymes. Disabling essential genes prevents organisms from growing, making it impossible to determine the genes role in metabolism. Starting with a list of known enzymes in understudied, unusual glycolytic pathway variants in *H. volcanii*, we have developed a dynamic metabolic control strategy to assess the metabolic function of uncharacterized enzymes. This approach involves tagging each enzyme with a small peptide enabling controlled protein degradation. Initially, the cells will be allowed to grow but, following induced proteolysis, the function of tagged enzymes will be removed. This will cause real time alterations in metabolite pools, which can be analyzed by mass spectrometry. Our approach allows for modular, highly scalable, dynamic assessment of essential metabolic enzymes and can be adapted for rapid elucidation of any unknown pathway.

Author Disclosure Block:

E.A. Moreb: None. **A. Schmid:** None. **M.D. Lynch:** None.

Poster Board Number:

SUNDAY-690

Publishing Title:

Rapid Identification of Protein-Protein Dimerization Sites Using Modified Error-Prone PCR Mutagenesis Coupled with Bacterial Two-Hybrid Screens

Author Block:

A. M. Ibrahim[1], Y. M. Ragab[2], K. A. Aly[1], M. A. Ramadan[2]; [1]Sinai Univ., North Sinai, Egypt, [2]Cairo Univ., Cairo, Egypt

Abstract Body:

Protein-protein interactions drive many critical events in the microbial cell. This led to recent surge in the quest for rapid methods to identify protein dimerization domains. Here, we took a systematic approach to identify the interaction site between two biomedically important *Staphylococcus aureus* proteins: EsxB and EsxD, which are known to strongly interact. Wild-type *esxD* was PCR-amplified using standard methods, followed by fusion to the C-terminal coding sequence of the *Bordetella pertussis adenylate cyclase* (*cya*) gene on the T25 expression vector (Euromedex). Next, *esxB* was amplified by modified error-prone PCR in the presence of 0.6 mM $MnCl_2$ and 20 mM $MgCl_2$ to create one point mutation per ~250 base pairs. Randomly mutated *esxB* from ten different mutagenesis reactions was consolidated and fused to the N-terminal coding sequence of the *B. pertussis cya* on the T18 vector (Euromedex). The two construct-carrying vectors were co-transformed into the *cya*-deficient *Escherichia coli* DHM1, followed by a blue-white two-hybrid screen. Cells were grown in the presence of antibiotics for vector propagation, IPTG for fusion gene inductions and the chromogenic substrate X-gal. The two-hybrid screen revealed the presence of three different color species: 1- Dark blue (no mutation in the EsxB-EsxD dimerization domain), 2- Light blue (possible mutation in a non-dimerization site that may have rendered EsxB less stable), and 3- White (no EsxB-EsxD interaction). The *esxB* gene from white colonies was subjected to classical PCR, followed by DNA sequencing to identify the point mutation that abolished EsxB-EsxD interaction. A single *esxB* point mutation at position 157 coding for asparagine was identified. N53D substitution had no impact on EsxB stability, but only inhibited EsxB-EsxD interaction as judged by SDS-PAGE of the variant protein. Taken together, this novel combination of error-prone PCR mutagenesis and bacterial two-hybrid screens provides a powerful platform for the rapid and robust identification of protein-protein interaction sites, and opens numerous avenues of future microbial biochemistry research.

Author Disclosure Block:

A.M. Ibrahim: None. **Y.M. Ragab:** None. **K.A. Aly:** None. **M.A. Ramadan:** None.

Poster Board Number:

SUNDAY-691

Publishing Title:

Optogenetic Investigation of *Neisseria* Species Motility During the Formation of Microcolonies

Author Block:

I. Eugenis[1], K. Gardner[2], N. Biais[1]; [1]Brooklyn Coll. of the City Univ. of New York, Brooklyn, NY, [2]City Coll. of the City Univ. of New York, New York, NY

Abstract Body:

Bacterial motility plays an important role in the attachment to and colonization of a host. However, the twitching motility of bacteria caused by the retraction of their Type IV pili is an understudied biological phenomenon. Here, an optogenetic system was cloned into the genome of both *Neisseria gonorrhoeae* and *Neisseria elongata* to study both their motility and to assay the role of bacterial movement during the formation of microcolonies, the precursors to biofilms. Optogenetics, a technique commonly used in neurons to regulate the expression of genes with light, was applied to bacteria in this experiment. Using this method, pilT, an ATPase motor protein responsible for the retraction of pili and thus the twitching motility, was put under the control of a promoter responding to blue light. Gibson Assembly, a molecular tool that allows for the joining of DNA fragments, was used to create DNA constructs containing the genes encoding pilT and EL222, a light-sensitive protein that binds to a specific sequence of DNA and allows for the transcription of downstream genes. This tool allows for the precise spatial and temporal control of the motility behavior of the bacteria upon the activation of the pilT gene. The optogenetic control of pilT, and thus of the twitching motility, in *N. gonorrhoeae* and *N. elongata* also allows for the measurement of the dynamics of pili turnover.

Author Disclosure Block:

I. Eugenis: None. **K. Gardner:** None. **N. Biais:** None.

Poster Board Number:

SUNDAY-692

Publishing Title:

FactorsInfluencing In Vitro Transformation Efficiency of *Neisseria lactamica*

Author Block:

J. R. Laver, **Z. C. Pounce**, R. C. Read; Univ. of Southampton, Southampton, United Kingdom

Abstract Body:

Natural competence in the Neisseriaceae is mediated by recognition of DNA uptake sequences (DUS) in extracellular DNA, active translocation of DNA into the cell by Type IV pili and its homologous recombination into the chromosome. Despite being naturally competent, the human commensal *Neisseria lactamica* (Nlac) has, until now, proven refractory to targeted genetic manipulation. The genome of Nlac strain Y92-1009 encodes multiple restriction/modification systems, including NlaIII, which cuts DNA at frequently occurring 5'- CATG – 3' sequences. We posit that Nlac restriction enzymes degrade horizontally acquired genetic material prior to homologous recombination and present the most substantial barrier to targeted mutagenesis.To develop a protocol for the genetic modification of Nlac, we designed gene constructs to incorporate kanamycin resistance gene *aphA3* at specific chromosomal loci of Nlac Y92-1009. Constructs contain at least one DUS and sequences of homology to the Nlac genome. Using a modified plate-based transformation method exploiting the natural competence of Nlac, we recovered kanamycin resistant colonies, demonstrating the targeted mutagenesis of this important commensal for the first time.Transformation efficiency was significantly influenced by the presence of CATG restriction sites in the donor material [$p<0.0001$]. This supports a role for restriction activity in disrupting the horizontal acquisition of genes by Nlac. Using hypermethylated (i.e. restriction resistant) PCR products as donor material, we observed both a 1000-fold increase in transformation efficiency [$p<0.0001$] and ablation of the effect of CATG sequences.Based on studies of other naturally competent bacteria, we hypothesized that a variety of additional factors could influence the efficiency of Nlac transformation. In an attempt to optimize these parameters, we showed that transformation efficiency increases proportionately with the length of sequence homologous to the chromosomal insertion locus [$p<0.0001$] and that transformation efficiency is significantly enhanced when constructs contain more than one DUS [$p=0.002$].In summary we have developed and refined a novel, highly efficient methodology for the targeted mutagenesis of Nlac.

Author Disclosure Block:

J.R. Laver: None. **Z.C. Pounce:** None. **R.C. Read:** None.

Poster Board Number:

SUNDAY-693

Publishing Title:

Targeted Genetic Manipulation of *Neisseria lactamica*: A Novel System for Expression of Heterologous Antigen and Potential Therapy

Author Block:

J. R. Laver, Z. C. Pounce, R. C. Read; Univ. of Southampton, Southampton, United Kingdom

Abstract Body:

The human commensal bacterium, *Neisseria lactamica* (Nlac) has approximately 67 % genetic identity with the highly pathogenic *Neisseria meningitidis*. Experimental intranasal inoculation of adult human volunteers results in up to 6 months of asymptomatic nasopharyngeal carriage of Nlac, an event that prevents meningococcal acquisition. Genetic manipulation of Nlac therefore presents an opportunity for microbiome-modifying bacteriotherapy, or novel vaccination. However, despite the natural competence of the Neisseriaceae, attempts to transform Nlac have thus far been unsuccessful, likely due to expression of multiple restriction systems. Using a novel, hypermethylated PCR-based strategy, we demonstrate for the first time the targeted mutagenesis of this important commensal organism.Against a background of Nlac strain Y92-1009 (WT), we have deleted the native β-galactosidase (β-gal) gene (*lacZ*), generating Nlac strain *ΔlacZ* and ablating β-gal Specific Activity ($p<0.0001$ cf. WT). Subsequently, we re-introduced a functional copy of the *lacZ* gene, under the control of the Nlac-derived *porB* promoter, into *Neisseria* Heterologous Construct Insertion Site number 1 (NHCIS1), an intergenic chromosomal locus that minimises disruption to Nlac's existing genetic architecture. With the intention to develop a potential probiotic, the recovery of β-gal activity in strain *ΔlacZ* NHCIS1::*lacZ* indicated that *lacZ* could be used as a marker in antibiotic resistance-free cloning. Using this method, we have successfully introduced the gene encoding the immunodominant meningococcal outer membrane protein, Porin A (*porA*) into NHCIS1, which under the control of its native promoter can be detected on the surface of recombinant Nlac by flow cytometry.The ability to perform targeted mutagenesis of and to introduce heterologous antigen into the Nlac genome has therapeutic potential, both in the development of recombinant outer membrane vesicle (OMV)-based vaccines, and for the derivation of strains suitable for use in either experimental human challenge or as a recombinant commensal prophylactic against diseases of the upper respiratory tract.

Author Disclosure Block:

J.R. Laver: None. **Z.C. Pounce:** None. **R.C. Read:** None.

Poster Board Number:

SUNDAY-694

Publishing Title:

A Simple Recombineering-Based Protocol for Rapid Allelic Replacement in *Escherichia albertii,* an Attaching and Effacing Pathogen

Author Block:

M. Egan, J. Ramirez, C. Xander, S. Bhatt, Department of Biology, Department of Mathematics; Saint Joseph's Univ., Philadelphia, PA

Abstract Body:

The genetic engineering technique of recombineering allows bacterial pathogeneticists to rapidly and reliably mutagenize the genomes of pathogens to identify virulence factors. In recombineering, linear double- or single-stranded DNA substrates with two terminal homology arms are transformed into hyperrecombinogenic bacteria that express phage-encoded recombinases. The recombinase catalyzes the replacement of the endogenous allele with the exogenous allele by a double crossover event to generate selectable or screenable recombinants. In particular, lambda red recombinase played a pioneering role in constructing mutants to characterize the virulence factors of the attaching and effacing (A/E) pathogens enteropathogenic *Escherichia coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), and *Citrobacter rodentium. Escherichia albertii* is another member of this group; however, its virulence remains unexplored despite a report from the Centers for Disease Control and Prevention (CDC) that *E. albertii* is an emerging pathogen. Recent studies show that many EPEC and EHEC isolates (~15%), which were previously incriminated in outbreaks, are taxonomically *E. albertii*. Thus, there is urgency to engineer mutations in *E. albertii* and comprehensively characterize its virulence. To date, not a single chromosomal gene has mutagenized in *E. albertii* since it was first isolated almost 25 years ago. This is disconcerting because an *E. albertii* outbreak could have devastating consequences due to our inadequate understanding of its virulence. Herein, we describe a modified lambda red recombineering protocol to mutagenize *E. albertii*. We demonstrate its usefulness by successfully deleting three distinct virulence-associated genetic loci - *ler*, *grlRA*, and *hfq* - and replacing each wild type allele by a mutant allele with an encodable drug resistance cassette bracketed by FRT sites. Subsequently, the FRT-site flanked drug resistance marker was evicted by transient expression of the FLP recombinase to generate excisants containing a solitary FRT site. Our protocol will enable researchers to construct virtually any kind of mutation in the genome of *E. albertii,* which will decipher its pathogenic mechanisms to develop appropriate ways to combat *E. albertii* outbreaks.

Author Disclosure Block:

M. Egan: None. **J. Ramirez:** None. **C. Xander:** None. **S. Bhatt:** None.

Poster Board Number:

SUNDAY-695

Publishing Title:

Development of Novel *Clostridium difficile* Genetic Tools

Author Block:

K. N. McAllister, J. A. Sorg; Texas A&M Univ., College Station, TX

Abstract Body:

Clostridium difficile is a Gram-positive, anaerobic, spore-forming bacterium and a significant concern as a nosocomial pathogen. The tools available to genetically manipulate this organism are limited, but include: TargeTron (ClosTron) system, Allelic-Coupled Exchange/CodA systems and Mariner transposition. The most widely used system is the TargeTron system which relies on the re-targeting of mobilizable group II introns and the use of retrotransposable activated markers (RAM). Though RAM markers allow for the easy identification of potential mutants, unfortunately, the TargeTron system only creates an insertion mutation which prevents the introduction of more precise mutations into the *C. difficile* genome and can have off-target effects if the preferential site exists elsewhere in the genome. Our own experiences with the allelic exchange systems have been unreliable and time consuming, which prevents the efficient introduction of site-directed, non-polar mutations. Our lab is currently trying to apply other genetic systems to *C. difficile* research with the hope of rapidly introducing markerless, site-directed, non-polar insertions or deletions into the *C. difficile* genome.

Author Disclosure Block:

K.N. McAllister: None. **J.A. Sorg:** None.

Poster Board Number:

SUNDAY-696

Publishing Title:

Development of an Integratable Vector for G*eobacillus* spp

Author Block:

T. J. Marks[1], P. T. Hamilton[2]; [1]Campbell Univ., Buies Creek, NC, [2]North Carolina State Univ., Raleigh, NC

Abstract Body:

Geobacillus species are gram-positive thermophilic bacteria that have potential for biofuel production because they are capable of fermenting C5 and C6 sugars to mixed acids and ethanol. Some species are also able to produce enzymes of industrial interest, including amylases, proteases, and lipases and some have demonstrated the ability to degrade hydrocarbons and cellulose. Unfortunately, there is a need for additional genetic tools for these organisms due to a lack of gene transfer and protein expression vectors that are useful in *Geobacillus*. The diversity among bacteriophages represents a vast set of potential tools that can be developed to exchange, alter and express genes and gene products from an organism of interest. We have characterized two bacteriophages, designated GBK1 and GBK2, that infect *G. kaustophilus* (ATCC #8005) and have the potential to enhance the tools available to study and modify *Geobacillus* species for various industrial applications. GBK2 is a lytic phage with a circularly permuted dsDNA genome containing 39,078 bp and 64 identified ORFs. Of note are ORFs 39 and 40, which encode proteins with homology to RecE and RecT proteins from *B. subtilis* phage SPP1. RecE/RecT have been used to develop recombineering systems in other bacteria. GBK1 is a temperate phage with a linear dsDNA genome containing 45,439 bp and 63 identified ORFs. A 40 bp attP/attB site was identified in GBK1/*G. kaustophilus.* Slightly downstream of the attP site in GBK1 is ORF 47 which has homology to tyrosine integrase/recombinase. We have shown that the integrase is active by isolating GBK1 lysogens inserted into *G. kaustophilus*. Our current studies involve the development of a suicide plasmid, pUCG5.2INT, that replicates in *E. coli*, confers kanamycin resistance in both *E. coli* and *Geobacillus* spp., and acts as an integratable vector in potentially four different *Geobacillus* species. The development of pUCG5.2INT involved the cloning of the attP/integrase from GBK1 into an existing shuttle plasmid (pUCG18) and removal of the origin of replication for geobacilli. Future studies will involve the integration of the recE and recT genes from GBK2 into a host strain by way of the pUCG5.2INT plasmid to explore the possibility of a functional recombineering system in geobacilli.

Author Disclosure Block:

T.J. Marks: None. **P.T. Hamilton:** None.

Poster Board Number:

SUNDAY-697

Publishing Title:

The IntXO-*PSL* Recombination System is a Key Component of the Second Maintenance System for *Bacillus anthracis* Plasmid pXOA1

Author Block:

A. P. Pomerantsev, C. Rappole, Z. Chang, S. H. Leppla; NIH/NIAID, Bethesda, MD

Abstract Body:

Background: We previously identified three noncontiguous regions on *Bacillus anthracis* plasmid pXO1 that comprise a system for accurate plasmid partition and maintenance. However, deletion of these regions did not decrease retention of the certain shortened pXO1 plasmids during vegetative growth. **Methods:** Using two genetic tools developed for DNA manipulation in *B. anthracis* (Cre-*loxP* and Flp-*FRT* systems), we found two other noncontiguous pXO1 regions that together are sufficient for plasmid stability.**Results:** This second pXO1 maintenance system includes the *tubZ* and *tubR* genes, characteristic of a type III partition system, and the *intXO* gene (GBAA_RS29165), encoding a tyrosine recombinase, along with its adjacent 37-bp *PSL* (perfect stem-loop) target. Insertion of either the *tubZ* and *tubR* genes or the IntXO-*PSL* system into an unstable mini-pXO1 plasmid did not restore plasmid stability. The need for the two components of the second pXO1-maintenance system follows from the sequential roles of IntXO-*PSL* in generating monomeric circular daughter pXO1 molecules (thereby presumably preventing dimer catastrophe), and of TubZ/TubR in partitioning the monomers during cell division. We show that the IntXO recombinase deletes DNA fragments located between two *PSL* sites in a manner similar to the actions of the Cre-*loxP* and Flp-*FRT* systems.**Conclusions:** These results demonstrate that tyrosine recombinases used in this study provide an approach for genome editing that may be preferred in some cases to alternative methods (e.g., CRISPR/Cas systems).

Author Disclosure Block:

A.P. Pomerantsev: None. **C. Rappole:** None. **Z. Chang:** None. **S.H. Leppla:** None.

Poster Board Number:

SUNDAY-698

Publishing Title:

Generation and *In Vivo* Characterization of an Unmarked *Hemo* Cluster Deletion Mutant of *Acinetobacter baumannii* Lac-4

Author Block:

P. J. Ewing[1], J. A. Garcia[1], W. Chen[2], H. Xu[1]; [1]California State Univ., Los Angeles, Los Angeles, CA, [2]Natl. Res. Council, Ottawa, ON, Canada

Abstract Body:

Background: *Acinetobacter baumannii* is one of the most problematic bacterial pathogens due to its emergence and spread of multi-drug resistance (MDR). We previously showed that a hospital outbreak MDR strain (LAC-4) of *A. baumannii* is hypervirulent in mice, exhibits high serum resistance and expresses a highly efficient heme utilization system. We also found that all *A. baumannii* strains possess a heme utilization gene cluster, while only some strains, including LAC-4, harbor a second gene cluster that contains a gene encoding for heme oxygenase (termed hemO cluster, HOC). However, systematic investigations of MDR *A. baumannii* have been hindered by a lack of facile genetic tools. Here we describe generation and in vivo characterization of an unmarked knockout (KO) mutant of LAC-4 in which the 9.5-kb eight-gene HOC was deleted.**Methods:** The HOC mutant was constructed via homologous recombination of the KO cassette cloned into the suicide vector pMo130. An apramycin (Apr) resistant gene was linked outside to two fused flanking regions of HOC via overlap extension PCR. After the initial transformant was isolated via single cross-over of the suicide plasmid, unmarked HOC mutant was obtained via second homologous recombination achieved by *sacB* counter selection and the use of *xylE* reporter gene. The virulence of the HOC mutant was evaluated in an established mouse model of bacterial pneumonia. **Results:** LAC-4 strain was found to be susceptible to Apr. Consequently, the Apr resistant gene was used for selection in genetic manipulations in this MDR strain. The *xylE* reporter gene was useful both in selection of co-integrants after initial transformation of LAC-4 with KO construct, and in screening for eventual HOC deletion mutant clones which have lost the plasmid backbone which contains *xylE* and *sacB* genes. Sequence-confirmed HOC mutant was found to be less virulent than LAC-4 strain with significantly lower bacterial burdens in the lung and spleen and milder bacteremia ($P<0.001$).**Conclusions:** Our findings indicate the use of *SacB* and *XylE* in conjunction with a construct design for unmarked deletion is an effective strategy to generate unmarked mutants. The hemO cluster appears to contribute to LAC-4's hypervirulence in mice.

Author Disclosure Block:

P.J. Ewing: None. **J.A. Garcia:** None. **W. Chen:** None. **H. Xu:** None.

Poster Board Number:

SUNDAY-699

Publishing Title:

A New Drug Selectable Marker For Rodent Malaria Parasites

Author Block:

A. C. Reese, E. E. Munoz, S. E. Lindner; Pennsylvania State Univ., University Park, PA

Abstract Body:

Sequential and conditional gene knockout approaches are valuable tools for reverse genetics in model species. However, studies of rodent malaria parasites (e.g. Plasmodium yoelii) suffer from having only one drug-selectable marker, a variant of dihydrofolate reductase (DHFR), which is used to select for transgenic parasites in the presence of pyrimethamine. We predict that variants of dihydropterotate synthase (DHPS), another enzyme in the folate pathway, can be used to convey resistance to the drug sulfadiazine as they have conferred resistance in field isolates of human-infectious Plasmodium species. The purpose of this experiment is two-fold. First, to determine which variant of the DHPS sequence will provide the best resistance to sulfadiazine. Second, to determine if DHPS alone can confer resistance, as previous publications have utilized this cassette either with DHFR or with PPPK, the enzyme before DHPS in the folate pathway. To test the effectiveness of DHPS in conferring drug resistance, parasites were transfected with a plasmid that expresses a field variant of DHPS and GFP separately, and were selected by administering sulfadiazine via drinking water. Preliminary data indicates that the bifunctional PPPK-DHPS protein is sufficient to select for transgenic parasites at a dose of 1 mg/L sulfadiazine. Our ongoing experiments will determine at what concentrations of pyrimethamine and sulfadiazine it is possible to simultaneously select for parasites that possess both the DHFR and DHPS cassettes, if the DHPS domain alone is sufficient, and what field variant of DHPS confers the best therapeutic window. Once optimized, this system will provide an efficient means by which doubly transgenic parasites can be selected for by a drug cocktail.

Author Disclosure Block:

A.C. Reese: None. **E.E. Munoz:** None. **S.E. Lindner:** None.

Poster Board Number:

SUNDAY-700

Publishing Title:

Standardization of a Multifunctional Halotag Reporter System in Tritrichomonas Foetus

Author Block:

A. Kucknoor, **K. Arriola**, S. Ganguly; Lamar Univ., Beaumont, TX

Abstract Body:

The aim of this study is to standardize the multifunctional HaloTag reporter system in *Tritrichomonas foetus*, a cattle pathogen. Protein targeting and localization in trichomonad species is not well studied. Being extracellular parasites, trichomonads establish residence on host epithelial cells, and proteins that are necessary for the adherence of parasites are necessary for pathogenesis. In order to understand the functional role of proteins and how they interact within and outside of the cell it is very crucial to have a flexible reporter system, which will enable us to purify the protein, analyze its expression and localization, and perform further functional analyses. We have constructed an expression plasmid with HaloTag as the reporter gene, and standardized the transfection procedures in *T. foetus*. Following successful detection of HaloTag expression, we have constructed a fusion protein of Ferredoxin-HaloTag and verified the organelle specific localization of Ferredoxin gene using Halotag ligand system. Results from this study indicate that HaloTag system can be used for protein expression and localization studies in trichomonad species. Analyses of surface proteins that are important for host-pathogen interactions of *T. foetus* using the HaloTag system are underway.

Author Disclosure Block:

A. Kucknoor: None. **K. Arriola:** None. **S. Ganguly:** None.

Poster Board Number:

SUNDAY-701

Publishing Title:

Construction of Stable and Unstable Vectors for Use in Sphingomonads

Author Block:

S. S. Eleya, G. J. Zylstra; Rutgers Univ., New Brunswick, NJ

Abstract Body:

Sphingomonads are of a great interest in the bioremediation field due to their unusual capabilities to biodegrade and metabolize a wide range of monocyclic and polycyclic aromatic compounds including benzene, toluene, xylenes, phenols, biphenyl, naphthalene, phenanthrene, carbazole, dibenzofurans, and dibenzo-*p*-dioxins. *Sphingomonas yanoikuyae* strain B1 is one of the most interesting *Sphingomonas* strains because of its capability to degrade both monocyclic and polycyclic aromatic hydrocarbons such as biphenyl, naphthalene, phenanthrene, toluene, and *m*- and *p*-xylene as the sole source of carbon and energy. The complete sequence of the 32 kb *Sphingomonas yanoikuyae* B1 cryptic plasmid pKG2 revealed an origin of replication sequence (*oriR*) and two essential genes encoding for replication and partitioning components of the plasmid, named *repA* and *parA* respectively. We hypothesized that the *repA* gene along with the origin of replication (*oriR*) is the minimum required for replication of pKG2. We also hypothesized that *parA* is required for stability of the plasmid and proper partitioning during cell division. We designed and constructed two *Sphingomonas* shuttle vectors, one containing *repA* and *oriR* from pKG2 and the other containing *repA*, *oriR*, and *parA* from pkG2. Additionally the plasmids contained a tetracycline resistance gene, an origin of conjugal transfer (*oriT)*, the *E. coli* pMB1 *oriR*, and a *lacZ* gene with a multiple cloning site from pGEM5Z (f-). The newly constructed vectors were then examined for their ability to be maintained in *Sphingomonas* strains. Upon introduction into *S. yanoikuyae* B1 with tetracycline selection both new vectors caused the loss of plasmid pKG2 confirming the expected incompatability due to the identical *oriR* in both plasmids. Plasmid stability tests showed that the *repA*-*oriR*-*parA* vector was completely stable (0% loss) over six days of subculture without antibiotic selection. In contrast, the *repA*-*oriR* vector was lost at the rate of 10% for every 24 hr subculture. Our work demonstrates that the *repA*-*oriR* region is sufficient for replication and that *parA* is absolutely required for plasmid stability. These two cloning vectors in both a stable and unstable version are useful genetic tools for the manipulation of *Sphingomonas* strains.

Author Disclosure Block:

S.S. Eleya: None. **G.J. Zylstra:** None.

Poster Board Number:

SUNDAY-702

Publishing Title:

Assembly and Comparison of Whole Genomes from Single-Molecule Sequencing

Author Block:

A. Phillippy[1], S. Koren[1], B. Walenz[1], B. Ondov[2], T. Treangen[2]; [1]Natl. Human Genome Res. Inst., Bethesda, MD, [2]Natl. Biodefense Analysis and Countermeasures Ctr., Frederick, MD

Abstract Body:

Single-molecule sequencing with PacBio technology is now routinely used to assemble complete, high-quality microbial genomes, revealing population-scale structural variation. The newly released Oxford Nanopore MinION instrument provides a similar data type, but with a pay-per-run price model that eliminates instrument cost. This will enable small labs to generate their own genome sequences, in-house, and will demand efficient, usable software for the analysis of whole genomes. We have developed several methods aimed at assembling and comparing whole genomes derived from these data types.For genome assembly, we have developed a new whole-genome assembler, named Canu, that is specifically optimized for single-molecule sequencing. Canu represents a complete refactorization of the Celera Assembler that introduces support for Oxford Nanopore reads, lowers coverage requirements, and improves runtime. We have demonstrated that Canu can efficiently generate complete assemblies of microbial genomes using ~30X coverage of either PacBio or Nanopore data.For whole-genome comparisons, identifying closely related genomes is often the first step after sequencing a new genome. However, given a massive collection of sequences, pairwise alignment becomes infeasible for this task. To address this problem, we have developed a new tool, Mash, that reduces large genomes and metagenomes to small, representative sketches, from which pairwise genomic distances can be rapidly estimated. Mash distances correlate well with established metrics like Average Nucleotide Identity, and can be used to cluster thousands of genomes by species, cluster metagenomes by composition, reconstruct approximate phylogenies, and search large reference databases. For example, using Mash we were able to compress all 54,118 NCBI RefSeq genomes into a 93 MB sketch database, and using this reference database, correctly identify Ebola virus from 200 kb of MinION nanopore reads in just one second.Canu and Mash, as well as additional tools for metagenomics, genome assembly, and core-genome alignment, are freely available from https://github.com/marbl.

Author Disclosure Block:

A. Phillippy: None. **S. Koren:** None. **B. Walenz:** None. **B. Ondov:** None. **T. Treangen:** None.

Poster Board Number:

SUNDAY-703

Publishing Title:

Integrative Analysis Pipeline for Bacteria Genome Assembly and Methylation Analysis Using Smrt Sequencing and Hybrid Approach

Author Block:

h. nguyen, S. Leopold, L. R. Coy, S. Lek, B. Hanson, E. Sodergren, G. Weinstock; The Jackson Lab. for Genomic Med., Farmington, CT

Abstract Body:

With the rapid progress of Single-molecule real-time (SMRT®) DNA sequencing and computational methods, we can improve *de novo* genome assembly, and discover DNA modifications including N6-methyladenine, 5-methylcytosine and N4-methylcytosine at single-nucleotide resolution. We present an integrative analysis pipeline dedicated to bacterial genome assembly and methylation analysis using PacBio Sequencing and heterogeneous NGS bioinformatics resources (SMRTANALTYSIS, QUIVER, PILON, Celera). The pipeline contains four main iterative steps: (1) *de-novo* assembly using PacBio long reads, and PBcR and HGAP assembler, (2) error correction and gap filling of the draft assembly with high-quality of Illumina short reads, (3) utilization of GEPARD dotplots and illumina paired-reads mapped on PacBio contigs for assembly quality checks and circular validation, and (4) implementation of the draft reference genome and SMRT movie data for detecting the methylase motif including Dam GATC that plays a role in the initiation of bacterial replication. The ratio of the GATC motif detection in the movie data compared to the draft reference genome can also be used for validating the quality of raw data and of genome assembly. We have successfully applied this integrative pipeline for dozens of bacteria strains including *Klebsiella pneumoniae* carbapenemase, *Staphylococcus aureus*, and *Escherichia coli*.. Finally, we show that the combination of *de-novo* assembly processes and methylation analysis can help address a variety of biological questions concerning antibiotic resistance by bacterial pathogens.

Author Disclosure Block:

H. nguyen: None. **S. Leopold:** None. **L.R. Coy:** None. **S. Lek:** None. **B. Hanson:** None. **E. Sodergren:** None. **G. Weinstock:** None.

Poster Board Number:

SUNDAY-704

Publishing Title:

Absence of Cytomegalovirus in Transcriptome and High-Coverage DNA Sequencing Argues against Antiviral Treatment of Human Glioblastoma Multiforme

Author Block:

K-W. Tang; Univ. of Gothenburg, Gothenburg, Sweden

Abstract Body:

Viruses cause 10-15% of all human cancers. Massively parallel sequencing has recently proved effective for uncovering novel viruses and virus-tumour associations. We screened a diverse landscape of human cancer, encompassing 4,433 tumours and 19 cancer types, for known and novel expressed viruses based on >700 billion transcriptome sequencing reads from The Cancer Genome Atlas Research Network. The resulting map confirms and extends current knowledge. Our analysis argues strongly against viral aetiology in several cancers where this has frequently been proposed. For example were we unable to detect any cytomegalovirus (CMV) in 22.8 billion sequencing reads of mRNA from 167 glioblastoma multiforme tumors. We also utilized deep-coverage whole-genome sequencing data to detect latent CMV DNA and to assess the relative proportions of viral and human DNA. We did not find traces of CMV in 52.6 billion DNA sequencing reads from 34 glioblastomas. By statistical analysis, we conclude that should the virus be present in these tumors, the average CMV level does not exceed one virus per 240,000 tumor cells (99% CI). These findings together strongly argues against current antiviral treatment of glioblastoma multiforme.

Author Disclosure Block:

K. Tang: None.

Poster Board Number:

SUNDAY-705

Publishing Title:

Genome-Wide Analysis of Gene Function in the Hyperthermophilic Crenarchaeon *Sulfolobus islandicus* with Genetics and Genomics

Author Block:

C. Zhang, A. Phillips, R. Whitaker; Univ. of Illinois at Urbana-Champaign, Urbana, IL

Abstract Body:

Sulfolobus islandicus, an aerobic hyperthermophilic crenarchaeon which grows optimally at 80°C and pH 2 in terrestrial solfataric springs distributed around the world, has been developed as a novel model microorganism to study the unique biology of Crenarchaea. Here, a comprehensive genome-scale analysis of gene function by transposon mutagenesis coupled with deep sequencing methodology has been developed successfully in *S. islandicus*. Three independent transposon insertion libraries with around 100,000 colonies in total were collected, sequenced and then mapped to the reference genome. As a result, about 90,000 unique insertions (reads >3) were identified, allowing us to classify about 17% of the genome (~460 genes) as possible essential genes. The differences noted between our data with those predictors of essentiality led to several surprisingly discoveries. For example, the reverse gyrase-encoding gene, which is considered as a hallmark of hyperthermophiles and a prerequisite for hyperthermophilic life, is dispensable for cell survival as a couple of transposon insertions were found. This result was further confirmed by a successful disruption of the reverse gyrase gene via reverse genetics, suggesting that S. islandicus does not require reverse gyrase. Our investigation of essentiality of every gene will serve as a valuable resource to reveal unexplored genetic determinants and the underlying mechanisms of various biological processes especially the DNA repair, replication and recombination in the *S. islandicus*.

Author Disclosure Block:

C. Zhang: None. **A. Phillips:** None. **R. Whitaker:** None.

Poster Board Number:

SUNDAY-706

Publishing Title:

Dissecting the Genetic Basis of Species-Specific Essential Genes in Yeast

Author Block:

M. Sanchez[1], C. Payen[1], F. Cheong[1], B. Hovde[1], J. Skerker[2], R. Brem[2], A. Caudy[3], M. Dunham[1]; [1]Univ. of Washington, Seattle, WA, [2]Univ. of California Berkeley, Berkeley, CA, [3]Univ. of Toronto, Toronto, ON, Canada

Abstract Body:

To understand how complex genetic networks perform and regulate diverse cellular processes, the function of each individual component must be defined. One powerful approach to defining gene function is via genetics: phenotypes conferred by mutant alleles can be used to determine what processes depend on the normal function of a gene. This approach has been wildly successful in model organisms, in which comprehensive studies have been performed. These results are often translated to the increasing number of newly sequenced genomes by using sequence homology. However, sequence similarity does not always mean identical function or phenotype, suggesting that new methods are required to functionally annotate newly sequenced species. We have implemented comparative functional analysis by high-throughput experimental testing of gene function in a sequenced, but otherwise understudied species of yeast, *Saccharomyces uvarum*, a sister species of *S. cerevisiae.* We created a haploid and heterozygous diploid library containing Tn7 transposon insertions in *S. uvarum* to identify species-specific essential genes. Using deep sequencing, we identified 50,193 and 37,022 independent insertion sites found in 80% and 63% of orthologous coding sequences in the diploid and haploid libraries respectively. We predict 809 genes to be essential in *S. uvarum*, with 444 of those genes also known to be essential in *S. cerevisiae*. We predict 115 genes to be *S. cerevisiae*-specific essential genes (non-essential in *S. uvarum),* and confirmed 5 highly ranked genes. However, these genes are capable of cross-species complementation, demonstrating that differences in genetic background must contribute to differential gene essentiality. We are now mapping these genetic background elements to determine the molecular explanation for these differences. This data set provides direct experimental evidence of gene function across species, which can inform comparative genomic analyses, improve gene annotation and be applied across a diverse set of microorganisms to further our understanding of gene function evolution.

Author Disclosure Block:

M. Sanchez: None. **C. Payen:** None. **F. Cheong:** None. **B. Hovde:** None. **J. Skerker:** None. **R. Brem:** None. **A. Caudy:** None. **M. Dunham:** None.

Poster Board Number:

SUNDAY-707

Publishing Title:

Targeting Self-Resistance for Genomics-Based Discovery of Novel Antibiotics

Author Block:

X. Tang, J. Li, N. Millán-Aguiñaga, J. J. Zhang, E. C. O'Neill, J. A. Ugalde, P. R. Jensen, S. M. Mantovani, B. S. Moore; Scripps Inst. of Oceanography, UCSD, La Jolla, CA

Abstract Body:

Recent genome sequencing efforts have led to the rapid accumulation of uncharacterized or "orphaned" secondary metabolic biosynthesis gene clusters (BGCs) in public databases. This increase in DNA-sequenced big data has given rise to significant challenges in the applied field of natural product genome mining, including (*i*) how to prioritize the characterization of orphan BGCs, and (*ii*) how to rapidly connect genes to biosynthesized small molecules. Here we propose a new genomics-based strategy that by correlating putative antibiotic resistance genes with orphan biosynthetic gene clusters, we can predict the biological function of pathway specific small molecules before they have been revealed in a process we call target-directed genome mining. Combined with an approach based on transformation-associated recombination in yeast to directly assemble complete gene sets from genomic DNA, we can prioritize, capture, and express potential antibiotic producing biosynthetic gene clusters to identify antibiotic leads, their resistance genes, and their targets. To validate this approach, we queried the pan-genome of 86 *Salinispora* genomes for duplicated house-keeping genes co-localized with natural product biosynthetic gene clusters (BGCs) and prioritized an orphan polyketide synthase-nonribosomal peptide synthetase hybrid BGC (*tlm*) with a putative fatty acid synthase resistance gene. We then directly cloned *tlm* and the related *ttm* BGCs directly from genomic DNA and to heterologously express them in *Streptomyces* hosts. We show the production of a group of unusual thiotetronic acid natural products, including the well-known fatty acid synthase inhibitor thiolactomycin that was first described over 30 years ago, yet never at the genetic level in regards to biosynthesis and auto-resistance. This finding validates the target-directed genome mining strategy for the discovery of antibiotic producing gene clusters without *a priori* knowledge of the molecule synthesized and highlights a streamlined method for the coordinated discovery of antibiotics, their resistance genes, and their targets

Author Disclosure Block:

X. Tang: None.

Poster Board Number:

SUNDAY-708

Publishing Title:

Characterization of a Unique Gamma-Glutamylase Dependent Pathway for Ethanolamine Catabolism

Author Block:

B. San Francisco, X. Zhang, J. T. Bouvier, J. E. Cronan, J. A. Gerlt; Univ. of Illinois at Urbana-Champaign, Urbana, IL

Abstract Body:

Ethanolamine is abundant in nature as a component of phosphatidylethanolamine found in the cell membranes of eukaryotes and bacteria. Many species of bacteria derive carbon and/or nitrogen from ethanolamine via the well-characterized ethanolamine utilization (EUT) pathway. The central enzyme in the EUT pathway is the adenosylcobalamin-dependent ethanolamine ammonia-lyase, which cleaves ethanolamine to release ammonia and acetaldehyde. Here, we report functional and phylogenomic characterization of a new, gamma-glutamylase dependent pathway (termed EOA) for ethanolamine catabolism in bacteria. We use enzymology and microbiology guided by large-scale analyses of sequence-function relationships and genome context to delineate this four-enzyme pathway for ethanolamine catabolism that terminates in glycine, a source of cellular nitrogen. Analysis of the taxonomic distribution of the EOA pathway revealed that the pathway is found almost exclusively in Proteobacteria and Actinobacteria. Interestingly, the pathway in each phylum differs by the enzyme that catalyzes the final step. Proteobacteria utilize a previously uncharacterized hydrolase from the amidohydrolase superfamily while Actinobacteria utilize a member of the peptidase C26 enzyme family to catalyze the final step of the pathway. In total, the characterization of this pathway enabled the assignment of function to enzymes and proteins from at least ten different Pfam families encoded in the genomes of nearly 400 distinct species. We found that some species encode enzyme homologues of both the EUT and EOA pathways, and we provide evidence to suggest that organisms may prefer one pathway depending on microhabitat. We discuss possible modes of regulation and environmental pressures that may have been involved in pathway selection.

Author Disclosure Block:

B. San Francisco: None. **X. Zhang:** None. **J.T. Bouvier:** None. **J.E. Cronan:** None. **J.A. Gerlt:** None.

Poster Board Number:

SUNDAY-709

Publishing Title:

Single Molecule Real Time (SMRT) Sequencing of the *Prevotella intermedia* Methylome to Overcome the Restriction-Modification Barrier Using a Host Mimicking Strategy

Author Block:

C. D. Johnston, S. R. Rittling; The Forsyth Inst., Cambridge, MA

Abstract Body:

Prevotella intermedia is a major oral pathogen associated with periodontitis, endodontic infections and oro-facial gangrene. Additionally, *P. intermedia* is increasingly found in association with respiratory infections such as cystic fibrosis, a progressive and frequently fatal genetic disease. We previously identified a new virulence mechanism utilized by *P. intermedia* capable of efficiently disabling and killing neutrophils allowing persistent infections to become established. However, there is currently no system available for genetic manipulation of *P. intermedia,* so in order to elucidate this mechanism we attempted to use functional plasmids from related bacterial species (*Bacteroides*/*Porphyromonas/Prevotella*) but none of these conferred antibiotic resistance. The failure of these plasmids despite compatible replicative machinery led us to consider that restriction-modification (R-M) systems, which allow bacteria to distinguish between their own DNA and any foreign DNA, were inhibiting transformation. We demonstrate that *P. intermedia* genomic DNA (gDNA) is highly resistant to restriction endonucleases that recognize the short commonly occurring sequence $^{5'}$-GATC-$^{3'}$ (*Sau*3AI, *Mbo*I, *Dpn*I, and *Dpn*II), suggesting the presence of an active R-M system specific for the cytosine residue of this sequence (GAT^{m-}C). We hypothesize that this and other R-M systems are the root cause of the transformation-barrier in *P. intermedia* and that circumvention of these R-M systems is a prerequisite for the development of its genetic system. Fortunately, an innovative technology, Single Molecule Real-Time (SMRT) sequencing, has recently become available which allows rapid and sensitive detection of each methylation site, with single-base resolution, across an entire bacterial genome (i.e. the bacterial methylome). Here we utilize SMRT sequencing to define the complete methylomes of *P. intermedia* ATCC25611 and the clinical isolate strain-17 to identify specifically the cognate recognition sequences used by each of their R-M systems. Methylome analysis and the subsequent use of a host-mimicking plasmid (codon optimized *de-novo* synthesized plasmid DNA lacking each recognition sequence) represents an entirely novel and innovative approach to developing a genetic system for *P. intermedia*.

Author Disclosure Block:

C.D. Johnston: None. **S.R. Rittling:** None.

Poster Board Number:

SUNDAY-710

Publishing Title:

The Isolation of DNA from the Oral Metagenome Using Entrapment Vectors

Author Block:

S. Tansirichaiya, N. Antou, P. Mullany, A. P. Roberts; UCL Eastman Dental Inst., London, United Kingdom

Abstract Body:

Antibiotic resistance genes are often associated with transposable elements (TEs). TEs are segments of DNA capable of movement within a genome. By detecting TEs, we may discover associated resistance genes.Entrapment vectors are plasmids capable of capturing TEs based on their movement. They have previously been used to capture TEs within single bacterial isolates. As more than 99% of bacteria are not yet culturable, we constructed entrapment vectors for the direct isolation of TEs from human oral metagenomic DNA. Therefore, TEs from both culturable and not yet culturable could theoretically be captured by this system.Three entrapment vectors were used, each differing in their characteristics. We designed a small insert-high copy number (pUCpAK) and a large insert-low copy (but inducible to high copy) number (BACpAK) vectors based on the pAK1 entrapment vector. These vectors contain a *cI-tetA* positive selection cartridge. The *cI* gene encodes a repressor that inhibits the expression of *tetA*. Therefore, if there is disruption of *cI* gene by insertion of TE, it will allow *tetA* to be expressed and result in a tetracycline resistant phenotype, providing positive selection for insertional inactivation by a TE.The oral metagenome was either directly transformed into *Escherichia coli* competent cells containing an entrapment vector, or a library constructed in the vectors followed by transformation into *E. coli* and selection on tetracycline-containing media. Most of the tetracycline resistant clones contain different types of background mutation in *cI-tetA* (point mutation, deletion, and duplication) and some showed the insertion of TEs from the chromosome of the *E. coli* host. We also found the insertion of *Veillonella parvula* DNA and three different human DNAs at the same position between *cI* and *tetA*. These insertions are not likely to be a result of classical transposition and might represent a new mechanism of DNA recombination.

Author Disclosure Block:

S. Tansirichaiya: None. **N. Antou:** None. **P. Mullany:** None. **A.P. Roberts:** None.

Poster Board Number:

SUNDAY-711

Publishing Title:

A Novel Method for a More Complete Microbial Community Profile

Author Block:

C. L. Wright, M. Band, M. A. Mikel, A. G. Hernandez; Univ. of Illinois, Urbana, IL

Abstract Body:

Utilizing next generation sequencing (NGS) on 16S amplicons, one can classify all bacteria in a defined location and compare that community across different time-points, locations, treatments, or other conditions. Here, we present a novel approach to this common method that leverages NGS with microfluidics PCR to allow simultaneous amplification of multiple samples with not just the 16S bacterial locus, but also eukaryotic, archaeal, fungal, as well as functional-gene specific targets. The procedure takes less than 1 week with output ready for NGS sequencing.DNA samples are quantified and diluted to 2 ng/µl. A master mix is prepared using the Roche High Fidelity Fast Start Kit and 20x Access Array (AA) loading reagent according to Fluidigm AA protocol. Master mix, 1 µl DNA and 1 µl Fluidigm Illumina barcoded linkers are added to each sample well of a Fluidigm 48.48 AA IFC. Selected primer pairs are aliquoted separately. The AX controller (Fluidigm) performs microfluidic loading of all primer/sample combinations in the IFC followed by amplification on the Fluidigm Biomark HD. Following amplification, harvested products are quantified and run on a Fragment Analyzer (Advanced Analytics, Ames, IA) to confirm sizes. Samples are pooled, gel-size selected, and again checked on an Agilent Bioanalyzer. Up to 1536 barcoded samples can be combined into one pool for sequencing.The pool is quantitated by qPCR, denatured and diluted to 6 pM and spiked with 15% non-indexed PhiX. Sequencing is done on the Illumina MiSeq or V2 Rapid HiSeq based on the number of samples and primer-sets within each pool to target at least 20k sequencing reads per sample per primer. The fragments are sequenced for 250nt from each end.Cluster density averages ~700k/mm2 with ~90% of clusters passing filter. Raw .bcl files are run through the Illumina bcl2fastq pipeline using a custom pipeline to generate data by primer set and by index so that final data is ready to be analyzed in QIIME, mothur, IM-tornado, or other analysis software packages.This novel microbial profiling system is a fast, cost-effective, multi-targeted metagenomic approach for whole microbial community analysis. Surveying the entire microbial community gives an enhanced understanding of the complex nature of these communities. Here we share details with the broader research community to help raise microbial community studies to the next level.

Author Disclosure Block:

C.L. Wright: None. **M. Band:** None. **M.A. Mikel:** None. **A.G. Hernandez:** None.

Poster Board Number:

SUNDAY-712

Publishing Title:

The Impact of DNA Extraction and Selected 16S Primers on the Results of Gut Microbiota Composition Analysis

Author Block:

A. Rintala[1], E. Munukka[1], S. Pietilä[2], E. Eerola[1], J-P. Pursiheimo[1], A. Laiho[2], P. Huovinen[1]; [1]Turku Univ., Turku, Finland, [2]Turku Ctr. for Biotechnology, Turku, Finland

Abstract Body:

Next generation sequencing (NGS) has recently become the most popular method for investigating the gut microbiota composition. As the number of available methods is extensive, the comparability of different study results is often not guaranteed. In this study, we analyzed the impact of DNA extraction and selected 16S primers on the gut microbiota NGS results.Bacterial DNA from four fresh stool specimens was extracted with five commercial DNA extraction kits: QIAamp DNA Stool Mini Kit (Qiagen), QIAamp Fast DNA Stool Mini Kit (Qiagen), PowerFecal DNA Isolation Kit (MO BIO), GXT Stool Extraction Kit (Hain Lifescience), and MagNA Pure 96 DNA and Viral NA Large Volume Kit (Roche). The microbial composition was analyzed with two distinct protocols, one detecting the V3-V4 and the other detecting the V4-V5 area of the bacterial 16S rRNA gene. The V3-V4 area was amplified by following the Illumina 16S Metagenomic Sequencing Library Preparation Quide, while the V4-V5 area was amplified with indexed primers modified from Caporaso *et al.* (2011). NGS was performed with Illumina Miseq system, and the data were analysed with QIIME 1.9 pipeline (Caporaso *et al.* 2010).The DNA yield was highest with GXT Stool Extraction Kit, but the quantity and quality of the DNA was sufficient for 16S library preparation with all tested methods. The number of recognized operational taxonomic units (OTUs) in NGS did not significantly differ between the extraction methods, and no significant differences were found in the abundances of different bacterial phyla. Yet, the abundance of certain bacterial genera varied between the extraction protocols. The V3-V4 and V4-V5 sequencing results differed significantly from each other. The total OTU count was clearly higher in V4-V5 sequencing, but the average diversity of the samples was higher in the V3-V4 sequencing. The abundance of all bacterial phyla and several genera varied significantly between the protocols. The results indicate that the influence of the DNA extraction on the gut microbiota NGS analysis is relatively low, while the primers targeting the 16S rRNA gene have a huge impact on the gained results.

Author Disclosure Block:

A. Rintala: None. **E. Munukka:** None. **S. Pietilä:** None. **E. Eerola:** None. **J. Pursiheimo:** None. **A. Laiho:** None. **P. Huovinen:** None.

Poster Board Number:

SUNDAY-713

Publishing Title:

Minimization of Chimera Formation and Substitution Errors in Full-Length 16s Pcr Amplification

Author Block:

S. Oh, R. Hall, L. Hon, **C. Heiner**; Pacific BioSci.s, Menlo Park, CA

Abstract Body:

The constituents and intra-communal interactions of microbial populations have garnered increasing interest in areas such as water remediation, agriculture and human health. Amplification and sequencing of the evolutionarily conserved 16S rRNA gene is an efficient method of profiling communities. Currently, most targeted amplification focuses on short, hypervariable regions of the 16S sequence. Distinguishing information not spanned by the targeted region is lost, and species-level classification is often not possible. PacBio SMRT® Sequencing easily spans the entire 1.5 kb 16S gene in a single read, producing highly accurate single-molecule sequences that can improve the identification of individual species in a metapopulation.However, this process still relies upon PCR amplification from a mixture of similar sequences, which may result in chimeras, or recombinant molecules, at rates upwards of 20%. These PCR artifacts make it difficult to identify novel species, and reduce the amount of informative sequences. We investigated multiple factors that may contribute to chimera formation, such as template damage, denaturation time before and during thermocycling, polymerase extension time, and reaction volume. We found two related factors that contribute to chimera formation: the amount of input template into the PCR reaction, and the number of PCR cycles.A second problem that can confound analysis is sequence errors generated during amplification and sequencing. With the updated algorithm for circular consensus sequencing (CCS2), single-molecule reads can be filtered to 99.99% predicted accuracy. Substitution errors in these highly filtered reads may be dominated by mis-incorporations during amplification. Sequence differences in full-length 16S amplicons from several commercial high-fidelity PCR kits were compared.We show results of our experiments and describe our optimized protocol for full-length 16S amplification for SMRT Sequencing. These optimizations have broader implications for other applications that use PCR amplification to phase variations across targeted regions and generate highly accurate reference sequences.

Author Disclosure Block:

S. Oh: None. **R. Hall:** None. **L. Hon:** None. **C. Heiner:** None.

Poster Board Number:

SUNDAY-714

Publishing Title:

Development of a Metagenomics-Based Tool for Detection of Pathogens on Environmental and Clinical Samples

Author Block:

J. C. Castro[1], L. M. Rodriguez[1], J. K. Hatt[1], M. Carter[2], K. T. Konstantinidis[1]; [1]Georgia Inst. of Technology, Atlanta, GA, [2]United States Depertment of Agriculture, Albany, CA

Abstract Body:

Accurate detection of pathogenic bacteria plays a key role in the prevention of outbreak and spread of disease. Fresh produce and clinical isolates are commonplace for microorganisms, and in these conditions is crucial to be able to determine pathogen presence, in order to diminish the associated risks in our food supply and clinical environments. We aimed to develop a tool that allows for the detection of bacteria in metagenomic samples. In order to do this, we first determined the regions of the genome that had significant matches in another species; these "noisy" regions were flagged and discarded. We then created different sets of *in*-silico metagenomes, with variable amounts of reads generated from the E. coli genome. This served us to create a null model for the presence and absence of E. coli in the metagenome. Our tool uses a logistic model to estimate the probability of presence; based on the percentage of the genome that is recovered (sequencing breadth) and the number of times each region is sequences (sequencing depth). To test our model, we inoculated spinach leaves with different cell concentrations of this strain (80 to $8*10^5$ cells), and sequenced the resulting samples using the Illumina MiSEQ platform. Thus we achieved a simple yet effective bioinformatics approach and the associated wet-lab protocol to detect pathogen species based on metagenomics. Our methodology can easily be extended t other pathogens that may be present in fresh produce or clinical samples.

Author Disclosure Block:

J.C. Castro: None. **L.M. Rodriguez:** None. **J.K. Hatt:** None. **M. Carter:** None. **K.T. Konstantinidis:** None.

Poster Board Number:

SUNDAY-715

Publishing Title:

An Effect Size Meta-Analysis across Microbiome Studies

Author Block:

P. Vangay, D. Knights, 55455; Univ. of Minnesota, Minneapolis, MN

Abstract Body:

Microbial communities are critical contributors to maintaining balance in a variety of environments, such as deep-sea water vents, nutrient-rich farm soils, and throughout the human body. Despite large differences in microbiome studies, similar metrics are used for hypothesis testing throughout microbial ecology, including those such as alpha diversity, beta diversity, taxonomic differences, and functional pathway differences. Although many studies have calculated effect sizes for the purpose of power analysis when designing studies, these results typically include only one or two datasets and are not commonly published. There currently has not been an in depth meta-analysis of effect sizes across a large variety of microbiome studies. We present an analysis of effect sizes based on several well-known and newly published datasets in the microbiome field, and also take into account how factors such as data transformations, data filtering, rarefaction, and choice of statistical test influence the effect size of different group comparisons within a dataset. With this paper, we have also included access to an open-source R package with a public web interface for users to directly interact with, enabling reproduction of our results, the addition of new datasets, and direct use for their own studies.

Author Disclosure Block:

P. Vangay: None. **D. Knights:** None.

Poster Board Number:

SUNDAY-716

Publishing Title:

Multiplexing Strategies for Microbial Whole Genome Smrt® Sequencing

Author Block:

C. Lambert, W. Lee, J. Harting, P. Baybayan; PacBio, Menlo Park, CA

Abstract Body:

The increased throughput of the PacBio® RS II and Sequel Systems enables multiple microbes to be sequenced on a single SMRT® Cell. This multiplexing can be readily achieved by incorporating a unique barcode for each microbe into the SMRTbell™ adapters after shearing genomic DNA using a stream-lined library construction process. Incorporating a barcode without the requirement for PCR amplification prevents the loss of epigenetic information (e.g., methylation signatures), and the generation of chimeric sequences, while the modified protocol eliminates the need to build several individual SMRTbell libraries.We multiplexed up to 8 unique strains of *H. pylori*. Each strain was sheared, and processed through adapter ligation in a single, addition only reaction. The barcoded strains were then pooled in equimolar quantities, and processed through the remainder of the library preparation and purification steps.We demonstrate successful *de novo* microbial assembly and epigenetic analysis from all multiplexes (2 through 8-plex) using standard tools within SMRT Link Analysis using data generated from a single SMRTbell library, run on a single SMRT Cell. This process facilitates the sequencing of multiple microbial genomes in a single day, greatly increasing throughput and reducing costs per genome assembly.

Author Disclosure Block:

C. Lambert: D. Employee; Self; PacBio. **W. Lee:** D. Employee; Self; PacBio. **J. Harting:** D. Employee; Self; PacBio. **P. Baybayan:** D. Employee; Self; PacBio.

Poster Board Number:

SUNDAY-717

Publishing Title:

Complete Microbial Community Representation by Next-Generation Sequencing

Author Block:

M. Hymes, K. Cunningham; Swift BioSci.s, Inc., Ann Arbor, MI

Abstract Body:

Next generation sequencing (NGS) provides a powerful tool for metagenomics, however, multiple methods and are required in order to identify both single-stranded (ssDNA) and double-stranded DNA (dsDNA) organisms from a single sample. Here, we address a new method for NGS library preparation which utilizes a single preparation to analyze all microbial members in a metagenomic sample.In order to demonstrate the potential of capturing DNA from both ssDNA and dsDNA in a single NGS library preparation, we created a simplified phage community model comprised of ssDNA phage, PhiX174 and M13 mixed with dsDNA phage. A total of 10 different phage were pooled together in varying ratios across multiple samples in order to understand the impact of combining these organisms. In all cases, the abundance level as detected by NGS analysis aligned within 10% of the theoretical inputs. In a separate evaluation, harsh extraction methods of DNA were tested to determine the effects of the library preparation method on difficult to extract genomes. Two species, *Eggerthella sp* HPP0013 and *Facklamia sp.* HGF4, were subjected separately to either bead beating or NaOH boiling for DNA extraction followed by a ssDNA NGS library preparation. The *Eggerthella* samples produced comparable results with either method while the *Facklamia* samples provided a 30x increase in yield from using NaOH boiling for extraction. The experiment was repeated with a dsDNA library preparation method and resulted yields from the bead beating samples but not the NaOH boiled samples due to denaturing of DNA. The results of these studies demonstrate that the method used to extract and prepare the DNA library for sequencing influences the genomes which will be represented in the sample and must be carefully considered when designing NGS based experiments.

Author Disclosure Block:

M. Hymes: D. Employee; Self; Swift Biosciences, Inc. **K. Cunningham:** D. Employee; Self; Swift Biosciences Inc.

Poster Board Number:

SUNDAY-718

Publishing Title:

Profiling of Metagenomic Communities with Long Reads

Author Block:

A. Hernandez, C. Wright; Univ. of Illinois, Urbana, IL

Abstract Body:

Background: Next-generation DNA sequencing has allowed massive gains in microbial studies. Assembly of individual genomes and metagenomes and the characterization of metabolic pathways is an important step for elucidating the structure and function of these communities and in evaluating how they change in response to physiological, pathological or environmental conditions. However, this is still a daunting task using short-read sequencing technologies.The aim of this project is the optimization of tools for assembling and profiling metagenomic communities in a timely and cost-effective manner. In this regard, we have beta tested and optimized a novel application that produces long sequence reads with high coverage from short Illumina reads. These *in-silico* long reads enable the accurate reconstruction of individual genomes allowing more comprehensive profiling of the strains that compose the community as well as characterization of the genes present. The application, known as TruSeq Synthetic Long Reads (Illumina, CA) is the only existing method for production of contiguous long reads with very low error rate.**Methods:**To start, 500ng of DNA from a metagenomic community were sheared to an average size of 8kb with a g-Tube (Covaris). The fragments were blunt-ended, 3'-end A tailed, ligated to adaptors, size selected on a 0.8% agarose gel and quantitated by qPCR. 1,500fg of fragments were robotically dispensed on a 384-well plate, which results in ~ 300 fragments per well. Fragments were amplified with long-range PCR, tagmented and amplified with one barcoded primer per well. The products were pooled, quantitated by qPCR and sequenced on one lane on a HiSeq 2500 with paired-reads 150nt in length. The reads were assembled with the TSLR application in the BaseSpace cloud environment (Illumina).**Results:**The sequencing produced over 288 million reads (43 Gbases), which resulted in 541 Mbases of assembled reads with an N50 read length of 7.8kb and a distribution from 1.4kb to 11kb.**Conclusions:**Utilizing the TSLR library preparation method produced long reads up to 11kb in length with very high depth of short reads with no associated bioinformatics cost or labor. Efforts are underway to integrate these reads into assemblies done from shotgun and mate-pair library sequencing. We present our work on this novel application to help move this field forward and enhance researcher efforts in metagenomic studies.

Author Disclosure Block:

A. Hernandez: None. **C. Wright:** None.

Poster Board Number:

SUNDAY-719

Publishing Title:

Workflow for Processing High-throughput, Single Molecule, Real-time Sequencing Data, for Analyzing the Microbiome of Patients Undergoing Fecal Microbiota Transplantation

Author Block:

R. Hall[1], C. Heiner[1], S. Oh[1], C. Staley[2], M. Hamilton[2], A. Khoruts[3], C. Kelly[4], L. Brandt[5], M. Sadowsky[3]; [1]Pacific BioSci.s, Menlo Park, CA, [2]BioTechnology Inst., St Paul, MN, [3]Univ. of Minnesota, St Paul, MN, [4]The Warren Alpert Med. Sch. of Brown Univ., Providence, RI, [5]Albert Einstein Coll. of Med., New York, CA

Abstract Body:

There are many sequencing-based approaches to understanding complex metagenomic communities, the most common employing either targeted amplification (e.g., multiple 16S regions) or whole-sample shotgun sequencing. While targeted approaches provide valuable data at low sequencing depth, they are limited by primer design and PCR. Traditionally, whole-sample shotgun experiments have relied on high-throughput, short-read sequencing, which results in data processing difficulties. For example, reads less than 500 bp in length will rarely cover a complete gene or region of interest, and will require assembly. In complex communities comprised of many similar organisms, this not only introduces the possibility of incorrectly combining sequence from different community members, it requires a high depth of coverage. As such, rare community members may not be represented in the resulting assembly. Circular-consensus, single molecule, real-time (SMRT®) Sequencing reads in the 1-3 kb range, with >99% accuracy can be efficiently generated. For reads >1 kb at >99% accuracy, it is reasonable to expect a high percentage to include gene fragments useful for analysis without the need for *de novo* assembly. Recent improvements in instrumentation in the Sequel System, have resulted in a 7x increase in the data generated per run compared with the previous generation PacBio RS II. Samples from a blinded, placebo-controlled trial have been sequenced on the Sequel System and include those from pre-FMT patients, post-placebo patients at 2 and 8 weeks, post-open-label patients at 2 and 8 weeks, and the donor control. We demonstrate that our workflow allows a high resolution analysis of the differences between microbial communities, at the level of both abundance and function.

Author Disclosure Block:

R. Hall: D. Employee; Self; Pacific Biosciences. K. Shareholder (excluding diversitied mutual funds); Self; Pacific Biosciences. **C. Heiner:** D. Employee; Self; Pacific Biosciences. K. Shareholder (excluding diversitied mutual funds); Self; Pacific Biosciences. **S. Oh:** D. Employee; Self; Pacific Biosciences. K. Shareholder (excluding diversitied mutual funds); Self;

Pacific Biosciences. **C. Staley:** None. **M. Hamilton:** None. **A. Khoruts:** None. **C. Kelly:** None. **L. Brandt:** None. **M. Sadowsky:** None.

Poster Board Number:

SUNDAY-720

Publishing Title:

Vgrg and Paar Proteins Define Distinct Versions of a Functional Type VI Secretion System

Author Block:

F. R. Cianfanelli, M. Guo, M. Trost, S. J. Coulthurst; Univ. of Dundee, Dundee, United Kingdom

Abstract Body:

The Type VI secretion system (T6SS) is a macromolecular machinery, widespread in Gram negative bacteria, which is used to deliver toxic proteins ("effectors") into target eukaryotic or prokaryotic cells. As a result, this system plays a crucial role both in direct pathogenicity and in interbacterial interactions, including polymicrobial infections. T6SS are encoded within large gene clusters containing, at least, fourteen conserved core components. These form a large trans-envelope machinery including an expelled puncturing device consisting of a tube formed by hexameric rings of Hcp, topped by a VgrG trimer and further sharpened by a PAAR-repeat protein. Anti-bacterial T6SS can deliver a variety of effectors targeting different compartments of the recipient cell, such as the peptidoglycan cell wall, the cell membrane and nucleic acid. Current models suggest that a variety of effectors can be injected in one single lethal shot, each either non-covalently associated or directly fused with one of the proteins forming the puncturing device. *Serratia marcescens* is an opportunistic pathogen that possesses a potent anti-bacterial T6SS. This T6SS is encoded by a large cluster in addition to several other loci encoding non-essential components and multiple effectors. Two different VgrG homologues and three PAAR-repeat proteins are encoded in the genome of *S. marcescens* Db10. Here, we used a combination of genetic, proteomic and phenotypic approaches to determine the role of the two VgrG and the three PAAR-repeat proteins in the functionality of the T6SS. We identified specific VgrG-PAAR combinations able to mediate active secretion and displaying different anti-bacterial activity, and we further showed that this latter difference could be due to delivery of specific subsets of antibacterial effectors.

Author Disclosure Block:

F.R. Cianfanelli: None. **M. Guo:** None. **M. Trost:** None. **S.J. Coulthurst:** None.

Poster Board Number:

SUNDAY-722

Publishing Title:

Transformation of *Chlamydia trachomatis* with a Plasmid Containing Epitope-tagged Translocator and Chaperone Protein

Author Block:

D. Bulir, S. Liang, J. B. Mahony; McMaster Univ., Hamilton, ON, Canada

Abstract Body:

Background: *Chlamydia trachomatis* is the leading cause of preventable blindness worldwide, and the most common bacterial sexually transmitted infection in North America. Undiagnosed infections can lead to pelvic inflammatory disease (PID) and infertility. *C. trachomatis* is believed to utilize a type III secretion system (T3SS) to infect the host cell. The translocator proteins of the T3SS, *Chlamydia* outer protein (Cop) B and D are believed to be essential for infection of host cells. To examine the possible function of the translocator proteins, epitope-tagged translocator proteins and their putative chaperone, LcrH_1, were transformed into a plasmidless strain of *C. trachomatis* using the recently developed transformation system of *C. trachomatis*.**Methods:** Using overlapping PCR, restriction sites and epitope tags were added to LcrH_1, CopB, and CopD for cloning into the shuttle vector pGFP::SW2, generating pGFP-LBD. Prior to transformation into *C. trachomatis*, protein expression was first confirmed in E. Coli. Transformation of *C. trachomatis* L2 was performed as described by Barta *et. al.* (2015). Immunofluorescence and western blot analysis was performed to examine the production, and localization of the epitope-tagged proteins throughout the chlamydial life cycle.**Results:** Cloning into pGFP::SW2 produced a plasmid containing 6xHis-LcrH_1, 3xFLAG-CopB, HA-CopD, as well as the native transcription start site for the operon containing LcrH_1, CopB, CopD. Using western blot analysis, expression of the tagged proteins were confirmed in *E. coli*. We successfully transformed *C. trachomatis* L2 with the pGFP-LBD vector, as western blot and immunofluorescence confirmed the expression of the epitope-tagged translocator and chaperone proteins.**Conclusion:** *Chlamydiae* are obligate intracellular pathogens requiring the T3SS to infect the host. The translocator proteins are believed to be an essential component of the infection machinery, yet, there is limited information regarding the localization and function of the translocators within an infected cell. In the absence of techniques to knock genes out and propagate *Chlamydia* in the absence of cells, transformation of *Chlamydia* with epitope-tagged fusion proteins allows for better understanding of the function of chlamydial proteins in the life cycle of *C. trachomatis* infections.

Author Disclosure Block:

D. Bulir: None. **S. Liang:** None. **J.B. Mahony:** None.

Poster Board Number:

SUNDAY-723

Publishing Title:

The C-Terminus of the *Staphylococcus aureus* Esat-6 Virulence Component Esxc Is a Calcium-Dependent Homodimerization Domain

Author Block:

R. M. Abd El-Fatah[1], N. M. Mesbah[2], D. M. Abo-elmatty[2], K. A. Aly[1]; [1]Sinai Univ., North Sinai, Egypt, [2]Suez Canal Univ., Ismailia, Egypt

Abstract Body:

The human pathogen *Staphylococcus aureus* poses a serious threat to the global healthcare system. It recruits a specialized pathway, termed the ESAT-6 Secretion System (Ess), for full virulence. The Ess pathway is a membrane-spanning complex that coordinates the secretion of 4 non-canonical substrates: EsxA, EsxB, EsxC and EsxD into the extracellular environment. EsxC is a 17 kDa homodimer that lacks a classical topogenic sequence. Previous studies have shown that EsxC is required for the secretion of all 4 substrates, thereby revealing its biomedical importance. Here, a modified bacterial two-hybrid screen (BTH) shows that EsxC homodimerization is abolished in the presence of EDTA. Homodimer restoration was tried using several cations, and was found to be successful in the presence of the divalent calcium, suggesting that EsxC dimerization is calcium-dependent. To gain insights into the nature of EsxC homodimerization, N- and C-terminal truncations were constructed in blocks of 5 amino acids each, followed by BTH analysis of the EsxC homodimerization pattern. Results show that the last 5 amino acids of EsxC are required for self-association. To further validate the BTH data, N-terminal 6x-His-tagged wild-type (WT) EsxC and an EsxC variant lacking the last 5 amino acids (EsxCΔ5C) were purified by immobilized metal affinity chromatography (IMAC) over an FPLC-operated HisTrap column. Ultrapure proteins were subjected to chemical crosslinking using the homobifunctional crosslinker disuccinimidyl suberate. SDS-PAGE of the cross-linked WT EsxC shows monomeric and dimeric bands at 17 kDa and 34 kDa, respectively. In contrast, EsxCΔ5C revealed only a monomeric signal, validating the importance of the EsxC C-terminus in homodimerization. We are currently studying the role of the calcium-dependent C-terminal homodimerization domain of EsxC in Ess substrate secretion, the outcome of which will have a profound impact in the areas of microbial biochemistry and drug discovery.

Author Disclosure Block:

R.M. Abd El-Fatah: None. **N.M. Mesbah:** None. **D.M. Abo-elmatty:** None. **K.A. Aly:** None.

Poster Board Number:

SUNDAY-725

Publishing Title:

Identification of the Regulators of the Type Vi Secretion System *In Vibrio Harveyi*

Author Block:

S. G. Olney, J. van Kessel; Indiana Univ., Bloomington, IN

Abstract Body:

Quorum sensing is a bacterial communication mechanism that controls the switch between individual and group behaviors in response to changes in cell density. Quorum sensing enables bacteria to synchronously alter expression of genes that produce many different behaviors such as biofilm formation, bioluminescence, and virulence factor production. The type VI secretion system (T6SS) in *Vibrio harveyi* is controlled by the master regulator LuxR. T6SS is a virulence factor and defense mechanism that delivers effector proteins via an injector complex to both bacterial and phagocytic eukaryotic cells. In *V. harveyi*, LuxR binds to the three T6SS promoter regions to directly activate transcription of five gene operons. However, recent work has suggested that other proteins likely work together with LuxR to drive transcription activation. Thus our goals were to (1) identify other novel regulators of this system and (2) examine the link between T6SS and the quorum sensing regulation pathway. A reporter plasmid was constructed with the T6SS promoter and downstream gene *tssD* transcriptionally fused to *lacZ* (P_{tssD}-*tssD*-*lacZ*) to measure expression. We generated a transposon library in wild-type *V. harveyi*, and the reporter plasmid was subsequently introduced into this library. We screened for mutants with either higher or lower levels of β-galactosidase activity compared to wild-type *V. harveyi*. We identified several novel regulators which will be further characterized to elucidate their roles in regulation of the T6SS genes.

Author Disclosure Block:

S.G. Olney: None. **J. van Kessel:** None.

Poster Board Number:

SUNDAY-727

Publishing Title:

Differential Regulation and Functional Redundancy of *esxb* Duplications in *M. marinum*

Author Block:

R. E. Bosserman, M. M. Champion, P. A. Champion; Univ. of Notre Dame, Notre Dame, IN

Abstract Body:

Mycobacterium marinum is a widely used model organism for studying Esx-1 (ESAT-6 System 1), a protein export system required for virulence in pathogenic mycobacteria. Two major substrates of the Esx-1 system are EsxB and EsxA, a pair of tightly interacting proteins. EsxB and EsxA are encoded by *esxBA* within the *esx-1* locus. Interestingly, the *M. marinum* genome has two duplications of *esxBA* outside of the *esx-1* locus. *esx* gene duplications are widespread in *M. marinum* and *M. tuberculosis (M. tb)* genomes, but the role of these genes remain unknown. This study examines an *esxBA* duplication in *M. marinum,* which is not conserved in *M. tb,* and its involvement with the Esx-1 system. The duplicate locus encodes for an EsxB paralog, EsxB_1, which is 100% identical to EsxB at the amino acid level. Because EsxB_1 is identical to a major virulence factor in mycobacteria, we sought to understand why *M. marinum* has two *esxB* genes and whether EsxB_1 contributes to Esx-1-mediated virulence. To study this locus, we created a clean deletion of *esxB_1* and employed molecular techniques, genetics, and mass spectrometry. We found that the *esxB_1* gene is transcribed and translated however at levels lower than the *esxB* locus. Deletion of *esxB* results in a loss of Esx-1-mediated export and virulence despite the presence of an intact *esxB_1* locus. Although deletion of *esxB* abrogates Esx-1 function, we found that deletion of *esxB_1* does not, indicating distinct roles. Yet, our data show that over-expression of the *esxB_1* locus can functionally complement Esx-1 function in the Δ*esxBA* strain. These data show that the two loci can substitute for each other when regulation is decoupled. We therefore hypothesize that the two loci are differentially regulated. Future work will focus on transcriptional regulation of the *esxB_1* locus. We conclude that EsxB_1 and its putative partner EsxA_1 can be secreted by Esx-1 in *M. marinum*. Moreover, the *esxB_1* locus is functionally redundant to the Esx-1 locus, *esxBA*, when over-expressed. However, the *esxBA* locus, not *esxB_1*, is the primary contributor to Esx-1 function and virulence. This study shows that by considering *esx*- gene duplications, Esx-1 secretion may be more complex than previously thought.

Author Disclosure Block:

R.E. Bosserman: None. **M.M. Champion:** None. **P.A. Champion:** None.

Poster Board Number:

SUNDAY-729

Publishing Title:

The Conjugative Relaxase Trwc Delivers DNA into Human Cells and Promotes Its Integration in the Human Genome

Author Block:

c. gonzalez, M. Llosa; Univ. of Cantabria, Santander, Spain

Abstract Body:

Bacterial conjugation consists on the transfer of relaxase-DNA complexes between bacteria through a Type IV secretion system (T4SS). These systems stand out for their plasticity in terms of translocated substrates (proteins and/or DNA) and secretion target (prokaryotic or eukaryotic cells). We exploited this versatility to secrete a conjugative T4SS substrate through a T4SS required for effector translocation into human cells, resulting in efficient translocation of the relaxase-DNA into human cells through the VirB/D4 T4SS of *Bartonella henselae* (Fernández-González *et al*., 2011). This accomplishment suggests that natural transkingdom DNA transfer may occur through T4SS of human pathogens (Llosa *et al.*, 2012).The DNA introduced in the human cell is led by TrwC, the conjugative relaxase of plasmid R388, responsible for DNA processing during bacterial conjugation. Apart from its role in conjugation, TrwC is also a site-specific integrase being able to catalyse integration of the mobilized DNA into a copy of its target sequence (*oriT*) present in the recipient cell (Agúndez *et al*., 2012). We have characterized integration of TrwC-driven DNA molecules into the recipient genome when a target oriT copy is not available, both in bacterial and in human genomes.We have selected stable integration events of the mobilized DNA into the human genome. TrwC was found to facilitate such integration by more than 3 logs. We have characterized the integration pattern mediated by TrwC using linear amplification-mediated PCR. We have identified at least one site-specific integration event mediated by TrwC, implying TrwC is active in human cells; however, the vast majority of integrants represent random integration events of recircularized plasmid. Our results suggest that TrwC stabilizes the incoming DNA molecules, probably by protecting both ends, favouring their long-term presence and subsequent genomic integration through host-mediated mechanisms. Due to that, TrwC could be used in conjunction with a site-specific nuclease to accomplish targeted genomic modification of human cells.Agúndez, L., González-Prieto, C., Machón, C. & Llosa, M. 2012. PLOS One 7, e31047.Fernández-González, E., de Paz, H. D., Alperi, A., Agúndez, L., Faustmann, M., Sangari, F., Dehio, C. and Llosa, M. 2011. J Bacteriol 193, 6257-6265.Llosa, M., Schroder, G. & Dehio, C. 2012. Trends in Microbiology 20, 355-359.

Author Disclosure Block:

C. gonzalez: None. **M. Llosa:** None.

Poster Board Number:

SUNDAY-730

Publishing Title:

Characterization of a Spore-associated Protein to Study Assembly of *Streptomyces* Spores

Author Block:

Z. J. Resko, M. Kocher, C. Moore, J. R. McCormick; Duquesne Univ., Pittsburgh, PA

Abstract Body:

Streptomyces coelicolor is a model non-pathogenic, filamentous bacterium that produces chains of spores from aerial hyphae. Linear plasmid SCP1 contains *sapC*, *sapE*, and *sapD*, which are genes that code for spore-associated proteins. Spore-associated protein SapC (17 kDa, no putative conserved domains) does not contain a normal secretion signal nor is the mechanism of secretion known. Sap proteins were identified because they can be extracted from the spore surface using a non-lethal detergent wash and analyzed via SDS-PAGE, Coomassie blue staining and/or by Western Blot. In our preliminary studies, our strategy to study secretion was to use recombineering to fuse a passenger protein to the C-terminus of a spore-coat protein, SapC. We chose a passenger protein that we could detect with an antibody, the β subunit of the *E. coli* heat labile toxin (LTB). Results characterizing this fusion suggest that SapC-LTB was secreted and assembled on the spore surface. A current goal of this ongoing project is to analyze several constructed C-terminal truncations of SapC (SapC'-LTB) to locate the secretion and/or spore surface assembly signals of SapC. To further characterize SapC protein secretion, an N-terminal fusion of LTB to SapC was also constructed and LTB-SapC secretion is being analyzed. Finally, N-terminal truncations of SapC (LTB-`SapC) are being constructed to complement the C-terminal truncation study to potentially locate and understand the nature of the secretion signal. In the future, the use of Sap protein fusions to passenger proteins might lead to an additional method for vaccine delivery by assembling recombinant *Streptomyces* spores with epitopes displayed on the spore surface.

Author Disclosure Block:

Z.J. Resko: None. **M. Kocher:** None. **C. Moore:** None. **J.R. McCormick:** None.

Poster Board Number:

SUNDAY-731

Publishing Title:

Cooperation of Two Key Virulence Factors in Bordetella

Author Block:

Z. Nash, P. Cotter; Univ. of North Carolina - Chapel Hill, Chapel Hill, NC

Abstract Body:

Pertussis is a human respiratory illness caused by the Gram-negative bacterium *Bordetella pertussis*. Two main virulence factors of *B. pertussis* are filamentous hemagglutinin (FHA) and adenylate cyclase toxin (ACT). FHA is an adhesin and immunomodulator formed from FhaB after regulated cleavage of its C-terminal prodomain (PD). Before removal from FhaB, the PD appears to function in protein folding and prevention of bacterial clearance by the immune system. Bacteria lacking the extreme C-terminus of the PD rapidly convert all FhaB to FHA and can adhere *in vivo* but cannot persist. Alternatively, processing of FhaB that lacks the PD N-terminus results in FHA with a misfolded and non-functional mature C-terminal domain (MCD), the domain necessary for bacterial adherence to host cells. Recent work has shown that the MCD can bind purified ACT, a toxin that inserts into membranes of phagocytes to create pores and inhibit host signaling. Additionally, *fhaB*-null cultures have reduced bacteria-localized adenylate cyclase activity in comparison to wild type. Instead, activity is mostly detected in the supernatant, suggesting that FHA promotes retention of ACT to the bacterial surface. Because FHA is an adhesin with a role in immunomodulation and ACT requires bacteria-host adherence to transfer the toxin, this implies a role for ACT-FHA association in delivery of ACT to host cells *in vivo*.We sought to explore ACT and FHA *in vitro* localization in wild type and *fhaB*-null bacteria using immunoblotting techniques. We initially degraded bacterial surface proteins with Proteinase K then examined ACT and FHA production and localization over time. We found minimal membrane-localized ACT in *fhaB*-null bacteria. Instead, most of the ACT was located in supernatant. Additionally, the appearance of supernatant-localized ACT was delayed in wild type bacteria, occurring only after membrane-bound FHA was saturated with bound ACT. These data indicate that ACT preferentially binds to FHA following export instead of releasing into the supernatant. We propose a model in which ACT binds to the MCD of FHA in order to gain proximity to the host cell. This suggests that two well-studied virulence factors work together to enhance pathogenesis.

Author Disclosure Block:

Z. Nash: None. **P. Cotter:** None.

Poster Board Number:

SUNDAY-732

Publishing Title:

Mapping the Sorting Signals of the Cytoplasmic Domain of Atg27

Author Block:

M. A. Trujillo; High Point Univ., High Point, NC

Abstract Body:

Proteins perform different cellular functions such as structural support and transport of solutes. It is essential that these proteins be delivered to where they are needed. Membrane trafficking is an important process that allows for the distribution of specific macromolecules, such as proteins, throughout the cell. Protein transport is carried out with the help of sorting signals, which are short stretches of amino acids that mediate the transport of proteins to their designated compartment or organelle. Autophagy is a specialized type of membrane trafficking during which unneeded or damaged cytoplasmic components are taken to the degradative organelle of the cell for recycling. Irregularities in autophagy can lead to human diseases like cancer and neurodegenerative disease. In addition, certain pathogenic bacteria are known to escape targeting by harnessing autophagy. Because autophagy is highly conserved in eukaryotes, the eukaryotic microbe *Saccharomyces cerevisiae* (baker's yeast) has been a great model system to better understand autophagy and its connection to human diseases. One of the key trafficked proteins in autophagy is Atg27, a membrane protein with a single cytoplasmic domain. In order to map the sorting signals of this protein, we have generated multiple fluorescent Atg27 cytoplasmic domain deletion mutants. Our current work is focused on dissecting the effects of these mutations on Atg27 localization and function during autophagy.

Author Disclosure Block:

M.A. Trujillo: None.

Poster Board Number:

SUNDAY-733

Publishing Title:

Development of Vacuole Targeted-delivery System by Signal Peptide Sequence of Lysosomal Proteins in Yeast

Author Block:

D. Park, Y-H. Kim, J. Min; Chonbuk Natl. Univ., Jeonju, Korea, Republic of

Abstract Body:

All of the proteins consists one or more long chains of amino acid residues with signal peptide sequence. They have different delivery mechanism according to bind what kinds of signal sequence. It is important to delivery proteins to the organelles in eukaryotes for mass transfer. In our study we focused on vacuole which consists single membrane and have several of hydrolytic enzymes to discompose macromolecule from the outside of the cells in yeast. Those vacuole proteins also have signal peptide sequence in protein residue. We tried to transport cytosolic protein to vacuole by developing two type of vacuole targeted-delivery vector using signal sequence of vacuole proteins, respectably. First type of signal peptide exists in front of vacuole proteins and other type is in the middle of the protein. Thus our result indicated that development of vacuole targeted delivery system is useful tool that specific proteins are able to transport organelles by signal peptide sequence in eukaryotic cells. This work was carried out with the support of "Cooperative Research Program for agriculture Science & Technology Development (Project title: Development of Target-specific Antimicrobial and Neutralizing Agents for Livestock Biological Hazardous Factors, Project No:PJ01052701)' Rural Development Administration, Republic of Korea. The authors are grateful for their support.

Author Disclosure Block:

D. Park: None. **Y. Kim:** None. **J. Min:** None.

Poster Board Number:

SUNDAY-734

Publishing Title:

Who Will Be My Next Host? New Approach to Determine Possible Hosts of Unknown Mobile Plasmids

Author Block:

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Abstract Body:

Increased incidence of antimicrobial resistance (AMR) in bacteria has raised global awareness and will continue to do so, due to the decreased rate of introduction of new antibiotics. One approach to treat AMR infections is to use the most appropriate antibiotic combinations that inhibit the transfer of AMR genes, since inappropriate treatments increase AMR incidence. In human pathogens, AMR genes spread frequently by transfer of mobile conjugative plasmids from the large environmental AMR pool. Each plasmid can be hosted in a particular repertoire of appropriate bacterial hosts, characterized by the plasmid's MOB group. Currently to determine the MOB group, a mobile plasmid must be sequenced, the sequence of its relaxase must be determined and conjugation experiments must performed. We have presently considered the following. The most important components for plasmid transfer are oriT regions and conjugative relaxases. The oriT site is a substrate for the relaxase, which nicks the DNA and initiates transfer. Since each relaxase is specific to one type of oriT, DNA in each oriT region must have specific physicochemical properties that most efficiently attract and enable activity of the particular relaxase. We thus developed a bioinformatic framework, incorporating prediction of DNA physicochemical properties of oriT regions according to current models and machine learning algorithms. Based on our computational analysis of physicochemical properties of oriT regions, we were able to successfully sort 64 mobile plasmids into their corresponding MOB groups (classification accuracy of 99%). By using this method we only needed to obtain the identity of a DNA sequence up to 240 bp long, with which the most probable hosts can be determined. In the future, this approach will help clinicians determine the right antibiotic treatment based on the present mobile elements and their host ranges, as well as develop new approaches for inhibition of horizontal AMR gene transfer within polymicrobial infections.

Author Disclosure Block:

J. Zrimec: None. **T. Rijavec:** None. **A. Lapanje:** None.

Poster Board Number:

SUNDAY-735

Publishing Title:

Toxin-Antitoxin Systems Inside the Antibiotic Resistance Plasmid World

Author Block:

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Abstract Body:

Plasmids are key players in the current antibiotic resistance (AbR) emergency, especially within Enterobacteriaceae. They have the ability to acquire and spread either single or multiple AbR genes, which has become a problem and an obstacle to successful therapeutic treatment. The factors that allow certain plasmid types to maintain AbR genes, to remain stable in bacterial populations and to successfully spread to different bacterial strains remain unclear. However, successful stability has been attributed to the presence of toxin-antitoxin (TA) systems (along with multimer resolution systems and partition systems) and their role in plasmid maintenance. TA systems are small genetic elements composed of a toxin gene and its cognate antitoxin and are both in bacterial plasmids and chromosomes. They are diverse both in the nature of their constituents (protein or RNA) and attributed functions. Historically, a role in plasmid maintenance has been attributed to plasmidial TA systems, but there are indications that their role could be more complex. The real contribution and the diversity of TA systems to the success of AbR dissemination have not yet been fully explored, and it is not known whether specific TA systems are associated with any particular type of plasmid. Our main aim is to elucidate the diversity of TA systems encoded in AbR plasmids in *E. coli* and *K. pneumoniae*, and specifically to characterize a novel putative TA system encoded on an IncX4 plasmid. Using bioinformatic tools a novel putative TA system was identified in pJIE143, an IncX4 plasmid carrying $bla_{\text{CTX-M-15}}$ from an *E. coli* ST131 isolate. We analyzed its genetic and molecular characteristics and the effect of its expression on *E. coli* growth, as well as its contribution to plasmid maintenance. Bioinformatic analysis revealed that it is exclusively present in IncX plasmids. According to bacterial two-hybrid and co-purification assays, the toxin and the antitoxin do not form a protein complex. Toxin overexpression affected the density of *E. coli* cultures and induced cell elongation, which was associated with diminish FtsZ levels. Overall, our results show that AbR plasmids could contain an underappreciated diversity of TA systems, some of them with novel characteristics, toxin targets and functions.

Author Disclosure Block:

P. Bustamante: None. **J. Iredell:** None.

Poster Board Number:

SUNDAY-736

Publishing Title:

High Level of Resistance to Ciprofloxacin Conferred by a Plasmid Carrying a *qnrS1* Gene from an *Escherichia coli* Clinical Isolate

Author Block:

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Abstract Body:

It has been described that QnrS1 confers a low level of resistance to fluoroquinolones, and is able to increase 12.3-fold the MIC of ciprofloxacin, and 24- to 49-fold under induced expression.[1] We studied the ciprofloxacin resistance conferred by plasmids carrying *qnr* genes, extracted from *Enterobacteriaceae* strains that had been isolated from urine samples of patients in an Italian hospital.Twenty clinical isolates of *E. coli* and 15 of *Proteus mirabilis* were studied. The presence of *qnr* (A, B, C, D, S) and *aac(6´)-Ib-cr* genes was assessed by PCR and sequencing. The antibiotic MICs were determined by the microdilution method. The resistance conferred by plasmids carrying *qnr* genes was determined by conjugation assays, using *E. coli* J53 rifampicin-resistant as recipient strain. The transcript levels of *qnr* genes were studied by reverse transcription of RNA.The MIC50, MIC90 and Range of ciprofloxacin MICs in the 35 studied strains were respectively 2 µg/ml, 32 µg/ml and 0.625 - 128 µg/ml. Plasmids with *qnr* genes were only detected in eight *E. coli* strains, 6 *qnrS1*-positive, 1 *qnrB1*-positive and a plasmid with *qnrS1* and *qnrB1* genes. No other type of *qnr* gene was detected. The *qnrS1* and *qnrB1*- positive plasmid increased 32-fold ciprofloxacin MIC in J53 strain. Furthermore, the increase in ciprofloxacin MICs in the recipient strain that were conferred by either *qnrS1*-positive or *qnrB1*-positive plasmids ranged 16- to 32-fold, with the exception of the plasmid 27-MD that only carried *qnrS1* and increased 128-fold the ciprofloxacin MIC in J53 strain (from 1 to 128 µg/ml). The ciprofloxacin MIC in the donor strain 27-MD was 32 µg/ml and the plasmid 27-MD that was extracted from the same strain, was not found to carry *aac(6´)-Ib-cr* gene. In addition, the transcript level of *qnrS1* in the plasmid 27-MD in the transconjugant was 15- to 21-fold higher than those of the *qnrS1* genes in the remaining plasmids when they were expressed in J53 strain.The increased transcript level of *qnrS1* gene in a native *E. coli* plasmid was responsible for a high level of resistance to ciprofloxacin in a susceptible recipient strain.

Author Disclosure Block:

I. Anacarso: None. **M.M. Tavío:** None.

Poster Board Number:

SUNDAY-737

Publishing Title:

IncA/C_2 plasmids Act as Suppressor of Cyclic-Di-GMP Dependent Biofilm Formation in S*almonella enterica* Serovar Typhimurium

Author Block:

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Abstract Body:

Background: Conjugative plasmids of the A/C incompatibility group (IncA/C) are well known for spreading multidrug resistance among pathogenic *Enterobacteriaceae*. Bacterial biofilm formation has been broadly studied in *S.*Typhimurium (ST). While conjugative plasmids have often been correlated with enhanced biofilm production, the influence of IncA/C plasmids on biofilm formation is still unclear. Here we report that the type 2 IncA/C_2 plasmid pVCR94 subdues the biofilm formation in ST by depleting the cellular secondary messenger c-di-GMP.**Methods:** ST SR11 was used as the wildtype strain. For biofilm analyses in solid/liquid state, ST was grown in saltless Luria agar or broth (LB) at 28ºC & followed for 3 days. For red dry and rough (*rdar*) morphotype and cellulose detection, Luria agar was supplemented with congo red and calcofluor, respectively. Biofilms on liquid-air interphase from static LB culture were stained with crystal violet to examine the adherent cellulose on glass tubes.**Results:** On saltless Luria agar, pVCR94-bearing ST produced a brown dry and rough (*bdar*) morphotype distinct from the wildtype strain which produced a *rdar* morphotype. On calcofluor plates, the presence of pVCR94 resulted in weak cellulose production when analyzed under UV light. Crystal violet staining revealed that pVCR94-bearing ST produced less adherent cellulose on air-liquid interface than the wildtype strain in LB culture favoring biofilm formation. Interestingly, all pVCR94 driven biofilm phenotypes in ST vanished upon deletion of the master activator *acaCD*, which controls conjugative transfer of IncA/C plasmids, reverting to the wildtype level. Deletion of the conserved predicted c-di-GMP phosphodiesterase gene, *pdeAC*, which is embedded in one of the four clusters of transfer genes of IncA/C_2 plasmids, also abolished pVCR94 driven biofilm phenotypes.**Conclusions:** Loss of rugoseness and *rdar* morphotype in the presence of pVCR94 and subsequent restoration of these phenotypes after *acaCD* deletion suggested that the suppression of biofilm in *S.* Typhimurium was solely pVCR94 driven. These findings unveil a direct association between IncA/C_2 plasmids and c-di-GMP-regulated biofilm formation, thereby suggesting a possible role of IncA/C_2 plasmids in the virulence of pathogenic *Enterobacteriaceae*.

Author Disclosure Block:

S.F. Rouf: None. **S. Rodrigue:** None. **V. Burrus:** None.

Poster Board Number:

SUNDAY-738

Publishing Title:

The *Salmonella* Genomic Island 1 Reshapes the Conjugative Apparatus Encoded by IncA/C Plasmids

Author Block:

N. Carraro, **R. Durand**, N. Rivard, C. Anquetil, V. Burrus; Lab. of bacterial molecular genetics, Université de Sherbrooke, Sherbrooke, QC, Canada

Abstract Body:

IncA/C conjugative plasmids (ACPs) and the *Salmonella* genomic island 1 (SGI1) or its variants are frequently associated with multidrug resistance in clinical isolates of pathogenic *Enterobacteriaceae*. SGI1 is not self-transmissible and has been shown to be specifically mobilized in *trans* by ACPs[1]. Excision of SGI1 from the chromosome is triggered by the transcriptional activator complex AcaCD that is encoded by ACPs[2]. Seven genes of SGI1 are preceded by predicted AcaCD boxes. Three of these genes, namely *trhN*, *trhH* and *trhG*, code for distant orthologues of the ACP-encoded proteins TraN, TraH and TraG, respectively, which are putative mating pore subunits. We engineered a mini-SGI1 element that only contains the conserved sequences shared by all SGI1-like elements, including the orthologous *tra* genes, and confirmed its functionality. Using combinations of deletion mutants of mini-SGI1 and the helper ACP pVCR94, we explored the role of each Tra and Trh subunit in conjugative transfer and the complex interactions between mini-SGI1 and pVCR94. We found that TraN/G/H are essential for ACP conjugative transfer and that TrhN/G/H are functional proteins, which are expressed under the control of AcaCD. Complementation assays of individual *tra*/*trh* mutants revealed that not only do TrhN/G/H replace TraN/G/H in the mating pore encoded by ACPs but also that *trhG* is required for mini-SGI1 transfer whereas *trhH* strongly improves mini-SGI1 transfer. TraN and TrhN were found to be equivalent *vis-à-vis* pVCR94 or SGI1 transfer efficiency. These results indicate that SGI1 alters the mating pore encoded by ACPs to selectively enhance its own transfer by a mechanism that remains to be determined. Known mobilizable genomic islands rarely code for mating pore components; instead they usually hijack the mating pore encoded by their helper conjugative element. Whether this strategy of mating pore alteration confers a competitive advantage to SGI1 over ACPs for dissemination in clinical population of enteric pathogens remains to be determined.

Author Disclosure Block:

N. Carraro: None. **R. Durand:** None. **N. Rivard:** None. **C. Anquetil:** None. **V. Burrus:** None.

Poster Board Number:

SUNDAY-739

Publishing Title:

Unusual Class 1 Integrons Located on Plasmids of Canine *Escherichia coli*

Author Block:

A. K. Siqueira[1], G. B. Michael[1], M. G. Ferraz[2], M. G. Ribeiro[3], S. Schwarz[1], **D. S. Leite**[2];
[1]Friedrich-Loeffler-Inst., Neustadt-Mariensee, Germany, [2]UNICAMP, Campinas, Brazil,
[3]UNESP, Botucatu, Brazil

Abstract Body:

Escherichia coli is a common cause of canine urinary tract infections. There is a growing number of studies that report unusual class 1 integrons lacking the 3´-conserved segment and displaying a genetic linkage with the sulphonamide resistance gene *sul3*. The aim of this study was to investigate the presence of such integrons among clinical and non-clinical *E. coli* isolated from dogs (n=158). PCRs were performed for the detection of class 1 integron-integrase gene (*intI1)*, *sul3*, *sul1*, truncated quaternary ammonium resistance gene (*qacE1)* and for the variable region of usual class 1 integrons. Two isolates, the 16C from faeces from an apparently healthy dog and 87U from a canine urinary tract infection, were *intI1*- and *sul3*-positive but showed negative results for *qacE1*, *sul1* and the variable region of usual integrons. Based on the antimicrobial susceptibility testing and reference sequences, linkage PCRs between *intI1* and *dfrA12*, *dfrA12* and *cmlA*, *cmlA* and *qacH* as well as *qacH* and *sul3* were performed. They were positive and the sequence analysis confirmed that both unusual class 1 integrons carried the gene cassette array *dfrA12*-orfF-*aadA2*-*cmlA1*-*aadA1*. Plasmid profiling was done by S1-PFGE and one 35-kb plasmid was found in each isolate. The isolate 87U carried an additional 220-kb plasmid. After electrotransformation, PCRs and sequence analysis the location of the *intI1* and *sul3* and their linkage to the gene cassette array *dfrA12*-orfF-*aadA2*-*cmlA1*-*aadA1* on the 35-kb transformed plasmids was confirmed. Electrotransformants displayed also the same antimicrobial multi-resistance pattern (chloramphenicol, streptomycin, sulfonamide and trimethoprim). According to the replicon typing, the 35-kb plasmids belonged to the incompatibility groups IncF+IncFIC. Similar restriction patterns were seen with the PstI and HindIII-digested plasmids. However, XbaI-PFGE of the isolates 16C and 87U revealed distinctly different patterns. In this study, unusual class 1 integrons were located on similar plasmids of unrelated isolates which may indicate a horizontal dissemination of such multi-resistance plasmids among apparently healthy and diseased dogs.

Author Disclosure Block:

A.K. Siqueira: None. **G.B. Michael:** None. **M.G. Ferraz:** None. **M.G. Ribeiro:** None. **S. Schwarz:** None. **D.S. Leite:** None.

Poster Board Number:

SUNDAY-740

Publishing Title:

New Small Plasmid Harboring *bla*$_{kpc-2}$ in Two Different *Pseudomonas aeruginosa*

Author Block:

R. Galetti[1], L. N. Andrade[1], M. Chandler[2], A. M. Varani[3], A. L. C. Darini[1]; [1]Univ. of Sao Paulo, Ribeirao Preto, Brazil, [2]Ctr. Natl. de la Recherche Scientifique, Ramonville, France, [3]Univ.e Estadual Paulista "Julio de Mesquita Filho", Jaboticabal, Brazil

Abstract Body:

First report of KPC-producing *Pseudomonas aeruginosa* was 10 years ago, in Colombia, and *bla*$_{KPC-2}$ gene was chromosomal localized, with subsequent worldwide reports on plasmid and in chromosomal genetic contexts. In Brazil, the first KPC-producing *P. aeruginosa* was reported in 2012, however, up to date, there are no data regarding genetic localization. The aim of this study was to characterize the genetic context of *bla*$_{KPC-2}$ in two *P. aeruginosa* (BH6 and BH9 isolates) from Brazil. These bacteria were isolated from inpatients in Belo Horizonte city, in 2011, and the isolates were characterized as KPC-2 producers multidrug-resistant (MDR) and belonged to ST244 (BH6) and to ST381 (BH9). Furthermore, genomic localization of *bla*$_{KPC-2}$ gene was determined by hybridization after I-*Ceu*-I-PFGE using *bla*$_{KPC-2}$ probe as well as *S1*-PFGE using 16S rDNA probe. In addition, plasmids were searched following the PCR-based replicon typing (PBRT) scheme targeting replicons of the major incompatibility (Inc) groups of plasmids occurring in *Enterobacteriaceae*, however no Inc group was detected. Besides, the investigation of plasmids DNA content was carry out using Midiprep Kit, according to the manufacturer's protocol, and the plasmid DNA purified was completely sequenced using Ion PGM™ procedures. The set of experiments mentioned above confirmed that *bla*$_{KPC-2}$ was harbored in a single small plasmid in BH6 isolate and there is no copy of this carbapenemase-encoding gene in its chromosome. The complete sequencing revealed a new plasmid, characterized here as pBH6, of 3,642 bp including an IS*Swi1* resolvase, *bla*$_{KPC-2}$ gene, a partial IS*Kpn6* and an origin of replication (*ori*). No *rep* gene was present in the pBH6. However, in BH9 isolate, *bla*$_{KPC-2}$ was detected inserted in the chromosomal DNA. Thus, the entire genomic DNA of BH9 isolate was sequenced using Ion Proton™ procedure. Analyzes of genomic content of BH9 showed that the same genetic element, pBH6, was totally inserted in its chromosomal DNA. The genetic context of *bla*$_{KPC-2}$ gene here evaluated is different from those already described. In our study, *bla*$_{KPC-2}$ was harbored in a new small plasmid, living as a "free" element in BH6 isolate and inserted in the chromosomal DNA of BH9 isolate.

Author Disclosure Block:

R. Galetti: None. **L.N. Andrade:** None. **M. Chandler:** None. **A.M. Varani:** None. **A.L.C. Darini:** None.

Poster Board Number:

SUNDAY-741

Publishing Title:

A New Family of Multidrug Resistance Islands Found Among Clinical *Vibrio cholerae* non-O1/non-O139 in Haiti are Mobilizable by IncA/C Conjugative Plasmids

Author Block:

N. Rivard[1], N. Carraro[1], D. Ceccarelli[2], R. R. Colwell[3], V. Burrus[1]; [1]Lab. of bacterial molecular genetics, Université de Sherbrooke, Sherbrooke, QC, Canada, [2]Central Vet. Inst. of Wageningen UR, Lelystad, Lelystad, Netherlands, [3]Maryland Pathogen Res. Inst., Univ. of Maryland, Coll. Park, College Park, MD

Abstract Body:

IncA/C conjugative plasmids (ACPs) are efficient vehicles for dissemination of multidrug resistance genes in modern pathogenic bacteria. Mobility of ACPs ultimately depends on the expression of multiple ACP-borne operons activated by the master activator AcaCD[1]. Beyond ACP transfer, AcaCD also activates the expression of genes located in the unrelated mobilizable genomic islands MGI*Vmi1* of *Vibrio mimicus* and SGI1 of *Salmonella enterica*.While ACPs have been rarely reported in *Vibrio cholerae*, a recent study[2] described their presence in a 2012 clinical O1 isolate from Haiti. Here we investigated the presence of ACP-activated genomic islands in Haitian *V. cholerae* and discovered MGI*Vch*Hai6, a novel genomic island integrated into the 3' end of *trmE* in the genome of non-O1/non-O139 clinical isolates recovered in 2010. MGI*Vch*Hai6 contains the mercury resistance transposon Tn*5053* and an In104-like multidrug resistance element similar to the one inserted in SGI1. MGI*Vch*Hai6 carries *mobI*- and *xis*-like genes preceded by an AcaCD box. *mobI* and *xis* genes code for a putative auxiliary protein required for the transfer of several conjugative elements and a recombination directionality factor, respectively. We showed that MGI*Vch*Hai6 excises from the chromosome, transfers at high frequency in an ACP-dependent manner and confers resistance to β-lactams, sulfamethoxazole, trimethoprim, tetracycline, chloramphenicol, streptomycin and spectinomycin. *In silico* analyses revealed that MGI*Vch*Hai6-like elements are also found in environmental and clinical strains recovered from North and South America, and from the Indian subcontinent.ACPs have influenced the evolution of Haitian *V. cholerae* strains by propagating genomic islands bearing AcaCD-activated genes. ACP-driven circulation of MGI*Vch*Hai6-like elements should be carefully monitored as they serve as a potential reservoir of transmissible multidrug resistance genes.

Author Disclosure Block:

N. Rivard: None. **N. Carraro:** None. **D. Ceccarelli:** None. **R.R. Colwell:** None. **V. Burrus:** None.

Poster Board Number:

SUNDAY-742

Publishing Title:

Toxin-Antitoxin ParE/ParD of Conjugative Plasmid Controls Plasmid Copy Number in *Pseudoalteromonas rubra*

Author Block:

Baiyuan Li, Jianyun Yao, Pengxia Wang, **X. Wang**; South China Sea Inst. of Oceanology, Guangzhou, China

Abstract Body:

The toxin-antitoxin systems were originally identified as plasmid maintenance or stability modules, and can eliminate plasmid-free cells due to a mechanism known as post-segragational killing. Here we characterized a type II toxin/antitoxin pair ParE/ParD on a low-copy-number conjugative plasmid in marine bacteria *Pseudoalteromonas rubra*. The size of the conjugative plasmid is 69.9 kb, containing genes that are responsible for plasmid replication, partition and conjugative transfer. The copy number of the conjugative plasmid is maintained at one copy per cell. Deletion of the toxin/antitoxin pair from the plasmid greatly increased the copy number of plasmid, reaching up to 60 copies per cell. However, deletion of the toxin alone did not affect the copy number, indicating a possible role of antitoxin in controlling the copy number. Bioinformatics analysis showed that the toxin belongs to the ParE family, and overproduction of the toxin caused filamentous growth in *E. coli* host, and caused cell inflation and cell lysis in *Pseudoalteromonas rubra* host. The antitoxin contains a CopG-like domain. Co-production of the antitoxin neutralized the toxic effect of the toxin, and toxin and antitoxin interact directly at the protein level. Toxin is very stable but the antitoxin is labile. Thus they consists of a typical type II toxin/antitoxin system. Our EMSA results showed that the antitoxin can bind to its own promoter and autoregulate the expression of the toxin/antitoxin operon. Additionally, it can also bind and shift the promoter the replication gene *repA* of the plasmid. Further investigation on how this toxin/antitoxin pair affects plasmid stability, segregation and dissemination is needed.

Author Disclosure Block:

X. Wang: None.

Poster Board Number:

SUNDAY-743

Publishing Title:

A Large Ciprofloxacin-Resistant Plasmid from Nigeria Confers Resistance to Multiple Antibacterials without a Carrying Cost

Author Block:

R. Monárrez[1], M. Braun[1], O. Coburn-Flynn[1], B. W. Odeotyin[2], A. O. Aboderin[2], I. N. Okeke[3]; [1]Haverford Coll., Haverford, PA, [2]Obafemi Awolowo Univ., Ile-Ife, Nigeria, [3]Univ. of Ibadan, Ibadan, Nigeria

Abstract Body:

Background: Antimicrobial resistance is a large and growing global problem. We screened 97 fecal fluoroquinolone resistant *Escherichia* isolates from Nigeria for plasmid-mediated quinolone resistance genes. Sixteen of the isolates harbored at least one of these genes and three were positive for *aac(6′)-Ib-cr*. In one strain, *aac(6′)-Ib-cr* was mapped to a self-transmissible 125-kb IncFII plasmid, pMB2, which also bears CTX-M-15 and seven other resistance genes. We hypothesized that pMB2 had been selected by antimicrobials and that its large size would confer a growth disadvantage. **Methods**: We studied growth rates of laboratory strains carrying pMB2 and found they grew as fast as, or faster than, isogenic strains lacking the plasmid in both rich and minimal media. We excised a 32-kb fragment, not including most resistance or conjugative genes, and studied growth and nutrient utilization of the resultant 93-kb mini-plasmid. **Results**: There was a carrying cost for the 93-kb mini-plasmid in all tested media and phenotypic microarrays revealed that utilization of certain amino acids was slower in the deletion. The deleted region included *sitABCD* genes, *nikM* and several open-reading frames of unknown function. Complementation of growth defects with the excised portion on a separate plasmid was unsuccessful, potentially due to copy-number effects. However, trans-complementation with the cloned *sitABCD* genes alone confirmed that these genes account for the growth advantage conferred by pMB2 in iron-depleted media. **Conclusion**: Nutritional factors encoded on a large and transmissible multiresistance plasmid allow it to be maintained successfully without a carrying cost, worsening the trajectory for antimicrobial resistance.

Author Disclosure Block:

R. Monárrez: None. **M. Braun:** None. **O. Coburn-Flynn:** None. **B.W. Odeotyin:** None. **A.O. Aboderin:** None. **I.N. Okeke:** None.

Poster Board Number:

SUNDAY-744

Publishing Title:

Single-cell (Meta-)genomics of a Dimorphic Candidatus Thiomargarita Nelsonii Reveals Extraordinary Genomic Plasticity and a Mite Bearing Group I Intron

Author Block:

B. E. FLOOD[1], P. Fliss[1], D. S. Jones[1], G. J. Dick[2], S. Jain[2], A-K. Kastor[3], M. Winkel[4], M. Mußmann[5], J. V. Bailey[1]; [1]Univ. of Minnesota, Minneapolis, MN, [2]Univ. of Michigan, Ann Harbor, MI, [3]Leibniz Inst. DSMZ, Braunschweig, Germany, [4]GFZ German Res. Ctr. for GeoSci.s, Potsdam, Germany, [5]Max Planck Inst. for Marine Microbiol., Bremen, Germany

Abstract Body:

The genus *Thiomargarita* includes the world's largest bacteria. But as uncultured organisms, their physiology, metabolism, and basis for their gigantism are not well understood. Thus a genomics approach, applied to a single *Candidatus* Thiomargarita nelsonii cell was employed to explore the genetic potential of one of these enigmatic giant bacteria. The *Thiomargarita* cell was obtained from an assemblage of budding *Ca.* T. nelsonii attached to a provannid gastropod shell from Hydrate Ridge, a methane seep offshore of Oregon, USA. Here we present a manually curated genome of Bud S10 resulting from a hybrid assembly of long Pacific Biosciences and short Illumina sequencing reads. Surprisingly, the genome included the largest number of metacaspases and introns ever reported in a bacterium. In eukaryotes, metacaspases are involved with programmed cell death, but in bacteria they are poorly understood. Both group I and group II introns have been previously observed in the rRNA genes of giant vacuolated sulfur bacteria, but never in protein-coding genes. In the Bud S10 genome we observed a high number of these introns and other repetitive and palindromic elements, such as transposons and miniature inverted-repeat transposable elements (MITEs). In some cases, mobile genetic elements disrupt key genes in metabolic pathways. For example, a MITE interrupts *hupL*, the key gene in hydrogen oxidation. Moreover, we detected a group I intron in one of the most critical genes in the sulfur oxidation pathway, *dsrA.* Group I introns in bacteria are extremely rare and hardly ever disrupt protein encoding genes. This group I intron also carries a MITE sequence found throughout the genome. We hypothesize that the Group I intron plays a role in dispersal of the MITE sequences and is a new form of molecular symbiosis. The presence of a high degree of mobile elements in genes central to *Thiomargarita*'s core metabolism has not been previously reported in bacteria and suggests a highly mutable genome where the transcription of introns and other mobile elements may mediate cellular responses.

Author Disclosure Block:

B.E. Flood: None. **P. Fliss:** None. **D.S. Jones:** None. **G.J. Dick:** None. **S. Jain:** None. **A. Kastor:** None. **M. Winkel:** None. **M. Mußmann:** None. **J.V. Bailey:** None.

Poster Board Number:

SUNDAY-745

Publishing Title:

Application of a Plasmid Classification System to Determine Prevalence of Replicon Familiesamong Multidrug Resistant *Enterococci*

Author Block:

C. R. Jackson[1], J. B. Barrett[1], L. M. Hiott[1], T. A. Woodley[1], S. Cho[2], J. G. Frye[1]; [1]USDA-ARS, Athens, GA, [2]Univ. of Georgia, Athens, GA

Abstract Body:

Background: The presence and transfer of plasmids from commensal bacteria to more pathogenic bacteria may contribute to dissemination of antimicrobial resistance. However, prevalence of plasmids from commensal bacteria in food animals such as the enterococci remains largely unknown. In this study, the prevalence of plasmid families from multidrug resistant (MDR; resistance to three or more antimicrobials) enterococci from poultry carcasses was determined. Plasmid positive MDR enterococci were also tested for the ability to transfer plasmids and resistance to other enterococci. **Methods:** *Enterococcus faecalis* (n=98) and *Enterococcus faecium* (n=702) used in this study were isolated from poultry carcass rinsates between 2004 and 2011. Isolates were identified to species and analyzed for antimicrobial susceptibility as part of the animal arm of the National Antimicrobial Resistance Monitoring System (NARMS)—Enteric Bacteria at the US Department of Agriculture—Agricultural Research Service (USDA-ARS), Athens, GA. MDR enterococci were tested for the presence of 19 plasmid replicon (*rep*) families using multiplex PCR. Transfer of plasmids and antimicrobial resistance was determined by filter mating.**Results:** Approximately 48% of *E. faecalis* (47/98) and 17% of *E. faecium* (116/702) were positive for at least one *rep*-family. Fourteen *rep*-families were detected indicating the presence of 14 different plasmids among the isolates. The most common MDR pattern among the isolates was GenKanLinTet and LinNitTet. The *rep*-family 9 (rep_9) was predominant among both *E. faecalis* and *E. faecium* for all years tested. Host range for *E. faecalis* and *E. faecium* was different as 13 *rep*-families were found among *E. faecalis* and 12 among *E. faecium*. The rep_7 and rep_{17} family were unique to *E. faecalis*, while the rep_5 family was unique to *E. faecium*. The most number of *rep*-families detected was in 2005 (n=10) and the least in 2009 (n=1). No transfer of tetracycline or erythromycin resistance was detected among selected isolates.**Conclusion:** Results from this study show that *E. faecalis* and *E. faecium* from poultry carcasses contain numerous and diverse *rep*-families. Additional conjugation studies are ongoing to determine if the plasmids can transfer into other enterococci.

Author Disclosure Block:

C.R. Jackson: None. **J.B. Barrett:** None. **L.M. Hiott:** None. **T.A. Woodley:** None. **S. Cho:** None. **J.G. Frye:** None.

Poster Board Number:

SUNDAY-746

Publishing Title:

Increase in IS*256* Transposition in Different *S. aureus* Lineages after Antibiotic Selective Pressure

Author Block:

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Abstract Body:

Background: In *S. aureus*, transposition of IS*256* has been described to play an important role in biofilm formation and antibiotic resistance. Previously, we reported an augment in IS*256* transposition associated with the selection of hVISA/VISA strains, both in vivo and in vitro, indicating that VAN pressure could enhance IS256 transposition, and eventually the loss of *agr* function. In this study we aim to characterize the effect of antibiotic selective pressure in IS*256* transposition of *S. aureus* isolates belonging to different lineages.**Methods:** Mutant strains were independently selected on increasing concentrations of vancomycin (ST8, ST100) and tigecycline (ST5, ST239). Isolates were genotypically characterized by PFGE, MLST, *spa*, SCC*mec*, *agr*, and southern hybridization using IS*256* and *agr*AC specific probes. The *agr* locus functionality was determined by delta-heamolysis assay. The *sig*B and *rsb*U regulatory genes were mapped by PCR. Additionally, the transcription of IS*256* transposase (*tnp*) gene was evaluated by RTqPCR for ST5 and ST239 strains. Data was expressed as normalized relative quantities (NRQ) using *gyr*B and *pta* as reference genes. All experiments were performed by triplicate. Statistical analysis of the data was accomplished using Student t test (*alfa*=0.05).**Results:** Isogenicity between mutant and parental strains was confirmed by PFGE. An increase in IS*256* copies was demonstrated in mutants selected with vancomycin (MRSA-ST100-IV_{nv}-t*002-agrII*, and MSSA-ST8-t*008-agrI*), and tigecycline (MRSA-ST239-III-t*654-agrI*) by southern blot hybridization. No copy of IS*256* was inserted into *sig*B and *rsb*U genes. NRQ values of *tnp* gene were significantly higher in ST239 mutant strain when compared to parental strain ($p<0.0082$). On the other hand, an increase was also detected for MRSA-ST5-IV-t*002-agr*II mutant strain but it was no significantly different ($p=0.2616$).A loss of *agr* function was determined for ST5, ST100 and ST8 mutant strains but it could not be correlated to IS*256* transposition.**Conclusions:** Selective pressure of vancomycin and tigecycline produce an increase in IS*256* transposition in strains belonging to ST100, ST8 and ST239. This phenomenon could generate diverse genetic rearrangements, which may contribute to the flexibility and evolution of different lineages of this pathogen

Author Disclosure Block:

S. Di Gregorio: None. **M. Herrera:** None. **S. Fernandez:** None. **J. Di Conza:** None. **Á. Famiglietti:** None. **M. Mollerach:** None.

Poster Board Number:

SUNDAY-747

Publishing Title:

Analysis of Mobile Genetic Elements Encoding Macrolide Resistance in *Streptococcus suis*

Author Block:

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Abstract Body:

Streptococcus suis is a major pathogen of pigs and is responsible for a variety of diseases including meningitis, arthritis and pneumonia. Macrolide resistance emerged globally during the 1990's in streptococci including *S. suis*. The mechanism to macrolide resistance is mainly due to modification of the methylase-mediated target site by the product of *erm*(B) and followed by drug efflux by products of *mef*(A) and *msr*(D). To better understand the mobile genetic elements (MGEs) response for macrolide resistance, a total of 80 clinical isolates of *S. suis* and 22 complete genome sequenced strains from NCBI database were analyzed by comparative genomics followed by PCR, sequencing, and epidemiological analysis. ICE*Sa*2603-like integrative and conjugative elements (ICEs) and Φm46.1-like phages were the main elements carrying *erm*(B) and *mef*(A), respectively. The *erm*(B)-carrying ICE*Sa*2603-like ICEs specifically recognized a 15-bp *att* sites at the 3'-end of *rplL*, which was highly conserved in genus *Streptococcus*. Although *mef*(A)-carrying Φm46.1-like phages have been described in *Streptococcus pyogenes* and *Streptococcus pneumoniae*, here we first reported it in *S. suis*. Further, a novel ICE-phage composite MGEs was identified to disseminating macrolide, tetracycline, and aminoglycoside resistance in China. Fitness assay of the clinical isolates of MGEs and non-MGEs showed little or no fitness cost which may explain why those MGEs-carrying isolates emerge and disseminate. The data indicate that *erm*(B) and *mef*(A) is widely disseminated in streptococci through ICEs and phages, respectively, and the elements are undergo recombination to form new composite elements. Thus *S. suis* is an important antibiotic resistance reservoir that can contribute to the spread of macrolide resistance genes to the streptococci.

Author Disclosure Block:

J. Huang: None. **Y. Liang:** None. **L. Wang:** None.

Poster Board Number:

SUNDAY-748

Publishing Title:

Characterization of Two Novel Composite Staphylococcal Cassette Chromosome *mec* Elements of Methicillin-Resistant *Staphylococcus lugdunenesis*

Author Block:

S-C. Chang, C-F. Yeh, C-W. Cheng, T-P. Liu, J-F. Lin, M-C. Ge, Y-T. Chou, M-H. Lee, J-J. Lu; Chang Gung Mem. Hosp. at Linkou, Taoyuan, Taiwan

Abstract Body:

Methicillin resistance in staphylococci is mediated by the acquisition of *mecA* gene, which is carried by mobile genetic element staphylococcal cassette chromosome *mec* (SCC*mec*). SCC*mec* elements in coagulase-negative staphylococci (CoNS) are highly diverse and may act as a reservoir of SCC*mec* for *Staphylococcus aureus* to form methicillin resistance. However, little is known about the SCC*mec* structure of methicillin-resistant CoNs. Here, we used whole-genome sequencing to determine the composite SCC structures of two clinical *Staphylococcus lugdunenesis* isolates CMUH-SL22 and CMUH-SL25, which were assigned to SCC*mec* type Vt and V, respectively, as the common methicillin-resistant *Staphylococcus lugdunenesis* clones prevalent in Taiwan by multiplex PCR. Each of these elements had a unique mosaic structure but shared the common feature of integration at an identical integration site within the 3' end of *orfx* gene flanked by direct and inverted repeats. Although sequences located between *orfx* and IS*Sau4*-like transposase of both elements were similar to those found in *Staphylococcus aureus* PM1, sequences of CMUH-SL25 harbored a novel *ccrC* complex, and a C2-like *mec* complex in opposite orientation similar to that in *Staphylococcus aureus* WIS. Besides, a *ccrA4B4* gene, and parts of sequences within J1 and J2 regions of CMUH-SL25 were similar to those elements found in *Staphylococcus haemolyticu*s. In contrast, J1 region of CMUH-SL22 contained several regions that were highly similar to *Staphylococcus epidermidis* or *Staphylococcus aureus.* These observations suggested that elements found in CMUH-SL25 and CMUH-SL22 evolved separately and assembled through independent recombination events or horizontal gene transfer between staphylococcal species.

Author Disclosure Block:

S. Chang: None. **C. Yeh:** None. **C. Cheng:** None. **T. Liu:** None. **J. Lin:** None. **M. Ge:** None. **Y. Chou:** None. **M. Lee:** None. **J. Lu:** None.

Poster Board Number:

SUNDAY-749

Publishing Title:

Whole Genome Sequencing Reveals an Insertional Mutation Disrupting A*grc* in a Methicillin Resistant *Staphylococcus aureus* Isolate of St239 Lineage

Author Block:

A. M. N. Botelho[1], M. Nicolás[2], A. M. S. Figueiredo[1]; [1]Inst. of Microbiol., Rio de Janeiro, Brazil, [2]Natl. Lab. for Scientific Computing, Petropolis, Brazil

Abstract Body:

Background: Multiresistant isolates of the ST239-SCC*mec*III lineage are the most frequently detected methicillin-resistant *Staphylococcus aureus* (MRSA) in hospital infections. The balance between fitness and virulence capability could be a key factor in the MRSA clonal shift resulting from the success of one specific clone over another. The accessory gene regulator (Agr) is the major staphylococcal quorum sensing system, and it plays an important role in the global regulation of virulence factors. In this study, Agr-dysfunctional isolates were investigated. **Methods:** Agr activity was assessed using MALDI-TOF in a collection of ST239 clinical isolates (n=100). Whole-genome sequencing (WGS) was carried out using Roche 454 for four isolates displaying natural Agr attenuation. PCR was used to detect specific DNA sequences in the bacterial genomes, whereas real time qRT-PCR was used for transcript quantification. Finally, biofilm was tested using a microplate-based method. **Results:** A percentage of 30% of Agr dysfunction was detected. The WGS revealed one isolate (HC1335) carried a transposase element inserted in the *agrC*, resulting in complete Agr inactivation. This same insertion was found in 13% of all Agr-dysfunctional isolates. No mutation was found in the *agr* locus of other sequenced genomes, indicating the defect was upstream *agr*. In fact, these ST239 isolates displayed low *sarA* transcripts (n=2) or increased *saeRS* expression (n=1), which are positive or negative regulators of *agr*, respectively. Additionally, a greater number of Agr-functional isolates was collected from infection than from colonization ($p<0.001$). Also, the amount of biofilm accumulated positively correlated with Agr function ($p<0.05$). **Conclusion:** These results demonstrate that an IS*256* element can quickly re-edit the bacterial global regulation by inactivating the master regulator RNAIII. The importance of the *agr* on/off mechanism in ST239 isolates remains unclear. However, recent studies associated Agr dysfunction with fitness improvement and persistent bacteremia. Thus, it is possible that this switch represents a significant mechanism of genetic variability in the adaptation of this MRSA as a successful hospital pathogen.

Author Disclosure Block:

A.M.N. Botelho: None. **M. Nicolás:** None. **A.M.S. Figueiredo:** None.

Poster Board Number:

SUNDAY-750

Publishing Title:

Gene Transfer Agents in *Heliconia phytotelmata* and Microbial Mats

Author Block:

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Abstract Body:

Microbial mats and heliconia phytotelmata are micro-ecosystem which fosters a highly diverse microbiota and present a complex nutrient cycle, in which horizontal gene transfer mechanisms should be playing an important role regulating the ecological structure and dynamics. Besides conjugation and transformation, an important and novel genetic exchange process carried out through constitutive transduction by a phage-like element is called gene transfer agents (GTAs). The function and importance of GTAs in the population structure and maintenance of the metabolic capabilities in microbial mats and heliconia phytotelmata is poorly understood. The purpose of this research is to determine the presence of GTAs elements in Purple Non Sulfur Bacteria (PNSBs) isolated from these two aquatic environments. To test the presence of GTAs in our environmental collection of PNSBs, a PCR using GTA *gp5*-specific primers was performed and further analyzed *in silico*. Amplicons from the isolates were obtained using specific primers for the major capsid protein *gp*5. The putative presence of the transduction-like gene transfer agent (GTA) were observed in genome sequences of four out of eight isolates from heliconia phytotelmata and in several isolates from microbial mats. *In silico* analysis suggests the presence of *gp*5 sequences derived from genome-sequenced Rhodobacterales. The presence of the GTA in our samples will allow the development of new research questions about the physiological and ecological roles that the GTA-mediated gene exchange may have to these microbial groups in adaptation to transient environments like microbial mats and phytothelmata.

Author Disclosure Block:

M. De Jesus-Cruz: None. **C. Rios-Velazquez:** None.

Poster Board Number:

SUNDAY-751

Publishing Title:

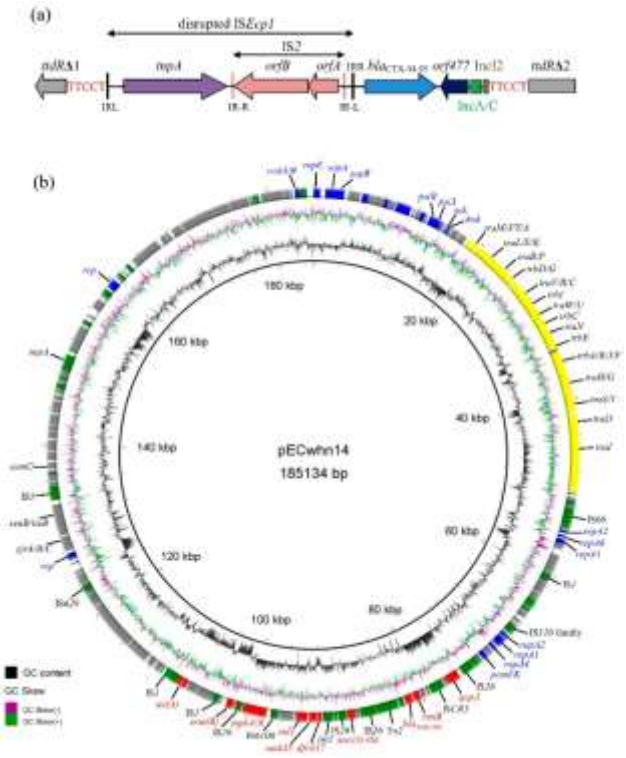
Chromosomal Location of the *Bla*$_{\text{ctx-M-55}}$ Gene in a Multidrug Resistant *Escherichia coli* ST167 Isolated from Healthy Chicken in China

Author Block:

C-W. Lei, B-H. Liu, H-N. Wang, A-Y. Zhang; Sichuan Univ., Chengdu, China

Abstract Body:

Background: CTX-M-55 extended-spectrum beta-lactamase (ESBL), like CTX-M-15, mediates resistance to the third-generation cephalosporins and enhances the hydrolytic activity against ceftazidime. It is generally carried by IncI2 plasmids and has become one of the most prevalent ESBLs in China. Here we first characterized the chromosomal location of *bla*$_{\text{CTX-M-55}}$ gene in a multidrug resistant *Escherichia coli* isolated from healthy chicken in China.**Methods:** *E. coli* strain ECwhn14 was isolated from a healthy chicken in Hebei province. Antimicrobial susceptibility test and conjugation experiments were performed. Whole genome sequencing was performed on the Illumina MiSeq using a 400-bp paired-end library with 210-fold average coverage. The paired-end reads were assembled de novo and the gaps among contigs were filled in by PCR-linkage.**Results:** Strain ECwhn14 displayed multidrug resistant profile. The Sequence Type of ECwhn14 was ST167. An amikacin-resistant plasmid, named pECwhn14, could be transferred to *E. coli* EC600 with 5.5×10^{-4} frequency. However, the ceftazidime-resistant determinant could not be transferred. Sequence analysis showed the *bla*$_{\text{CTX-M-55}}$ was integrated into the chromosomal gene *ttdR* encoding LysR family transcriptional regulator (Fig. 1a). The 4482-bp IS*Ecp1*-mediated transposition unit might come from the IncI2 plasmid. The 1331-bp insertion sequence IS*2* that contained *orfA* and *orfB* transposase genes was inserted into the IS*Ecp1*. The 185134-bp plasmid pECwhn14, which belonged to F36:A4:B1 and carried eleven resistance genes, was also characterized (Fig. 1b).**Conclusions:** This is the first report of the chromosomal location of *bla*$_{\text{CTX-M-55}}$ and IS*Ecp1-bla*$_{\text{CTX-M-55}}$ region disrupted by IS*2*.



Author Disclosure Block:

C. Lei: None. **B. Liu:** None. **H. Wang:** None. **A. Zhang:** None.

Poster Board Number:

SUNDAY-752

Publishing Title:

Mycobacterial Sufb Intein as Environmental Sensor

Author Block:

C. Green, N. Topilina, M. Belfort; Univ. at Albany, SUNY, Albany, NY

Abstract Body:

Inteins are mobile genetic elements that self-splice at the protein level from their flanking extein fragments. Mycobacteria have inteins inserted into several important genes, including those corresponding to the iron-sulfur cluster assembly protein SufB. Curiously, the SufB inteins are found primarily in mycobacterial species that are potential human pathogens. Experiments with an *Escherichia coli*-derived reporter system and the *Mycobacterium tuberculosis* SufB precursor revealed that reactive oxygen species and reactive nitrogen species inhibit SufB extein ligation. Such exceptional sensitivity of SufB intein splicing to oxidative and nitrosative stresses is a result of predisposition of the intein's catalytic cysteine residues to oxidative modification. Our data suggest that reactive oxygen and reactive nitrogen species interference with SufB splicing is a dynamic process, where the concentration of these species affects the extent of splicing inhibition and the outcome of precursor processing. These dynamics depend on whether the catalytic cysteine residues participate in splicing before they become modified through oxidative reactions. We suggest that oxidative modifications inhibit the splicing pathway and diminish activity of the iron-sulfur cluster assembly machinery. We propose that splicing inhibition is an immediate, post-translational regulatory response that can be either reversible, by inducing precursor accumulation, or irreversible, by inducing N-terminal cleavage, which may potentially channel mycobacteria into dormancy under extreme oxidative and nitrosative stresses.

Author Disclosure Block:

C. Green: None. **N. Topilina:** None. **M. Belfort:** None.

Poster Board Number:

SUNDAY-753

Publishing Title:

Characterization of pHeBE7, an IncFII Type Virulence-Resistance Plasmid Carrying *bla*$_{\text{ctx-M-98b}}$, *bla*$_{\text{tem-1}}$ and *rmtb* Gene in *Escherichia coli* from Chicken in China

Author Block:

A. Zhang, Y. Yang, H. Wang; Sichuan Univ., Chengdu, China

Abstract Body:

Background: Recentely, a novel variant of the CTX-M enzyme, CTX-M-98, was detected in *Escherichia coli* isolates from food animals in China. Notably, the *bla*CTX-M-98 gene was also found in *E. coli* strains from patients and healthy individuals in China, which indicated the blaCTX-M-98 gene might spread between *E. coli* strains from food animals and human beings. **Methods:** An *E. coli* HeB7 strain, positive for A *bla*$_{\text{CTX-M-98b}}$, *bla*$_{\text{TEM-1}}$, *rmtB* and *traT* gene, was isolated from a diseased chicken. Whole-genome sequencing was performed with Illumina MiSeq using a 350-bp paired-end library. Sequence reads for the plasmid were assembled by using the GS De Novo Assembler v2.8. The contigs were closed by PCR with specific primers. The transferability of pHeBE7 was investigated by mating-out assays. Genetic stability tests and growth competition experiments were conducted as previously described.**Results:** The assembled plasmid, named pHeBE7, belonged to F2:A-:B-, is a complete molecule of 86,015-bp with a GC content of 51.9% and 84 open reading frames (ORFs).The pHeBE7 can be divided into two parts consisting of a -67.2 kb backbone structure and a -18.8 kb mosaic region. The backbone structure contains replication operon (4 ORFs of *repA2-repA3-repA1-repA4*) and coding regions that are involved in the plasmid stability (17 ORFs) and plasmid transfer (33 ORFs). The mosaic regions of pHeBE7, which only part related to several other plasmid sequenced so far, consist of two specific transposon units that are associated with antimicrobial resistance. The first unit is the Tn*3* transposon haboring *bla*$_{\text{TEM-1}}$ and 16S rRNA methylases *rmtB* gene. BLASTn searches revealed that a similar structure was found in some other IncFII type plasmids, such as pMC-NDM and pXZ. The other unit is a typical (IS*Ecp1-bla*$_{\text{CTX-M}}$-IS*903*) transposition unit inserted into a Tn*1721* haboring *bla*$_{\text{CTX-M-98b}}$ gene,which shows a great genetic diversity in other plasmids due to carrying different resistance genes. Conjugation and growth competition experiments indicated that pHeBE7 could be transmitted effectively with low fitness cost. **Conclusions:** This is the first completely resolved sequence of plasmid carrying *bla*$_{\text{CTX-M-98.}}$ The genetic characteristion of pHeBE7 may help to better understand the dissemination of resistance and virulence genes in enterobacter.

Author Disclosure Block:

A. Zhang: None. **Y. Yang:** None. **H. Wang:** None.

Poster Board Number:

SUNDAY-754

Publishing Title:

YhgA-Like Proteins of *Escherichia coli* K-12 and Their Contribution to RecA-Independent Horizontal Transfer

Author Block:

A. W. Kingston; New England Biolabs, Ipswich, MA

Abstract Body:

In bacteria, mechanisms that incorporate DNA into a genome without strand-transfer proteins such as RecA play a major role in generating novelty by horizontal gene transfer. We have developed a conjugal DNA transfer system in *Escherichia coli* to identify, compare, and analyze novel gene acquisition mechanisms. Using this system, we identified an unexpected RecA-independent recombination mechanism in which a large segment of the recipient chromosome is replaced with genomic DNA from the donor. This process occurs at low frequency (~10^{-10}/recipient/h) and a single event can exchange over two megabases of continuous DNA.We then used this conjugal system to investigate YhgA-like proteins, which were predicted to contribute to horizontal gene transfer based on their distribution patterns among bacteria. The *E. coli* K-12 genome encodes five YhgA-like paralogs (YjiP, YfcI, YadD, YhgA and YfaD). When overexpressed in the recipient of our conjugal system, all but the last of these increased the frequency of RecA-independent recombination events, were toxic to the cell, and induced expression of a reporter of DNA damage. Mutagenesis of predicted active site residues in YhgA abolished or diminished these effects. Purified YhgA exhibits endonuclease activity *in vitro* as well. We propose a working model in which YhgA-like proteins promote RecA-independent recombination by cleaving genomic DNA *in vivo*, and help connect one end to a strand of foreign DNA via sequence microhomology.

Author Disclosure Block:

A.W. Kingston: None.

Poster Board Number:

SUNDAY-755

Publishing Title:

High Incidence of *lux* Merodiploids Indicates Frequent Horizontal Gene Transfer in Vibrios

Author Block:

K. Venkler, C. F. Wimpee; Univ. of Wisconsin-Milwaukee, Milwaukee, WI

Abstract Body:

This laboratory has been carrying out a long-term study of bacterial bioluminescence as a model system for bacterial molecular evolution and interspecific gene mobility. In a diversity study of bioluminescent bacteria from Puerto Rico, we encountered a surprising number of Vibrio isolates possessing two copies of *luxA*. These merodiploids were initially detected by restriction endonuclease fingerprinting of *luxA* PCR products, in which the restriction fragments generated from certain strains added up to twice the size of the *luxA* amplicon. In addition, sequencing of amplified *luxA* from these strains revealed numerous mixed sites. Fingerprinting of multiple single colonies from each of these strains demonstrates that the two copies are not a result of contaminating single-copy lux strains. These merodiploids are not identical. Among 96 isolates from 2 ml of seawater, the diversity of *luxA* restriction patterns indicates that there are five different strain types carrying two copies. Merodiploids might arise by either gene duplication or by horizontal gene transfer (HGT). To distinguish between these two possibilities, PCR-amplified *luxA* genes from each merodiploid type were cloned, and multiple separate clones were fingerprinted and sequenced. Phylogenetic analysis of the cloned *luxA* sequences indicates that the merodiploids did not arise by gene duplication, but rather by import of *luxA* from other strains (HGT). Furthermore, we have identified the closest relatives for the individual *luxA* genes of the merodiploids, and find them among the single-copy lux strains we have isolated in the same water sample. These merodiploids thus represent intermediates in the process of HGT. The diversity of merodiploids that we find in only 2 ml of seawater supports the hypothesis that horizontal transfer of *lux* genes is a frequent and ongoing process in Vibrios. It is therefore likely that many other Vibrio genes that are less easily detected undergo frequent horizontal transfer, as well.

Author Disclosure Block:

K. Venkler: None. **C.F. Wimpee:** None.

Poster Board Number:

SUNDAY-756

Publishing Title:

Transfer of Plasmids from *Escherichia coli* to *Clostridium difficile* is Sensitive to DNAse

Author Block:

L. Khodadoost, P. Mullany; UCL Eastman Dental Inst., London, United Kingdom

Abstract Body:

Background: Horizontal gene transfer (HGT) between bacterial cells is an important mediator of bacterial evolution and adaptation to various environments. Three mechanisms of HGT in bacteria are known: conjugation, transduction and transformation. HGT is also used to transfer genetic constructs made in organisms that have relatively advanced genetic technologies, such as *Escherichia coli*, to organisms that are not as amenable to genetic manipulation. Commonly plasmid RK2 is used to mobilise plasmids having a compatible origin of transfer *oriT*. This system is used for genetically modifying the important human pathogen *Clostridium difficile.* In this work we demonstrated *oriT* is not required for transfer between *E. coli* and *C. difficile* and that transfer is abolished in the presence of DNase indicating that a possible cell-to-cell transformation-like mechanism is responsible for transfer. **Objectives:** To investigate the mechanisms of transfer of plasmids between *E. coli* and *C. difficile.* **Methods:** *E. coli* donor strain CA434 (HB101 carrying the IncPß conjugative plasmid, R702) was transformed with pMTL9301 or pMTL9301Δ*oriT*. *E. coli* donors containing the plasmids was mixed with *C. difficile* CD37 and incubated anaerobically without selection for 18 hours then transferred onto selective plates, supplemented with the antibiotic to select for the plasmid-encoded resistance, with counter-selection against the *E. coli* donor. **Conclusions:** Deletion of *oriT* lowered the transfer frequency of pMTL9301 but did not stop it indicating that pMTL9301 can be transferred by a mechanism different from conjugation. In support of this pMTL9301Δ*oriT* could not transfer to *C. difficile* in the presence of DNase. We hypothesize that an unknown and undefined DNA uptake system, possibly a cell-to-cell transformation-like mechanism is involved.

Author Disclosure Block:

L. Khodadoost: None. **P. Mullany:** None.

Poster Board Number:

SUNDAY-757

Publishing Title:

Htra1 Functions to Induce *Comga* Transcription and Increase Transformation Efficiency in *Staphylococcus aureus*

Author Block:

K. L. Maliszewski, **P. Roy**, P. D. Fey; Univ. of Nebraska Med. Ctr., Omaha, NE

Abstract Body:

Natural bacterial transformation, which occurs during a physiological state called competence, is responsible for a major proportion of horizontal gene transfer and evolution in certain species. *Staphylococcus aureus*, thought to evolve primarily through point mutation and acquisition of mobile genetic elements via conjugation and transduction, has never been considered a naturally transformable species until recently. The DNA binding and uptake apparatus, encoded by *com* genes, are conserved in *S. aureus*. Moreover, competence regulation in *S. aureus* have been found to be dependent on the alternative sigma factor σ^H, its overexpression facilitating the uptake of both plasmid and chromosomal DNA. However, the genes that function with σ^H are unknown. Hence this study was designed to identify genes that function with σ^H to regulate *comGA* transcription and induce competent state in *S. aureu*s.The Nebraska Transposon Mutant Library was utilized to identify genes that regulate transcription of *comGA*. The phage library was transduced into *S. aureus* RN4220 *PcomGA::lacZ* reporter strain. β-galactosidase activity of the mutants with enhanced *comGA* transcriptional activity was confirmed by the modified Miller assay. qRT-PCR was performed to evaluate *sigH* and *comGA* expression and select for mutants of interest. Lastly, to determine the function of *htrA1* and *htrA2* in transformation frequency, σ^H was overexpressed and transformation experiments were performed on N315, N315 *htrA1*, N315 *htrA2*, and N315 *htrA1 htrA2* with or without the *sigH* overexpressed plasmid (pNF285).The screen identified 117 genes that induced transcription of *comGA* including *htrA1* and *htrA2* encoding serine proteases previously linked to competence induction in *S. pneumoniae.* A transformation frequency of ~10^{-6} was detected in N315/pNF285, while the transformation frequency was increased two orders of magnitude (10^{-4}) in N315 *htrA1*/pNF285 and N315 *htrA1 htrA2*/pNF285; no change was observed in N315 *htrA2*/pNF285. Complementation with plasmid encoded *htrA1* decreased N315 htrA1/pNF285 transformation frequency, suggesting a function for HtrA1 in regulating competence induction in *S. aureus.*In conclusion, our data suggests that HtrA1 functions to regulate σ^H activity, and subsequently *comGA* transcription, thus regulating transformation efficiency in *S. aureus*.

Author Disclosure Block:

K.L. Maliszewski: None. **P. Roy:** None. **P.D. Fey:** None.

Poster Board Number:

SUNDAY-758

Publishing Title:

Hyper Efficient Horizontal Genome Transfer Between *Streptomyces* Strains by Insertion of Conjugative Transfer Genes on the Chromosome

Author Block:

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Abstract Body:

In the nature, lateral gene transfer (LGT) was thought to take an important role in bacterial evolution. Little is known about the mechanism how the large DNA fragment could be transferred in bacterial genome evolution. The case of Hfr of the F factor in *Escherichia coli* is exceptionally well known mechanism of genome level transfer where the whole genome is transferred with high frequency. We proposed that Hfr like mechanism would be working in bacterial evolution. *Streptomyces* is major soil bacteria and known as a major antibiotic producer. They have frequent small conjugative circular plasmids. We have studied the conjugation mechanism of the pSN22, a small conjugative plasmid replicated via rolling circle mechanism, from *S. nigrifaciens* (*S. flavovirens*). pSN22 transfer depends on the tra genes consisted of *traR, traA, traB* and *spdB*, and the *cis*-element *clt*. The pSN22 transfer is accompanied with chromosomal mobilization. After conjugation between *S. lividans*, the pSN22 derivatives transferred nearly 100% recipient cells in donor excess condition, and up to 10^{-3} recipients were chromosome recombinants. To test the Hfr like behavior of the pSN22 *tra* system, the DNA fragment encoded the *traR, traA, traB* genes and *clt* locus was inserted *S. lividans* TK21 genome using phiC31 integration system with Neomycin resistant gene. The resultant strain was crossed with *S.lividans* TK24 derivative (Sm^r). Surprisingly over 10% recipient cells showed Neomycin resistant, indicating that chromosomal transfer from donor cell occurred with very high frequency. This Chromosomal Mobilizing Activity (CMA) was lost by mutations within *traB* or in the absence of the *clt* region. The lack of *traA* resulted in the decrease of CMA to 10^{-4} likely in the case of plasmid transfer. To test the application possibility of this system in genomic engineering in *Streptomyces*, we tested the inter-species genome transfer from *S. lividans* to the other *Streptomyces* strains. Although the frequency was decreased, enough chromosomal recombinants were obtained. The potential of this technique in genome engineering will be discussed.

Author Disclosure Block:

M. Kataoka: None. **T. Miyatake:** None. **J. Kim:** None. **K. Murabata:** None.

Poster Board Number:

SUNDAY-759

Publishing Title:

Detecting Lateral Gene Transfer Events in Host-associated Microbial Communities

Author Block:

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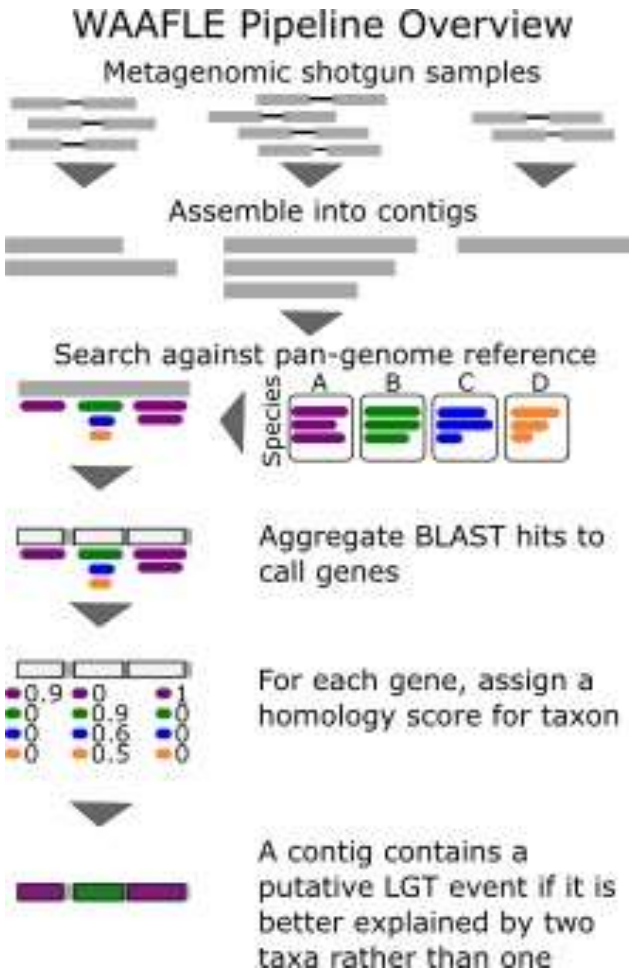
Abstract Body:

Background: Lateral gene transfer (LGT) is a key mechanism for adaptation and maintenance of gene diversity in microbial communities. Currently, methods to detect LGT are time consuming and optimized for whole genomes. However, many microbial communities are profiled using metagenomic shotgun sequencing, which generates short, fragmentary assemblies. **Methods:** Here, we present a new method to detect recent LGTs in shotgun metagenomes, titled WAAFLE (Workflow for Annotating Assemblies and Finding LGT Events). Shotgun reads are first assembled into contigs, which are then searched against a reference database consisting of pan-genomes. This can provide a reference-based gene annotation for contigs by grouping overlapping homologous regions. Homology to pangenome elements is then used to assign and score taxa of origin for each gene. We then compute how likely the contig is explained by one taxon versus multiple. Sufficiently high multiple-taxon scores indicate a likely LGT. **Results:** WAAFLE achieved median true positive rates from 86.4-91.2% and maintained median false positive rates from 0-0.6% on a set of 1,000 synthetic contigs containing LGT events spanning 8 phylogenetic levels. To date, we have further analyzed 12 body sites' metagenomes from one female in the Human Microbiome Project (HMP). Genus-level LGT events ranged from 0 events out of 1552 contigs (anterior nares) to 434 events out of 139878 contigs (stool). LGT analyses are ongoing for 265 individuals on 6 major body sites at up to 3 time points in the HMP. **Conclusion:** WAAFLE provides a validated method for identifying recent LGT events, donor and recipient taxa, and transferred functions in shotgun metagenomes.



Author Disclosure Block:

T.Y. Hsu: None. **E.A. Franzosa:** None. **C. Luo:** None. **D.H. Wong:** None. **M.G.I. Langille:** None. **R.G. Beiko:** None. **C. Huttenhower:** None.

Poster Board Number:

SUNDAY-760

Publishing Title:

A Functional Analysis Of The Stator Complex Of The Flagellar Motor From Alkaliphilic Bacillus Trypoxylicola

Author Block:

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Abstract Body:

Many bacteria swim by rotating their flagella. The flagella are filamentous organelles that extend from the cell surface. A flagellum consists of three parts, the filament (helical propeller), the hook (universal joint), and the basal body (rotary motor). Intensive genetic and biochemical studies of the flagellum have been conducted in *Salmonella* and *Escherichia coli*, and more than 50 gene products are known to be involved in the flagellar assembly and function. The flagellar motor is energized by either a proton (H^+) or sodium ion (Na^+) motive force, and its torque is generated by electrostatic interaction between the rotor (FliG) and stator interface. In general, there are three kinds of stator complexes: MotAB-type stators use protons, while MotPS- and PomAB-type stators use Na^+ as coupling ions. In 2012, we reported that alkaliphilic *Bacillus alcalophilus* AV1934 was first identified as a bacterium which uses Na^+, potassium ion (K^+) and rubidium ion (Rb^+) as coupling ions for flagellar rotation (1). This finding suggested that coupling ions other than H^+ and Na^+ are available to power the bacterial flagellar motor. Recently, we surveyed another example of a bacterium which can use K^+ as a coupling ion for bacterial flagellar rotation. Alkaliphilic *Bacillus trypoxylicola* was selected as a candidate bacterium because this bacterium can grow at high alkaline pH and K^+ rich conditions. In this study, we describe the flagellar stator protein (BT-MotPS) from *B. trypoxylicola* that is coupled to both Na^+ and K^+. In the absence of Na^+ or K^+, no swimming was observed in *B. trypoxylicola*. A stator-free *E. coli* mutant expressing BT-MotPS complemented a deficiency in motility in the presence of Na^+ or K^+. Thus, the operating principles of flagellar motors and molecular mechanisms of ion selectivity are clarified.

Author Disclosure Block:

M. Ito: None. **R. Imazawa:** None. **S. Naganawa:** None.

Poster Board Number:

SUNDAY-761

Publishing Title:

Energy And Aerotaxis Receptors In *pseudomonas*: Phylogenetics And Functional Characterization In *p. Pseudoalcaligenes* Kf707

Author Block:

S. C. Booth, R. J. Turner; Univ. of Calgary, Calgary, AB, Canada

Abstract Body:

Energy-, aero- and chemo-taxis allow bacteria to swim towards optimum growth environments. Taxis towards specific chemoattractants has been attributed to their binding to cognate, membrane-bound receptors called Methyl-accepting Chemotaxis Proteins (MCPs). Recently, ligands for many MCPs have been identified in various *Pseudomonas* species, though the majority remain unknown (1). This is partially because energy and aerotaxis have been found to mask the function of some MCPs as they enable cells to swim towards optimum environments for generating energy, be it ideal concentrations of oxygen or metabolizable carbon source (2). The MCPs *aer* (homologous to the MCP in *E. coli)* and *aer-2* were identified in *P. aeruginosa* and have been respectively characterized as energy and aerotaxis receptors (3), though the function of *aer-2* has been disputed (4). Conversely, 3 homologs of *aer* were found in *P. putida* of which only one was functional in energy taxis, with the role of the other homologs remaining unknown (5). No homolog of *aer-2* was found. As taxis is important for the ability of various *Pseudomonas* species to swim towards attractants in pathogenesis, plant-growth promoting and bioremediation scenarios it would be beneficial to understand the function of these genes and their distribution within the genus in order to elucidate whether they are redundant in function or not.Phylogenetic analysis of available *Pseudomonas* genomes has revealed that neither *aer* nor *aer-2* are part of the core genome and that the presence and number of *aer* homologs is widely variable. Despite this variability, the genomic environments of each of the homologs are conserved implying conserved function. There are four homologs of *aer* of which some species possess up to 3. Few species possessed both multiple *aer* homologs and *aer-2*. To investigate possible redundancy or alternative functions for these genes, deletion mutants were constructed in *P. pseudoalcaligenes* KF707 as it possesses 3 *aer* homologs and *aer-2*. Using all possible combinations of single and multiple deletions a semi-redundant function has been revealed for these genes. Swim diameters in soft-agar stab plates showed that the most ancestral homolog of *aer* is responsible for most energy taxis but functions in conjunction with the other homologs and also, surprisingly, with *aer-2*.

Author Disclosure Block:

S.C. Booth: None. **R.J. Turner:** None.

Poster Board Number:

SUNDAY-762

Publishing Title:

Organization of Flim and Fliy in the Flagellar Switch Complex of *Bacillus subtilis*

Author Block:

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Abstract Body:

The flagellar switch complex is a protein assembly that regulates the direction of rotation of the flagellar filament in response to changes in cellular levels of phospho-CheY, regulated by the chemotaxis pathway. The complex is made up of multiple copies of the proteins FliG and FliM, together with either FliY (in Gram-positive organisms) or FliN (in Gram-negative organisms). Despite the clear homology of the switch proteins in *E. coli* and *Bacillus subtilis,* the switches respond oppositely to phospho-CheY, giving CW rotation in *E. coli* but CCW rotation in *B. subtilis*. Further, FliY has an additional large middle domain that is not present in *E. coli* FliN, suggesting significant differences in subunit organization. In the present work, we used cross-linking, two-hybrid assays of protein interaction, and systematic mutagenesis to examine the organization of FliM and FliY in *Bacillus subtilis.* Two-hybrid and crosslinking data indicate that the middle domain of FliM ($FliM_M$) interacts with itself and is organized similarly to the corresponding domain in *E. coli*. The large $FliY_M$ domain that is not present in Gram-negative organisms also shows a self-interaction in two-hybrid assays, but does not interact with $FliM_M$, and thus appears to form an assembly distinct from the $FliM_M$ array. Disulfide-crosslinking results indicate significant differences in the organization of the bottom (membrane-distal) part of the complex: Whereas in *E. coli* this is formed from an alternating array of $FliM_C$ domains and FliN multimers, in *B. subtilis* the $FliM_C$ and $FliY_C$ domains can form an intertwined dimer, structurally similar to a $FliY_C$ dimer observed in crystal structures (from *Thermotoga maritima*). This intertwining of $FliM_C$ and $FliY_C$ might account for the closer homology of these domains in *B. subtilis* than in *E. coli* or other Gram-negative species, and would imply a 1:1 ratio of FliM and FliY subunits in the switch. Two-hybrid data and switch- biased phenotypes of certain mutations indicate a probable site of interaction for phospho-CheY on the $FliM_M$ domain. The candidate CheY binding site corresponds with that proposed previously on the basis of NMR studies of Dahlquist and co-workers, using proteins of *T. maritima*. Overall, our findings provide a preliminary picture of the organization of the middle and lower parts of the *B. subtilis* switch complex. Future work will aim to extend these results to the upper part, containing FliG.

Author Disclosure Block:

E. Ward: None. **D. Blair:** None.

Poster Board Number:

SUNDAY-763

Publishing Title:

YbgC Is Involved in Motility of *Shewanella Oneidensiss*

Author Block:

T. Gao, H. Gao; Zhejiang Univ., Hang Zhou, China

Abstract Body:

Background: *Shewanella oneidensis*, a facultative γ-proteobacterium, is extensively studied for redox transformations. Now it is becoming a research model for many other studies such as biofilm formation, respiration diversity, fatty acid metabolism and motility. *S. oneidensis* is motile by a single polar flagellum. The flagellar genes are organized into a four-tiered transcriptional system. However, it is clear that the whole flagellar system is complex and there may be other regulator elements involved in flagellar transcription, assembly and function.**Methods:**In frame mutant construction and complementation, motility assay, microscope observation, site-directed mutagensis**Results:** In this study, we identified a role of *S. oneidensis* YbgC (*So*YbgC, SO_2752) in flagellar gene expression and motility. YbgC proteins have been proposed to be functionally associated with the Tol-pal system which is critical to division because its coding gene is co-transcribed with *tol-pal* genes. Unlike *tol-pal* mutants, Δ*SoybgC* results in no growth defect but increased motility, which is confirmed by genetic complementation. Promoter activity assay shows that expression of *flgKL*, whose products are hook proteins to which the filament is attached, increases 2-fold. These data indicate *So*YbgC can improve the stability of filament. YbgC proteins from *Helicobacter pylori* (*Hp*YbgC) and *Haemophilus influenza* (*Hi*YbgC) have thioesterase activity, and *Hi*YbgC^{D18N} does not show this activity. Our experiments show that *So*YbgC^{D15N} can not decrease motility, indicating that Asp-15 is important for YbgC's function in motility.**Conclusions:***So*YbgC may improve the stability of filament. *So*YbgC^{D15N} can not decrease motility, indicating that Asp-15 is important for YbgC's function in motility.To our knowledge, this is the first report for the role of YbgC in motility. It provides new insights into to our understanding of the bacterial locomotion by flagallar systems.

Author Disclosure Block:

T. Gao: None. **H. Gao:** None.

Poster Board Number:

SUNDAY-764

Publishing Title:

***Helicobacter pylori* Chemoreceptor Tlpb Mediates Tactic Responses to Acid, Not Urea**

Author Block:

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Abstract Body:

Background: *Helicobacter pylori* utilize natural pH gradients within gastric mucus to direct initial colonization in establishing lifelong infections. Gastric *Helicobacter* species express acid sensing chemoreceptor TlpB (also senses AI-2) that promotes negative chemotactic responses. A recent study reported that TlpB promotes urease-dependent positive chemotactic responses to urea which enables colonization and persistence. We initiated this study to formally test these two competing hypotheses. **Methods:** Chemotactic behavior of *H. pylori* and mutants (*ureAB, ureI*, and carbonic anhydrase, AC) were tracked by video microscopy in flat micro-capillary tubes (population based assays) and by syringe-based tactic assays (bacterial enumeration). Threshold tactic responses were determined over pH ranges from 1 - 8 and for urea from 10 microM to 10 mM. Competition assays (urea + HCl) tested tactic dominance and site-directed mutagenesis was used to identify periplasmic domain mutants defective in taxis.**Results:** Most bacterial species show no chemotactic response to urea and our studies confirmed that *H. pylori* and mutant strains exhibited no chemotactic response to urea. In contrast, *H. pylori* strains and *ureAB, ureI* and *CA* mutants displayed acid avoidance pH taxis. Since CA is primarily responsible for buffering of the periplasm, we determined that these mutants exhibited more robust tactic responses to external acid challenge than did WT strains. Since the urease system maintains a relatively pH-neutral periplasm (pH ~6.1) at external pH below 6.0, we next examined how TlpB responds to small changes in periplasmic pH. Mutation of two conserved histidine residues in the periplasmic domain of TlpB (H72A and H172A) correlated with loss of pH taxis. **Conclusions:** In an integrated model of acid acclimation and pH sensing, we show that TpB responds to strong acid via the PAS domains (<pH 5) and to weak acid by histidine protonation/deprotonation (>pH 5). We show that the urea-taxis phenotype results from urease mediated alkalization of the weakly buffered chemotaxis medium, creating a pH gradient (pH taxis). Our studies confirm that *H. pylori* utilize natural pH gradients to colonize the gastric mucosa and that TlpB competes successfully with the urease-dependent acid acclimation system that buffers the periplasm to mediate pH tactic behavior.

Author Disclosure Block:

P.S. Hoffman: None. **C. Gineste:** None. **R. Razzaq:** None. **S. Shukla:** None.

Poster Board Number:

SUNDAY-766

Publishing Title:

Functional Annotation of Chemoreceptors Using Recombinant Sensor Domain Ligand Screening

Author Block:

D. Martin-Mora[1], V. Garcia[2], J. A. Reyes-Darias[1], A. Ortega[1], B. Morel[1], A. Corral-Lugo[1], M. A. Matilla[1], T. Krell[1]; [1]Estación Experimental del Zaidín - Superior Council for Scientific Res., Granada, Spain, [2]Ctr. for Biomolecular Sci., Nottingham, United Kingdom

Abstract Body:

Many bacterial signal transduction processes are initiated by the recognition of signal molecules by sensor proteins. However, most of the sensor proteins are functionally unannotated and their cognate ligands remain unknown, which represents currently a major bottleneck.To identify ligand that bind to chemoreceptors, we have been using *in vitro* ligand screening approaches using recombinantly produced sensor domains. We report here the functional annotation of two chemoreceptors from the saprophytic bacterium *Pseudomonas putida* KT2440, termed McpP and McpQ. Using isothermal titration calorimetry studies we show that the McpP sensor domain binds acetate, pyruvate, propionate and l-lactate whereas the corresponding domain of McpQ recognizes specifically citrate and citrate/metal complexes. McpQ complements the ligand profile of the previously identified McpQ paralogue McpS, which binds many TCA cycle intermediates but no citrate/metal complexes. Chemotaxis assays showed that the mutation of the *mcpP* and *mcpQ* genes resulted in a strong reduction of the response towards the corresponding ligands identified. McpP is predicted to have a CACHE sensor domain and evidence is presented suggesting that this domain type may be associated with the recognition of pyruvate and acetate. The response to TCA cycle intermediates is thus mediated by the concerted action of two paralogous receptors, McpS and McpQ, that possess a broad and complementing narrow ligand specificity.The approach was successfully used to annotate chemoreceptors but can also be employed to annotate other of sensor proteins.

Author Disclosure Block:

D. Martin-Mora: None. **V. Garcia:** None. **J.A. Reyes-Darias:** None. **A. Ortega:** None. **B. Morel:** None. **A. Corral-Lugo:** None. **M.A. Matilla:** None. **T. Krell:** None.

Poster Board Number:

SUNDAY-767

Publishing Title:

Physiological and Transcriptomic Characterization of *Mvat mvau osro* Knockout Mutants Devoid of Pf4 Prophage in *Pseudomonas aeruginosa* Pao1

Author Block:

G. Li[1], C-D. Lu[2], L. Yang[3], J-Y. Li[2]; [1]Univ. of Massachusetts Lowell, Lowell, MA, [2]Georgia State Univ., Atlanta, GA, [3]Nanyang Technological Univ., Singapore, Singapore

Abstract Body:

MvaT and MvaU are two redundant xenogeneic silencing proteins of the H-NS family in *Pseudomonas aeruginosa*. Previous studies to investigate the physiological consequences of *mvaT* and *mvaU* depletion were hampered by activation of Pf4 prophage in the resulting mutants. In this study, an *mvaT mvaU* double knockout mutant (PAOΔTU) was constructed in a strain of PAO1 (Δpf4) devoid of the Pf4 prophage on the chromosome. In comparison to its parent strain and the wild type PAO1, PAOΔTU exhibited several distinctive phenotypes including loss of pyocyanin production, retarded motilities in swimming, swarming and twitching, and a slower growth rate in 2YT broth (generation time 15 minutes v.s. 9 minutes of PAO1). Transcriptome analysis by GeneChip (Affymetrix) revealed that over 227 genes were found up-regulated in PAOΔTU, including several multi-gene loci for type III and type VI protein secretion systems, O-antigen, exopolysaccharide, pili assembly, and many others of unknown functions. In addition, a relatively unexplored locus (PA2222-PA2228) including *qsrO* and *vqsM* for quorum sensing and virulence that exists only in PAO1 and some strains of *P. aeruginosa* was found highly induced in PAOΔTU. In comparison, about 54 genes including *rhlLR* and *pqsABCDE-phnAB* in quorum sensing were down-regulated in PAOΔTU. A triple knockout mutant of mvaT/mvaU/qsrO was constructed, and this mutant partly recovered the capability on pyocyanin production and motilities. Transcriptome analysis indicated that the expression levels of *pqsABCDE, phnAB, rhIL/rhIR* and some unknown genes were reverted back to the level as in its parental PAO1. Similar patterns of gene expression were also observed by RNA-seq analysis. Among ncRNA of known functions, only *crcZ* in carbon catabolite repression and *phrS* in PQS were found reduced significantly in PAOΔTU. These results support the global effects of MvaT and MvaU as gene silencers, and provide the evidence to link down-regulation of PQS and pyocyanin synthesis to the strain-specific QsrO protein as part of the MvaT/MvaU regulon.

Author Disclosure Block:

G. Li: None. **C. Lu:** None. **L. Yang:** None. **J. Li:** None.

Poster Board Number:

SUNDAY-768

Publishing Title:

Strain-Specific Regulation of Flagella Genes in *Helicobacter pylori*: A Complex Interplay Between Csra and Rpon

Author Block:

Y. T. Huang, C. Y. Kao, **J. J. Wu**; Natl. Cheng Kung Univ. Med. Coll., Tainan, Taiwan

Abstract Body:

Helicobacter pylori infects 50% of the population worldwide, and increases the risk for developing peptic and duodenal ulcer, gastric adenocarcinoma and mucosa-associated lymphoid tissue lymphoma (MALToma). Previous studies indicated that carbon storage regulator A (CsrA) is necessary for *H. pylori* full motility and infection ability. Moreover, CsrA controls *H. pylori* motility through regulating *rpoN* expression and flagella formation. In the present study, we further demonstrated the strain-specific regulation of flagellar-related genes expression, especially focused on the interplay between CsrA and RpoN. The results showed that both *csrA* mutant J99 and 26695c8 displayed deficient motility compared to the wild-type. Transmission electron micrographs showed that no flagella were observed in the *csrA* mutant J99 and 26695c8 compared to the wild-type strains. Only 57% *flaA*, 29% *flaB*, and 57% *rpoN* mRNA expression in the *csrA* mutant J99 determined by RT-qPCR ($p < 0.001$, < 0.001 and < 0.01, respectively). Interestingly, 210% *flaA*, 57% *flaB*, and 59% *rpoN* mRNA expression were determined in the *csrA* mutant 26695c8 compared to its wild-type ($p < 0.01$, < 0.05 and < 0.01, respectively). In addition, the FlaA and FlaB proteins were decreased in the *csrA* mutant J99 determined by western blotting, whereas the FlaA protein was no such change in the *csrA* mutant 26695c8. Furthermore, RT-qPCR results showed that the *flaA* mRNA expression was decreased more prominent in the *rpoN* mutant J99 compared to the wild-type, whereas *flaA* mRNA expression dramatically increased in the *rpoN* mutant 26695c8. These results suggest that the regulation of flagella/motility is strain-specific in *H. pylori*.

Author Disclosure Block:

Y.T. Huang: None. **C.Y. Kao:** None. **J.J. Wu:** None.

Poster Board Number:

SUNDAY-769

Publishing Title:

Altered Swarming Motility and Quorum-sensing Exhibited by a Mutant Lacking *lrp* in *Pseudomonas aeruginosa*

Author Block:

B. L. Moses, M. C. Griffin; Kennesaw State Univ., Kennesaw, GA

Abstract Body:

The leucine responsive regulatory protein, Lrp, has been widely characterized in the intestinal bacterium, *Escherichia coli*. Lrp belongs to the feast or famine protein family, responsible for gene repression and regulation based on the presence or absence of amino acid co-factors. Through extensive mutant and microarray analysis, it is projected to regulate up to 10% of the *E. coli* genome. Little is known about the Lrp homologue in *Pseudomonas aeruginosa*. Currently, only the *dadAX* operon involved in alanine catabolism has been demonstrated to be regulated by Lrp in *P. aeruginosa* (1). We speculate that Lrp is well-positioned to play a more global role for *P. aeruginosa* as it is found in many diverse environments (ie. water, soil and humans) thus requiring a versatile metabolism. We report here an altered environmental response, swarming motility, by an *lrp* knockout mutant. This complex motility is specifically-adapted for a viscous environment. When grown on swarming agar directly from a rich media, wild-type PAO1 swarms across the surface in an even concentric pattern away from the inoculation site. Conversely, an *lrp* knockout mutant has a ruffled and uneven, advancing margin morphology. Furthermore, when WT is grown side by side with the mutant on the same assay plate, an antagonistic effect is observed against WT. The growth margins of wild-type are repulsed or broken down nearest to the *lrp* mutant. This suggests that the mutant is producing an inhibitory or degradative product that is likely normally suppressed by the wild-type Lrp protein. SDS-PAGE analysis of supernatants from both strains indicates the presence of an extracellular protein present in *lrp*- supernatants only. Identification of this product is underway. The regulation of swarming motility by quorum-sensing (qs) guide bacteria to switch forms of motility (swarming, surface, gliding) in response to environmental conditions. Using a *Chromobacterium violaceum* acyl-homoserine lactones (AHL) signal-deficient mutant, we observed that a *C. violaceum* qs-induced violet pigment could be induced by AHLs from *P. aeruginosa* and an enhanced induction of *C. violaceum* pigment was seen for the *lrp* mutant in comparison to WT. These phenotypes have not been reported for Lrp in *P. aeruginosa* and may indicate more roles for this protein in *P. aeruginosa* than the regulation of alanine as previously reported.

Author Disclosure Block:

B.L. Moses: None. **M.C. Griffin:** None.

Poster Board Number:

SUNDAY-770

Publishing Title:

Citrate, an Organic Acid, Increases Biofilm Formation in *Pseudomonas fluorescens* Through the Diguanylate Cyclase, GcbC, and Limiting Adhesin Proteolysis by LapG

Author Block:

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Abstract Body:

The intracellular signaling molecule, cyclic diguanosine monophosphate (c-di-GMP) is a potent modulator that influences a wide range of bacterial processes. High levels of c-di-GMP shift bacteria from a planktonic life style to sessile life style, forming a biofilm. Biofilms are formed in response to environmental cues. These cues are of great medical and industrial interest due to the capability of enhancing antibiotic resistance, biofouling, and bioleaching. *Pseudomonas fluorescens*, a model organism of biofilm formation, requires cell surface localization of the large adhesin, LapA to promote biofilm formation. The level of LapA at the cell surface reflects the amount of c-di-GMP within the cell. In conditions unfavorable for biofilm formation, LapA is cleaved by the calcium-dependent protease, LapG, which inhibits biofilm formation. The mechanism of biofilm formation in *P. fluorescens* is well understood, however the environmental cues regulating this process are unknown. We hypothesized that citrate, an organic acid secreted by plants, is sensed by the diguanylate cyclase, GcbC, to promote biofilm formation. GcbC contains a calcium channel chemotaxis receptor (CACHE) domain, which are known to sense small environmental ligands. The calcium chelator, citrate, was previously shown to increase biofilm formation by inhibiting proteolytic activity of LapG. Here we propose a two-tiered role of citrate 1) enhances biofilm formation through inhibiting LapA proteolysis by LapG and 2) increases GcbC activity, thereby increasing c-di-GMP production and enhancing LapA levels at the cell surface.

Author Disclosure Block:

D. Giacalone: None. **T. Smith:** None. **L.J. Bergeron:** None. **G.A. O'Toole:** None.

Poster Board Number:

SUNDAY-771

Publishing Title:

Altered Levels of the Housekeeping Protein DNAq Affect Multiple Traits in *Pseudomonas aeruginosa*

Author Block:

D. A. Meireles[1], Y. Apidianakis[2], A. P. B. Nascimento[1], J. He[3], L. G. Rahme[3], R. L. Baldini[1]; [1]Inst. of Chemistry USP, Sao Paulo, Brazil, [2]Univ. of Cyprus, Nicosia, Cyprus, [3]Massachusetts Gen. Hosp., Boston, MA

Abstract Body:

Background: *P. aeruginosa* is an ubiquitous and metabolic versatile proteobacterium and PA14 is a highly virulent strain with the ability to infect several phylogenetically distinct hosts. The attenuated-in-virulence *kerV* deletion mutant strain displays a pleiotropic phenotype that includes higher production of pyocyanin and impaired motility. *kerV*, which encodes a putative S-adenosyl-L-methionine (SAM) methyltransferase, is located immediately upstream of *rnhA* and *dnaQ*, both related with DNA replication and repair. The organization of this locus is well conserved across several proteobacteria (1), suggesting that its genes products might function in correlate pathways. DnaQ is the ε-subunit of DNA polymerase III that has 3'-->5' exonuclease activity for proofreading in DNA replication and it is important to improve the catalytic efficiency of DNA polymerase III *in vitro* by its association with α-subunit (DnaE). **Methods:** The methods include detection of Transcriptional Start Sites (TSSs) of individual genes by 5'RACE, analysis of gene expression by qRT-PCR, transcriptional *lacZ* fusions and immunoblot detection.Virulence was assessed by using both feeding and pricking *Drosophila* model of infection.**Results:** We detected independent TSSs for each gene of *kerV* locus and we also found that deletion of the *kerV* coding region affects *dnaQ* expression, since *kerV* mutant presented lower levels of both mRNA and DnaQ protein. Levels of *rnhA* mRNA are also affected, but this effect was not observed at the protein level. Attempts to complement *kerV* mutant strain showed that twitching and swarming motility and a feeding *Drosophila* model of infection were rescued not by KerV alone, but only when wild-type levels of DnaQ were restored. The complementation experiments support the conclusion that some phenotypes of *kerV* mutant strain are caused by decreased DnaQ levels and the coordinated expression of three genes of locus are necessary for full rescue of wild-type phenotype.**Conclusions:** Although essential genes are usually overlooked in respect to their role in virulence, a better understanding of their roles and regulation may uncover novel functions that increase the fitness of the pathogen in the environment provided by the host.

Author Disclosure Block:

D.A. Meireles: None. **Y. Apidianakis:** None. **A.P.B. Nascimento:** None. **J. He:** None. **L.G. Rahme:** None. **R.L. Baldini:** None.

Poster Board Number:

SUNDAY-772

Publishing Title:

Development of a Replicable oriC Plasmid for *Mycoplasma genitalium*

Author Block:

M. Hernandez-Solans, S. Torres-Puig, E. Querol, J. Piñol, O. Q. Pich; Univ. Autònoma de Barcelona, Bellaterra, Spain

Abstract Body:

Over the last two decades, replicative oriC plasmids have been successfully developed for several mollicutes. The oriC region of these shuttle vectors contains DnaA binding sites similar to the *E. coli* consensus sequence 5'-TTATCCACA-3'. Therefore, the presence of conserved DnaA boxes is a hallmark of mycoplasmal oriC sites, which actually facilitates the identification of the replication origin in the chromosome. So far, the identification of the *Mycoplasma genitalium* oriC region (Mge-oriC) has been elusive. The usual locations of the oriC, i.e. flanking the *dnaA* gene (MG_469) or between the *parA* (MG_470) and *dnaN* (MG_001) genes, are devoid of conserved DnaA boxes in this bacterium. In order to identify the Mge-oriC, we used a computational method aimed to predict stress-induced DNA destabilization sites (SIDD). Susceptibility to stress-induced destabilization is closely associated with several classes of DNA regulatory regions, including promoters, terminators or replication origins. Using the SIDD method, we identified a putative oriC region between the *parA* and *dnaN* genes. We found that plasmids containing the putative oriC region were capable to replicate when introduced into *M. genitalium*. Transformation efficiencies obtained with the Mge-oriC plasmid were similar to those obtained with transposons. When the replicative oriC plasmid was introduced into the reference strain G37, it rapidly integrated into the chromosome at a high frequency. In contrast, the plasmid remained episomic when introduced into a *recA* null mutant. The absence of sequences with less than two mismatches with respect to the DnaA box consensus within the identified Mge-oriC indicates that DnaA binding sites of *M. genitalium* are significantly divergent. Of note, the Mge-oriC contains four instances of a 10-bp long palindromic sequence that is only found seven additional times in the chromosome. Deletion of some of these sequences abrogated the self-replicating capacity of the oriC plasmid. This result suggests the existence of novel regulatory sequences controlling replication initiation in *M. genitalium*. Work is in progress to pinpoint the DnaA binding sites and to establish the participation of additional sequences in the replication of the *M. genitalium* chromosome.

Author Disclosure Block:

M. Hernandez-Solans: None. **S. Torres-Puig:** None. **E. Querol:** None. **J. Piñol:** None. **O.Q. Pich:** None.

Poster Board Number:

SUNDAY-773

Publishing Title:

Human Cytomegalovirus Localization Pattern of Nascent Viral DNA Along with Replication Proteins in Human Fibroblasts Using Fluorescent Imaging

Author Block:

S. Manska, C. Rossetto, V. Gutierrez; Univ. of Nevada, Reno, NV

Abstract Body:

Human Cytomegalovirus (HCMV), a member of the herpesvirus family, is a highly prevalent pathogen that asymptomatically infects the majority of the population by adulthood. HCMV was originally thought to be an opportunistic infection in immunologically compromised individuals, predominantly in organ transplant recipients and HIV/AIDS patients. Congenital infection with HCMV is the number one viral cause of birth defects and leads to developmental disabilities such as diminished mental capability, deafness, and motor skill deficiencies. As this area of research continues to grow, it has been demonstrated that HCMV can also cause or exacerbate disease in healthy individuals. For HCMV DNA replication to take place, host cell machinery along with viral encoded factors are required for viral replication, leading to production of infectious virions and ultimately establishment of a lifelong latent infection in the host. Therefore, identification of the proteins required for DNA replication is essential towards understanding mechanisms of infection. Preliminary experiments performed using iPOND (identification of Proteins On Nascent DNA) with HCMV infected human fibroblasts followed by mass spectrometry (LC-MS/MS) have identified viral and cellular proteins that are associated with viral DNA synthesis. iPOND experiments identified known viral encoded factors required for viral DNA replication but also identified novel cellular and viral proteins associating with nascent DNA. Although these proteins have proven to be fundamental in viral replication, the details in protein and DNA co-localization were unknown. Using Click-iT® EdU Alexa Fluor® 594 which incorporates a fluorescently labeled nucleoside analog into replicating DNA, we now show nascent DNA synthesis in infected (AD169) vs. non-infected human fibroblasts. Furthermore, we use an Immunofluorescent Assay (IFA) to detect HCMV and cellular replication proteins along with nascent DNA to determine patterns of co-localization.

Author Disclosure Block:

S. Manska: None. **C. Rossetto:** None. **V. Gutierrez:** None.

Poster Board Number:

SUNDAY-774

Publishing Title:

An Accessory Helicase Pcra and Recombination Proteins Mitigate Replication-Transcription Collisions in *Bacillus subtilis*

Author Block:

P. Yeesin[1], A. Bittner[1], J. Peng[2], A. Medina[2], M. Schmitz[1], J. Wang[1]; [1]Univ. of Wisconsin-Madison, MADISON, WI, [2]Baylor Coll. of Med., Houston, TX

Abstract Body:

Since bacterial DNA replication and transcription concurrently take place on the same DNA template, collisions between replication machineries and RNA polymerase (RNAP) are inevitable and can lead to genome instability. Replication-transcription collisions can occur in either co-directional or head-on manners, depending upon specific gene orientation. Inversion of highly transcribed ribosomal RNA genes (*rrn*) creates strong head-on collisions and impairs DNA replication, which leads to loss of genome integrity and cell inviability in rich medium. Mechanisms by which bacterial cells cope with replication-transcription collisions during rapid growth remain poorly understood. In this work, we determined the roles of protein factors in alleviating replication-transcription conflicts in *Bacillus subtilis*. Using a candidate gene approach and plating efficiency, we identified an accessory helicase PcrA and multiple recombination proteins, namely RecA, RecFOR, and AddAB, which rescue *rrn*-inversion cells in rich medium. We further characterized PcrA ATPase activity and its C-terminus (an RNAP-interacting domain) and showed that both are indispensable for conflict mitigation. Interestingly, the rescue by PcrA is RecA-dependent, suggesting interactions between the two factors *in vivo*. To elucidate such interactions in *B. subtilis* cells, we used time-lapse fluorescence microscopy to monitor RecA dynamics in response to replication-transcription collisions in the presence or absence of PcrA. We found that PcrA promotes RecA-mediate homologous recombination after head-on collisions and prevents persistent RecA that could act as roadblocks to subsequent rounds of DNA replication. However, PcrA does not accelerate RecA disassembly after the homology-directed repair, implying that RecA displacement by PcrA (the mechanism demonstrated *in vitro*) is less likely in this case. Together, we propose a novel model in which PcrA and RecA interact *in vivo* to mitigate head-on replication-transcription collisions.

Author Disclosure Block:

P. Yeesin: None. **A. Bittner:** None. **J. Peng:** None. **A. Medina:** None. **M. Schmitz:** None. **J. Wang:** None.

Poster Board Number:

SUNDAY-775

Publishing Title:

SSB Recruitment of Exonuclease I Aborts Template-Switch Mutations in Bacteria

Author Block:

L. Laranjo, Stephen Gross, Danna Zeiger, Saie Mogre, Vincent A. Sutera Jr. and Susan T. Lovett; Brandeis Univ., Waltham, MA

Abstract Body:

Quasi-Palindromes (QPs) are short nucleic acid inverted repeats capable of forming hairpins and cruciform structures. Subsequently, these non-canonical nucleic acid structures can stall DNA polymerase and facilitate template switch mutations, double strand breaks and chromosomal rearrangements. Most of QP mutations result from template switching during DNA synthesis and they have been associated with several human diseases. Aiming to further understand the cellular avoidance mechanism of template-switch mutations, we have created QP reporters using a chromosomal QP in the *lacZ* gene. A frameshift mutation was created with the insertion of 4bp in one inverted repeat, causing a $lacZ^-$ phenotype. When a template-switch occurs onto the corresponding inverted repeat, the *lacZ* mutation is reverted and reports a $lacZ^+$ phenotype (blue colonies). In *E.coli*, the avoidance of QP related mutation involves novel genetic factors, including the two major exonucleases Exonuclease I (Exo I) and Exonuclease VII (Exo VII). Our results suggest that template switch mutations are stimulated by exonuclease-deficiency and different rates of mutation between the leading and lagging strand derives from more efficient avoidance of mutation through exonucleolytic degradation of the lagging strand instead of a more frequent occurrence of mutations on the leading strand. This work provides evidence of Exo I predominant role in aborting template-switch mutations and its required interaction with single stranded DNA binding (SSB). In addition we examined the effects of *xonA*-R148A, an Exo I mutant with no interaction with SSB, but with full exonuclease activity. Our results confirm that SSB interaction is absolutely necessary for ExoI to abort QP-associated mutagenesis on the lagging strand. Further studies will use QP reporters in yeast to assess QP mutation rates in eukaryotes.

Author Disclosure Block:

L. Laranjo: None.

Poster Board Number:

SUNDAY-776

Publishing Title:

Evolving Antimutators to Achieve Greater Genetic Stability for Synthetic Biology

Author Block:

D. Leon, D. Deatherage, J. E. Barrick; Univ. of Texas at Austin, Austin, TX

Abstract Body:

Biological systems are difficult to engineer because they are inherently unpredictable. Most synthetic devices that are encoded in the DNA of these "machines" decrease host fitness and are quickly inactivated, resulting in strong selection for mutations that lead to the loss of the designed function. To ensure the long-term reliability of synthetic devices, we must understand the mechanisms involved in genetic instability and engineer more stable host organisms. We propose that the stability of the host genome can be significantly improved by reducing the baseline mutation rate of the host organism, decreasing the probability that random mutations will render a device inactive. We have developed an iterative, universal method called Periodic Reselection for Evolutionarily Reliable Host Variants (PResERV), in which antimutator strains in a pool of mutants are enriched in a population over time by sorting for cells that maintain costly GFP expression. The final population is then sequenced to determine the causative mutations. An initial goal of this study was to address whether antimutator mechanisms are specific to the nature of the synthetic device and/or the strain background. In *Escherichia coli*, we assayed several K-12 derivatives (BW25113, MDS42, BL21, and TOP10) by expressing GFP on a pBR322-origin plasmid (pSKO4) or from a genomically integrated construct under control of T7 RNA polymerase. Our preliminary PResERV results using BW25113 + pSKO4 identified two antimutator mutations, in *polA* and *polB*, that are present in strains in which mutation rates on this plasmid are lower by a factor of 3- and 25-fold, respectively. We predict that the *polA* substitution results in a polymerase with higher fidelity and that the *polB* nonsense mutation leads to an antimutator phenotype by deleting this error-prone polymerase. Our work will provide essential knowledge and resources for engineering biological systems. Understanding the mechanisms involved in genetic stability will allow engineers to more rapidly tackle novel, challenging problems, without the hindrance of frequently inactivated parts.

Author Disclosure Block:

D. Leon: None. **D. Deatherage:** None. **J.E. Barrick:** None.

Poster Board Number:

SUNDAY-777

Publishing Title:

Polymorphism Associated with Fluoroquinolone Resistance Stabilizes *E. coli* MutM

Author Block:

B. D. Hornstein, A. C. Fluty, N. Sung, S. Lee, L. Zechiedrich; Baylor Coll. of Med., Houston, TX

Abstract Body:

A global problem, antibiotic-resistant bacteria infect 2 million people and kill 23,000 people each year in the U.S. alone. Only eight new antibiotics were approved by the FDA in the last two years, all of them target gram-positive bacteria, yet gram-negative infections are extremely problematic. Our goal is to prolong the use of the antibiotics that target gram-negative bacteria by better understanding resistance mechanisms. Toward this goal, since 1999, our laboratory has collected over 5,000 gram-negative clinical isolates, representing the full spectrum of known antibiotic-resistant phenotypes. Using this collection, we uncovered four single nucleotide polymorphisms (SNP) associated with fluoroquinolone resistance in *E. coli*, each of which encodes a modification in a DNA-binding domain. One of these resulting enzymes, MutM T127A, has an altered helix-capping residue in a helix-turn-helix motif. MutM is a DNA repair enzyme that excises 8-oxoguanine, a consequence of oxidative stress. We hypothesize that the threonine to alanine change in MutM stabilizes the helix-turn-helix motif and improves the ability of MutM to mitigate oxidative damage, which should affect antibiotic resistance. Supporting this hypothesis, a Δ*mutM* mutant strain complemented with *mutM(A127)* survived hydrogen peroxide exposure better than *mutM(T127)*, but *mutM(T127)* better survived UV exposure (1). After purifying both protein variants, we determined the protein stability using a thermofluor assay. We found that the melting temperature of MutM(A127) was consistently higher across 96 difference conditions than MutM(T127), with an average, statistically significant, difference of 1.5°C. DNA binding and catalysis studies are currently underway. The MutM(A127) variant is found in all fluoroquinolone-resistant *E. coli* isolates sequenced to date. The increased stability we detected may improve MutM function, making bacteria with this SNP better able to repair oxidative damage, which is a downstream mechanism of antibiotic activity.

Author Disclosure Block:

B.D. Hornstein: None. **A.C. Fluty:** None. **N. Sung:** None. **S. Lee:** None. **L. Zechiedrich:** None.

Poster Board Number:

SUNDAY-778

Publishing Title:

The Role of H-Ns in Nucleoid Compaction in *Escherichia coli*

Author Block:

C. Pina, C. Weitzel, S. T. Lovett; Brandeis Univ., Waltham, MA

Abstract Body:

H-NS is an abundant nucleoid associated protein (NAP) with many functions within the *E. coli* cell. It exists largely as a dimer but is able to oligomerize along DNA. Notably, H-NS has been implicated in DNA structure and organization as well as possible chromosome integrity. Over-expression of H-NS has been shown to produce a condensed toroidal nucleoid. In an attempt to further evaluate the toroidal structure (that has previously been suggested to be "unnatural"), we have re-examined the nucleoid using deconvolution fluorescence microscopy. We illustrate that the toroidal nucleoid is a physiologically relevant structure, appearing during exposure to protein synthesis inhibitors and entry into stationary growth phase. Moderate over-expression of H-NS is required to reproduce nucleoid condensation. We have demonstrated that over-expression of several abundant NAPs are unable to generate the same nucleoid structure produced when H-NS is over-expressed. This compaction is prevented by treatment with rifampicin, suggesting a dependence on transcription. Three individual mutations within *hns* were shown to have differing abilities to produce a toroidal nucleoid when their protein is over-expressed. Future experiments seek to examine H-NS mutants through live cell imaging and evaluate the consequences of mutant H-NS within the cell.

Author Disclosure Block:

C. Pina: None. **C. Weitzel:** None. **S.T. Lovett:** None.

Poster Board Number:

SUNDAY-779

Publishing Title:

Epigenetic Modification at the *vlha* Locus of *Mycoplasma synoviae* Wvu 1853^{t}

Author Block:

D. R. BROWN[1], G. F. KUTISH[2], M. A. MAY[3]; [1]UNIV OF FLORIDA, GAINESVILLE, FL, [2]UNIV OF CONNECTICUT, STORRS, CT, [3]UNIV OF NEW ENGLAND, BIDDEFORD, ME

Abstract Body:

Modified DNA nucleotides at the 78.5-kb *vlhA* locus of *Mycoplasma synoviae* WVU 1853^{T} were analyzed in an effort to identify candidate factors that control high-frequency unidirectional recombination between the single expression site of its primary adhesin VlhA and a contiguous reservoir of >60 promoterless *vlhA* pseudogenes. Methyl-6-adenine and methyl-4-cytosine residues identified by SMRT Analysis (pacb.com) were displayed using GenomeView (genomeview.org) and filtered for confidence score >30 (P<0.001) across the locus and flanking genes VY93_01145 *nanI* through VY93_01470 *gapDH*. Instances of genome-wide methylation motifs were mapped across the locus using the Insilicase degenerate motif finder (insilicase.co.uk). GC skew was visualized using GView (gview.ca). Motifs in the *vlhA* locus consistent with methylation by N-6 adenine-specific DNA methylases elsewhere throughout the 846-kb genome (GenBank CP011096) included GmATC, GAmAN$_6$TTT, AAmAN$_7$TTC, GAmAN$_5$YYCTTH, CTAmABN$_4$BNTTT and AAmANVN$_4$VTTAG. The locus was strikingly under-modified versus the rest of the genome. For example, only 33 of 208 (16%) sites recognizable by the Dam methylases VY93_01015 and VY93_02585 were modified in the locus versus 86% of 3,840 Dam sites modified genome-wide, and 23 of those 33 (70%) were only hemi-methylated, although the combined frequency of relative under-methylation at specific motifs did not reach statistical significance (Pearson chi-square P=0.0921). Its G+C content is 5.4 mol% higher than the whole genome, but no modifications by the N-4 cytosine-specific DNA methylase VY93_02380 were detected in the locus versus 742 instances found elsewhere. It is thought that *M. synoviae* acquired *vlhA* by horizontal transfer from *Mycoplasma gallisepticum*. If thus separated from species-specific enzymes or other factors necessary for its epigenetic modification, the resulting under-methylation could promote the high-frequency recombination and proliferation of *vlhA* pseudogenes observed in the new genomic context.

Author Disclosure Block:

D.R. Brown: None. **G.F. Kutish:** None. **M.A. May:** None.

Poster Board Number:

SUNDAY-780

Publishing Title:

Effect of FIS Deficiency on Radiation Survival of *Escherichia Coli*

Author Block:

M. B. Hussain, D. A. Hudman, **N. J. Sargentini**; ATSU/Kirksville Coll. of Osteopathic Med., Kirksville, MO

Abstract Body:

Background: The factor for inversion stimulation (*fis*) gene produces a nucleoid-associated protein (Fis) involved in growth regulation in *Escherichia coli*. Our previous, screening study suggested *fis*-associated radiation sensitivity. Our current study investigates this finding in more depth, and probes the role of Fis in DNA repair after UV- or X-irradiation, and in DNA recombination and in spontaneous and UV-radiation mutagenesis. **Methods:** A *fis779*::kan deletion mutation was introduced into *E. coli* K-12 AB1157 and related DNA repair-deficient strains (*uvrA, recA, recB, recC, recF*). Cells were grown in LB broth at 37^{o}C with aeration to log or stationary phase. Cell survival studies relied on plating assays after cells were treated with UV- (254 nm) or X- (160 kV) radiation. DNA recombination studies relied on an Hfr-dependent conjugation assay. Mutagenesis studies relied on an *argE3* (base-substitution) reversion assay. Statistical analysis was performed by interpreting radiation doses yielding 1% cell survival from triplicate, multi-dose survival curves, and t test was used to determine if differences were significant at $P \leq 0.05$. **Results:** Wild-type and *fis* mutant strains were compared for UV radiation survival in multi-dose studies. At 1% cell survival (for the wild-type strain), the *fis* mutation sensitized cells by 8 to 25-fold, if grown to log- or stationary-phase, respectively. After X-irradiation (stationary-phase only), *fis*-sensitization was much greater, i.e., 125-fold. To probe the *fis*-effect on specific DNA repair pathways, we compared the cell survival of stationary-phase cells (control vs. *fis* strain at 1% survival) after UV-irradiation. The *fis* mutation sensitized *uvrA* cells 7-fold ($P<0.001$). The *recF* strain was sensitized only 2-fold ($P=0.019$). However, *fis* did not sensitize the other *rec* strains tested, in fact *fis* protected the *recB* ($P=0.007$) and *recC* ($P=0.023$) strains about 2-fold. The *recA* strain was not protected ($P=0.069$). In associated studies, the *fis* mutant was 67% deficient in DNA recombination ability, normal for spontaneous mutagenesis, and 45% more susceptible to UV-radiation mutagenesis. **Conclusion:** Fis plays a greater role in the survival of X-irradiated vs. UV-irradiated cells, and its role in DNA repair most likely impacts recombinational repair (especially for DNA double-strand breaks) rather than nucleotide excision repair.

Author Disclosure Block:

M.B. Hussain: None. **D.A. Hudman:** None. **N.J. Sargentini:** None.

Poster Board Number:

SUNDAY-782

Publishing Title:

Rec220 and Rec534 Are Two Novel Regulators of RecA Function in the Human Pathogen *Mycoplasma genitalium*

Author Block:

S. Torres-Puig, C. Martínez-Torró, I. Granero-Moya, E. Querol, J. Pinyol, O. Q. Pich; Univ. Autònoma de Barcelona, Bellaterra, Spain

Abstract Body:

RecA is a highly conserved recombinase functioning in recombinational DNA repair and genome maintenance. It promotes the central steps of homologous recombination in bacteria, aligning and pairing two DNA molecules, and then promoting a strand switch by branch migration. To this end, RecA binds in long clusters to ssDNA to form a nucleoprotein filament. Both RecA loading onto DNA and filament extension are typically modulated by a broad network of accessory proteins, which can inhibit, promote or stabilize targeted assembly of recombinase filaments. Remarkably, the pathways that facilitate RecA loading onto ssDNA in many bacteria, e.g. RecBCD and RecFOR, seem to be missing in the streamlined bacterium *Mycoplasma genitalium.* In a recent study, we demonstrated that the alternative sigma factor MG428 is a master regulator of recombination. In addition to RecA and the Holliday junction resolvases, two previously uncharacterized proteins under the control of the MG428 regulator, MG_RS01295 and MG_RS02200 (hereinafter Rec220 and Rec534), were found to be necessary for homologous recombination in *M. genitalium*. In the present work, we analyzed the expression of a RecA-fluorescent protein fusion under the control of a constitutive strong promoter. Most of the cells of the isogenic mutant analyzed exhibited a diffuse fluorescence, but some mycoplasmas displaying a distinctive sharp fluorescence were also observed. These results are in agreement with the formation of RecA foci in a small subset of cells. Further analyses revealed that RecA foci formation required the expression of the Rec220 and Rec534 proteins. Additionally, we created mutants carrying Rec220- or Rec534-fluorescent protein fusions at their respective native loci. Of note, deletion of the *rec220* gene completely abrogated the presence of Rec534-fluorescent cells. In contrast, Rec220-fluorescent cells were still observed in *rec534* mutants, though the percentage of fluorescent cells was significantly reduced. Altogether, our data indicate that RecA, Rec220 and Rec534 interact *in vivo*, and that Rec220 and Rec534 have different roles in the regulation of RecA function. A model depicting the interplay of these three proteins to target RecA filamentation to specific sites is presented.

Author Disclosure Block:

S. Torres-Puig: None. **C. Martínez-Torró:** None. **I. Granero-Moya:** None. **E. Querol:** None. **J. Pinyol:** None. **O.Q. Pich:** None.

Poster Board Number:

SUNDAY-783

Publishing Title:

Inactivation of Sos System Reduces Atp Production and Membrane Integrity in Quinolone-resistant *Escherichia coli*

Author Block:

J-M. Rodriguez-Martinez[1], E. Recacha[2], J. Machuca[2], P. Diaz de Alba[2], M. Ramos Güelfo[2], F. Docobo Perez[1], J. Rodriguez Beltran[3], J. Blazquez[3], A. Pascual[1]; [1]Univ. of Seville, Seville, Spain, [2]Univ. Hosp. Virgen Macarena, Seville, Spain, [3]Inst. of Biomedicine of Seville, Seville, Spain

Abstract Body:

Background: SOS system has been postulated as target with potential impact in both quinolone resistance and mutant development. The aim of this study was to evaluate the impact of SOS system in resistance suppression in an isogenic collection of quinolone-resistant *Escherichia coli* strains using biochemical techniques. **Materials/methods**: Six isogenic strains were used: ATCC 25922 (wild-type), EC02 (*E. coli* ATCC 25922 plus Ser83Leu substitution in GyrA), EC04 (EC02 plus Ser80Arg substitution in ParC), EC08 (EC04 plus Asp87Asn substitution in GyrA), EC09 (EC08 plus deletion in *marR*) and EC59 (EC09 expressing QnrS1). To switch-off the SOS system, *recA* gene was inactivated (all strains) and *lexA* was replaced by *lexA1* (wild-type, EC04 and EC09). Ciprofloxacin concentrations assayed were: 1 mg/L (breakpoint for reduced susceptibility), 2.5 mg/L (human serum Cmax), 1xMIC for each isogenic group (on/off SOS system) and 0.5xMIC of the wild-type for SOS system for each isogenic group. Metabolic activity and viability were assayed by using the Luminescent BacTiter-Glo Microbial Cell Viability Assay (Promega) (ATP production) and LIVE/DEAD® BacLight™ Bacterial Viability Kit (Life Technologies) (membrane integrity), respectively. Fluorescence microscopy and *recA* reporter vector were also used. **Results**: *recA* reporter vector evidenced the lack of activation of SOS system in *recA* knockouts or *lexA1* mutants (at least 10-folds drop compared to wild-type SOS system). SOS inactivation produced a clear reduction in ATP production (about 100-folds after 24 hours) in all conditions assayed. Monitoring the viability of bacterial populations as a function of the membrane integrity of the cell showed a clear reduction (up to 95-folds after 4 hours of antimicrobial exposition) of surviving bacteria when SOS system was inactivated. These data were evidenced by fluorescent microscopy. **Conclusions**: This approach showed the sensitization of quinolone resistant *E. coli* (including both low level and high level resistant strains) by targeting *recA* or *lexA* genes. This strategy might have a potential role as adjuvant of antimicrobial treatment.

Author Disclosure Block:

J. Rodriguez-Martinez: None. **E. Recacha:** None. **J. Machuca:** None. **P. Diaz de Alba:** None. **M. Ramos Güelfo:** None. **F. Docobo Perez:** None. **J. Rodriguez Beltran:** None. **J. Blazquez:** None. **A. Pascual:** None.

Poster Board Number:

SUNDAY-784

Publishing Title:

Characterization of Mutations in *Acinetobacter baylyi* Adp1 *yqgf* Reveals an Unexpected Role in Enhancing Susceptibility to Dna Damage

Author Block:

D. J. McClung[1], **A. Calixto**[2], M. N. Mosera[2], S. J. Patel[2], E. L. Neidle[3], K. T. Elliott[2]; [1]Univ. of California Berkeley, Berkeley, CA, [2]The Coll. of New Jersey, Ewing, NJ, [3]Univ. of Georgia, Athens, GA

Abstract Body:

The gene *yqgF* is nearly ubiquitous in bacteria, and yet represents one of only 13 remaining essential genes of unknown function in *Escherichia coli*. We used the naturally transformable soil bacterium *Acinetobacter baylyi* ADP1 to begin to characterize *yqgF*, a predicted Holliday junction resolvase. Using deletion mutants and site-directed mutations in codons for predicted catalytic amino acids, we assessed the role of *yqgF* in comparison with *ruvC*, which encodes a known resolvase. Mutant strains were tested for effects on recombination-dependent phenotypes including the efficiency of allelic replacement by natural transformation, resistance to DNA damaging agents, and frequency of large spontaneous chromosomal deletions. We also surveyed sequences of novel DNA junctions resulting from spontaneous chromosomal deletions in these genetic backgrounds. Unexpectedly, Δ*yqgF* and Δ*ruvC* had opposite effects on each phenotype assessed. In addition, we found that the presence of a functional copy of *yqgF* resulted in increased susceptibility to DNA damage due to nalidixic acid or UV radiation. Despite the striking differences in phenotype between the Δ*yqgF* and Δ*ruvC* mutants, the amino acids predicted, based on similarity to RuvC, to participate in catalysis by YqgF were required for YqgF function. Taken together, these findings raise questions about the predicted function of YqgF as a Holliday junction resolvase. Further, our results establish *A. baylyi* ADP1 as a tractable system for molecular studies of this highly conserved but poorly characterized gene.

Author Disclosure Block:

D.J. McClung: None. **A. Calixto:** None. **M.N. Mosera:** None. **S.J. Patel:** None. **E.L. Neidle:** None. **K.T. Elliott:** None.

Poster Board Number:

SUNDAY-785

Publishing Title:

Does Recombineering Require a Replicating Dna Target?

Author Block:

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Abstract Body:

Recombineering, *in vivo* genetic engineering using bacteriophage recombination systems, is a powerful technique for making genetic modifications in bacteria. Two systems widely used in *Escherichia coli* are phage Red and the Rac prophage RecET. Both systems have a single-strand annealing protein and a 5'->3' double-strand DNA (dsDNA) exonuclease. Some experimental evidence suggests that recombineering requires a DNA target proficient for DNA replication. We further investigated the *in vivo* dependence of recombineering on DNA replication of recombining substrates. For the Red system, when replication of a target plasmid is prevented, Beta-mediated recombination with single-strand DNA (ssDNA) oligonucleotides is reduced nearly 1000-fold. The RecT recombination frequency is substantially less robust than that of Beta, but only when DNA replication is allowed. The Red Beta and Rac RecT recombination frequencies converge to similar levels when the plasmid target is not replicating. A very low level of plasmid by ssDNA recombination is observed in the absence of any phage functions and is independent of DNA replication. In contrast to these results, both the Red and RecET systems efficiently recombine a non-replicating linear dimer plasmid to yield a circular monomer. Our experiments show that for the Red system the requirement for DNA replication is substrate-dependent and consistent with Red recombination occurring primarily by single-strand annealing rather than by strand invasion.

Author Disclosure Block:

L.C. Thomason: None. **N. Costantino:** None. **D.L. Court:** None.

Poster Board Number:

SUNDAY-786

Publishing Title:

Sequence Analysis and Functional Studies of Xer Site-Specific Recombination Sites in *Klebsiella pneumoniae* Plasmids

Author Block:

M. Babayan[1], D. J. Sherratt[1], M. E. Tolmasky[2]; [1]Univ. of Oxford, Oxford, United Kingdom, [2]California State Univ. Fullerton, Fullerton, CA

Abstract Body:

Background: *Klebsiella pneumoniae* (Kp) has gained in importance as a hospital pathogen due to its acquired multidrug resistance. The sequencing of complete genomes of clinical isolates showed that Kp usually includes multiple plasmids that carry resistance genes, frequently as part of transposons. Most of these plasmids are poorly characterized despite their impact on the ability of the host strains to resist antibiotic treatment. An analysis of plasmids from two clinical isolates that were recently sequenced showed that most included regions highly related to Xer site-specific recombination (XSR) targets, which are essential for stable inheritance in several plasmids. **Methods:** Functionality of target sites was determined by dimer-resolution in vivo assays. *Escherichia coli* was transformed with dimers of recombinant clones harboring the sites. Dimers were prepared by transformation of the hyper-recombinogenic *E. coli* JC8679 followed by plasmid extraction and dimer purification. Site-directed mutagenesis was carried out using a commercial kit. **Results:** Inspection of the nucleotide sequences of XSR sites showed that many possess accessory sequences with a faulty ARG box, a locus that facilitates binding of one of the architectural proteins that help establishing the synaptic complex necessary for recombination. Others include variations in the distance between the core recombination site (crs) and accessory sequences, or in the lengths of the central region of the crs. Dimer resolution assays showed that these sites mediated resolution levels well below those necessary to stabilize the plasmids under laboratory conditions. In particular, mutagenesis of the ARG box indicated that the presence of the adequate nucleotides is essential for the mediation of recombination at high levels. **Conclusions:** We conclude that while XSR sites are present in most Kp plasmids, they are usually unable to mediate high levels of multimer resolution. It is therefore possible that they are essential only under specific growth conditions or that they mutated after becoming redundant, following the acquisition of replicative transposons that act as stabilizers through their own site-specific cointegrate resolution site. It is also possible that these sites have other still unknown functions.

Author Disclosure Block:

M. Babayan: None. **D.J. Sherratt:** None. **M.E. Tolmasky:** None.

Poster Board Number:

SUNDAY-787

Publishing Title:

Brujita Integrase: A Simple, Armless, Promiscuous Tyrosine Integrase System

Author Block:

B. L. Lunt, G. F. Hatfull; Univ. of Pittsburgh, Pittsburgh, PA

Abstract Body:

In most temperate phages lysogeny and immunity to superinfection are determined prior to integration into the host chromosome. However, mycobacteriophage Brujita is an unusual temperate phage in which establishment of immunity is dependent on chromosomal integration. Brujita integration is mediated by a non-canonical tyrosine integrase lacking an N-terminal domain typically associated with binding to arm-type sites within the phage attachment site (*attP*). This raises the questions of how these integrases (Ints) bind their DNA substrates, if they form higher order protein DNA complexes, and how site selection and recombinational directionality is determined. We used *in vivo* recombination and *in vitro* binding assays to explore these questions. Here we show that Brujita Integrase is a simple tyrosine recombinase, whose properties more closely resemble those of recombinases FLP and Cre than it does the canonical phage tyrosine integrases. Brujita Int uses relatively small DNA substrates, fails to discriminate between phage and bacterial attachment sites (*attP* and *attB*), cleaves attachment site DNA to form a 6-base overlap region, and lacks directional control. Brujita Int also has an unusual pattern of binding to its DNA substrates. It binds to two half sites (B and B') in *attB*, although binding to the B half site is strongly dependent on occupancy of B'. In contrast, binding to the P half site is not observed, even when Int is bound at P'. However, an additional Int binding site (P1) is displaced to the left of the crossover site in *attP*, is required for recombination, and is predicted to facilitate binding of Int to the P half site during synapsis. This simple tyrosine phage integrase system may reflect ancestral states of phage evolution with the complexities of higher-order complex formation and directional control representing subsequent adaptations.

Author Disclosure Block:

B.L. Lunt: None. **G.F. Hatfull:** None.

Poster Board Number:

SUNDAY-788

Publishing Title:

Mimicking *In Vivo* Stress Conditions Elicit Differential Responses from Several Strains of *M. tuberculosis* (*mtb*)

Author Block:

A. V. Veatch[1], J. Caskey[2], S. Mehra[3], D. Kaushal[1]; [1]Tulane Natl. Primate Res. Ctr., Covington, LA, [2]LSU Sch. of Vet. Med., Baton Rouge, LA, [3]LSU Sch. of Vetinary Med., Baton Rouge, LA

Abstract Body:

~1/3 of the human population is infected with *Mtb*, the causative agent of tuberculosis (TB). While most infections are latently controlled, 1.3M people die due to TB per year. *Mtb* is able to evade killing by phagocytes, survive in a dormant state within the granuloma, and able to re-activate to infect the next host. Phagocytes attempt to kill *Mtb* by using a variety of methods such as low pH, antimicrobial peptides, and oxidative stress. Appropriate response to stress is key to *Mtb*'s survival. Thus, *Mtb* must contend with hypoxia for its intra granulomatous survival. Of the major *Mtb* lineages, East Asian is associated with hyper virulence and lower immune responses. We hypothesized that variable virulence in *Mtb* strains may be due to differences in stress response. We therefore studied responses of *Mtb* strains CDC1551 (low virulence Euro Am), Erdman (high virulence Euro Am) and HN878 (high virulence East Asian) to hypoxia and oxidative stress using RNAseq. These strains are differentially susceptible to hypoxia and oxidative stress, correlating with virulence. Thus, high virulence strains Erdman and HN878 exhibit greater fitness in hypoxia, being able to replicate significantly better relative to CDC1551 in an *in-vitro* model of reactivation. During hypoxia the expression of i) a higher than expected number of tRNAs were induced in all strains, pointing to an interesting role for these genes in stress management; ii) greater than expected PE/PPE genes was down regulated in high relative to low virulence *Mtb* and iii) lipid biosynthesis genes was not enriched in the induced bin in the high relative to the low virulence strains. Reactivation to a higher magnitude may lead to a greater transmission, and thus higher virulence. Our results show that increased fitness in response to hypoxia imparts a greater magnitude of reactivation on high virulence *Mtb* and this response may explain the higher virulence of Erdman and HN878. In contrast, CDC1551 is highly susceptible to thiol oxidative stress while Erdman is resistant, with HN878 exhibiting an intermediate phenotype. Efficient phagocyte mediated killing of low virulence *Mtb* CDC1551 can explain its lower virulence as well as higher immunogenicity.

Author Disclosure Block:

A.V. Veatch: None. **J. Caskey:** None. **S. Mehra:** None. **D. Kaushal:** None.

Poster Board Number:

SUNDAY-789

Publishing Title:

Selective Activation of the Rv1625c Adenylyl Cyclase in *Mycobacterium tuberculosis*

Author Block:

R. Johnson[1], G. Bai[2], B. VanderVen[3], K. McDonough[1]; [1]SUNY Albany Sch. of Publ. Hlth., Albany, NY, [2]Albany Med. Coll., Albany, NY, [3]Cornell Univ., Ithaca, NY

Abstract Body:

Mycobacterium tuberculosis (Mtb) uses the 3'-5'-cyclic AMP (cAMP) second messenger to modulate expression of genes important to intracellular survival and pathogenesis in response to changing environmental conditions. cAMP signaling in Mtb is complex, potentially involving the activity of 15-17 adenylyl cyclases (ACs) and 10 putative cNMP binding effector proteins. The presence of many ACs has complicated the characterization of any individual cyclase within viable mycobacteria, and therefore most of our understanding of these enzymes is limited to biochemical characterization of purified recombinant proteins. Recently, a chemically diverse set of small molecules was found that inhibit mycobacterial growth both within macrophages and on cholesterol as a sole carbon source. Inhibition of Mtb growth was correlated with the production of high levels of cAMP, and multiple independent transposon insertions in *Rv1625c*, encoding a class III AC, conferred resistance to these drugs. Rv1625c has been of interest due to its homology to mammalian transmembrane ACs at both the sequence and structural levels. Here show that one of these compounds, V12-007958, specifically activates Rv1625c to convert ATP into cAMP, providing the first tool to selectively stimulate a single AC in live Mtb. We use both knock out and knock-in approaches to show that Rv1625c is necessary and sufficient for the compound to induce cAMP production in Mtb, *M. bovis* BCG and *M. smegmatis*. We rule out the potential for indirect effects mediated by Rv1625c on other mycobacterial ACs by expressing Rv1625c in *cya* deficient *E. coli*, showing that Rv1625c is responsible for producing the excess cAMP following V12-007958 treatment. cAMP was measured using a specific radioimmunoassay. In addition to their potential as therapeutics, V12-007958 and related compounds are the first small molecules identified capable of selectively activating a single AC in Mtb, and are powerful tools for studying cAMP signaling in this pathogen.

Author Disclosure Block:

R. Johnson: None. **G. Bai:** None. **B. VanderVen:** None. **K. McDonough:** None.

Poster Board Number:

SUNDAY-790

Publishing Title:

Characterization of a Third Transketolase in *Salmonella enterica*

Author Block:

J. A. Shaw, T. J. Bourret; Creighton Univ., Omaha, NE

Abstract Body:

Salmonella enterica serovar Typhimurium is a facultative intracellular pathogen that is a major cause of food-borne illness throughout the world. During infection of its host, *S.* Typhimurium must adapt to rapidly changing environments with fluxes in pH, nutrient availability, and osmolarity, as well as innate host defenses that include reactive oxygen species (ROS) and reactive nitrogen species (RNS). The pentose phosphate pathway (PPP) is notable for its contribution in maintaining cellular reducing power through the reduction of NADP+ to NADPH in the oxidative branch of the PPP (oxPPP), which enables *S.* Typhimurium to defend itself against ROS and RNS produced during infection. Downstream in the non-oxidative branch of the PPP (non-oxPPP), transketolases and transaldolases can either channel metabolites generated in the oxPPP to glycolysis, or divert carbon from glycolysis to generate precursors for nucleotide and aromatic amino acid biosynthesis. The *S.* Typhimurium genome encodes two transketolases that have been identified and characterized (TktA and TktB), along with third, putative transketolase (referred to here as TktC). TktC is hypothesized to be the product of two genes (*STM2340* and *STM2341*) that together form a functional transketolase enzyme. This study aims to explore the functional role of TktC in the PPP of *S.* Typhimurium. Analysis of growth in rich media revealed no major defects in strains lacking only one or two transketolases, while growth in the transketolase-deficient (Δ*tktA* Δ*tktB* Δ*tktC*) mutant was severely attenuated. Additionally, the transketolase-deficient strain was able to grow in minimal media with glucose as the sole carbon source, but failed to grow with ribose as the sole carbon source. This finding was indicative that the non-oxPPP was defective in the transketolase-deficient strain. These data suggest that not only are each of the enzymes able to compensate for the loss of the others, but also that TktC is capable of performing the transketolase reaction. This was supported by observations that 1) the expression of *STM2340* and *STM2341* is induced in strains lacking both *tktA* and *tktB*, 2) transketolase activity in the Δ*tktA* Δ*tktB* strain was similar to wild-type controls, and 3) that transketolase activity was absent in the strain lacking all three transketolases. Taken together, these data support the hypothesis that STM2340 and STM2341 produce a third functional transketolase in *S.* Typhimurium.

Author Disclosure Block:

J.A. Shaw: None. **T.J. Bourret:** None.

Poster Board Number:

SUNDAY-791

Publishing Title:

New Approach to Monitor Gamma-rays Effects on Different Bacteria in the Environment

Author Block:

A. Margaryan, D. Soghomonyan, S. Astanyan, K. Ohanyan, H. Badalyan, **A. Trchounian**; Yerevan State Univ., Yerevan, Armenia

Abstract Body:

Background: Development of mining of heavy metals and atomic technology lead to increasing of high-level radioactive wastes impacting on environment and can be dangerous for living organisms including bacteria. They are a basic part of biosphere having mechanisms to maintain cell integrity and activity upon environmental stress. Bacteria can be employed as sensors for monitoring of the environmental radiotoxicity.**Methods:** *Escherichia coli* M17 and *Pseudomonas aeruginosa* GRP3 wild types and accepted methods were used. Comparison analysis of bacterial cell morphology was carried out utilizing computational approach, using light and polarization microscopy and computer programs NOVA and LabView. As γ-rays source Cs^{137} with dose ranging from 30-35 μSv/h was emplyed.**Results:** The bacterial growth rate decreased 2-fold after 30 min γ-irradiation compared to control. The growth stationary phase of 30 min irradiated cells started 3 h later compared with non-irradiated cells. CFU of *P. aeruginosa* irradiated for 15 and 30 min with γ-rays decreased significantly by 10^1 and 10^4 times, respectively. It was found that γ-rays affected ATPase activity of *E. coli*: 15 min irradiation stimulated the *N,N'*-dicyclohexilcarbodiimide (DCCD) sensitive ATPase activity in 1.2-fold but 30 min irradiation had no effects. For *P. aeruginosa* the total H^+ efflux decreased on ~15% and ~30 % and the DCCD sensitive H^+ efflux suppressed on ~40 and ~50%, respectively, when compared with non-irradiated cells. Therefore, the F_0F_1-ATPase (for which DCCD is an inhibitor) could be a target for γ-rays, as for different environmental factors.Morphological alterations of *E. coli* and *P. aeroginosa* caused by γ-rays have been also detected using computational analysis. The average cell surface area decreased on 8% and 70% (15 min irradiated) and 3% and 50% (30 min irradiated), respectively, and bacterial cell perimeter (i.e. 2D projection of bacterial cell perimeter in photo image) - on 6% and 45% (15 min) and 2% and 35% (30 min), respectively. These changes were sensitive and statistically valid. They are stronger with *P. aeroginosa,* which could be used as a test strain.**Conclusions:** The comparison analysis points out γ-rays effects on different bacteria. As a rapid approach, computational analysis can be highly useful in monitoring of γ-rays effects on bacteria in the environment.

Author Disclosure Block:

A. Margaryan: None. **D. Soghomonyan:** None. **S. Astanyan:** None. **K. Ohanyan:** None. **H. Badalyan:** None. **A. Trchounian:** None.

Poster Board Number:

SUNDAY-792

Publishing Title:

Dinb is Structurally and Functionally Altered Upon Interaction with Reca

Author Block:

T. F. Tashjian, I. Lin, V. Belt, V. G. Godoy; Northeastern Univ., Boston, MA

Abstract Body:

Evolutionarily conserved translesion synthesis (TLS) DNA polymerases (pols) accommodate DNA lesions that otherwise cause replication-fork stalling and cell death. Consequently, TLS pols have an ample active site and relatively low fidelity. In *Escherichia* coli TLS pols' expression is regulated by the well-understood SOS response. However, the mechanisms underlying DNA Pol IV or DinB interaction with the cell's main recombinase, RecA, which increases DinB's fidelity, are unknown.Under non-damaging conditions DinB is approximately 10-fold more abundant than the replicative DNA pol III. It is possible that DinB could compete with pol III for access to RNA primers, which are readily available on the lagging strand. Because of DinB's relative low fidelity, this would result in high mutagenesis during normal DNA replication. The inherent ability of DinB to polymerize at RNA primers has not been previously studied.We purified both DinB and RecA in strains lacking the *dinB* and *recA* genes and separated the products of the DinB-RecA interaction by size exclusion chromatography. These were characterized by western blot, mass spectrometry, trypsin proteolysis, and circular dichroism spectroscopy. The activity of DinB upon interaction with RecA was tested by primer extension assays, where DinB incorporated nucleotides from a fluorescently labeled DNA or RNA primer, and compared to DinB alone.Remarkably, we find that DinB's secondary structure and trypsin proteolysis pattern is altered upon interaction with RecA. Moreover, DinB alone has a 2-fold preference to synthesize from a DNA primer compared to an RNA primer, which increases to a 20-fold preference upon interaction with RecA.In summary, DinB undergoes a structural change upon interaction with RecA. This change could be responsible for our previous observation of lowered DinB mutagenesis upon RecA interaction. RecA drastically increases DinB's preference for DNA primers, which could represent a regulatory mechanism for preventing DinB from synthesizing from RNA primers on the lagging strand during non-damaging conditions.

Author Disclosure Block:

T.F. Tashjian: None. **I. Lin:** None. **V. Belt:** None. **V.G. Godoy:** None.

Poster Board Number:

SUNDAY-793

Publishing Title:

Molecular Mechanism of *Pseudomonas Aeruginosa* Responses to Spermine Stress

Author Block:

Y-C. Peng[1], C-D. Lu[2]; [1]Georgia State Univ., Atlanta, GA, [2]Univ. of Massachusetts Lowell, Lowell, MA

Abstract Body:

Pseudomonas aeruginosa can grow efficiently on biogenic polyamines via the gamma-glutamylpolyamine synthetase (GPS) pathway. There are six GSP homologues encoded by *pauA1-pauA6* genes (polyamine utilization) scattered on the chromosome, and growth on spermine and spermidine was abolished completely in the *pauA2* knockout mutant. Without a functional PauA2, exogenous spermine exerts an adverse effect on growth as reflected on MIC measurements. Suppressors of the *pauA2* mutant against 10mM of spermine at pH 8.0 were isolated from the selection plates. These suppressors shared common changes on various phenotypes including delayed growth rate, retarded swarming motility, and pyocyanin overproduction. Transcriptome analysis of one such suppressor representative by GeneChip (Affymetrix) reveals that phosphate transporter genes (*pstS-pstCAB-phoU*) were highly induced over 5-20 fold. In addition, genome resequencing (Illumina) revealed that a unique C to T mutation at the *phoU* gene results in Ser to Leu amino acid substitution. Polyphosphate (polyP) accumulation can be visualized by Neisser stain. The reduction of polyP by the trans-acting polyphosphate phosphatase (PPX) increased the spermine susceptibility in the *pauA2* suppressor. Identical phenotypes were also observed in a Δ*pauA2*Δ*phoU* double knockout mutant. This study demonstrated polyphosphate accumulation as the potential protection mechanism against spermine toxicity in *P. aeruginosa*.

Author Disclosure Block:

Y. Peng: None. **C. Lu:** None.

Poster Board Number:

SUNDAY-794

Publishing Title:

Cell Envelope Stress Response Regulons & Adaptation to *n*-Butanol Stress in *Bacillus subtilis* 168

Author Block:

G. Mahipant[1], A. S. Vangnai[1], J. D. Helmann[2]; [1]Chulalongkorn Univ., Bangkok, Thailand, [2]Cornell Univ., Ithaca, NY

Abstract Body:

Biofuels such as *n*-butanol can be produced in appropriately engineered microbial hosts, but product toxicity remains a major obstacle. *Bacillus subtilis* 168 has a higher butanol tolerance than many other production strains. Here, we demonstrate that growth in the presence of 1%(v/v) butanol allows *B. subtilis* to better tolerate and grow in higher concentrations such as 1.6%. This suggests that *B. subtilis* may adapt to solvent stress, presumably by altering gene expression. The adapted cells are morphologically altered and have a much broader distribution of cell lengths compared to unstressed cells. We therefore hypothesized that *B. subtilis* adapts to butanol by activating the expression of one or more cell envelope stress responses (CESR). In *B. subtilis*, CESRs are regulated by extracytoplasmic function (ECF) σ factors and two-component systems (e.g. LiaRS, CssRS). Cell envelope homeostasis is also affected by other global regulators including σ^B, σ^D, σ^H, and σ^I. By monitoring the growth of various mutant strains in the presence of butanol, we have demonstrated that ECF σ factors are not the major regulators of adaptation. In fact, activity of σ^W, σ^X, and σ^M is decreased during growth in the presence of butanol, and mutants lacking these sigma factors are not impaired growth in butanol-containing medium. In contrast, mutants lacking σ^I (a positive regulator of *bcrC*, *mreBH*, and *lytE*) is more sensitive to butanol stress. These results are providing a picture of those genes and pathways that are most critical for adaptation of *B. subtilis* to *n*-butanol stress.

Author Disclosure Block:

G. Mahipant: None. **A.S. Vangnai:** None. **J.D. Helmann:** None.

Poster Board Number:

SUNDAY-795

Publishing Title:

A Novel Universal Compatible Solute of Halophilic and Halotolerant Bacteria: Trans-4-Hydroxy-L-Proline

Author Block:

K. Kim, H. Jeong, B. Jia, C. Jeon; Chung-Ang Univ., Seoul, Korea, Republic of

Abstract Body:

Halobacillus halophilus (*H. halophilus*), a moderate halophilic bacterium, has a capability to grow at a wide range of salinities. The strategy that accumulates compatible solutes to cope with osmotic stress in high salt environments has been well studied in *H.halophilus*, and glutamate, glutamine, and proline have been well known as compatible solutes. In this study, the accumulation of trans-4-hydroxy-L-proline (T4HP) was additionally identified from *H. halophilus* cells in the course of the analysis of osmolytes using ^{1}H-NMR. Accumulation of T4HP increased with the increase of salinity concentration in culture media. A gene that was annotated as multidrug DMT transporter permease (HBHAL_RS11735) in *H. halophilus* genome of GenBank was selected as a putative proline 4-hydroxylase (P4H) catalyzing the formation of T4PH by BLAST searches. To confirm the function of the putative P4H gene, the HBHAL_RS11735 gene was over-expressed in *E.coli* BL21(DE3) strain and the resulting recombinant strain showed the proline 4-hydroxylase enzyme activity, suggesting that the HBHAL_RS11735 gene is the P4H-coding gene in *H. halophilus*. RT-qPCR analysis showed that transcription levels of the HBHAL_RS11735 gene increased 80 times under 3M NaCl conditions compared to 0.4M NaCl. In addition, to confirm the presence of T4HP as a ubiquitous compatible solute, we analyzed T4HP from cell extracts of other halophilic or halotolerant bacteria that were cultivated at high salt conditions, which suggested that T4HP may be a novel universal compatible solute in halophilic or halotolerant bacteria.

Author Disclosure Block:

K. Kim: None. **H. Jeong:** None. **B. Jia:** None. **C. Jeon:** None.

Poster Board Number:

SUNDAY-796

Publishing Title:

Analyzing the Mechanism of Dipicolinic Acid Release during C*lostridium difficile* Spore Germination

Author Block:

M. B. Francis, J. A. Sorg; Texas A&M Univ., College Station, TX

Abstract Body:

Clostridium difficile is an anaerobic, spore forming bacterium that infects antibiotic-treated hosts. The vegetative form of *C. difficile* produces toxins that elicit the primary symptoms of disease but it is the spore form that is necessary for host to host transmission. Spores are metabolically dormant forms of bacteria that are resistant to harsh environmental conditions, including antibiotics. Spores sense changes in environmental conditions and begin the transition from dormancy to vegetative growth (germination) upon sensing appropriate signals (germinants). In *B. subtilis*, the initiation of germinant receptor-mediated spore germination is divided into two genetically separable stages. Stage I is characterized by the release of dipicolinic acid (DPA) from the spore core. Stage II is characterized by cortex degradation, and stage II is activated by the DPA released during stage I (DPA release precedes cortex hydrolysis during *B. subtilis* spore germination). We demonstrated previously that these stages I & II of germination are flipped during *C. difficile* spore germination (cortex hydrolysis precedes DPA release). To understand of the mechanism of DPA release, we investigated 3 proteins associated with the packaging and release of DPA in *B. subtilis*: SpoVAC, SpoVAD and SpoVAE. By creating in-frame deletions in the genes encoding these proteins and by using *B. subtilis* as a heterologous host, we hope to better understand how DPA is released from the *C. difficile* spore.

Author Disclosure Block:

M.B. Francis: None. **J.A. Sorg:** None.

Poster Board Number:

SUNDAY-797

Publishing Title:

Mitochondrial Activity and Cyr1 are Key Regulators of Ras1 Activation of *C. albicans* Virulence Pathways

Author Block:

N. Grahl[1], E. G. Demers[1], A. K. Lindsay[1], C. E. Harty[1], S. D. Willger[1], A. E. Piispanen[2], D. A. Hogan[1]; [1]Geisel Sch. of Med. at Dartmouth, Hanover, NH, [2]Franklin Pierce Univ., Rindge, NH

Abstract Body:

The human commensal and fungal pathogen *Candida albicans* is a major health concern and new therapies are desperately needed. Its morphological switch from yeast to hypha is associated with disease and the Ras1-Cyr1-PKA pathway is a major regulator of *C. albicans* morphogenesis as well as biofilm formation. Our previous studies of microbe-microbe interactions showed that bacterial phenazines and the analog methylene blue inhibit the yeast-to-hypha switch. Here, we show that mitochondrial inhibition by these compounds strongly decreased Ras1 GTP-binding and activity in *C. albicans* and other *Candida* species. Consistent with there being a connection between respiratory activity and GTP-Ras1 binding, mutants lacking complex I or complex IV grew as yeast in hypha-inducing conditions, had lower levels of GTP-Ras1, and Ras1 GTP-binding was unaffected by respiratory inhibitors. Mitochondria-perturbing agents decreased intracellular ATP concentrations and metabolomics analyses of cells grown with different respiratory inhibitors found consistent perturbation of pyruvate metabolism and the TCA cycle, changes in redox state, increased catabolism of lipids, and decreased sterol content which suggested increased AMP kinase activity. Biochemical and genetic experiments provide strong evidence for a model in which the activation of Ras1 is controlled by ATP levels in an AMP kinase independent manner. The Ras1 GTPase activating protein, Ira2, was required for the reduction of Ras1-GTP in response to inhibitor-mediated reduction of ATP levels. Furthermore, Cyr1, a well-characterized Ras1 effector, participated in the control of Ras1-GTP binding in response to decreased mitochondrial activity suggesting a revised model for Ras1 and Cyr1 signaling in which Cyr1 and Ras1 influence each other and, together with Ira2, seem to form a master-regulatory complex necessary to integrate different environmental and intracellular signals, including metabolic status, to decide the fate of cellular morphology. Future studies will show if this knowledge can be used to develop therapies that would favor benign host-*Candida* interactions by promoting low Ras1 activity.

Author Disclosure Block:

N. Grahl: None. **E.G. Demers:** None. **A.K. Lindsay:** None. **C.E. Harty:** None. **S.D. Willger:** None. **A.E. Piispanen:** None. **D.A. Hogan:** None.

Poster Board Number:

SUNDAY-798

Publishing Title:

***Saccharomyces cerevisiae* as a Model for Screening Volatile Organic Compounds**

Author Block:

S. U. Morath, C. E. Boland, J. W. Bennett; Rutgers Univ., New Brunswick, NJ

Abstract Body:

In indoor environments, volatile organic compounds (VOCs) are gaseous at room temperature, readily dissipate throughout the environment, and may be of anthropogenic or biogenic origin. VOCs, particularly those produced by microbes, have been correlated with "building related illness," a diagnosis with symptoms including fatigue and headache that resolve upon exiting the offending space. Additionally, the built environment provides a relatively closed ecosystem in which microbial VOCs may be present at increased concentrations and maintain their normal function as signaling molecules that alter population dynamics and behavior of the receiving organism, whose growth may be enhanced or inhibited. In this study, we used *Saccharomyces cerevisiae* to investigate the response to VOC exposure and the accompanying basic response mechanisms, which are often conserved in eukaryotes, from fungi to humans. We determined the sensitivity of *S. cerevisiae* in response to exposure to twenty-seven VOCs, collectively or singularly produced by bacteria, fungi, plants, and in industrial processes, and containing various chemical functional groups. Using a serial dilution spot assay with a wild-type yeast strain, the compounds were screened at 10ppm for 48hr in a shaking incubator maintained at 30°C to determine their impact on yeast growth. Mean relative growth of yeast was compared to the control after exposure. We found that the common industrial VOCs toluene and xylene had little effect on yeast growth, while gas-phase formaldehyde, three microbial VOCs, 1-octanol, 1-octen-3-one, and trans-2-octenal, and a common plant VOC, trans-2-hexen-1-al, completely inhibited yeast growth at 10 ppm. Several other compounds were significantly inhibitory: 1-octen-3-ol, benzene, and isobutyraldehyde. We also identified 2 common microbial VOCs, 3-methyl-1-butanol and 3-octanone, that significantly increased yeast growth. These results indicate that neither means of production nor chemical structure is indicative of the impact of these VOCs at the tested concentration on yeast growth. This study is the first study using yeast to screen the effects of VOCs in the gas-phase, and provides the basis for future studies to reveal whether there is a common mechanism of action of VOCs within the cell.

Author Disclosure Block:

S.U. Morath: None. **C.E. Boland:** None. **J.W. Bennett:** None.

Poster Board Number:

SUNDAY-799

Publishing Title:

Comparison of the Chemical Signaling of the Two Life Stages in the Coccolithophore *Emiliania huxley* Exposed to Visible Light Stress

Author Block:

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Abstract Body:

The cosmopolitan marine phytoplankton species *Emiliania huxleyi* presents a digenetic heteromorphic life cycle, with the non-motile diploid phase bearing coccoliths and the flagellated haploid phase being non-calcified. *E. huxleyi* contains high concentrations of dimethylsulphoniopropionate (DMSP), the precursor of dimethylsulphide (DMS). DMSP is a multifactorial compound; it acts as a compatible solute in cell metabolism and as a chemical signal influencing bacterial and protist behavior. In the atmosphere DMS enhances cloud formation influencing climate. However, little has been documented on *E. huxleyi* chemical signal responses to high light stress, and how this relates to the heteromorphic life cycle. To this end, low light acclimated cultures of both haploid and diploid *E. huxleyi* were exposed to high light for 2 hr and allowed to recover in low light for 2 hr. During and after these treatments, growth, photosynthetic efficiency (Fv/Fm), DMSP (intracellular and released) and cell chlorophyll content were measured. Our preliminary results suggest that presence of high light decreased Fv/Fm to a greater extent in haploid than in diploid (calcified) cells, while recovery of Fv/Fm was rapid in both life stages. The chlorophyll content and intracellular DMSP was not different in both life stages. However, the dissolved DMSP increased after light stress in diploid cells suggesting a possible advantage serves as antioxidant protection or another cellular function, such as grazing protection in this life stage.

Author Disclosure Block:

L.E. Valentin-Alvarado: None. **S. Strom:** None.

Poster Board Number:

SUNDAY-800

Publishing Title:

The Effects of Stress on Protein Translation in *Entamoeba histolytica*

Author Block:

M. A. Hapstack, H. M. Hendrick, B. H. Welter, L. A. Temesvari; Clemson Univ., Clemson, SC

Abstract Body:

Entamoeba histolytica is an intestinal parasite that is known to cause amoebic dysentery and liver abscess, resulting in 100,000 annual deaths worldwide. During infection, *E. histolytica* encounters a number of different stresses in the tissues of its host. Other eukaryotic organisms use a conserved response to stress, involving the phosphorylation of a serine residue (S59) on the alpha subunit of eukaryotic initiation factor2 (eIF2α). The result of this is down-regulation of global protein translation to conserve energy for the stress response. Although genomic data has revealed that *E. histolytica* possesses the components of this system, translational-control of stress has never been examined in this parasite. To better understand how *E. histolytica* counters stress, global protein translation after stress was analyzed by polyribosome profiling. Cells were stressed (long-term serum starvation, glucose deprivation, heat shock), and then cell lysates were resolved using continuous sucrose gradient fractionation. Fractions were taken and analyzed by spectroscopy (254 nm) to detect relative polyribosome abundance. Only long-term serum starvation showed a significant decrease in polyribosome abundance, indicating that *E. histolytica* utilizes the eIF2α phosphorylation pathway to survive this stress. This is the first example of translational control in *E. histolytica*. The data are consistent with other data that show a significant increase in the level of phospho-eIF2α during serum starvation in this pathogen. Experimentation with cell lines expressing phosphomimetic and non-phosphorylatable mutants of eIF2-α in *E. histolytica* will promote further understanding of stress response in this parasite, as well as in eukaryotic systems as a whole.

Author Disclosure Block:

M.A. Hapstack: None. **H.M. Hendrick:** None. **B.H. Welter:** None. **L.A. Temesvari:** None.

Poster Board Number:

SUNDAY-801

Publishing Title:

Career Needs of Students and Recent Graduates in the Field of Microbiology

Author Block:

S. Gadwal; American Society for Microbiol., Washington, DC

Abstract Body:

With the rising number of young scientists electing to pursue non-academic careers, universities and professional societies are increasingly concerned with how they can better serve the professional development needs of these individuals. To accommodate this shift in career choice, there is also increased focus on improving biomedical training for students in master's and doctoral programs. To better understand the skills and career resources young microbiologists are seeking in the current market, an online survey was conducted. Students, postdocs, and early-career microbiologists from various organizations around the world were invited to participate and approximately 940 surveys were collected. When asked what resources would be most helpful with regards to professional development, 48% of the respondents cited a mentorship program based on their career interests, 36% wanted information on applying and interviewing for academic positions, and 31% wanted more internship opportunities. Survey participants were also asked to explain the communication, managerial, and interpersonal skills they wanted to develop or improve. A majority of respondents indicated that the communication skills they seek are in writing scientific papers (57%) and grants (52%); for managerial skills, the top two responses were project management (34%) and leadership (32%); and for interpersonal skills, a majority identified negotiation skills (63%) and conflict resolution (51%). Participants were also asked to indicate the type of organization they wanted to work in. As predicted, the majority (60%) were interested in non-academic careers, such as industry, government, and hospitals or clinical settings, while 40% of the participants remain interested in working in academia. Some survey respondents felt a lack of information regarding non-academic careers and how to pursue them. Overall, our findings indicate that a significant percentage of students, postdocs, and early-career scientists are considering non-academic careers and seek better preparation for such career paths. The information gained from this study can be used to assist in planning of future programming activities and educational training programs to better prepare young scientists for a non-academic career.

Author Disclosure Block:

S. Gadwal: None.

Poster Board Number:

SUNDAY-802

Publishing Title:

A Survey of Microbiology Education in Nursing Practice

Author Block:

R. J. Durrant, J. P. Fenn, R. L. Buxton, A. R. Brooks, A. K. Doig; Univ. of Utah, Salt Lake City, UT

Abstract Body:

Background: Nurses must have sufficient education and training in microbiology to perform many roles within clinical nursing practice (e.g. administering antibiotics, collecting specimens, preparing specimens for transport and delivery, education of patients and families, communicating results to the healthcare team, and developing care plans based on results of microbiology studies and patient immunological status). It is unclear whether current microbiology courses in the United States focus on the topics that are most relevant to nursing practice. We sought to utilize an internet-based survey to inquire about educational background in microbiology and related topics, as well as nurses' use of microbiology knowledge in their current duties in order to gauge the relevance of their microbiology education to nursing practice. Our goal was to formulate suggestions on specific topics to improve the quality and applicability of microbiology education for nursing students.**Methods:** Three hundred and twenty six Registered Nurses completed a confidential, web-based survey that inquired about the participant's past microbiology education, types of microbiology specimens collected, nursing duties that require microbiology knowledge, and frequency of infectious diseases encountered in practice.**Results:** The topics deemed most relevant to current practice were infection control, hospital acquired infections, disease transmission, and collection and handling of patient specimens. Topics of least interest included the Gram stain procedure and using a microscope. Surprisingly, there was little interest in molecular testing methods. This may highlight a gap in understanding molecular microbiological test utilization that could be partially bridged in a microbiology course.**Conclusions:** We now have data supporting anecdotal evidence that nurses are most engaged in learning about microbiological topics that have the greatest impact to patient care. Information collected from this survey will be used to adapt the focus and emphasis of microbiology courses to focus on relevant topics for nursing practice.

Author Disclosure Block:

R.J. Durrant: None. **J.P. Fenn:** None. **R.L. Buxton:** None. **A.R. Brooks:** None. **A.K. Doig:** None.

Poster Board Number:

SUNDAY-803

Publishing Title:

Wadsworth Center Master's Degree in Laboratory Science: 4 Years Strong

Author Block:

L. Mingle, K. Chou, D. Kay, A. Fayo, K. Musser, M. Perry, C. Egan; Wadsworth Ctr., New York State Dept. of Hlth., Albany, NY

Abstract Body:

The New York State Department of Health's Wadsworth Center Master of Science in Laboratory Sciences (MLS) Program is designed to train the next generation of public health laboratory (PHL) scientists and supervisors. The program provides a comprehensive overview of PHL fundamentals and allows development of advanced technical and analytical skills, combining didactic lecture-based coursework with lab training. Students gain hands-on practical experience and training in the major PHL areas, including infectious disease, environmental health, genetics, clinical biochemistry, clinical/diagnostic immunology, biodefense/emergency preparedness, translational medicine and lab quality certification. The 2-year, 6-semester (60-credit) curriculum includes 9 core courses, 4 special topics courses, lab rotations, and culminates with a capstone project. By addressing a practical challenge in the PHL, the capstone project provides essential lab training and strengthens analytic, interpretive and writing skills. Unique aspects of the program include extensive development of communication skills; exposure to lab operations and management, including best practices, financial operations, and human resource management; hands-on experience with outbreak investigations and new assay development and validation; and access to core facilities using state-of-the-art instrumentation and technology. The MLS program is in its 4th year with 5 current matriculated students. Six graduates have worked in grant-funded positions at Wadsworth Center's newborn screening, biodefense, bacteriology/mycobacteriology, virology and bloodborne viruses labs. Several have earned competitive Emerging Infectious Diseases (EID), Oak Ridge Institute for Science and Education (ORISE) and Advanced Molecular Diagnostics Emerging Infections Program (AMD-EIP) fellowships sponsored by the Centers for Disease Control and Prevention (CDC) and Association of Public Health Laboratories (APHL). Graduates of the MLS program are well-equipped to conduct lab analysis with cutting-edge tools and technology, investigate complex public health questions and respond to everyday challenges in public health.

Author Disclosure Block:

L. Mingle: None. **K. Chou:** None. **D. Kay:** None. **A. Fayo:** None. **K. Musser:** None. **M. Perry:** None. **C. Egan:** None.

Poster Board Number:

SUNDAY-804

Publishing Title:

Teaching Matters: Making the Most of Teaching Training for Everyone's Benefit

Author Block:

B. Fahnert; Cardiff Univ., Cardiff, United Kingdom

Abstract Body:

Teaching professionalism benefits academic career establishment/advancement even though most developed economies do not yet require pedagogic training for Higher Education teachers. Many Higher Education Institutions encourage and offer teaching training, and demands for compulsory training have become stronger. For example a European Commission High Level Group recommended that everyone teaching in Higher Education needs to be trained by 2020, combined with mandatory continuing professional development and linking recruitment and promotion to teaching performance. Teaching skills can be developed e.g. in formal university courses, staff development programmes and through learned societies. Training teachers is perceived as valuable by students. Staff certainly benefit from being supported when developing their personal philosophy of teaching based on educational theory, analysing their teaching practice and attempting teaching innovation. Yet, ensuring long-term benefit of generic teaching training for all stakeholders depends on successfully making it part of individual continuing professional development and local departmental academic culture. A stakeholder and discourse analysis and a mixed method study were conducted, qualitatively analysing experiences of teaching training participants and non-participating colleagues in a biosciences department. Emerging important aspects were e.g. relevance and accessibility of pedagogy as a discipline new to quantitative scientists, not being trained by peers, and issues around accepting and learning how to use qualitative methodology and its findings. Presented outcomes of the study outline opportunities that help every academic to engage with pedagogic learning and to embed it in their professional identity in light of such challenges as high workload, local disciplinary contexts and issues of recognition, and that help managers and institutions to support it. Understanding the opportunities and challenges crucially informs provision of training and tailoring communication with all stakeholders in order to optimally implement teaching training initiatives and to generally relate training of individuals to organisational learning, and to manage the respective change.

Author Disclosure Block:

B. Fahnert: None.

Poster Board Number:

SUNDAY-805

Publishing Title:

Insights Into the Academic Interests of Undergraduates Identified During Undergraduate Initiation of an Asm Student Chapter

Author Block:

J. A. Lucci, G. Tollefson; Univ. of Rhode Island, Kingston, RI

Abstract Body:

Background: In the fall of 2012, the University of Rhode Island consolidated its Microbiology, Biotechnology, and Biochemistry majors into an all-encompassing Cell & Molecular Biology (CMB) major. The the previously separate majors were organized into subcategories of this collective CMB major. To help students to reap the benefits of this departmental consolidation, a hand selected group of CMB students worked together to organize a student chapter of the American Society for Microbiology (ASM) to encourage increased unity and cooperation between a diverse group of students in the combined CMB program. At the inaugural meeting, the chapter took a poll to determine the greatest areas of interest for CMB students. This information could be used as valuable feedback to aid faculty in providing resources and coursework that is more closely aligned with our student population. **Methods:** To identify the groups interests, copies of the 'Scientific Areas' section from the *Scientific Background* section of the Directory Profile of the ASM website were provided at the next meeting. Chapter members indicated areas of interest on these sheets which were collected and analyzed by the chapter board. **Results:** Significant findings include that 11 of 18 (61%) members expressed interest in viruses and 10 out of 18 (55%) expressed interest in biodefense. 8 out of 18 (44%) members indicated interest in Immunology as well as Cell Biology. Fortunately for our undergraduates, these topics are explored in the new CMB curriculum. **Conclusions:** The variable nature of recorded areas of interest exemplifies the many focuses and applications of cell and molecular biology to industry, health, and academic research. We aim to share this data with the faculty of the University of Rhode Island CMB department to encourage personalized course material. We will also use this data to help us plan meeting content that will be helpful and interesting to our ASM student chapter.Data

Topics listed in Scientific Areas	# of Freshmen Out of 18 who Indicated Interest
Viruses	11
Biodefense/ Biotechnology/ Infectious Disease	10
Cell biology/Emerging diseases/Immunology	8

Bacteria/Bacteriophages/Extremophiles/ Pathogenesis	7
Host defense/Virulence	6
Antimicrobials/Clinical/Fungi/Genetics/Humans	5
Animal /Cultures/Ecology/ Parasites/ Plants/ Proteins/ Water	4
Anaerobes/Biophysics/Metabolism/Public Health/Quorum-Sensing/Space	3

Author Disclosure Block:

J.A. Lucci: None. **G. Tollefson:** None.

Poster Board Number:

SUNDAY-806

Publishing Title:

Cybernetic Girls Can Be Pinky: Empowering Biologists into Computational Fields

Author Block:

L. Casillas, L. Casillas-Santiago; UPR-Humacao, Humacao, Puerto Rico

Abstract Body:

Recent studies by the National Science Foundation indicate that women's participation in certain areas such as Computational Sciences (CS) is less than 18%. This number is lower in Latinas, with only 4.9% participation. A problem alluring Hispanics females to join careers in CS is that femininity and achievements in men-dominated careers are opposed. Consequently, we have engaged in a **Teaching to Increase Diversity and Equity in Science** (TIDES) initiative to increase the number of Latinas entering into CS. More specifically, our empowering program "*Cybernetic Girls can be Pinky*" is alluring Latinas into Computational Biology. Our program consist of changes at the curricular level revamping three Biology core courses with more quantitative analysis experiments, the offering of R and Phyton programming workshops to Biology students, and gender-related conferences for undergraduates. Comparison of the pre and post-tests that measures critical-thinking skills in the revamped courses indicated Hake's Gain up to 66% in five of the nine skills tested. In the Molecular and Cell Biology Course we implemented the Small World Initiative and students were able to identify to the molecular level more than 25 unique isolates with antibiotic activities. Students also had an active participation in oral reports, class discussions and final poster preparations. Overall evaluation of the programming workshops was 4.3 (with 5 as excellent), students only faced problems making graphs using ggplot2 and in how to simulate different distributions in a study. To fully empower our students we provided a Women in Science Course that emphasize the accomplishments of Latinas in Science. In all courses we provided students with a pre and post questionnaire named: What do you think about quantitative analysis and computational biology? We asked students perception toward female scientists specifically in Computational Biology. Interestingly, most students were not aware of the salary differences, masculine environments and how motherhood was considered an obstacle for success. We observed changes in male perceptions, for example, in the post tests male students reported stronger disagreement with the idea that one of the basic responsibilities of a professional woman is to raise her children. We plan to incorporate the changes, surveys and programming and gender workshops as part of our curriculum.

Author Disclosure Block:

L. Casillas: None. **L. Casillas-Santiago:** None.

Poster Board Number:

SUNDAY-807

Publishing Title:

Gram Stain Workshop Improves Technique Comprehension and Interpretation

Author Block:

M. S. Delfiner, Luis R. Martinez, Charles S. Pavia; NYIT Coll. of Osteopathic Med., Old Westbury, NY

Abstract Body:

Introduction: Laboratory diagnostic tests have an essential role in patient care, and the increasing number of medical schools that teach laboratory medicine reflect this importance. However, it has not been shown if pedagogical method influences competency in comprehension and interpretation of these tests. The Gram stain is a frequently used diagnostic test that helps categorize bacteria based on cell wall structure. Here, a randomized control study is used to determine if there is a difference when students learn Gram staining via a hands-on workshop, a small group discussion, or a lecture.**Methods:** Twenty-seven volunteers, selected from the first-year class at NYIT College of Osteopathic Medicine (COM), were randomly placed into three groups: a lecture group, a workshop group, and a discussion group. All participants attended a lecture where they were instructed on the structure of bacterial cell walls and the basic principles of the Gram stain procedure**.** Additionally, the workshop participants performed the Gram stain procedure and observed their stained slides under a light microscope. The discussion group participants interpreted pre-stained slides and discussed their findings. One week later, all participants took a multiple-choice quiz, which assessed understanding of staining procedure, importance, and interpretation. Mean scores were calculated and compared with analysis of variance (ANOVA) and the Bonferonni test. Chi-square analysis was used for categorical variables. One outlier was removed since it fell over two standard deviations from the mean.**Results:** Ten participants each were assigned to the workshop and the lecture groups, while seven were assigned to the discussion group. There was no significant difference between groups in regards to age, gender, and race. The mean quiz score for the workshop group was 13.1 out of 15 (SD=1.76), for the discussion group was 10.29 (1.98), and for the lecture group was 9.2 (2.44). There were significant differences between the workshop and lecture groups ($p=0.04$) and between the workshop and discussion groups ($p=0.002$). There was no difference between the discussion and lecture groups.**Conclusion:** This study shows that there is an improvement in Gram stain interpretation when students have hands-on experience. These results suggest importance in including a laboratory component in medical school curricula.

Author Disclosure Block:

M.S. Delfiner: None.

Poster Board Number:

SUNDAY-808

Publishing Title:

International Workshop and Evaluation of Science in Countries of the Caucasus Region

Author Block:

F. Perler[1], **E. Becraft**[2], J. Bird[3], D. McCrimmon[4], D. Polosukhina[5], R. Ramaley[6], J. Spear[7], W. Swingley[8], L. Ziolkowski[4], T. A. Vishnivetskaya[3]; [1]Perls of Wisdom Biotech Consulting, Brookline, MA, [2]Bigelow Lab. for Ocean Sci., East Boothbay, ME, [3]Univ. of Tennessee, Knoxville, TN, [4]Univ. of South Carolina, Columbia, SC, [5]Vanderbilt Univ., Nashville, TN, [6]Univ. of Nebraska Med. Ctr., Omaha, NE, [7]Colorado Sch. of Mines, Golden, CO, [8]Northern Illinois Univ., DeKalb, IL

Abstract Body:

The Caucasus Mountains provide a unique opportunity for investigating microbial diversity and microbial processes in non-volcanic thermal regions. Though numerous hot springs are found in the Caucasus, their biology is just beginning to be tapped. The natural mineral waters contain indigenous microorganisms with valuable characteristics involved in biosynthetic and biodegradative pathways, heavy metal resistance, and bioremediation of toxic metals and explosives. In 2015 an international workshop was held in Georgia and Armenia for the purpose of discussing the potential for establishing beneficial new collaborations, as well as opportunities for expanding educational interchange. The workshop format was similar in both countries with a symposium comprising local and U.S. speakers, visits to local scientific institutions, field trips to natural environments, and cultural components. The NSF expert panel consisted of ten U.S. scientists, mostly with basic science backgrounds, running the gamut from graduate students to professors to retired personnel. The Armenian participants mirrored the composition of the U.S. panel with the addition of high level institutional and government representatives, while the Georgian participants consisted of distinguished senior scientists and government officials. The NSF expert panel evaluated (i) existing collections of thermophilic microorganisms, (ii) thermal natural resources, and (iii) basic or applied thermophilic microbiology research programs with respect to biotechnology. The workshop materials will be published as NSF report. This one-week workshop developed strong international networks among scientists from participating countries, establishing a new niche for innovative collaborative research on Caucasus natural resources amongst faculty and students from U.S. and Armenian or Georgian universities. It provides a blueprint for establishing similar connections within other countries.

Author Disclosure Block:

F. Perler: None. **E. Becraft:** None. **J. Bird:** None. **D. McCrimmon:** None. **D. Polosukhina:** None. **R. Ramaley:** None. **J. Spear:** None. **W. Swingley:** None. **L. Ziolkowski:** None. **T.A. Vishnivetskaya:** None.

Poster Board Number:

SUNDAY-809

Publishing Title:

Hiv Outbreak in Scott County, Indiana: The Laboratory Response

Author Block:

J. Madlem, S. Dalenberg, J. Gentry, M. Cruz, E. Vecchio, M. Cross, S. Matheson, M. Glazier; Indiana State Dept. of Hlth., Indianapolis, IN

Abstract Body:

Background: In late 2015, cases of Hepatitis C and HIV increased to outbreak levels in a small southern Indiana county. A state of emergency was declared, and all available resources from the Indiana State Department of Health (ISDH) HIV, Preparedness, Laboratory and other divisions were mobilized to Scott County. In a matter of days, a Community Outreach Center was opened, providing services to residents of Scott and surrounding counties. Services included immunizations, drivers' licenses, birth certificates, needle exchange, and HIV testing. **Methods:**The ISDH Laboratory increased surge capacity for HIV and Hepatitis B/C testing, and provided emergency on-site phlebotomy training to disease investigators from the Centers for Disease Control and Prevention (CDC) and other state health departments. These investigators responded to the call for assistance in controlling this outbreak. **Results:** Phlebotomy is becoming an essential service of public health during outbreak situations. Rapid collection of serology specimens is critical to confirming and controlling disease outbreaks. Disease investigators, public health nurses, and other essential personnel have been called upon to perform phlebotomy more often in recent years. Unfortunately, a majority of these personnel have not had proper training to perform this function. The ISDH Laboratories Outreach and Training Team developed a single-day phlebotomy refresher course to meet this critical need. During eight training sessions in Scott County, 48 disease investigators were trained. Using the Kirkpatrick Model of evaluation, there was a 66% increase in the post test scores. Evaluations from this training indicated the highest level of satisfaction with this course. **Conclusions:** The ISDH Laboratory tested a total of 1681 specimens from Scott and surrounding counties. Of those, 181 (11%) tested positive for HIV, 618 (38%) tested positive for Hepatitis C, and 30 (3%) tested positive for Hepatitis B. Furthermore, the ISDH HIV Program initiated an HIV Testing Blitz in November 2015 in an effort to test associates of known positive cases. 152 additional specimens were received, and of those, 150 were tested. The November testing blitz detected 5 (3%) cases of HIV, along with 83 (55%) cases of Hepatitis C.

Author Disclosure Block:

J. Madlem: None. **S. Dalenberg:** None. **J. Gentry:** None. **M. Cruz:** None. **E. Vecchio:** None. **M. Cross:** None. **S. Matheson:** None. **M. Glazier:** None.

Poster Board Number:

SUNDAY-810

Publishing Title:

Forgotten: The Spanish Influenza Pandemic of 1918

Author Block:

S. C. Pal; Univ. of California at Davis, Davis, CA

Abstract Body:

The Spanish Influenza pandemic of 1918 killed more individuals globally than World War I, yet it is often mentioned as a corollary to the war, rather than its own event. The disease played a part during the First World War, but it also affected civilians and baffled medical professionals. Despite this, the pandemic gathered little popular attention and failed to leave a literary or scientific mark, causing its near erasure in the American collective memory. The virus was strange because of its virulence and atypical patterns of infection. While soldiers struggled to define the flu and keep it from spreading, medical professionals tried to quell the rapidly growing public health crisis caused by outbreaks. Privately, doctors and nurses wrote of the disease, but publicly published little, even years after the pandemic ended. This research extensively uses primary sources to show that the pandemic of 1918 was largely forgotten because dying in war due to influenza or any other disease was seen as less honorable than dying due to combat, as evidenced by the lack of memorials for flu victims. The violent military deaths in combat overshadowed the death of soldiers dying of influenza. Consequently, those mourning felt guilty for their sorrows in juxtaposition to the War and placed less importance on the pandemic and more importance on "worthier" causes, such as the war effort. The pandemic wasn't written on in scientific publications until the late 1900s because there wasn't an understanding to address it. In 1918, the scientific advancements just weren't advanced enough to explain the virus. Disease is often seen as an inevitable and natural. This, although true, does not merit the dismissive attitude that much of history has placed on the Spanish Influenza pandemic of 1918. How a population views a pandemic after its occurrence says much about how disease is viewed in society and in memory.

Author Disclosure Block:

S.C. Pal: None.

Poster Board Number:

SUNDAY-811

Publishing Title:

The Practice of Smallpox Inoculation in Colonial Boston and the Response from the Medical and Religious Communities in the Colonies and Britain, 1721-30

Author Block:

S. M. Richart; Azusa Pacific Univ., Azusa, CA

Abstract Body:

Centuries prior to Jenner's cowpox vaccination, the Chinese practiced inoculation of smallpox material on healthy people as a means of preventing smallpox disease. As this practice spread to India, Turkey, and parts of Africa, it came to the attention of the European world in the early 18th century. Enlisting the aid of local Boston physician Zebediah Boylston, famous Puritan minister Cotton Mather championed the implementation of the smallpox inoculation following an epidemic in Boston starting in 1721 that resulted in about 50% of the city's inhabitants developing smallpox disease. Boylston's practice of inoculation sparked an intense public debate that played out from the newspapers to the pulpit. While ultimately a compelling pragmatic argument arose in support of inoculation when data showed lowered smallpox death rates in people who had been inoculated, many other ethical considerations and concerns were raised within both the medical and religious communities before inoculation became more widely embraced. Here, using primary documents from Boston and Britain written between 1721-30, we examine some of the major ethical concerns to inoculation raised during this pivotal time period. While many of these concerns illustrate a pre-Germ theory conception of disease, some are recognizable in the current vaccination debate.

Author Disclosure Block:

S.M. Richart: None.

Poster Board Number:

SUNDAY-812

Publishing Title:

Stockhausen's Review of Beijerinck's Work: Valuable Thoughts from 1907 Concerning the Niche, Enrichments and Microbial Ecology

Author Block:

D. KLEIN; COLORADO STATE Univ., FORT COLLINS, CO

Abstract Body:

In 1907, Ferdinand Stockhausen published a summary of M. W. Beijerinck's work, entitled "Ecology, enrichments according to Beijerinck," or in German, "Ökoligie, Anhäufungen nach Beijerinck." This brief book contains many insights concerning the nature of enrichments and their use to better understand the characteristics of the microbial niche. The book begins with a discussion of different approaches that can be used to obtain "pure" cultures. Pasteur's approach was considered to be "mechanical," while that of Delbrueck was based on natural enrichments, the approach that was further developed by Beijerinck. Following this introduction, there is an extended discussion concerning the niveau (niche). A niche can be developed based on a series of factors: this can involve physical separation (for motile organisms) the formation of gradients, as well as niche creation through symbiotic processes, light, and negative factors. A major emphasis was placed in the role of nutrients, oxygen and temperature (p. 42) as determinants of niche characteristics over space and time . He also noted that niches could shift their physical location, appear and also disappear. The use of carefully designed enrichments for a wide range of microbes, especially concerned with the nitrogen and sulfur cycles, made it possible for him to draw out valuable general ecological principles. One of the most valuable was his observation (p. 177) that the slightest changes in environmental conditions can lead to differences in microbes ;that become dominant in a specific environment, and that it is possible to predict outcomes of varied environmental manipulations, in essence allowing the description of the niche for a specific microbe. In spite of the centrality of ecology to the book, the word ecology, itself, only appears to be used in the title, along with the word enrichment. The message? All enrichments, whether created in the laboratory or as they occur in nature, are ecological, involving in situ active, interacting microbes. The foundational concepts provided by Beijerinck, as summarized by Ferdinand Stockhausen, provide valuable insights, for understanding how the field of microbial ecology developed to the present, and to assist in shaping the future.

Author Disclosure Block:

D. Klein: F. Investigator; Self; professor.

Poster Board Number:

SUNDAY-813

Publishing Title:

"Sir Almost Right": The Prodigious but Colorful Career of Sir Almroth Wright (1861-1947)

Author Block:

B. J. Freij[1], J. B. Freij[2]; [1]Beaumont Children's Hosp., ROYAL OAK, MI, [2]Johns Hopkins Bloomberg Sch. of Publ. Hlth., Baltimore, MD

Abstract Body:

Almroth Wright (AR), "brought up in scholastic surroundings and comparative poverty", graduated at 22 years with degrees in literature and medicine. AR eschewed clinical practice for medical science. Early research was on coagulation, citration of blood, and diabetes. In 1892, he became Chair of Pathology at the Army Medical School, Netley. He developed an agglutination test for Malta fever. From the bacteriologist Haffkine AR learned of live/killed bacteria use as vaccines. He made a Malta fever killed vaccine, injected himself, and was then inoculated with a live culture. It failed and AR suffered from Malta fever for weeks. AR focused on typhoid fever, a major killer of British soldiers. He injected himself and "surgeons-on-probation" with killed typhoid bacilli and showed that blood's killing power against live bacteria increased 50-fold. The Boer War allowed for a limited field study in volunteers. AR pushed for vaccinating all soldiers. A heated debate arose with Karl Pearson in the *BMJ* pages. Pearson wanted a controlled trial, but AR was unwilling to wait. The War Office was pondering vaccinating all soldiers, before definitive proof of efficacy. AR was young and not well known. He was knighted to shore up his standing to push for the vaccine! AR moved to St. Mary's Hospital in 1902 where he worked on opsonization and the "opsonic index", paving the way for use of therapeutic vaccines; this failed. Because of lack of funds, AR financed vaccine production by collaborating with Parke Davis for 40 years. He raised the ire of medical practitioners with attacks on Harley Street and views on the "bankruptcy of medicine". Critics dubbed him "Sir Almost Right". Later research included saline treatment of wounds and pneumococcal vaccination of miners. AR was a fierce critic of the suffragette movement. He believed in "an illimitable gulf between men and women" and that diacritical judgment was "notoriously lacking in women". An anti-suffragette letter in the *The Times* (March 28, 1912) espoused views such as "the mind of woman is always threatened with danger from the reverberations of her physiologic emergencies" and "there is mixed up with the woman's movement much mental disorder". *The Unexpurgated Case Against Woman Suffrage* followed, a book justifying his opinions. By the time of his death, AR had published about 150 books and papers.

Author Disclosure Block:

B.J. Freij: None. **J.B. Freij:** None.

Poster Board Number:

SUNDAY-814

Publishing Title:

The History And Use Of The Terms Endospore And Spore

Author Block:

R. Wolff; South Univ., Columbia, SC

Abstract Body:

‘Spore’ is a word with a great history and includes both reproductive structures and structures which are able to survive by resistance to environmental conditions. Organisms considered to have ‘spores’ include protozoa, fungi, plants, algae and bacteria. In 1876 the term endospore was coined for the highly resistant structures found in some bacteria. The usage of endospore has varied over the years, but they are generally regarded as occurring only in some bacteria, and they must contain dipicolinic acid to be a true endospore. Most of the true endospores occur in the Phylum Firmicutes, in medically important genera such as *Bacillus* and *Clostridium*. There are other spore types in bacteria including myxospores, but these are reproductive. Some misuses of the term endospore are attributed to Cyanobacteria and Algae. The more general term ‘spore’ is used for various structures in a wide variety of organisms. In the fungi there are many types of spores and their use is very specific (Based on structure are sporangiospores, zygospores, ascospores, basidiospores, aeciospores, urediniospores, teliospores, oospores, carpospores and tetraspores; or function such as chlamydospores; or life cycle including meiospores and mitospores; or by mobility are zoospores, autospores, and ballistospores). The term cyst is also confusing as it has been defined as a protective capsule and/or resting stage in some animals, plants and protozoa, as well as bacteria. The bacteria *Azotobacter* is described as forming a cyst of dormant cells with thickened cell walls. The history of ‘spore’ usage for bacteriology needs to be clarified, and most importantly, in the age of computer searches it is critical that the term ‘endospore’ be used whenever research papers are published on endospores so that that proper retrieval of articles may be accomplished. Endospore is a very useful and precise term and proper usage needs to be encouraged.

Author Disclosure Block:

R. Wolff: None.

Poster Board Number:

MONDAY-001

Publishing Title:

Activity of Omadacycline against Clinical Isolates of *Neisseria gonorrhoeae*, Including Ciprofloxacin-Resistant Isolates

Author Block:

D. Sweeney, D. Hall, D. Shinabarger, **C. Pillar**; Micromyx, Kalamazoo, MI

Abstract Body:

Background: The prevalence and ease of transmission of gonococcal infection coupled with the high degree of resistance among *N. gonorrhoeae* (GC) to former front-line therapies (penicillin, tetracycline, and ciprofloxacin) and recent emergence of cephalosporin resistance has caused concern about gonorrhea control going forward. Omadacycline (OMC), a new once daily oral and intravenous aminomethylcycline currently in phase 3 development for the treatment of skin and respiratory infections, retains potent *in vitro* activity against bacteria resistant to legacy tetracyclines. In this study, the *in vitro* activity of OMC against recent clinical isolates of GC including ciprofloxacin-resistant (CIP-R) isolates was evaluated.**Methods:** MICs of OMC, CIP, and ceftriaxone (CRO) were determined by agar dilution in accordance with CLSI guidelines (CLSI M7 and M100) against 52 clinical isolates of GC, 24 of which were CIP-R. In addition, the impact of testing susceptibility by agar dilution relative to broth microdilution using GC medium was evaluated using concurrent inocula of *N. gonorrhoeae* ATCC 49226.**Results:** The activity (µg/mL) of OMC and comparators against GC is shown in the table below.

Test Agent	Overall (n=52)		CIP-S (n=28)		CIP-R (n=28)	
	MIC range	MIC_{50}/MIC_{90}	MIC range	MIC_{50}/MIC_{90}	MIC range	MIC_{50}/MIC_{90}
OMC	0.25-4	2/4	0.25-2	1/2	1-4	4/4
CIP	0.002->8	0.008/>8	0.002-0.015	0.004/0.008	2->8	>8/>8
CRO	0.002-0.12	0.008/0.06	0.002-0.015	0.004/0.008	0.004-0.12	0.03/0.06

OMC had an overall $MIC_{50/90}$ of 2/4 µg/mL and maintained activity against CIP-R GC with an MIC_{90} within 2-fold that observed for CIP-S GC. CRO also maintained activity against CIP-R GC, though the MIC_{90} was reduced 8-fold relative to CIP-S GC. The OMC agar dilution MIC against *N. gonorrhoeae* ATCC 49226 (2 µg/mL) was 4-fold higher than the OMC broth microdilution MIC (0.5 µg/mL).**Conclusions:** OMC exhibited *in vitro* activity against the evaluated GC isolates and maintained potency against CIP-R isolates which are commonly encountered clinically. The variation in potency of OMC against GC by broth microdilution relative to agar dilution suggests variability in activity by test method.

Author Disclosure Block:

D. Sweeney: H. Research Contractor; Self; Paratek. **D. Hall:** H. Research Contractor; Self; Paratek. **D. Shinabarger:** H. Research Contractor; Self; Paratek. **C. Pillar:** H. Research Contractor; Self; Paratek.

Poster Board Number:

MONDAY-002

Publishing Title:

***In Vitro* Activity of Two New Topoisomerase Inhibitors Against Biodefense Pathogens**

Author Block:

S. Demons[1], L. Miller[1], S. Halasohoris[1], B. Somerville[1], F. Danel[2], B. Gaucher[2], M. Page[2]; [1]United States Army Med. Res. Inst. of Infectious Diseases, Frederick, MD, [2]Basilea Pharmaceutica Intl. Ltd, Postfach, Switzerland

Abstract Body:

Background: BAL1 and BAL2 are novel tricyclic topoisomerase inhibitors with potent activity against multi-drug resistant bacteria causing life-threatening infections. They were evaluated *in vitro* at the United States Army Medical Research Institute of Infectious Diseases against 30 isolates of geographically and genetically diverse sets of each of the following Tier 1 bacteria: *Bacillus anthracis*, the causative agent of anthrax, *Francisella tularensis*, the causative agent of tularemia, *Yersinia pestis*, the causative agent of plague, *Burkholderia mallei*, the causative agent of glanders, and *Burkholderia pseudomallei*, the causative agent of melioidosis.**Methods:** Minimum inhibitory concentrations (MICs) were determined according to Clinical and Laboratory Standards Institute microtiter-based methods. BAL1, BAL2, and comparator antibiotics were serially diluted in cation-adjusted Mueller-Hinton broth. The antibiotic range tested for both compounds was 0.015 - 32 µg/ml, with a final well volume of 100 µl. Plates were incubated for 18-48 h, depending on the organism. Quality control of antibiotic stocks was established using *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, and *S. aureus* ATCC 29213.**Results:** BAL2 was active against *B. anthracis*, *F. tularensis*, *Y. pestis*, *B. mallei*, and *B. pseudomallei* with MIC_{90} values of ≤ 2µg/ml. BAL1 had a MIC_{90} of ≤ 2µg/ml against *B. anthracis*, *F. tularensis*, *Y. pestis*, and *B. mallei*. Lesser activity was seen against *B. pseudomallei* with a MIC_{90} of 8µg/ml. The MIC values were generally similar to the clinical comparators ciprofloxacin, azithromycin, and ceftazidime.**Conclusions:** Overall both compounds show potential to expand the armamentarium for treating anthrax, tularemia, plague, glanders, and melioidosis based on the *in vitro* data. Their efficacy should be assessed in murine aerosol models.

Author Disclosure Block:

S. Demons: None. **L. Miller:** None. **S. Halasohoris:** None. **B. Somerville:** None. **F. Danel:** None. **B. Gaucher:** None. **M. Page:** None.

Poster Board Number:

MONDAY-003

Publishing Title:

Broth Microdilution Testing of S-649266 Against *Acinetobacter baumannii* and Appropriate Interpretation of Mic Endpoints

Author Block:

M. Hackel[1], M. Tsuji[2], R. Echols[3], D. Sahm[1]; [1]IHMA, Inc., Schaumburg, IL, [2]Shionogi and Co., Ltd., Osaka, Japan, [3]Shionogi Inc., Florham, NJ

Abstract Body:

Background: S-649266 is a novel parenteral catechol-substituted siderophore cephalosporin that is active against carbapenem-resistant Gram-negative bacteria. Accurate *in vitro* testing of S-649266 by broth microdilution (BMD) requires the use of iron-depleted (ID) conditions to mimic the condition in mammalian hosts. Indistinct MIC endpoints (trailing) have been observed when testing *Acinetobacter baumannii.* This study was designed to ascertain the frequency and reproducibility of trailing endpoints, and to establish proposed guidelines for determining MIC values for S-649266.**Methods**: All MICs were determined by BMD according to CLSI guidelines except cation-adjusted Mueller Hinton broth (CAMHB) was replaced with ID-CAMHB made iron deficient by pretreatment with Chelex-resin (Bio-Rad Laboratories) with replenishment of Ca, Mg and Zn. Technologists were instructed to confirm that there was strong growth control (i.e. a button of > 2 mm) before proceeding to read the MICs. MIC endpoints were defined as the first well in which growth was significantly reduced (i.e. a button of < 1 mm or light/faint turbidity) relative to control. Trailing was noted when present. To determine the frequency of trailing, 240 isolates (200 *A. baumannii*, 20 *P. aeruginosa*, 20 *Enterobacteriaceae*) were tested. In addition, 20 *A. baumannii* were read by 8 technologists, who were instructed to follow the proposed guidelines. Twenty isolates, including *A. baumannii* with some degree of trailing, were retested 10 times over four days using 3 media lots to determine whether trailing was reproducible.**Results**: Trailing was noted in 64/200 (32%) of *A. baumannii* isolates (no trailing was noted in *P. aeruginosa* or *Enterobacteriaceae*), however using the proposed guidelines, it was possible to assign an MIC value for 199/200 (99.5%) isolates. When read by 8 different technologists, 17/20 (85%) *A. baumannii* MICs were within one doubling dilution. When 10 replicates of 20 isolates were read by a single technologist, 94.2% of MICs were +/- 1 doubling dilution, whereas trailing was not consistently reproducible.**Conclusions**: Trailing occurs inconsistently when testing *A. baumannii*, however the proposed interpretive criteria guidelines for reading S-649266 MIC endpoints are effective when trailing is encountered.

Author Disclosure Block:

M. Hackel: M. Independent Contractor; Self; IHMA, Inc. **M. Tsuji:** D. Employee; Self; Shionogi and Co. **R. Echols:** D. Employee; Self; Shionogi Inc. **D. Sahm:** M. Independent Contractor; Self; IHMA, Inc..

Poster Board Number:

MONDAY-004

Publishing Title:

Activity of Ceftazidime-Avibactam (Ca) and Ceftolozane and Tazobactam (Ct) Against Gram-negative Bacteremic Isolates Exhibiting Various Antimicrobial-resistant Phenotypes

Author Block:

S. Burgio, D. Amsterdam; Erie County Med. Ctr., Buffalo, NY

Abstract Body:

The armamentarium of antimicrobial agents directed against Gram-negative microorganisms is limited and the potency of newly developed agents (CA and CT) against various antimicrobial resistant phenotypes is questionable. We investigated the comparative susceptibility of CA and CT against a variety of Gram-negative antimicrobial resistant phenotypes. Bacterial identification and antimicrobial susceptibility studies were performed with the MicroScan (Beckman Coulter) system using CLSI interpretive guidelines. A total of 94 bacteremic resistant phenotypes recovered from 2011-2015 were evaluated. They comprised: 32 *Klebsiella pneumoniae*; 30. *Escherichia coli*; 5 *Klebsiella oxytoca*; 5 *Enterobacter spp*.; 4 *Citrobacter* spp.; 11 *Acinetobacter baumannii*; 3 *Pseudomonas aeruginosa*; and 4 other *Enterobacteriaceae*. Of the 8 antimicrobial agents initially evaluated for these bacteremic isolates: cefepime, ceftriaxone, doripenem, ertapenem, meropenem, piperacillin/tazobactam, tobramycin, and tigecycline; only tigecycline exhibited near uniform (100%) susceptibility. Based upon most recent CLSI guidelines, all carbapenemase producing organisms were classified as CREs. ESBLs were confirmed with clavulanic acid via MicroScan or manually. E-Test strips were used to determine MICs for CA and CT using manufacturer FDA interpretations for susceptibility(S) and resistance(R). CA interpretations are applicable for *Enterobacteriaceae* and *Pseudomonas* and are the same for both ≤ 8/4 (S) and >16/4 (R). CT interpretations are ≤ 2/4 (S) for *Enterobacteriaceae* and ≤4/4 for *Pseudomonas*. Interpretive guidelines are unavailable for either antibiotic for *Acinetobacter*. Of the 76 *Enterobacteriaceae* tested, all were uniformly susceptible to CA and 56.6% to CT. Among the CA susceptible species were 20 CREs, 47 ESBLS, and 9 CRE/ESBL. CT displayed limited activity, i.e. less than 50%; ESBL resistant phenotypes exhibited greater susceptibility. Resistant phenotypes that were not susceptible to CT included ESBLs and CREs. The addition of CA and CT to the antimicrobial arsenal has been long awaited. It remains to be determined if they can sustain the in vitro potency.

Author Disclosure Block:

S. Burgio: None. **D. Amsterdam:** None.

Poster Board Number:

MONDAY-005

Publishing Title:

***In Vitro* Activity of Eravacycline and Comparators Against *Enterobacteriaceae*, Including Strains Resistant to Carbapenems Or 3rd/4th Generation Cephalosporins in the US**

Author Block:

Y. Doi[1], G. R. Corey[2], I. Morrissey[3], T. Grossman[4], K. Luepke[4], **P. Scoble**[4], J. Sutcliffe[4]; [1]Univ. of Pittsburgh Med. Ctr., Pittsburgh, PA, [2]Duke Univ. Med. Ctr., Durham, NC, [3]IHMA Europe Sarl, Epalinges, Switzerland, [4]Tetraphase Pharmaceuticals, Inc., Watertown, MA

Abstract Body:

Background: Eravacycline (ERV) is a novel, fully synthetic fluorocycline antibiotic of the tetracycline class with broad-spectrum activity in development for the treatment of serious infections, including those caused by multidrug-resistant (MDR) pathogens. The purpose of this study was to evaluate the *in vitro* activity of ERV and comparators against *Enterobacteriaceae* (ENT), including isolates resistant to carbapenems (CR) or 3rd/4th generation cephalosporins (GC-R) in the US.**Methods:** Clinical isolates were collected from various body sites in patients in US hospitals from 2013-14. MIC results for ERV and comparators against 2,723 ENT isolates were determined by CLSI broth microdilution. Susceptibility was determined with CLSI 2015 breakpoints, except for tigecycline (TGC) where FDA breakpoints were used. GC-R was defined as resistant to ceftriaxone, cefotaxime, ceftazidime, or cefepime (FEP). CR was defined as resistant to imipenem (IPM).**Results**: The ERV $MIC_{50/90}$ for all ENT was 0.5/2 mg/L. The ERV $MIC_{50/90}$ of GC-R and CR isolates were 0.5/2 and 1/2 mg/L, respectively. ERV and comparator MIC results (mg/L) for *Escherichia coli, Klebsiella pneumoniae,* and *Serratia marcescens* are shown in the table below. Most GC-R-resistant ENT were susceptible to TGC (91.8%), however susceptibilities decreased in CR isolates (61.4%).

Organism (N)	**ERV**	**TGC**	**IPM**	**FEP**
	$MIC_{50/90}$	**$MIC_{50/90}$**	**$MIC_{50/90}$**	**$MIC_{50/90}$**
E. coli (349)	0.12/0.25	0.25/0.5	≤0.25/0.5	≤0.25/4
GC-R (50)	0.25/0.5	0.25/0.5	≤0.25/1	16/>16
K. pneumoniae (350)	0.5/1	0.5/2	≤0.25/1	≤0.25/4
GC-R (50)	1/2	1/2	0.5/8	16/>16
CR (20)	0.5/2	1/2	>8/>8	>16/>16
S. marcescens (347)	1/2	2/2	1/2	≤0.25/0.5
GC-R (26)	2/2	2/2	1/4	1/16
CR (6)	1/2	1/2	4/>8	≤0.25/>16

$MIC_{50/90}$, minimum inhibitory concentration required to inhibit growth of 50/90% of isolates (mg/L)**Conclusion:** Overall, ERV MIC_{90} for ENT isolates ranged from 0.25-2 mg/L, and was the same or within two-fold for GC-R and CR isolates. Potency of ERV was comparable to 2-fold greater than TGC. ERV was up to 4-fold and 8-fold greater for IPM for FEP, respectively. ERV shows promising activity against ENT including those with resistant phenotypes isolated from US patients.

Author Disclosure Block:

Y. Doi: J. Scientific Advisor (Review Panel or Advisory Committee); Self; Tetraphase Pharmaceuticals, Inc. **G.R. Corey:** J. Scientific Advisor (Review Panel or Advisory Committee); Self; Tetraphase Pharmaceuticals, Inc. **I. Morrissey:** C. Consultant; Self; Tetraphase Pharmaceuticals, Inc. **T. Grossman:** D. Employee; Self; Tetraphase Pharmaceuticals, Inc. **K. Luepke:** D. Employee; Self; Tetraphase Pharmaceuticals, Inc. **P. Scoble:** D. Employee; Self; Tetraphase Pharmaceuticals, Inc. **J. Sutcliffe:** D. Employee; Self; Tetraphase Pharmaceuticals, Inc..

Poster Board Number:

MONDAY-006

Publishing Title:

Susceptibility Survey of Ceftazidime-Avibactam (C-A) Against Multidrug Resistant (Mdr) Gram-negative Clinical Isolates from a Single Academic Medical Center

Author Block:

L. Donohue, H. L. Cox, J. Carroll, A. Cheruvanky, A. J. Mathers; Univ. of Virginia Hlth.System, Charlottesville, VA

Abstract Body:

Background: C-A has in vitro activity against both *Klebsiella pneumoniae* carbapenemase-producing organisms (KPCO) and MDR *Pseudomonas aeruginosa*, but a widely available antimicrobial susceptibility testing (AST) method for clinical use is unestablished. AST is important when both resistant and susceptible clinical isolates exist, but little resistance has been described. We sought to evaluate C-A susceptibility among MDR *Gammaproteobacteria* to assess the importance of AST prior to use. **Methods:** Forty MDR *Gammaproteobacteria* clinical isolates, most KPCO, were selected from those collected at our institution since August 2008. Isolates included: *Enterobacter cloaca*e (n=15), *Klebsiella pneumoniae* (n=7), *K. oxytoca* (n=5), *Serratia marcescens* (n=2), *Aeromonas hydrophila* (n=1), and *P. aeruginosa* (*P*sA, n=10). All but one (an *E. cloacae)* non-*PsA* were KPCO by PCR and all were CB-nonsusceptible (all resistant), as were 90% of *PsA* (8 resistant, 1 intermediate). Thirty-one (78%) isolates were FEP-nonsusceptible (23 resistant, 8 intermediate). VITEK2 was the primary testing modality for CB and FEP susceptibility, with disk diffusion (DD) performed for 2 isolates. AST was performed using manufacturer-supplied E-test strips and disks per manufacturer and CLSI standards. C-A zones and MICs were interpreted by FDA breakpoints.**Results:** In non-*PsA*, all isolates were C-A susceptible by E-test (MIC range 0.5 - 2 µg/mL, MIC_{90} 1.5 µg/mL). One *K. pneumoniae* was resistant by DD (19 mm). Of the 10 *PsA*, 4 were resistant by E-test (MIC range 2 - ≥256 µg/mL, MIC_{90} 64 µg/mL). Zones ranged from 0 - 25 mm, with 3 resistant by DD. DD exhibited 95% categorical agreement to E-test. **Conclusions:** Multiple species of KPCO exhibited *in vitro* susceptibility to C-A. However, the *K. pneumoniae* isolate resistant by DD warrants further investigation. C-A resistant *PsA* isolates likely resulted from mechanisms other than class A or class C β-lactamase overproduction but this requires further study. Given the limited availability and prolonged turnaround time of broth dilution, these results re-iterate the need for reliable and accessible AST methods prior to clinical use.

Author Disclosure Block:

L. Donohue: None. **H.L. Cox:** None. **J. Carroll:** None. **A. Cheruvanky:** None. **A.J. Mathers:** None.

Poster Board Number:

MONDAY-007

Publishing Title:

***In Vitro* Activity of Eravacycline and Comparators Against *Acinetobacter baumannii*, Including Carbapenem-resistant Strains, and *Stenotrophomonas maltophilia* Isolated from Patients in the US**

Author Block:

Y. Doi[1], G. R. Corey[2], I. Morrissey[3], T. Grossman[4], K. Luepke[4], **P. Scoble**[4], J. Sutcliffe[4]; [1]Univ. of Pittsburgh Med. Ctr., Pittsburgh, PA, [2]Duke Univ. Med. Ctr., Durham, NC, [3]IHMA Europe Sarl, Epalinges, Switzerland, [4]Tetraphase Pharmaceuticals, Inc., Watertown, MA

Abstract Body:

Background: Eravacycline (ERV) is a novel, fully synthetic fluorocycline antibiotic of the tetracycline class with broad-spectrum activity in development for the treatment of serious infections, including those caused by multidrug-resistant (MDR) pathogens. The purpose of this study was to evaluate *in vitro* activity of ERV and comparators against *Acinetobacter baumannii* (AB), including strains with a carbapenem-resistant (CR) phenotype, and *Stenotrophomonas maltophilia* (SM) isolated from US patients. **Methods:** Non-duplicate, non-consecutive, single-patient clinical isolates of AB and SM were collected from US patients in 2013-2014. MICs for ERV and comparators against 380 isolates from both species were determined by CLSI broth microdilution. Susceptibility was determined with CLSI 2015 breakpoints, except for tigecycline (TGC) where FDA breakpoints were used. CR in AB (CRAB) was defined as resistant to imipenem.**Results**: ERV *in vitro* activity against AB and SM is shown in the following Table. ERV MIC_{90} for all AB and SM isolates was 1 mg/L, and 2 mg/L for CRAB. TGC, imipenem (IPM), and colistin (CST) MIC_{90} for AB were 4, >8, and 2 mg/L, respectively, and remained consistent for CRAB. Susceptibility to CST for all AB was 95%, while for CRAB was 93%. Susceptibility to IPM for all AB and CRAB was poor (39.4%, 0%, respectively). TGC, IPM, and CST MIC_{90} for SM were 2, >8, and 4 mg/L, respectively. There were no CR SM isolates collected.

Organism (N)	**ERV**	**TGC**	**IPM**	**CST**
	$MIC_{50/90}$	**$MIC_{50/90}$**	**$MIC_{50/90}$**	**$MIC_{50/90}$**
A baumannii (349)	0.5/1	1/4	>8/>8	1/2
CRAB (207)	0.5/2	2/4	>8/>8	1/2
S. maltophilia (31)	0.5/1	1/2	>8/>8	1/4

$MIC_{50/90}$ = minimum inhibitory concentration required to inhibit growth of 50/90% of isolates (mg/L).**Conclusions:** Overall, ERV MIC_{90} for AB and SM was 1 mg/L (2 mg/L for CRAB), and

was 2-4 fold more potent than TGC or CST, and up to 8-fold more potent than IPM. ERV shows promising activity against AB and SM, including CRAB, isolated from US patients.

Author Disclosure Block:

Y. Doi: J. Scientific Advisor (Review Panel or Advisory Committee); Self; Tetraphase Pharmaceuticals, Inc. **G.R. Corey:** J. Scientific Advisor (Review Panel or Advisory Committee); Self; Tetraphase Pharmaceuticals, Inc. **I. Morrissey:** C. Consultant; Self; Tetraphase Pharmaceuticals, Inc. **T. Grossman:** D. Employee; Self; Tetraphase Pharmaceuticals, Inc. **K. Luepke:** D. Employee; Self; Tetraphase Pharmaceuticals, Inc. **P. Scoble:** D. Employee; Self; Tetraphase Pharmaceuticals, Inc. **J. Sutcliffe:** D. Employee; Self; Tetraphase Pharmaceuticals, Inc..

Poster Board Number:

MONDAY-008

Publishing Title:

Eravacycline *In Vitro* Activity Against Clinical Isolates Obtained from Genitourinary (Gu) and Gastrointestinal (Gi) Sources from Patients in the US

Author Block:

G. R. Corey[1], Y. Doi[2], I. Morrissey[3], T. Grossman[4], M. Olesky[4], **H. Patel**[4], J. Sutcliffe[4]; [1]Duke Univ. Med. Ctr., Durham, NC, [2]Univ. of Pittsburgh Med. Ctr., Pittsburgh, PA, [3]IHMA Europe Sarl, Epalinges, Switzerland, [4]Tetraphase Pharmaceuticals, Inc., Watertown, MA

Abstract Body:

Background: Eravacycline (ERV) is a novel, fully synthetic fluorocycline antibiotic of the tetracycline class with broad-spectrum activity being developed for the treatment of serious infections, including those caused by multidrug-resistant (MDR) pathogens. ERV has been evaluated in studies for the treatment of complicated intra-abdominal infections (cIAI) and complicated urinary tract infections (cUTI), including pyelonephritis. The purpose of this study was to evaluate the activity of ERV against key pathogens from the US isolated from GU and GI sources.**Methods:** MIC results for ERV and comparator agents were determined by CLSI broth microdilution against 1004 GI and 1199 GU isolates collected from US hospitals in 2013-2014. MDR was defined as resistant to ≥3 from cefepime/ceftazidime/ceftriaxone (any one), gentamicin, imipenem, levofloxacin, piperacillin-tazobactam or tetracycline. **Results:** ERV MIC data for species where N ≥ 30 from each infection type are shown in the Table.

GI (n)			**GU (n)**		
	$MIC_{50/90}$	**MIC range**		**$MIC_{50/90}$**	**MIC range**
Gram-negatives					
C. freundii (36)	0.25/1	0.06-2	*A. baumannii* (43)	0.25/1	0.03-4
E. cloacae (77)	0.5/0.5	0.12-8	*C. freundii* (45)	0.25/0.5	0.12-1
E. coli (170)	0.12/0.25	0.06-2	*E. aerogenes* (102)	0.5/1	0.12-8
K. oxytoca (63)	0.25/1	0.12-8	*E. cloacae* (133)	0.5/1	0.12-8
K. pneumoniae (189)	0.5/2	0.12-8	*K. oxytoca* (101)	025/0.25	0.06-1
P. mirabilis (54)	1/2	0.25-4	*M. morganii* (30)	2/2	0.25-4
P. aeruginosa (145)	8/16	2-32	*P. mirabilis* (104)	1/2	0.25-4
MDR *P. aeruginosa* (40)	8/32	8-32	*P. aeruginosa* (63)	8/16	2-32
S. marcescens (31)	1/2	1-4	*S. marcescens* (98)	1/2	0.5-8

Gram-positives					
E. faecium (40)	0.06/0.12	0.03-0.5	*E. faecalis* (187)	0.06-0.06	0.008-0.12
VR *E. faecium* (26)	0.06/0.12	0.03-0.5	*E. faecium* (104)	0.06-0.06	0.008-0.5
S. aureus (56)	0.06/0.12	0.03-0.5	VR *E. faecium* (79)	0.06-0.06	0.03-0.5
MRSA (37)	0.06/0.12	0.03-0.5	*S. agalactiae* (47)	0.03/0.03	0.008-0.06

$MIC_{50/90}$, minimum inhibitory concentration (mg/L) required to inhibit growth of 50/90% of isolates; MRSA, methicillin-resistant *S. aureus*; VR, vancomycin-resistant**Conclusions:** For GI and GU isolates, the ERV MIC_{90} was $\leq$ 2 mg/L, except for *P. aeruginosa*. ERV showed activity against clinically-important Gram-negative and -positive isolates from GU or GI infections from the US.

Author Disclosure Block:

G.R. Corey: J. Scientific Advisor (Review Panel or Advisory Committee); Self; Tetraphase Pharmaceuticals, Inc. **Y. Doi:** J. Scientific Advisor (Review Panel or Advisory Committee); Self; Tetraphase Pharmaceuticals, Inc. **I. Morrissey:** C. Consultant; Self; Tetraphase Pharmaceuticals, Inc. **T. Grossman:** D. Employee; Self; Tetraphase Pharmaceuticals, Inc. **M. Olesky:** D. Employee; Self; Tetraphase Pharmaceuticals, Inc. **H. Patel:** D. Employee; Self; Tetraphase Pharmaceuticals, Inc. **J. Sutcliffe:** D. Employee; Self; Tetraphase Pharmaceuticals, Inc..

Poster Board Number:

MONDAY-009

Publishing Title:

Comparison of Fosfomycin *In Vitro* Susceptibility Testing Methods - Disc Diffusion, Etest and Vitek 2 - Against *Escherichia coli* and *Enterococcus* Urinary Isolates

Author Block:

T. T. Nguyen, D. C. Vinh, T. C. Lee, V. G. Loo; McGill Univ. Hlth.Ctr., Montreal, QC, Canada

Abstract Body:

Background: Fosfomycin (FM) has been reintroduced in many jurisdictions for the treatment of urinary tract infections. Laboratories will need to periodically test and report susceptibility. To determine if the antimicrobial susceptibility testing method influences results, we compared 3 *in Vitro* tests on urinary isolates. **Methods:** Consecutive urinary isolates of *E. coli* and *Enterococcus* were collected from February to August 2015 at our Canadian university-affiliated tertiary care center, targeting 150 samples. FM susceptibility was tested in parallel on both genera using CLSI's disc diffusion (DD) method, by Etest, and for *E. coli*, by VITEK 2 AST-N263. Although FM interpretive criteria for *E. faecium* are not defined by CLSI, those for *E. faecalis* were applied for comparison. Correlation between testing methods was evaluated as rates of categorical agreement (CA) and error rates. **Results:** Fosfomycin susceptibility by method is presented in Table 1. Most isolates of *E. coli* and *E. faecalis* were susceptible to FM. *E. faecium* had lower susceptibility. For *E. coli*, CA of VITEK 2 and Etest relative to DD were 99.3% and 98.6%, with minor errors in 0.7% and 1.4%, respectively. For *Enterococcus*, CA of Etest relative to DD was 93.8%, with minor errors in 6.2%. There were no major or very major errors.

Table 1. Susceptibility to fosfomycin by different methods[a]

Organism (N) / Method	Susceptible [N (%)]	Intermediate [N (%)]	Resistant [N (%)]	$MIC_{50/90}$ (µg/mL)
E. coli (146)				
• DD	141 (96.5%)	2 (1.4%)	3 (2.1%)	N/A
• Etest	140 (95.8%)	3 (2.1%)	3 (2.1%)	0.75/16
• VITEK 2	141 (96.5%)	2 (1.4%)	3 (2.1%)	<16/<16
ESBL *E. coli* (17)				
• DD	16 (94.1%)	0 (0%)	1 (5.9%)	N/A
• Etest	15 (88.2%)	0 (0%)	2 (11.8%)	0.75/48
• VITEK 2	15 (88.2%)	0 (0%)	2 (11.8%)	<16/<16
Enterococcus (145)				
• DD	134 (92.4%)	11 (7.6%)	0 (0%)	N/A
• Etest	126 (86.9%)	18 (12.4%)	1 (0.7%)	32/96
E. faecalis (126)				
• DD	125 (99.2%)	1 (0.8%)	0 (0%)	N/A
• Etest	120 (95.2%)	6 (4.8%)	0 (0%)	32/48
Non-VRE *E. faecium* (9)				
• DD	5 (55.6%)	4 (44.4%)	0 (0%)	N/A
• Etest	3 (33.3%)	6 (66.7%)	0 (0%)	96/196
VRE *E. faecium* (10)				
• DD	4 (40.0%)	6 (60.0%)	0 (0%)	N/A
• Etest	3 (30.0%)	6 (60.0%)	1 (10.0%)	96/196

[a]DD, disc diffusion; $MIC_{50/90}$, MIC that inhibited 50%/90% of isolates (susceptible, ≤64 µg/ml; intermediate, 128 µg/ml; resistant, ≥256 µg/ml); VRE, vancomycin-resistant *Enterococcus*.

Conclusions: For *E. coli*, there was excellent agreement among the 3 methods tested. For *Enterococcus*, there was a higher rate of minor errors, which still falls in the <10% range at which CLSI considers a test adequate. We suggest that any of the three methods of susceptibility testing can be utilized for *E. coli* and *E. faecalis*, but that further evaluation of breakpoints and testing methods for *E. faecium* are warranted.

Author Disclosure Block:

T.T. Nguyen: None. **D.C. Vinh:** None. **T.C. Lee:** None. **V.G. Loo:** None.

Poster Board Number:

MONDAY-010

Publishing Title:

Fosfomycin Has the Lowest Resistance Rate among Oral Antimicrobial Agents Tested Against Community Uropathogens

Author Block:

S. E. Farhat[1], I. Coelho[1], M. Bhatti[1], G. Lim[1], B. Shingala[1], W. P. Shih[1], M. Pandya[1], B. Shea[1], B. Premraj[1], A. E. Simor[2]; [1]Alpha Lab., Toronto, ON, Canada, [2]Sunnybrook Hlth.Sci. Ctr., Toronto, ON, Canada

Abstract Body:

Background: Fosfomycin (FOS) has recently attracted increasing interest, largely because of its good activity *in vitro* against multidrug-resistant (MDR) pathogens. However, there have been few studies of FOS in North America, mostly with *Escherichia coli*. In light of current antimicrobial resistance (R) in the community, we sought to assess the R rate of FOS among community urinary isolates, in comparison to those of other oral antimicrobial agents commonly used for the treatment of uncomplicated urinary tract infections (UTIs) in non-hospitalized patients.**Methods:** Consecutive isolates were identified by conventional methods from urine cultures processed over a 9 week period ending on January 30, 2015. Organisms not recommended for routine testing (e.g., streptococci) were excluded from the study. Isolates were tested by disk diffusion or the Vitek-2 system (bioMérieux), in accordance with CLSI guidelines, against ampicillin (AM), cefazolin (KZ), ciprofloxacin (CIP), nitrofurantoin (FM), trimethoprim/ sulfamethoxazole (SXT), and FOS. Due to lack of FOS interpretive criteria for all organisms, CLSI *E. coli* and *E. faecalis* breakpoints were applied for all organisms, similar to recently published investigations.**Results:** Of 11,853 urine specimens processed, a total of 2,455 non-duplicate isolates were tested, including *E. coli* (*n* = 1,617), *E. faecalis* (343), *Klebsiella* (192), *Proteus mirabilis* (144), *Citrobacter* (56), *Enterobacter* (39), *Morganella morganii* (35), *Staphylococcus aureus* (17), *Pseudomonas aeruginosa* (4), *Acinetobacter* (3), *Serratia* (3), *Providencia* (1) species, and *E. faecium* (1). R rates for AM, KZ, CIP, FM, SXT, and FOS were 43%, 25%, 12%, 10%, 30%, and 2%, respectively. FOS R rates among MDR isolates that were R to ≥ 3 antimicrobial classes (*n* = 268), and ESBL producing strains (90 *E. coli*, 2 *Klebsiella*), were 6% and 1%, respectively.**Conclusions:** Of the six oral antimicrobial agents reported in this study, FOS had the lowest resistance rate among community urinary isolates. These results provide support for FOS as a useful agent for the treatment of UTIs caused by various organisms.

Author Disclosure Block:

S.E. Farhat: None. **I. Coelho:** None. **M. Bhatti:** None. **G. Lim:** None. **B. Shingala:** None. **W.P. Shih:** None. **M. Pandya:** None. **B. Shea:** None. **B. Premraj:** None. **A.E. Simor:** None.

Poster Board Number:

MONDAY-011

Publishing Title:

CLSI M23 Tier 2 Study to Establish Disk Diffusion and Broth Microdilution Quality Control Ranges for Lactone Ketolide Nafithromycin (WCK 4873)

Author Block:

M. Hackel[1], S. Bhagwat[2], M. Patel[2], D. Sahm[1]; [1]IHMA, Inc., Schaumburg, IL, [2]Wockhardt Res. Ctr., Aurangabad, India

Abstract Body:

Background: Nafithromycin (WCK 4873) is a new lactone ketolide discovered at Wockhardt having completed Phase 1 studies in US and Europe. This study was performed to establish quality control (QC) broth microdilution and disk diffusion ranges for WCK 4873 against specific ATCC QC organisms as recommended by the Clinical and Laboratory Standards Institute (CLSI).**Methods**: Five ATCC QC organisms within the spectrum of WCK 4873 activity were tested: *Haemophilus influenzae* ATCC 49247 (disk and broth), *Streptococcus pneumoniae* ATCC 49619 (disk and broth), *Staphylococcus aureus* ATCC 29213 (broth only), *S. aureus* ATCC 25923 (disk only), and *Enterococcus faecalis* ATCC 29212 (broth only). All strains were tested following CLSI guidelines for broth microdilution and disk diffusion. WCK 4873 and a control agent (telithromycin) were tested against all QC strains. This study design met the CLSI M23-A3 requirements for Tier 2 QC studies: seven laboratories/independent sites; three media lots (different manufacturers); two disk lots; 10 replicates of each QC strain per laboratory (70 total test points per medium lot); and 210 test points per QC strain/drug combination.**Results**: Greater than 95% of all MIC values reported from at least seven laboratories were within the ranges shown in the table.**Summary of Approved CLSI Quality Control Ranges for WCK 4873**

	WCK 4873 Approved CLSI Quality Control Range	
Organism	Broth Microdilution (µg/mL)	Disk Diffusion (mm)
S. aureus ATCC 29213	0.06 - 0.25	NA
S. aureus ATCC 25923	NA	25 - 31
E. faecalis ATCC 29212	0.015 - 0.12	NA
S. pneumoniae ATCC 49619	0.008 - 0.03	25 - 31
H. influenzae ATCC 49247	2 - 8	16 - 20

NA- not applicable**Conclusions**: All proposed quality control ranges were accepted by the CLSI at the June 2015 meeting and should be used to monitor the quality control whenever testing WCK 4873 by broth microdilution or disk diffusion.

Author Disclosure Block:

M. Hackel: M. Independent Contractor; Self; IHMA, Inc. **S. Bhagwat:** D. Employee; Self; Wockhardt. **M. Patel:** D. Employee; Self; Wockhardt. **D. Sahm:** M. Independent Contractor; Self; IHMA, Inc..

Poster Board Number:

MONDAY-012

Publishing Title:

In Vitro Activity Of Wck4873, A Second Generation Ketolide, Against Chlamydia Pneumoniae

Author Block:

S. A. Kohlhoff, M. R. Hammerschlag; SUNY Downstate Med. Ctr., Brooklyn, NY

Abstract Body:

Background: WCK4873 is an advanced generation, lactone ketolide antibiotic currently being evaluated for treatment of community acquired bacterial pneumonia (CABP). WCK 4873 shows potent activity for all the typical and atypical respiratory pathogens including telithromycin-non-susceptible pneumococci. The drug recently received qualified infectious disease product status from the FDA.**Methods:** We tested 10 isolates of *C. pneumoniae* against WCK4873, azithromycin, docycycline and levofloxacin. Isolates tested were: TW 183, AR 39, CM-1, T 2040, and 6 isolates from bronchoalveolar lavage specimens from patients with human immunodeficiency virus infection and pneumonia from the United States (BAL15, BAL16, BAL 18, BAL 19, BAL 37, BAL 62). Susceptibility testing of *C. pneumoniae* was performed in cell culture using HEp-2 cells grown in supplemented DMEM media followed by infection with 10^3 - 10^4 IFU/ml. After incubation at 35C for 72 h, cultures were fixed and stained for inclusions with fluorescein-conjugated antibody to the chlamydial lipopolysaccharide genus-specific antigen. The minimum inhibitory concentration (MIC) was the lowest antibiotic concentration at which no inclusions were seen.**Results:** The MIC_{50} and MIC_{90} for WCK4873 were 0.03 and 0.25 g/ml, respectively (range 0.03-1 g/ml). The MIC_{50} and MIC_{90} for azithromycin were 0.0075- 0.015 (range 0.003-0.03), for doxycycline, 0.06 and 0.125 (range 0.03-0.25), and levofloxacin, 0.25 and 0.25 (range 0.03-25).The in vitro activity of WCK4873 was comparable to that of several antibiotics with proven clinical efficacy for treatment of infections due to *C. pneumoniae*. Activity was also comparable to solithromycin (MIC_{90} 0.25 g/ml), another ketolide that is now in phase 3 clinical trials for CABP. Furthermore, the in vitro susceptibility testing protocol used for this study is the only one that has been shown to correlate with clinical outcome and microbiologic eradication for infections caused by *C. pneumoniae*.**Conclusion:** Based on favourable PK-PD demonstrated for telithromycin-non-susceptible pneumococci and potent intracellular activity (shown against Legionella), WCK4873 is expected to provide therapeutic efficacy for *C. pneumoniae* infections.

Author Disclosure Block:

S.A. Kohlhoff: None. **M.R. Hammerschlag:** H. Research Contractor; Self; Principal investigator.

Poster Board Number:

MONDAY-013

Publishing Title:

A Multicenter Evaluation of *In Vitro* Activity of Ceftazidime-Avibactam Against Selected Isolates from the Chicago Area

Author Block:

A. Harrington, E. Stolar, E. Wenzler, S. Collier, P. Schreckenberger, B. Blumer, M. Costello, K. Singh, D. Schora, P. Patel, R. Thomson, D. Shabib, A. Charnot-Katsikas; Chicago Micro Directors Roundtable Organization (CMDRO), Chicago, IL

Abstract Body:

Background: There are a limited number of antimicrobial agents available for the treatment of carbapenem resistant *Enterobacteriaceae* (CRE) and other carbapenem resistant organisms (CRO). Ceftazidime-avibactam (CTZ) is a new antibiotic recently approved by the FDA to treat adults with complicated intra-abdominal and urinary tract infections. Avibactam is a member of a novel class of non-β-lactam β-lactamase inhibitors that has activity against class A (KPC), class C (AmpC), and some class D (OXA) β-lactamases. It does not have activity against class B (MBL). Although there have been larger evaluations of the *in vitro* activity of CTZ against isolates recovered by clinical microbiology laboratories, the data are not specified by geographic region. The purpose of this study was to gather data specific to the Chicago area. **Methods**: A convenience sample of isolates from 6 clinical microbiology laboratories affiliated with academic medical centers or large hospital networks in the Chicago area was tested using E-test to determine the *in vitro* activity of CTZ. **Results**: A total of 210 isolates were evaluated: 11 *E. coli*; 12 *Pseudomonas spp.* (1 *P. putida*, 11 *P. aeruginosa*); 1 *Acinetobacter baumannii*; 1 *Serratia marcescens*; 4 *Enterobacter cloacae*; 2 *Citrobacter freundii*; 161 *Klebsiella pneumoniae.* The MIC distribution for all isolates ranged from 0.047 to >256 μg/ml. 10 isolates were identified as carrying MBL against which CTZ had no activity. 4 isolates were carrying an OXA mechanism, and the MICs ranged from 1 to 16. For *Pseudomonas* isolates, where the mechanism of resistance was not identified, the MICs ranged from 3 to 24. Overall susceptibility of CTZ to CRO was 96% (192/200) excluding organisms possessing MBLs. 185 isolates were positive by the modified-hodge test or identified as CRE. The MICs for those isolates ranged from 0.047 to 96 (MIC_{90} $\leq$4), where 4 isolates had MIC values considered resistant ($\geq$16). Confirmation of resistance is pending. **Conclusions**: CTZ demonstrated good activity *in vitro* to a large collection of isolates specifically from the Chicago area. Region-specific susceptibility data may impact utilization strategies for these new antimicrobial agents.

Author Disclosure Block:

A. Harrington: None. **E. Stolar:** None. **E. Wenzler:** None. **S. Collier:** None. **P. Schreckenberger:** J. Scientific Advisor (Review Panel or Advisory Committee); Self; Advisory

Board Member. **B. Blumer:** None. **M. Costello:** None. **K. Singh:** None. **D. Schora:** None. **P. Patel:** None. **R. Thomson:** None. **D. Shabib:** None. **A. Charnot-Katsikas:** None.

Poster Board Number:

MONDAY-014

Publishing Title:

***In Vitro* Activity of Ceftazidime/Avibactam and Ceftolozane/Tazobactam Against *Pseudomonas aeruginosa* Isolates from Cystic Fibrosis Patients**

Author Block:

D. C. Karkow[1], B. A. Ford[2], E. J. Ernst[1]; [1]Univ. of Iowa Coll. of Pharmacy, Iowa City, IA, [2]Univ. of Iowa Hosp. and Clinics, Iowa City, IA

Abstract Body:

Background: Ceftazidime/avibactam (CZA) and ceftolozane/tazobactam (C/T) are two new antimicrobial agents with activity against *P. aeruginosa*, including multidrug resistant (MDR) strains. There is limited data regarding activity against strains isolated from cystic fibrosis (CF) patients and no studies directly comparing the activities of these agents. The purpose of this study was to determine the activities of CZA and C/T against resistant *P. aeruginosa* isolated from CF patients.Methods: A total of 54 isolates obtained from the sputum of 24 CF patients aged 9 to 64 years were collected from March to December 2015. Included strains were either resistant to meropenem [n = 41], resistant to two beta-lactams from different classes [n = 50], or resistant to one agent in three different classes (MDR) [n = 47]. Strains resistant to agents in all classes tested were further categorized as extensively drug resistant (XDR) [n = 7]. Both mucoid and non-mucoid phenotypes were included. MICs for CZA and C/T were determined by investigational E-tests provided by the drug manufacturers. Tests were performed in duplicate or triplicate to reconcile discordant results. The MIC_{50}, MIC_{90}, and susceptibility percentages were determined using FDA breakpoints for CZA and C/T. Susceptibility for all other antibiotics was determined with the Trek Sensititre GN3F panel, using current CLSI breakpoints.Results: A summary of the activity of CZA and C/T are provided in the table below. Of 10 resistant isolates, 5 were resistant to CZA, 3 were resistant to C/T, and 2 were resistant to both. No XDR pathogens were resistant to CZA, whereas 3 were resistant to C/T.

Summary of CZA and C/T activity against P. aeruginosa isolates from CF patients

CZA	MIC_{50} (µg/ml)	MIC_{90} (µg/ml)	Range (µg/ml)	%S/%I/%R
All Isolates (n = 54)	4	16	0.5-32	87.0/-/13.0
MDR Strains (n = 47)	4	16	0.5-32	85.1/-/14.9
Mucoid Strains (n = 17)	4	16	0.5-32	82.4/-/17.6
C/T	MIC_{50} (µg/ml)	MIC_{90} (µg/ml)	Range (µg/ml)	%S/%I/%R
All Isolates (n = 54)	2	8	0.5-256	88.9/1.8/9.3
MDR Strains (n = 47)	2	8	0.5-256	87.2/2.1/10.7
Mucoid Strains (n = 17)	1	4	0.5-16	88.2/5.9/5.9

Conclusions: Both agents demonstrated good activity against *P. aeruginosa* strains isolated from CF patients. Fewer isolates were resistant to C/T and MICs were generally lower compared to CZA. C/T was more active against mucoid strains, while CZA was more active against XDR strains.

Author Disclosure Block:

D.C. Karkow: None. **B.A. Ford:** None. **E.J. Ernst:** None.

Poster Board Number:

MONDAY-015

Publishing Title:

Oritavancin Susceptibility Testing among Vancomycin Resistant *Enterococcus faecium* Blood Isolates

Author Block:

A. Paskovaty, N. Babady, Y-W. Tang, M. Kamboj, S. Seo; Mem. Sloan Kettering Cancer Ctr., NY, NY

Abstract Body:

Background: Vancomycin resistant *enterococcus* (VRE) is the most common gram-positive bacteria isolated from febrile neutropenic patients and those with pre-engraftment bacteremia after allogenic stem cell transplant SCT. Treatment of invasive infections due to this organism is challenging , no drugs have received FDA approval for treatment of VRE bacteremia. Daptomycin is commonly used to treat VRE infections but non -suspectibiltiy to this drug is a growing problem. Oritavancin, a synthetic lipopeptide has shown an in-vitro activity against VRE. The goal of this study is to test in-vitro susceptibilities of oritavancin against VRE blood isolates with a daptomycin MIC of ≥ 4 mcg/mL.**Methods:** Susceptibility testing on 50 VREF blood isolates from patients who developed bacteremia at MSKCC between 2009-2013 were included in the study. Testing by broth microdilution was simultaneously performed for the following drugs: vancomycin, range: 0.25 to 16 mcg/mL; linezolid, range: 0.5 to 8 mcg/mL; daptomycin, range: 0.12 to 4 mcg/mL; teicoplanin, range: 0.12 to 16 mcg/mL; oritavancin, range: 0.004 to 4 mcg/mL; high-level gentamicin, 500 mcg/mL; high-level streptomycin, 500 to 1,000 mcg/mL; penicillin, range: 0.12 to 8 mcg/mL. Testing was performed in duplicate according to CLSI M07-A10 and CLSI M100-S25 standards for broth microdilution method and interpretation. The project was supported by a research grant from the Medicine Company.**Results:** 18/50 (36%) isolates had daptomycin MIC 90 of > 4 mcg/mL. Median oritavancin MIC 90 was 0.25 mcg/mL (range: 0.06-0.5). Median linezolid MIC 90 was 4 mcg/mL (range 1->8), 50 (100%) of isolates had vancomycin and teicoplanin MIC 90 >16 mcg/mL, penicillin MIC 90 > 8 mcg/mL; 31(62%) had high-level gentamicin MIC 90 >500; and 30 (60%) had high-level streptomycin MIC 90 >1,000 mcg/mL.**Conclusions:** For VRE isolates with high MIC to daptomycin, oritavancin median MIC 90 was 0.25 mcg/mL. Oritavancin may have a role in management of serious VRE infections with limited treatment options. Further data are needed to evaluate oritavancin utility in clinical practice.

Author Disclosure Block:

A. Paskovaty: F. Investigator; Self; The Medicines Company. **N. Babady:** None. **Y. Tang:** None. **M. Kamboj:** None. **S. Seo:** None.

Poster Board Number:

MONDAY-016

Publishing Title:

Evaluation of a New Rapid Tool for Reading Antibiotic Susceptibility Testing by the Disk Diffusion Technique

Author Block:

S. Le Page, G. Dubourg, J-M. Rolain; IHU Méditerranée Infection; Aix-Marseille Université, Marseille, France

Abstract Body:

Background: In clinical microbiology, some instruments are able to automatically read inhibition zone diameters for Antibiotic Susceptibility Testing (AST) performed by the disk diffusion (DD) method but its low quality resolution and the time necessary for results of AST became too long around 18 to 24 hours. High-resolution scanners have been developed to improve measurement of inhibition of growth like the Scan® 1200 (Interscience, Saint Nom, France). We evaluate and compare the reading and interpretation of AST by DD by the Scan® 1200 to validate this new instrument for its implementation in the routine laboratory. Furthermore, we tried to read AST earlier after 6h and 8h of incubation. **Methods:** The validation of the technique was assessed on 3 reference strains, as usually requested by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and on 211 clinical isolates with 2 instruments: the Scan® 1200 and the Sirscan system (reference method). For the earlier reading of AST, we measured and interpreted reading zone diameters in a series of 121 Gram-negative bacteria with rapid growthafter 6h, 8h and 24 h of incubation. For both, correlation, statistical and medico-economic analyses are realized.**Results:** The correlation between the 2 instruments, assessed using 211 clinical isolates (n= 2439 zones of growth inhibition measured) was excellent, with a correlation of 0.97 and 1.3% of discrepant results. We obtained a correlation of 0.96 with 3% of discrepant results for 6h of incubation and after 8h the correlation was 0.98 with only 1% of discrepant results. No significant difference was found between 6-8h of incubation and 24h (all p value > 0.05).**Conclusions:**The Scan® 1200 with the complete validation of the technique, its small size, the resolution of the camera, the rapidity of use and its cost represents a robust alternate tool for reading AST for routine use in clinical microbiology laboratories. Moreover, the Scan® 1200 could improve results of AST in a very rapid turnaround time (6h to 8h) in Gram-negative bacteria thanks to the high-resolution imaging.

Author Disclosure Block:

S. Le Page: None. **G. Dubourg:** None. **J. Rolain:** None.

Poster Board Number:

MONDAY-017

Publishing Title:

Zone Size Variation of Meropenem Discs Maintained at Four Temperatures

Author Block:

E. L. Rank, Ph.D.; Quest Diagnostics, Inc., Teterboro, NJ

Abstract Body:

Background: We observed a series of resistant meropenem (M) disc zone (DZ) sizes when tested against a strain of *Haemophilus parainfluenzae*. We retested the organism using fresh M discs. The observed DZ sizes indicated sensitivity. Storage directions for M discs state that discs can be maintained from -20°C to 8°C. Discs routinely are returned to the refrigerator until the next usage. Thus, we systematically investigated whether specific holding temperatures can lead to decreased performance over time for discs with M and other antimicrobials (OA).**Methods:** A 6-month study measured DZ for M and OA against 4 microorganisms: *Escherichia coli* ATCC 25922 (Ec), *Haemophilus influenzae* ATCC 49247 (Hi), *H. parainfluenzae* (Hp), and *Staphylococcus aureus* ATCC 25923 (Sa). OA were: amoxicillin/clavulanate (AC), ampicillin (Amp), ampicillin/sulbactam (AS), aztreonam (Azt), ceftriaxone (Cef), clarithromycin (C), levofloxacin (L), tetracycline (T), and sulfamethoxazole-trimethoprim (ST). Four sets of antimicrobials were opened and stored at 37°C, 22°C to 25°C, 2°C to 8°C, and -20°C. Standard Bauer-Kirby (BK) methodology was employed. Testing was performed once per week. Ec and Sa were grown on BD Mueller-Hinton II agar media in ambient air. Hi and Hp were grown on BD HTM agar media under CO_2. Measured DZ were averaged and compared.**Results:** The percent variation in M DZ size increased as storage temperature increased (Table). DZ variation for OA was also evident. AC, Amp, and AS for Ec and Hi had the greatest average DZ percentage variations. Azt, Cef, L, T, and ST had the least DZ size variation. Average measured DZ sizes were largest for M and OA held at -20°C but decreased as the temperature increased to 37°C. Antimicrobial deterioration was not scientifically confirmed but erratic DZ measurements were photographed along with happenstance patient findings.**Conclusion:** Our study suggests that M discs specifically, but OA as well, should be held at -20°C to ensure consistent performance.

Meropenem Percent Variation in Zone Size				
Temperature	*E.coli*	*H. influenzae*	*H. parainfluenzae*	*S. aureus*
37°C	14.8	25.5	13.0	29.8
22-25°C	9.3	25.0	7.6	19.2
2-8°C	4.6	1.2	5.1	8.6
-20°C	0.0	0.0	0.0	0.0

Author Disclosure Block:

E.L. Rank: None.

Poster Board Number:

MONDAY-018

Publishing Title:

WCK 4282 (Cefepime-Tazobactam) Disk Diffusion Quality Control Ranges Using a Multi-Laboratory Study Design

Author Block:

J. E. Ross, R. K. Flamm, R. N. Jones, H. S. Sader; JMI Lab., North Liberty, IA

Abstract Body:

Background: We conducted a study to establish disk diffusion (DD) quality control (QC) ranges for WCK 4282 (cefepime-tazobactam) using a 30/20-µg disk (2 manufacturers) and the reference DD method. WCK 4282 is under clinical development for the treatment of serious Gram-negative infections.**Methods**: An eight laboratory study design followed CLSI M23-A3 guidelines. Five QC strains were tested (*Escherichia coli* ATCC 25922 [EC25922], *E. coli* NCTC 13353 [EC13353], *Klebsiella pneumoniae* ATCC 700603 [KPN700603], *Pseudomonas aeruginosa* ATCC 27853 [PSA27853], and *Staphylococcus aureus* ATCC 25923 [SA25923]), using three agar lots (three manufacturers). Ten replicate tests/disk lot/media lot/site were performed for each QC organism generating 480 zone diameters/QC strain (2,400 total results). Cefepime and piperacillin-tazobactam were used as control agents.**Results**: The zone diameter QC range for cefepime-tazobactam disk test from eight laboratories when testing EC25922 was 32 - 37 mm which included 97.9% of the reported results. (See Table). EC13353 is a CTX-M-15 producer and was included to properly evaluate tazobactam inhibition effect. The proposed zone diameter QC range of 27 - 31 mm for EC13353 included 96.7% of results. The KPN700603 strain, a SHV-18 producer, provided a six mm QC range of 25 - 30 mm with 99.4% of the results included. Another narrow range was proposed for PSA27853 of 27 - 31 mm which included 97.3% of all reported zone diameters. The SA25923 QC strain demonstrated a range of 24 - 30 mm which included 99.0% of reported results. Using Range Finder statistical program, there were no laboratories or mediums identified as an outlier. All cefepime and piperacillin-tazobactam disk zones generated for the internal controls were within the CLSI published QC ranges.**Conclusions:** These disk QC ranges for WCK 4282 (cefepime-tazobactam) should accurately guide clinical or reference laboratories participating in the testing of clinical trial isolates, and facilitate the regulatory review process for this investigational antimicrobial combination.

QC organism (Reference no.)	**WCK 4282a zone diameter (mm) QC ranges (% of results in range):**
E. coli (ATCC 25922)	32 - 37 (97.9)
E. coli (NCTC 13353)	27 - 31 (96.7)

K. pneumoniae (ATCC 700603)	25 - 30 (99.4)
P. aeruginosa (ATCC 27853)	27 - 31 (97.3)
S. aureus (ATCC 25923)	24- 30 (99.0)
a. Cefepime-tazobactam disk concentration 30/20-µg	

Author Disclosure Block:

J.E. Ross: H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Wockhardt. **R.K. Flamm:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Wockhardt. **R.N. Jones:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Wockhardt. **H.S. Sader:** H. Research Contractor; Self; The study and abstract presentation were funded by a research grant from Wockhardt.

Poster Board Number:

MONDAY-019

Publishing Title:

Rapid Growth-Based Detection of Carbapenem-Resistant Gram-Negative Bacteria Using the Real-Time Light-Scattering Method

Author Block:

E. A. Idelevich[1], M. Hoy[1], M. Borowski[2], D. Görlich[2], B. Grünastel[1], D. Knaack[1], G. Peters[1], K. Becker[1]; [1]Univ. Hosp. Münster, Münster, Germany, [2]Univ. of Münster, Münster, Germany

Abstract Body:

Background: The prevalence of multidrug resistant Gram-negative bacteria is increasing worldwide. Therefore, rapid detection of carbapenem resistance is critical for early initiation of an appropriate antimicrobial treatment. **Methods:** Growth-based detection of meropenem resistance was performed by the light-scattering method using BacterioScan^{TM}216R device for real-time monitoring of microbial growth (BacterioScan Inc., St. Louis, MO). Ten meropenem-resistant and ten meropenem-susceptible clinical isolates of Gram-negative rods (seven *Pseudomonas aeruginosa* and three *Klebsiella pneumoniae* isolates in each group) were used. The inoculum of $5x10^5$ cfu/ml was prepared in cation-adjusted Mueller-Hinton broth. The samples were incubated in the BacterioScan instrument for 6 hours at 35°C with and without meropenem in breakpoint concentration 2 µg/ml. Each sample was automatically measured every 3 minutes. ROC analyses were performed considering the ratio of bacterial growth trends (bacteria without antibiotic vs. corresponding sample with antibiotic) for all time points, where growth trends were estimated using a recently developed moving window regression procedure. Broth microdilution method was performed for determination of meropenem minimum inhibitory concentrations (MIC).**Results:** The estimated sensitivity and specificity for detection of meropenem-resistant Gram-negative bacteria achieved both 100% after 200 minutes of incubation. Due to the particularly early response of susceptible *K. pneumoniae* isolates to meropenem, the differentiation of resistant vs. susceptible isolates was possible already after 80 minutes for this species. According to the broth microdilution, MIC_{50}/MIC_{90} were 16/16 µg/ml and 0.25/0.5 µg/ml for meropenem-resistant and meropenem-susceptible isolates, respectively.**Conclusions:** The light-scattering method is promising for the early detection of carbapenem-resistant Gram-negative bacteria. The time to result seems to be particularly short for *Enterobacteriaceae*. The performance of this method warrants further investigation on large number of isolates.

Author Disclosure Block:

E.A. Idelevich: None. **M. Hoy:** None. **M. Borowski:** None. **D. Görlich:** None. **B. Grünastel:** None. **D. Knaack:** None. **G. Peters:** None. **K. Becker:** None.

Poster Board Number:

MONDAY-020

Publishing Title:

CLSI M23 Tier 2 Study to Establish Disk Diffusion Quality Control Ranges for S-649266

Author Block:

M. Hackel[1], D. Dressel[1], M. Tsuji[2], D. Sahm[1]; [1]IHMA, Inc., Schaumburg, IL, [2]Shionogi and Co., Ltd., Osaka, Japan

Abstract Body:

Background: S-649226 is a novel parenteral siderophore cephalosporin with potent activity against Gram-negative pathogens including carbapenem-resistant isolates and is currently in clinical development with Shionogi & Co., Ltd. This study was performed to establish quality control (QC) disk diffusion ranges for S-649266 against specific ATCC QC organisms utilized by the Clinical and Laboratory Standards Institute (CLSI).**Methods:** Two ATCC QC organisms within the spectrum of S-649266 activity were tested: *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853. S-649266 and a control agent (cefepime) were tested against both QC strains following CLSI guidelines for disk diffusion. This study design met the CLSI M23-A3 requirements for Tier 2 QC studies: seven laboratories/independent sites; three media lots (different manufacturers); two disk lots; 10 replicates of each QC strain per laboratory (at least 70 total test points per medium lot); and at least 210 test points per QC strain/drug combination. Calculation of the proposed QC ranges was based on the Gavan statistic.**Results:** Greater than 95% of all test values reported from nine laboratories were within the ranges shown in the table. All cefepime results were within the established CLSI ranges.**Summary of Proposed CLSI M23 Tier 2 Quality Control Ranges for S-649266**

Organism	**Gavan Statistic Proposed QC Range (mm)**	**Size of range**	**(%) in Range**
E. coli ATCC 25922, All 3 media	23-31	9	95.3 (515/540)
P. aeruginosa ATCC 27853, All 3 media	19-31	13	95.0 (513/540)
P. aeruginosa ATCC 27853, Media A & B only	19-28	10	96.3 (347/360)

Conclusions: Using M23-A3 criteria, a nine millimeter range of 23 - 31 mm included 95.3% of all reported results for *E. coli* ATCC 25922. The Gavan statistic calculated a 13 millimeter range of 19 - 31 mm for *P. aeruginosa* ATCC 27853, which included 95.0% of all reported results. It was noted that a significant number of test results for *P. aeruginosa* ATCC 27853 on Media C

were at the higher end (larger zones) of the calculated range, therefore the analysis was repeated including data from Media A and Media B only, yielding a 10 mm range of 19-28 mm.

Author Disclosure Block:

M. Hackel: M. Independent Contractor; Self; IHMA, Inc. **D. Dressel:** M. Independent Contractor; Self; IHMA, Inc. **M. Tsuji:** D. Employee; Self; Shionogi and Co. **D. Sahm:** M. Independent Contractor; Self; IHMA, Inc..

Poster Board Number:

MONDAY-021

Publishing Title:

Validation of the Check-MDR 103XL Assay Utilizing the CDC/FDA Antimicrobial Resistance Isolate Bank Gram-Negative Carbapenemase Detection Panel

Author Block:

E. Powell, **J. Mortensen**; Cincinnati Children's Hosp., Cincinnati, OH

Abstract Body:

Background: Multi-drug resistant Gram-negative bacilli are being reported more frequently worldwide. The challenge to the clinical microbiology laboratory is how to best detect and monitor these isolates. Including a genotypic component along with the traditional phenotypic antimicrobial resistance testing may add clarity for isolates that are highly resistant or have unclear or difficult to interpret antimicrobial resistance patterns. One such genotypic testing platform is the Check-points system using the CT103XL panel (CPXL). This platform involves a multiplex PCR to amplify resistance genes followed by a microarray for detection. The purpose of this study was to begin the validation process for the CPXL for use in the clinical microbiology laboratory using the CDC-FDA Antimicrobial Resistant Isolate Bank: Gram-Negative Carbapenemase Detection Panel. **Methods:** The panel consists of eighty Gram-negative bacilli with reduced susceptibility to carbapenems via a variety of previously identified mechanisms. DNA was isolated using the NucliSENS easyMAG extractor and the presence of various beta-lactamases—including penicillinases, AmpCs, extended spectrum beta-lactamases, and carbapenemases—was determined using the CPXL test.**Results:** The CPXL detected most reported resistance genes that were included in the CPXL (73 genes in 64 isolates). 6 isolates without reported resistance markers were negative by CPXL. 4 reported genes were not detected by Check-points: chromosomal AmpC genes were not detected in 2 isolates of *Enterobacter aerogenes*, only OXA-24 was detected in 1 isolate with OXA-23 and 24, and the OXA-23 was misidentified as OXA-58 in a second isolate. Additionally, 4 isolates in which there were no reported resistance genes were positive on CPXL: 1 isolate with a wild-type TEM penicillinase, 1 isolate with SHV with 238S and 240K point mutations, 1 isolate with OXA-24, and 1 isolate with NDM and CTX-M-1, group 15 like.**Conclusions:** As clinical microbiology laboratories incorporate more molecular based methods for the determination of antibiotic resistance, the challenges of validating these systems compared to traditional phenotypic systems will be many. Discrepant results may need further phenotypic characterization. Overall, The CPXL performed well in this first trial and warrants further study.

Author Disclosure Block:

E. Powell: None. **J. Mortensen:** None.

Poster Board Number:

MONDAY-022

Publishing Title:

Multi-site Evaluation of Dalbavancin and Vancomycin Mic Test Strip Compared to Broth Microdilution Mic

Author Block:

L. M. Koeth[1], D. J. Hardy[2], T. R. Walsh[3]; [1]Lab. Specialists, Inc., Westlake, OH, [2]Univ. of Rochester Med. Ctr., Rochester, NY, [3]Cardiff Univ., Cardiff, United Kingdom

Abstract Body:

Background: Dalbavancin and vancomycin are antibiotics used for treatment of acute bacterial skin and skin structure infections caused by Gram-positive organisms. This study was performed to evaluate the performance of the dalbavancin and vancomycin MIC test strips (MTS) from Liofilchem, Roseto degli Abruzzi, Italy compared to a broth microdilution method (BMD) for a FDA 510(k) submission.**Methods:** Clinical and challenge isolates were tested by BMD with frozen panels and by MTS. For dalbavancin, 311 *S. aureus* from recent clinical sources were collected and tested at 3 sites, 76 *S. aureus* challenge isolates were tested at 1 site, and for reproducibility 10 *S. aureus* were tested 10 times at 3 sites. For vancomycin, 312 *S. aureus*, 20 *S. epidermidis*, 378 *E. faecalis*, and 62 *E. faecium* from recent clinical sources were collected and tested at 3 sites, 76 challenge isolates (41 *S. aureus*, 5 *S. epidermidis*, 11 *E. faecalis* and 24 *E. faecium*) were tested at 1 site, and for reproducibility 10 isolates were tested 10 times at 3 sites. QC strains (*S. aureus* ATCC 29213, *E. faecalis* ATCC 29212) were tested a minimum of 20 times by each site.**Results:** As shown in the table, dalbavancin and vancomycin MTS MIC results for consolidated clinical and challenge organisms were within +/- one doubling dilution (essential agreement) of BMD MIC results for all isolates with only one exception.

Agent	Organism	N	% Essential Agreement	% Category Agreement
Dalbavancin	*S. aureus*	387	100%	99.7%
Vancomycin	*S. aureus*	353	99.7	98.6
Vancomycin	*S. epidermidis*	65	100	98.5
Vancomycin	*E. faecalis*	389	100	99.7
Vancomycin	*E. faecium*	81	100	97.5

For reproducibility strains, 100% of dalbavancin and 98.1% of vancomycin MTS results were within a doubling dilution of BMD results. All dalbavancin and vancomycin QC results were within CLSI ranges**Conclusions:** The dalbavancin MTS against *S. aureus* and the vancomycin

MTS against *S. aureus, S. epidermidis, E. faecalis* and *E. faecium* performs similar to the reference broth microdilution method.

Author Disclosure Block:

L.M. Koeth: H. Research Contractor; Self; Allergan. **D.J. Hardy:** None. **T.R. Walsh:** None.

Poster Board Number:

MONDAY-023

Publishing Title:

Reproducibility of the Accelerate Id/Ast Blood Culture Assay at Multiple Clinical Sites

Author Block:

M. S. Keller[1], B. Mochon[1], K. R. Kastner[1], M. A. Morgan[2], R. C. Chan[2], Y. Urquiza[2], P. A. Granato[3], M. Degilio[3], P. Schreckenberger[4], S. DesJarlais[4], S. Collier[4], S. M. Ihde[5], B. L. Dylla[5], P. C. Kohner[5], R. Patel[5], B. W. Buchon[6], M. L. Faron[6], N. A. Ledeboer[6], P. Pancholi[7], M. Jindra[7], R. Humphries[8], S. Miller[8], J. A. Hindler[8], B. Ford[9], W. Howard[9], C. Harms[9], **S. W. Metzger**[10]; [1]Banner Gateway Med. Ctr., Gilbert, AZ, [2]Cedars-Sinai Med. Ctr., Los Angeles, CA, [3]Lab. Alliance of Central New York, Liverpool, NY, [4]Loyola Univ. Med. Ctr., Maywood, IL, [5]Mayo Clinic, Rochester, MN, [6]Med. Coll. of Wisconsin, Milwaukee, WI, [7]The Ohio State Univ. Wexner Med. Ctr., Columbus, OH, [8]UCLA, Los Angeles, CA, [9]Univ. of Iowa Hosp. and Clinics, Iowa City, IA, [10]Accelerate Diagnostics, Inc., Tucson, AZ

Abstract Body:

Background: Interlaboratory reproducibility of antimicrobial susceptibility results has been identified by CLSI and CAP as a significant challenge. In this multi-center study, we evaluated the reproducibility of a novel integrated ID and AST system that can provide molecular-based ID and automated microscopy-based phenotypic AST results directly from positive blood cultures.**Methods:** Five bacterial isolates (*S. aureus*, *E. faecalis*, *K. pneumoniae*, *A. baumannii*, and *P. aeruginosa*) were tested at 9 clinical sites. Isolates were seeded into BACT/Alert SA Standard Aerobic, BACTEC Plus Aerobic/F Medium or VersaTREK Redox 1 Aerobic Media with healthy donor blood, incubated until positivity, and run on the Accelerate ID/AST System. ID agreement and AST reproducibility for 24 antibiotics (amikacin, ampicillin, ampicillin-sulbactam, aztreonam, cefazolin, cefepime, ceftaroline, ceftazidime, ceftriaxone, ciprofloxacin, colistin, daptomycin, doxycycline, ertapenem, erythromycin, gentamicin, imipenem, linezolid, meropenem, minocycline, piperacillin-tazobactam, tobramycin, trimethoprim-sulfamethoxazole and vancomycin) and 4 resistance phenotypes (MRSA/MSA, MLSb, high-level gentamicin and streptomycin) were determined.**Results:** Overall ID sensitivity and specificity were 97.8% (44/45) and 100% (765/765), respectively, with 1 false-negative *S. aureus* result. Interlaboratory AST reproducibility (MIC values within 1 dilution of the mode) was 98.4% (443/450).**Conclusions:** The Accelerate ID/AST Blood Culture Assay produced highly reproducible ID and AST results between all clinical laboratory sites using several types of blood culture systems.

Author Disclosure Block:

M.S. Keller: None. **B. Mochon:** H. Research Contractor; Self; Accelerate Diagnostics, Inc.. **K.R. Kastner:** None. **M.A. Morgan:** H. Research Contractor; Self; Accelerate Diagnostics,

Inc.. L. Speaker's Bureau; Self; Accelerate Diagnostics, Inc.. **R.C. Chan:** None. **Y. Urquiza:** None. **P.A. Granato:** H. Research Contractor; Self; Accelerate Diagnostics, Inc.. **M. Degilio:** None. **P. Schreckenberger:** H. Research Contractor; Self; Accelerate Diagnostics, Inc.. L. Speaker's Bureau; Self; Accelerate Diagnostics, Inc.. **S. DesJarlais:** None. **S. Collier:** None. **S.M. Ihde:** None. **B.L. Dylla:** None. **P.C. Kohner:** None. **R. Patel:** H. Research Contractor; Self; Accelerate Diagnostics, Inc. **B.W. Buchon:** H. Research Contractor; Self; Accelerate Diagnostics, Inc.. **M.L. Faron:** None. **N.A. Ledeboer:** None. **P. Pancholi:** H. Research Contractor; Self; Accelerate Diagnostics, Inc.. **M. Jindra:** None. **R. Humphries:** C. Consultant; Self; Nanosphere, Cepheid. H. Research Contractor; Self; Accelerate Diagnostics, Inc.. **S. Miller:** None. **J.A. Hindler:** C. Consultant; Self; Accelerate Diagnostics, Inc. **B. Ford:** H. Research Contractor; Self; Accelerate Diagnostics, Inc.. **W. Howard:** None. **C. Harms:** None. **S.W. Metzger:** D. Employee; Self; Accelerate Diagnostics, Inc.. K. Shareholder (excluding diversitied mutual funds); Self; Accelerate Diagnostics, Inc..

Poster Board Number:

MONDAY-024

Publishing Title:

Rapid Antibiotic Susceptibility Tests by Mass on 96-Well Plates

Author Block:

C. Schneider, P. Harris, K. Babcock, S. Strenn, S. Markakis; Affinity Biosensors, Santa Barbara, CA

Abstract Body:

A rapid phenotype-based antibiotic susceptibility test (AST) would positively impact medical outcomes and antibiotic stewardship, provided that it is amenable to clinical workflow. This work was carried out using a recently-developed resonant mass method, (Burg), in which a mechanically resonant microchannel is used to monitor microbial growth in broth microdilutions by detecting and measuring the mass of individual microbes at high throughput. This technique has been automated and applied to standard 96 well plates so as to provide rapid testing against numerous antibiotics, using sample preparation nearly identical to the CLSI broth microdilution reference method. We tested this rapid AST for repeatability and reproducibility on Gram negative quality control (QC) strains, and assessed its ability to determine the correct minimum inhibitory concentration (MIC) in three hours or less.Gram negative QC strains *Escherichia coli* ATCC 25922, *Escherichia coli* ATCC 35218, *Klebsiella pneumoniae* ATCC 700603, and *Pseudomonas aeruginosa* ATCC 27853 were used to inoculate standard MIC well plates (Thermo Scientific Sensititre GN2F, GN3F, and GN4F). Each well plate contains between 20 and 24 antibiotics at various concentrations. Additional testing was carried out using manual dilutions of a subset of antibiotics. In all cases well plates were inoculated at a microbe concentration of $2x10^5$/ml and incubated for two to three hours, then measured. Wells that showed less than 10x growth from the inoculation concentration were labelled as not growing (susceptible) at that antibiotic concentration. For this study, each microbe/well plate combination was tested three times on each of two instruments.All results obtained with the Sensititre plates were within quality control MIC ranges. Repeated tests and instrument comparisons were in agreement in 95% of the cases, and within a single 2-fold dilution for the remainder. For the manual dilutions, MICs were within the QC range in 90% of the cases, and within a single 2-fold dilution for the remainder.The results indicate that a rapid AST based on microbe mass detection produces the correct and repeatable AST result on Gram negative strains in standard antibiotic panels in under 3 hours. Ongoing work seeks to expand these results to Gram positive strains and to validate the method on clinical samples from positive blood cultures.

Author Disclosure Block:

C. Schneider: D. Employee; Self; Affinity Biosensors. D. Employee; Spouse/Life Partner; Affinity Biosensors. K. Shareholder (excluding diversitied mutual funds); Spouse/Life Partner;

Affinity Biosensors. **P. Harris:** D. Employee; Self; Affinity Biosensors. **K. Babcock:** D. Employee; Self; Affinity Biosensors. D. Employee; Spouse/Life Partner; Affinity Biosensors. K. Shareholder (excluding diversitied mutual funds); Self; Affinity Biosensors. **S. Strenn:** M. Independent Contractor; Self; Affinity Biosensors. **S. Markakis:** D. Employee; Self; Affinity Biosensors.

Poster Board Number:

MONDAY-025

Publishing Title:

Value of Beckman Coulter Microscan Antimicrobial Susceptibility Panels to Detect Resistance to Carbapenems in *Enterobacteriaceae* in South Africa, Report from National Antimicrobial Reference Laboratory

Author Block:

O. Perovic, A. Singh-Moodley; Natl. Inst. for Communicable Diseases, Johannesburg, South Africa

Abstract Body:

Background: Since the first case of carbapenemases producing Enterobacteriaceae (CPE) in 2010, the Antimicrobial Resistance Laboratory at the National Institute for Communicable Diseases has received isolates for confirmatory antimicrobial susceptibility testing (AST) and molecular analysis. We aimed to validate AST panels in estimating resistance to carbapenems. **Methods:** Confirmatory identification was done on MALDI-TOF. AST was performed by the MicroScan® automated Walkaway system (Beckman Coulter, USA) using Gram-negative MIC Panel Type 44 (ertapenem 0.5-1µg/mL; doripenem 1-4µg/mL; imipenem 1-8 µg/mL; meropenem 1-8 µg/mL), the interpretation was based on the Clinical and Laboratory Standards Institute (CLSI) guidelines. DNA was extracted from the phenotypically confirmed CPEs by a crude boiling method. The supernatant was screened for bla_{NDM}, bla_{KPC}, $bla_{\text{OXA-48}}$ and its variants, $bla_{\text{GES,}}$ bla_{IMP} and bla_{VIM} using real-time polymerase chain reaction (PCR) (Light Cycler 480 II, Roche Applied Science, Light Cycler 480 Probes Master kit and the individual LightMix Modular kits (Roche Diagnostics, IN, USA). Validity of the panels was analysed using STATA. **Results:** A total of 1621 carbapenem non-susceptible isolates from various specimen types were received. MicroScan panels confirmed non-susceptibility to carbapenems in 88% from a total of 1241 Enterobacteriaceae and 90% in 746 *Klebsiella pneumoniae*. By PCR (gold standard) we confirmed CPEs in 1082 (67%) isolates. Sensitivity of ertapenem was 93.7%; specificity, 28.6%; positive predictive value (PPV), 71% and negative predictive value (NPV), 63%. For imipenem/meropenem and doripenem, the sensitivity was 75% and 76%, retrospectively; specificity was 69 and 72%, PPV 83 and 87% and NPV 57 and 55%. *Klebsiella pneumoniae* was the predominant organism 1114 (61%) and carbapenemases were detected in 721 (72%) isolates which included 442 (42%) NDM as the most predominate gene with a substantial increase in the positivity rate from 33% in 2014 to 52% in 2015. **Conclusions:** MicroScan panel showed excellent sensitivity with ertapenem and good specificity with the other three carbapenems. It can be recommended for screening and confirmation of CPE when molecular tests are not available.

Author Disclosure Block:

O. Perovic: None. **A. Singh-Moodley:** None.

Poster Board Number:

MONDAY-026

Publishing Title:

Antibiograms in Five Pipetting Steps: Precise Dilution Assays in Sub Microliter Volumes with a Conventional Pipette

Author Block:

L. Derszi[1], T. S. Kaminski[2], **P. Garstecki**[2]; [1]Univ. of Padova, Dept. of Physics and Astronomy, Padova, Italy, [2]Inst. of Physical Chemistry, Polish Academy of Sci., Warsaw, Poland

Abstract Body:

Here we demonstrate a microfluidic chip that allows to execute an antibiotic susceptibility assay in an array of nanoliter droplets with the use of a standard automatic pipette, requiring only five pipetting steps. The system passively prepares a series of dilutions of a chemical compound and mixes them with portions of the sample and all the liquid handling operations are hard-wired into the geometry of the microfluidic chip. The precision of metering, merging, mixing, and splitting of discrete portions of liquid samples roots in the passive capillary action in microfluidic traps and not in the precision of dosing with a pipette. We demonstrate exemplary use of the system in determination of minimum inhibitory concentration (MIC) of ampicillin against *E. coli* ATCC 25922. The technology may significantly lessen the manual work load required to execute the most commonly performed assay in clinical microbiology.

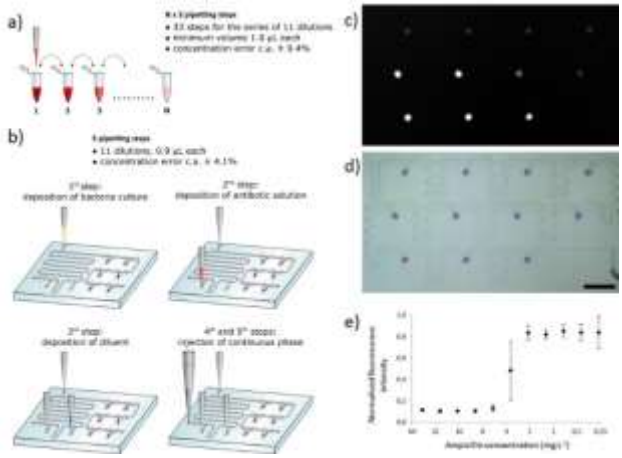


Fig. 1. a) Classic MIC assay requires 3N pipetting steps: aliquoting of pure broth, dilution of antibiotic, addition of bacteria, **b)** microfluidic chip requires 5 pipetting steps, regardless of the total number of dilutions. Fluorescent **(c)** brightfield **(d)** image show conversion of resazurin to resorufin in test-droplets after incubation, **e)** the MIC plot for ampicillin against ATCC 25922.

Author Disclosure Block:

L. Derszi: None. **T.S. Kaminski:** None. **P. Garstecki:** None.

Poster Board Number:

MONDAY-027

Publishing Title:

Discrepant Susceptibility to Gentamicin by Vitek 2 in *Klebsiella pneumoniae* Induced by *Arma*

Author Block:

J. H. Ko[1], **J. Y. Baek**[2], K. R. Peck[1], S. H. Kim[2], H. Seok[1], G. E. Park[1], S. Y. Cho[1], Y. E. Ha[1], C-I. Kang[1], D. R. Chung[1], H. J. Huh[1], N. Y. Lee[1], J. H. Song[2]; [1]Samsung Med. Ctr., Sungkyunkwan Univ. Sch. of Med., Seoul, Korea, Republic of, [2]Asia Pacific Fndn. for Infectious Diseases, Seoul, Korea, Republic of

Abstract Body:

Background: In the era of increasing beta-lactam resistance of *Enterobacteriaceae*, aminoglycoside antibiotics become important treatment option for multi-drug resistant *Enterobacteriaceae.* Although VITEK 2 automated system plays major role in clinical practice measuring antibiotics susceptibility, there were reports that high-level resistance to aminoglycosides could be falsely reported as susceptible. We evaluated true antibiotics susceptibility and presence of 16S rRNA methylase gene in *K. pneumoniae* with this unique aminoglycoside susceptibility pattern presented by VITEK 2. **Methods:** We screened *K. pneumoniae* blood isolates from January 2015 to December 2015 at a 1950-bed tertiary care center. MIC values and interpretations reported by VITEK 2 system were reviewed and isolates with gentamicin non-resistance despite amikacin resistance were selected. We substantiated MICs of selected isolates by broth microdilution method. Presence of 16S rRNA methylase genes including *armA, rmtB, rmtC, rmtE,* and *rmtF* were investigated by PCR. **Results:** A total of 564 blood isolates of *K. pneumoniae* was identified, 550 isolates (97.3%) were susceptible, 2 (0.4%) were intermediate, and 12 (2.1%) were resistant to amikacin by VITEK 2 system. Among 12 amikacin resistant isolates, 9 (75%) were susceptible (4 isolates) or intermediate (5 isolates) to gentamicin, all of which were tested for MIC and 16S rRNA methylase genes. All of gentamicin intermediate isolates (100%) and 3 of gentamicin susceptible isolates (75%) showed high-level resistance to both amikacin and gentamicin (MIC > 128μg/ml and ≥ 256μg/ml, respectively) by broth microdilution method and were positive to *armA* gene. **Conclusion:** Discrepant susceptibility to gentamicin despite amikacin resistance in *K. pneumoniae* by VITEK 2 was related with false susceptibility induced by *armA*. This finding presents important information in selecting antibiotics especially for multi-drug resistant pathogens, and gentamicin susceptibility of *K. pneumoniae* isolates with discrepant aminoglycoside susceptibility pattern presented by VITEK system should be confirmed by alternative susceptibility testing.

Author Disclosure Block:

J.H. Ko: None. **J.Y. Baek:** None. **K.R. Peck:** None. **S.H. Kim:** None. **H. Seok:** None. **G.E. Park:** None. **S.Y. Cho:** None. **Y.E. Ha:** None. **C. Kang:** None. **D.R. Chung:** None. **H.J. Huh:** None. **N.Y. Lee:** None. **J.H. Song:** None.

Poster Board Number:

MONDAY-028

Publishing Title:

S-649266 Mic Quality Control Ranges In Iron-Depleted Cation-Adjusted Mueller-Hinton Broth Using A Multi-Laboratory Study Design

Author Block:

J. E. Ross[1], A. Ito[2], M. Tsuji[2], R. K. Flamm[1], R. N. Jones[1], H. S. Sader[1]; [1]JMI Lab., North Liberty, IA, [2]Shionogi & Co., Ltd., Toyonaka, Osaka, Japan

Abstract Body:

Background: We conducted a study to establish MIC quality control (QC) ranges for S-649266, a siderophore cephalosporin, in iron-depleted cation-adjusted Mueller-Hinton broth (MHB). Chelex treatment depletes iron from the medium and enhances the uptake of S-649266, which can enter the bacterial cell through iron transport mechanisms in Gram-negative (GN) bacteria. S-649266 exhibits potent efficacy against various GN bacteria including carbapenem-resistant strains.**Methods:** An eight laboratory study design followed CLSI M23-A3 guidelines. Two QC strains were tested (*Escherichia coli* ATCC 25922 [EC25922] and *Pseudomonas aeruginosa* ATCC 27853 [PSA27853], using four media lots (three manufacturers) of MHB treated with a chelating agent. MHB was treated for 2 hours with Chelex, filtered, pH adjusted, and levels of calcium and magnesium were adjusted to CLSI specifications. Zinc was adjusted to 0.5 mg/mL; 10µM. Ten replicate tests were performed for each QC organism generating 320 broth microdilution values/QC strain. Cefepime was used as a control agent.**Results:** S-649266 MIC QC range of 0.06 - 0.5 µg/mL was proposed for EC25922, which included 99.7% of results and a mode at 0.25 µg/mL. A MIC "shoulder" at 0.12 µg/mL included 53.1% of the number of MIC results compared to the number of MIC values at the modal 0.25 µg/mL, thus causing a need for a four $\log_2$ dilution range. PSA27853 also needed a four $\log_2$ dilution range of 0.06 - 0.5 µg/mL to include 95.0% of all reported results for S-649266. This distribution had a modal value at 0.25 µg/mL with a "shoulder" at 0.12 µg/mL which included 83.2% of the number of MICs compared to the modal value. One medium lot was identified as an outlier when tested against EC25922 with S-649266 having a modal MIC value of two $\log_2$ dilutions (0.06 µg/mL) lower than the other three lots (0.25 µg/mL). Medium variations occurred with the S-649266 compound only. All MIC values generated for cefepime were within the CLSI published QC range.**Conclusions:** These proposed MIC QC ranges for S-649266 when tested in MHB (iron depleted by Chelex treatment) should accurately guide clinical or reference laboratories participating in the testing of clinical trial isolates, and facilitate the regulatory review process for this investigational antimicrobial agent.

Author Disclosure Block:

J.E. Ross: H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Shionogi & Co., Ltd. **A. Ito:** D. Employee; Self; Employee of Shionogi & Co., Ltd. **M. Tsuji:** D. Employee; Self; Employee of Shionogi & Co., Ltd. **R.K. Flamm:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Shionogi & Co., Ltd. **R.N. Jones:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Shionogi & Co., Ltd. **H.S. Sader:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Shionogi & Co., Ltd..

Poster Board Number:

MONDAY-029

Publishing Title:

Performance Of Two Methods For Detection Of Carbapenem Resistance Mechanism Among Enterobacteriaceae

Author Block:

S. Miller[1], T. Wagner[2], **R. M. Humphries**[1]; [1]UCLA, Los Angeles, CA, [2]OpGen, Gaithersburg, MD

Abstract Body:

Background: Carbapenem resistance is conferred by the presence of extended spectrum beta-lactamases (ESBL), AmpC enzymes and/or carbapenemases, often in combination with a permeability defect. Knowledge of the resistance mechanism is critical to epidemiological studies, but few laboratories have the capacity to routinely evaluate for mechanisms of resistance among carbapenem resistant Enterobacteriaceae (CRE).**Methods:** The performance of the OpGen Acuitas® Resistome Test (Opgen Inc. Gaithersburg, MD) was compared to a laboratory-developed TaqMan real-time polymerase chain reaction (RT-PCR) that detects the presence of *bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{IMP}$, *bla*$_{VIM}$, *bla*$_{SME}$ and *bla*$_{OXA-48-like}$ carbapenemase genes, for a collection of 20 clinical isolates. All isolates had imipenem and/or meropenem MICs >1 μg/ml. The Acuitas Resistome Test applies a microfluidic PCR array that analyzes isolates of Gram-negative bacilli for approximately 50 resistance mechanisms, including carbapenemases, ESBLs, and AmpC.**Results:** 18/20 isolates yielded concordant carbapenemase results by the Acuitas Resistome Test and RT- PCR. These included 1 KPC, 2 IMP, 2 NDM and 2 OXA-48-like enzymes, and 11 isolates negative for carbapenemase genes targeted in the RT-PCR. A *Providencia rettgeri* was positive for KPC, NDM, SHV ESBL and TEM by the Acuitas Resistome Test, but NDM alone by the RT-PCR. Repeat testing yielded NDM alone by both tests. A *Citrobacter freundii* was positive for VIM, CTX-M-1, SHV, TEM and CMY/MOX by the Acuitas Resistome Test and negative by the RT-PCR. Upon repeat testing, only CTX-M-1 and CMY/MOX AmpC were detected by Acuitas Resistome Test and the RT-PCR was negative. We hypothesize these 2 discrepancies may be due to plasmid loss and/or heterogeneous populations of the cultured isolates. For the carbapenemase-negative isolates by the RT-PCR, plasmid AmpC was detected in 5, ESBL in 3, OXA-23 and OXA-51 in 1 and no beta-lactamases in 2, by the Acuitas Resistome Test.**Conclusion:** The Acuitas Resistome Test performed comparably to RT-PCR for the detection of carbapenemase genes. Laboratories must be aware that discordant results might be observed in CRE, one possibility being due to plasmid loss. Furthermore, this test provides data on resistance mechanisms outside the presence of carbapenemases.

Author Disclosure Block:

S. Miller: None. **T. Wagner:** D. Employee; Self; OpGen. **R.M. Humphries:** I. Research Relationship; Self; Cepheid, Curetis, Accelerate, OpGen, bioMerieux, BD, BeckMan Coulter, GenMark. J. Scientific Advisor (Review Panel or Advisory Committee); Self; Merck, Nanosphere, Cepheid, Roche. L. Speaker's Bureau; Self; Merck, Allergan.

Poster Board Number:

MONDAY-030

Publishing Title:

Using a Novel Atp Bioluminescence Assay (Atpb) with Elimination of Extracellular (Ec) Atp to Predict Quantitative Counts in Time-Kill Studies (Tks) within 15 Minutes Against Extensively Drug Resistant (Xdr) Gram Negative Bacteria (Gnb)

Author Block:

Y. Cai[1], J. Ng[2], L. Hui[1], S-X. Tan[1], T-P. Lim[1], J. Teo[1], W. Lee[1], T-T. Tan[1], T-H. Koh[1], A-L. Kwa[1]; [1]Singapore Gen. Hosp., SG, Singapore, [2]Natl. Univ. of Singapore, SG, Singapore

Abstract Body:

TKS is commonly employed to determine bactericidal antibiotic (abx) combinations (combi) for XDR GNB. However, bacterial count quantification in TKS is laborious & requires 24h incubation. Bacterial ATP has been proposed as a surrogate for viable count, but its utility is confounded by EC-ATP released upon exposure to abx. We developed a novel ATPB assay with EC-ATP elimination & demonstrated its use in quantifying kill in TKS within 15min. **Methods:** 6 clinical XDR GNB [2 *A. baumannii* (AB), 2 *K. pneumoniae* (KP), 2 *P. aeruginosa* (PA)] were used. 24hTKS were conducted with 5log CFU/mL at baseline with clinical concentrations of amikacin (65), levofloxacin (8), meropenem (20) tigecycline, polymyxin B (2) & aztreonam (24) singly & in 2-abx combi. At each timepoint (0, 2, 4 6, 8, 24h), the following samples were obtained: 1) direct measurement of total ATP; 2) elimination of EC-ATP using apyrase (1U/mL) for 15min at 37°C with repeated washings & ATP measurement; 3) filtration with ATP measurement to determine EC-ATP. Bacterial counts by viable plating was determined. Correlation (r^2) between counts (CFU/mL) & ATP measurements (RLU/100uL) were determined. **Results:** Moderate correlation ($r^2 = 0.62$) was observed between viable counts & direct ATP measurements when all XDR GNB were collectively analyzed (operating range of 10^2 to 10^8 CFU/mL). When correlation between viable counts & direct ATP measurements was determined for each species, correlation was highest for KP & lowest for PA (KP $r^2 = 0.71$, AB $r^2 = 0.69$, PA $r^2 = 0.48$). Significantly higher correlation of ATP measurements to viable counts was observed in apyrase-treated samples ($r^2 = 0.82$) when all XDR GNB were collectively analyzed. Improved correlation was also seen in each species (KP $r^2 = 0.90$, AB $r^2 = 0.84$, PA $r^2 = 0.74$). Measurements of EC-ATP showed that the amount EC-ATP varied widely upon abx exposure, accounting for the high variability in direct ATP measurements. **Conclusions:** Our ATPB assay with EC-ATP removal can accurately predict bacterial counts to determine killing in TKS within 15min. This short turn-around time allows TKS to be employed to guide the timely selection of abx combi for clinical use.

Author Disclosure Block:

Y. Cai: None. **J. Ng:** None. **L. Hui:** None. **S. Tan:** None. **T. Lim:** None. **J. Teo:** None. **W. Lee:** None. **T. Tan:** None. **T. Koh:** None. **A. Kwa:** None.

Poster Board Number:

MONDAY-031

Publishing Title:

Evaluation of Antibiotic Susceptibility by Headspace Gas Analysis

Author Block:

J. D. S. Newman, E. M. Becker, M. Krause, **J. L. Lucas**, J. A. Trimboli; MRIGlobal, Rockville, MD

Abstract Body:

Recent analyses of bacterial headspace samples have demonstrated that microorganisms can be identified by profiling a range of volatile organic compounds (VOCs), including distinguishing between antibiotic susceptible and resistant sub-strains of *Staphylococcus aureus* (Bean et al, 2014). We have identified the VOC profiles of two strains of *Bacillus anthracis*, the causative agent of anthrax, using a non-invasive, micro-extraction technique. *B. anthracis* Sterne 1:6 is resistant to ciprofloxacin, while the parent strain, Sterne, is susceptible. Our approach involves culturing the *B. anthracis* strains in the presence and absence of ciprofloxacin, the primary treatment for anthrax. The culture's headspace is then sampled with solid-phase microextraction (SPME), and the chemical constituents analyzed with gas chromatography-mass spectrometry (GC-MS). To date, we have characterized the signatures associated with *B. anthracis* Sterne and *B. anthracis* Sterne 1:6 (AMR). Two fiber compositions (PDMS/Carboxen and PDMS/Carboxen/Divinylbenzene) have been tested as well as three culture media (tryptic soy broth (TSB), Mueller-Hinton (MH) and LB broth). Our study identified compounds unique to the *B. anthracis* AMR strain, either in the presence or absence of ciprofloxacin. One fiber-media combination (PDMS/Carboxen and TSB) resulted in the discovery of a single compound, 3-hydroxy-2-butanone, which was only present in the AMR strain. This compound is capable of differentiating the AMR strain from the susceptible strain in both the presence and absence of ciprofloxacin. These results support the further development of VOC profiles in bacteria species as a novel approach for antibiotic susceptibility testing.

Author Disclosure Block:

J.D.S. Newman: None. **E.M. Becker:** None. **M. Krause:** None. **J.L. Lucas:** None. **J.A. Trimboli:** None.

Poster Board Number:

MONDAY-032

Publishing Title:

Evaluation of Ceftolozane-Tazobactam Mic Test Strip Compared to Broth Microdilution Mic for *Enterobacteriaceae* and *Pseudomonas aeruginosa*

Author Block:

L. M. Koeth, J. DiFranco-Fisher; Lab. Specialists, Inc., Westlake, OH

Abstract Body:

Background: Ceftolozane-tazobactam (C-T) is a cephalosporin - beta lactamase inhibitor combination antimicrobial agent that was approved by the Food and Drug Administration (FDA) for the treatment of complicated urinary tract infections (cUTI), including pyelonephritis and in combination with metronidazole for the treatment of complicated intra-abdominal infections (cIAI). This study was performed to evaluate the performance of a newly developed gradient strip, the ceftolozane-tazobactam MIC Test Strip (MTS) from Liofilchem, Roseto degli Abruzzi, Italy compared to a broth microdilution method against indicated Gram negative isolates. **Methods:** The study isolates (50 *Enterobacteriacieae* [21 *E. coli*, 13 *K. pneumoniae*, 9 *E. cloacae*, 4 *P. mirabilis* and 3 *K. oxytoca*] and 50 *P. aeruginosa*) were recent clinical isolates primarily from indicated sources and chosen to include a wide range of C-T MIC results (0.12-4 to >32-4 µg/mL). Each isolate was tested for C-T MIC by broth microdilution (BMD; LSI prepared frozen panels) and by C-T MTS on 100 mm Mueller Hinton agar (MHA) plates (Becton Dickinson, Sparks, MD). QC strains (*E. coli* ATCC 25922, *E. coli* ATCC 35218, *P. aeruginosa* ATCC 27853 and *K. pneumoniae* ATCC 700603) were tested on 9 days with MHA from 2 suppliers (Becton Dickinson and Hardy Diagnostics, Santa Maria, CA) and results compared to CLSI expected ranges. **Results:** As shown in the table, C-T MTS and BMD results were within +/- one doubling dilution (essential agreement) for 94.0% of *Enterobacteriaceae* and 96.2% of *P. aeruginosa*. Quality control results were within CLSI established ranges, with exception of one outlier result for *E. coli* ATCC 35218 on Hardy MHA.

Organism	Dilution difference of ceftolozane-tazobactam MTS-BMD (n)							Total
	-3	-2	-1	0	1	2	OS>	
Enterobacteriaceae	2	1	11	16	14	0	6	50
P. aeruginosa	2		6	12	28		5	53

OS: Off-scale (> highest concentration tested)**Conclusions:** This initial evaluation of the C-T MTS showed good correlation to BMD MIC. Further testing with additional isolates at multiple sites and with media from multiple manufacturers is warranted.

Author Disclosure Block:

L.M. Koeth: H. Research Contractor; Self; Liofilchem and Merck. **J. DiFranco-Fisher:** None.

Poster Board Number:

MONDAY-033

Publishing Title:

Comparison of Modified Hodge Test, Neo-Rapid Carb, and Xpert Carba-R for the Detection of Carbapenemases in the *Enterobacteriaceae*

Author Block:

C. Doern, C. Bender, T. Neal, B. Forbes; Virginia Commonwealth Univ. Hlth.System, Richmond, VA

Abstract Body:

Historically, laboratories have relied on phenotypic methods such as the Modified Hodge Test (MHT) to confirm carbapenemase production. The limitations of this method in both sensitivity and specificity have been well documented and newer methods such as the Neo-Rapid CARB Kit (NRC)) (Rosco Diagnostica) and the Xpert Carba-R (XCR) (Cepheid) promise to improve the accuracy of carbapenemase detection. NRC is a phenotypic test that detects carbapenemase activity through carbapenem hydrolysis in the presence of an indicator. XCR is a multiplex PCR assay that detects genes encoding KPC, VIM, OXA-48, IMP-1, and NDM carbapenemases. The goal of this study was to compare the performance of the XCR and NRC assays to that of MHT for carbapenemase detection in isolates of *Enterobacteriaceae.* Herein we compare the results of meropenem disk diffusion (MDD), MHT, NRC, and the XCR testing for a challenge set of 95 isolates (14 *E. coli*, 19 *Enterobacter* spp., 41 *Klebsiella* spp., 12 *Citrobacter* spp., 3 *Providencia* spp., 2 *Proteus mirabilis*, 2 *Serratia marcesens*, and 2 *Morganella morganii*) with reduced susceptibility to either 3rd generation cephalosporins and/or carbapenems. Forty-seven isolates were obtained from clinical specimens and 48 with known mechanisms of resistance were purchased. Forty-three isolates yielded negative MHT results. Of these, all but 4 were negative by NRC and all were negative by the XCR. MDD using current CLSI breakpoints yielded 9 resistant and 15 intermediate results within this group. Fifty-two isolates were MHT positive. Of these, 32 were positive by XCR (30 KPC, 2 VIM) and 44 were positive by NRC. MDD yielded susceptible results in 8 isolates. When using MHT as the gold standard, these data show that both the XCR and NRC perform with high specificity (only 4 false positives; all by NRC). However, in MHT-positive isolates, significant discrepancies were observed. Eighteen and 8 MHT-positive isolates were negative by XCR and NRC, respectively. Three of these isolates were *Enterobacter* spp. which are known to harbor chromosomal *ampC* genes and four additional isolates were known to harbor ESBLs. These day may suggest that these are actually falsely positive MHT results. Further studies including reference broth microdilution and Check-Direct CPE are currently being performed to resolve these discrepancies.

Author Disclosure Block:

C. Doern: J. Scientific Advisor (Review Panel or Advisory Committee); Self; ThermoFisher. **C. Bender:** None. **T. Neal:** None. **B. Forbes:** None.

Poster Board Number:

MONDAY-034

Publishing Title:

Comparative Performance of the Modified Hodge Test (Mht) with a New Modified Rapid Carbar Kit (Rosco Tabs) for the Detection of Carbapenem Resistant Organisms (Cros)

Author Block:

J. Vedula, A. Clowes, M. Lam, T. Dingle, C. Hamula; Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract Body:

Background: The rise in prevalence of carbapenem resistant organisms (CROs) has precipitated the need for a rapid and accurate method to phenotypically identify these isolates. The CarbaNP and Modified Hodge test (MHT) are listed in the CLSI M100 guideline as the standard for carbapenemase detection. Both tests are laborious and present workflow issues for the clinical laboratory. A modified novel rapid carbapenemase detection kit based on the CarbaNP test (Neo-Rapid Carb Kit 98024, ROSCO Diagnostica) was compared to MHT in its performance by testing a collection of routine clinical isolates determined to be carbapenem resistant by the Vitek II (Biomerieux Diagnostics, France). **Methods:** We analyzed over 75 carbapenem resistant clinical isolates from the Mount Sinai Hospital Clinical Microbiology Laboratory in New York City. The isolates include 49 *Klebsiella* pneumonia, 15 *Pseudomonas aeruginosa*, 6 *Escherichia coli*, and 1 of each of *Serratia marcescens, Acinetobacter baumanii, Enterobacter aerogenes,* and *Enterobacter cloacae*. *Enterobacteriaciae* were selected based on resistance to both ertapenem and imipenem (VITEK II), and *P. aeruginosa* was selected based on resistance to ceftazidime, cefipime, meropenem, and imipenem(VITEK II). Strains were sub-cultured in the presence of a 10ug imipenem disk and the Neo-Rapid Carb Kit (NRC) was administered with a modified version of the manufacturer's protocol using 0.9% normal saline. The tabs were read at 30 minutes and 60 minutes. The MHT was set up concurrently and interpreted according to 2016 CLSI guidelines. **Results:** NRC successfully identified 100% of the carbapenemase producing organisms: 93% of isolates [70/75] were positive within the first 30 minutes while the remaining isolates [5/75] were positive by 60 minutes. Isolates tested 61% positive [46/75], 36% negative [27/75], and 3% [2/75] indeterminate by MHT. **Conclusions:** Further characterization of the isolates via PCR and comparison to results of the full-length CarbaNP protocol is needed. However, NRC is an accurate and reliable method of phenotypically identifying carbapenemase producing organisms (CPO). The kit can be administered and read in under 60 minutes providing a rapid alternative to the difficult to interpret, and labor- intensive MHT.

Author Disclosure Block:

J. Vedula: None. **A. Clowes:** None. **M. Lam:** None. **T. Dingle:** None. **C. Hamula:** None.

Poster Board Number:

MONDAY-035

Publishing Title:

Ability of the Bd Phoenix Emerge Nmic-140 Panel to Accurately Identify Extended Spectrum Beta-Lactamase (Esbl) Isolates

Author Block:

L. L. Steed, L. L. Gauld, G. E. Palmer-Long; Med. Univ of South Carolina, Charleston, SC

Abstract Body:

Routine ESBL testing is required at the Medical University of South Carolina (MUSC) Hospital as part of our Infection Prevention and Control's program to control multi-drug resistant organisms. An internal rule was used to supplement the expert system ESBL algorithm of our previous automated instrument to avoid overcalling resistance. We sought to determine if an internal rule was still necessary for use with the BD Phoenix Emerge NMIC-140 panels. This rule required that an isolate flagged by the Phoenix as being an ESBL must have the pattern cefazolin = R/cefoxitin = S/aztreonam, cefotaxime, ceftazidime, OR ceftriaxone = R. Isolates that fulfilled the internal rule were accepted as being ESBLs; isolates that did not were confirmed by double disk diffusion per the current CLSI M100. Between October, 2014, and July, 2015, 156 isolates were called ESBL by the Phoenix. Most isolates (85%) were from urine sources. Not unexpectedly, *Escherichia coli* predominated (80%), followed by *Klebsiella pneumoniae* (17%) and *Klebsiella oxytoca* (3%). A total of 88 isolates (56%) met the internal rule and were reported as ESBL without further testing. 58% of *E. coli* and *K. pneumoniae* isolates fulfilled the internal rule. Another 31 isolates (20%) were confirmed while 37 isolates (24%) did not confirm. While 27% of *K. pneumoniae* isolates that did not meet the internal rule confirmed as ESBL by double disk diffusion, 26% of *E coli* isolates that did not meet the internal rule failed to confirm as ESBL. Our use of an internal rule complementing the expert system ESBL algorithm used by the BD Phoenix prevented false reporting of 24% of isolates as ESBLs during the study period. Providing additional β-lactam antibiotic choices to clinicians when possible and reducing unnecessary use of contact isolation in our patients is worth the additional 18-24 hours needed for confirmatory double disk diffusion testing.

Author Disclosure Block:

L.L. Steed: None. **L.L. Gauld:** None. **G.E. Palmer-Long:** None.

Poster Board Number:

MONDAY-036

Publishing Title:

Tilmicosin Cannot be Used as the Veterinary Macrolide Class Representative to Predict Tulathromycin Activity

Author Block:

M. T. Sweeney[1], H. Moyaert[2], J. Watts[1]; [1]Zoetis, Kalamazoo, MI, [2]Zoetis, Zaventem, Belgium

Abstract Body:

Background: A number of diagnostic laboratories have begun to use tilmicosin as the class representative to predict the susceptibility for tulathromycin to the bovine respiratory disease pathogen *Mannheimia haemolytica* and other BRD and SRD bacterial species. However, no *in vitro* data are available that supports the use of tilmicosin as the class representative for tulathromycin or other veterinary macrolides. We report scattergram plots of tilmicosin-tulathromycin MIC values and calculated error-bound rates when using tilmicosin versus tulathromycin clinical breakpoints.**Methods:** Veterinary laboratories from the US and Canada provided *M. haemolytica* isolates recovered from diseased or dead beef or dairy cattle over an 11-year period (2004-2014). Each participating laboratory was requested to submit no more than 32 isolates per year in order to prevent over-representation from any one geographic area. *In vitro* MIC susceptibility values for the isolates against these drugs were generated at two laboratories following Clinical and Laboratory Standards Institute (CLSI) standardized methods.**Results:** Of 3819 *M. haemolytica* isolates tested, 302 isolates were determined to be tilmicosin resistant but tulathromycin susceptible based on established clinical breakpoints for tilmicosin and tulathromycin, resulting in a major error-bound rate of 7.9% which is above the 3% CLSI-recommended limit. Susceptibility values for another 448 isolates produced an 11.7% minor error-bound rate in which 359 isolates were tilmicosin-I/tulathromycin-S, 61 strains were tilmicosin-R/tulathromycin-I, 21 strains were tilmicosin-I/tulathromycin-R, and 7 strains were tilmicosin-S/tulathromycin-I.**Conclusions:** Since all veterinary macrolides have different substituted ring structures, as well as different *in vitro* and *in vivo* properties with regards to MIC activity, dosage regimen, and pharmacokinetics, any susceptibility results for tilmicosin to predict the activity and potential treatment outcome for tulathromycin is strongly discouraged.

Author Disclosure Block:

M.T. Sweeney: D. Employee; Self; Zoetis. **H. Moyaert:** D. Employee; Self; Zoetis. **J. Watts:** D. Employee; Self; Zoetis.

Poster Board Number:

MONDAY-037

Publishing Title:

A Universal Screening Medium Fo Polymyxin-Resistant Gram-Negative Bacteria

Author Block:

P. Nordmann, A. Jayol, L. Poirel; Univ. of Fribourg, Fribourg, Switzerland

Abstract Body:

Background: Multidrug resistant (MDR) Gram negatives usually remain susceptible to polymyxins (colistin and polymyxin B) however emergence of polymyxin resistance is increasingly reported. Polymyxin-based selective culture media have been developed for screening intrinsic polymyxin-resistant bacterial species but they are not adapted for screening Gram negatives with acquired resistance to polymyxins that display low- to high-level resistance to polymyxins. We developed here a selective culture medium to detect any type of polymyxin-resistant gram negative bacteria. **Materials/methods:** The SuperPolymyxin medium was prepared using an Eosine Methylene Blue agar (EMB)-based culture medium in which colistin or polymyxin B, daptomycin and amphotericin B were added. Performance of the medium was evaluated with a total of 82 Gram-negative strains from various enterobacterial and non-fermenters species. Some of those strains had well-characterized mechanisms of resistance, such as mutations in the *pmrA* or *pmrB* genes (*K. pneumoniae*, *A. baumannii*), alterations in the *mgrB* gene or its promoter sequences (*Klebsiella* spp.), or produced the plasmid-mediated MCR-1 colistin resistance determinant (*E. coli*). MICs of polymyxins were determined using the broth microdilution method according to the CLSI guidelines and results were interpreted according to the EUCAST breakpoints.**Results:** Polymyxin-resistant strains grew on the SuperPolymyxin medium in 24 h, except *P. aeruginosa* and *S. maltophilia* and the intrinsically polymyxin-resistant *Burkolderia* genus that grew in 24 to 48 h. The lowest limit of detection was below the cut-off value of 1×10^3 CFU/ml for all polymyxin-resistant strains, whereas the limit of detection of the polymyxin-susceptible strains was above 1×10^3 CFU/ml, being $\geq 1 \times 10^6$ CFU/ml. The sensitivity and specificity of the SuperPolymyxin medium for selecting polymyxin-resistant gram negatives were 100%.**Conclusion:** The SuperPolymyxin medium is the first screening medium that is aimed to detect intrinsic and acquired polymyxin resistant Gram negative rods. Its usefulness applies for both human and veterinary medicine. In human medicine, it will contribute to an early identification of carriers of polymyxin-resistant strains, therefore preventing and containing outbreaks due to polymyxin-resistant isolates.

Author Disclosure Block:

P. Nordmann: None. **A. Jayol:** None. **L. Poirel:** None.

Poster Board Number:

MONDAY-038

Publishing Title:

Activity of Tedizolid Tested Against Co_2-Dependent Small-Colony Variants of *Staphylococcus aureus*

Author Block:

E. Cercenado[1], A. Vindel[2], M. Marín[1], F. Román[2], E. Bouza[1]; [1]Hosp. Gen. Univ.rio Gregorio Marañón, Madrid, Spain, [2]Inst. de Salud Carlos III, Majadahonda, Spain

Abstract Body:

Background: Small-colony variants (SCVs) of *S. aureus* constitute a slow-growing subpopulation with a distinctive phenotype, usually recovered from chronic and relapsing infections. Most SCVs are auxotrophs for menadione, hemin, or thymidine, and rarely for CO_2. Tedizolid (TZD) is a novel oxazolidinone with broad-spectrum activity against Gram-positive organisms. We evaluate the in vitro activity of TZD tested against CO_2-dependent SCVs isolates of *S. aureus*. **Methods:** SCVs were identified phenotypically. Auxothrophy for hemin, menadione, and thymidine was tested with impregnated disks, and CO_2-dependency by incubation in 5% CO_2. The presence of the *mecA* gene was determined by PCR. Molecular characterization was achieved by PFGE, SCC*mec* and *spa* typing. TZD susceptibility testing was performed by the epsilon-test (Liofilchem, Italy) in Mueller-Hinton agar plates (Biomérieux, France). Readings were performed after incubation for 24 h at 37ºC in 5% CO_2. CLSI/EUCAST breakpoints were applied for TZD (susceptible ≤0.5 mg/L). *S. aureus* ATCC 29213 and *E. faecalis* ATCC 29212 were used as controls.**Results:** From January 2013 to August 2015, we recovered 54 SCVs isolates of *S. aureus*, all CO_2-dependent, from 54 patients. Among these, 35 were methicillin-resistant (MR) and 19 methicillin-susceptible (MS). The source of the isolates was respiratory tract (n=25), wounds/abscesses (n=21), blood (n=6), others (n=2). Among MRSA, all isolates carried SCC*mec* type IVc, PFGE showed 7 different profiles, and 29 isolates (82.8%) corresponded to the *spa* type t686. All MSSA showed different PFGE profiles and 8 isolates (42%) corresponded to the *spa* type t686. TZD inhibited 100% of isolates at an MIC of ≤0.75 mg/L. The TZD MIC50, MIC90, and range (mg/L) for all isolates tested were 0.38, 0.5, and 0.25-0.75 mg/L, respectively. The activity of TZD (MIC, mg/L) against MRSA and MSSA is shown in the table.**Conclusion:** This study shows potent in vitro activity of TZD against recent CO_2-dependent SCVs isolates of MRSA and MSSA.

***S. aureus* (No. isolates)**	**TZD MIC50**	**TZD MIC90**	**Range**
MRSA (n=35)	0.5	0.5	0.25-0.75
MSSA (n=19)	0.38	0.5	0.25-0.75

Author Disclosure Block:

E. Cercenado: None. **A. Vindel:** None. **M. Marín:** None. **F. Román:** None. **E. Bouza:** None.

Poster Board Number:

MONDAY-039

Publishing Title:

Clinical Isolates of *Helicobacter pylori* Demonstrate a High Rate of Combined Resistance to Clarithromycin and Elevated Metronidazole Mics

Author Block:

D. Chen, N. Cole, P. Kohner, R. Patel; Mayo Clinic, Rochester, MN

Abstract Body:

Background: Recommended treatment regimens for *H. pylori* are commonly based on clarithromycin or metronidazole. Treatment failures are often a result of antimicrobial resistance. Our clinical laboratory performs *H. pylori* antimicrobial susceptibility testing. The purpose of this study was to define current patterns of *H. pylori* antimicrobial resistance.**Methods:** Antimicrobial susceptibility testing was performed using agar dilution, as recommended by the Clinical and Laboratory Standards Institute (CLSI), on *H. pylori* isolated from routine clinical specimens and submitted for susceptibility testing from November 2011 to October 2015 at Mayo Clinic, Rochester, MN. Minimal inhibitory concentrations (MICs) were determined for clarithromycin, amoxicillin, metronidazole, tetracycline, and/or ciprofloxacin (ordered as a panel or individually). Clarithromycin results were interpreted based on CLSI breakpoints; the other four drugs do not have established interpretive criteria.**Results:** A total of 1,970 MICs were reported over the 4-year study period (clarithromycin n=412, amoxicillin n=410, metronidazole n=330, tetracycline n=409, and ciprofloxacin n=409). Clarithromycin was interpreted as resistant in 70% of isolates (n=290), intermediate in 2% (n=8), and susceptible in 28% (n=114). Metronidazole had a wide distribution of MICs (in μg/ml): ≤8 (n=58), 16 (n=24), 32 (n=66), 64 (n=116), 128 (n=63), 256 (n=2), >256 (n=1). There were 330 instances where both clarithromycin and metronidazole were tested on the same isolate. Of these, 61% (n=200) were both resistant to clarithromycin and had metronidazole MICs >8 μg/ml. The most frequent MICs for the other antibiotics were: amoxicillin ≤2 μg/ml (99%, n=405), tetracycline 1 μg/ml (60%, n=246), and ciprofloxacin >2 μg/ml (52%, n=211).**Conclusions:** A majority of clinical isolates submitted for *H. pylori* susceptibility testing showed elevated clarithromycin and metronidazole MICs; both agents are frequently used in *H. pylori* therapy. Furthermore, a substantial number of isolates showed high ciprofloxacin MICs.

Author Disclosure Block:

D. Chen: None. **N. Cole:** None. **P. Kohner:** None. **R. Patel:** E. Grant Investigator; Self; nanoMR, BioFire, Check-Points, Curetis, 3M, Merck, Actavis, Hutchison Biofilm Medical Solutions, Accelerate Diagnostics, Allergan, The Medicines Company. N. Other; Self; Dr. Patel has a patent Bordetella pertussis/parapertussis PCR with royalties paid to TIB, a patent Device/method for sonication with royalties paid to Samsung, and a patent anti-biofilm

substance issu, non-financial support from bioMerieux, Bruker, Abbott, Nanosphere, Siemens, BD, other from Curetis.

Poster Board Number:

MONDAY-040

Publishing Title:

Antimicrobial Susceptibility Profile of *Granulicatella* and *Abiotrophia* Species

Author Block:

A. Mushtaq, N. Cole, K. Greenwood-Quaintance, P. Kohner, S. Ihde, G. Strand, A. Virk, R. Patel; Mayo Clinic, Rochester, MN

Abstract Body:

Background: American Heart Association guidelines for treatment of *Abiotrophia* and *Granulicatella* endocarditis are based on penicillin susceptibility. For penicillin-susceptible strains, monotherapy with either penicillin or ceftriaxone is recommended. Ceftriaxone has the advantage of once-daily dosing and hence, is often selected. We have recently observed penicillin-susceptible, but ceftriaxone-non-susceptible isolates. The objective of our study was to 1) determine MICs of a collection of *Abiotrophia* and *Granulicatella* species against a panel of antibiotics and, 2) describe the proportion of ceftriaxone non-susceptible strains in the penicillin susceptible sub-group. **Methods:** Fifteen isolates of *Abiotrophia defectiva* and 72 isolates of *Granulicatella adiacens* from the clinical microbiology laboratory of Mayo Clinic, Rochester, MN, recovered from January 1980 to November 2015 were studied. Isolates were identified to the species level using MALDI-TOF MS or 16S rRNA gene sequencing. MIC values were determined by broth microdilution using the Sensititre® commercial system (Trek Diagnostics Systems®, Oakwood Village, OH) per the manufacturer's guidelines. When available, CLSI breakpoints were used to interpret results.**Results:** The percentages of isolates susceptible to penicillin and ceftriaxone were 38% (33/87) and 31% (27/87), respectively. For *A. defectiva*, all 4 penicillin-susceptible isolates were also ceftriaxone susceptible. For *G. adiacens* penicillin-susceptible isolates, only eight were ceftriaxone susceptible, while 21 were ceftriaxone non-susceptible. High susceptibility rates were found for vancomycin (100%, 83/83), levofloxacin (99%, 76/77), and meropenem (85%, 61/72). Low susceptibility rates were found for erythromycin (49%, 36/73), cefotaxime (22%, 10/46) and cefepime (12%, 9/74). A subgroup of 46 isolates was also tested against linezolid (MIC_{50} and MIC_{90}, 2 μg/mL), daptomycin (MIC_{50} and MIC_{90}, >2 μg/mL), and tigecycline (MIC_{50} and MIC_{90}, $\leq$0.015 μg/mL).**Conclusions:** *Abiotrophia* and *Granulicatella* species have low susceptibility rates to penicillin and ceftriaxone, and 72% (21/29) of penicillin-susceptible *G. adiacens* isolates test ceftriaxone non-susceptible.

Author Disclosure Block:

A. Mushtaq: None. **N. Cole:** None. **K. Greenwood-Quaintance:** None. **P. Kohner:** None. **S. Ihde:** None. **G. Strand:** None. **A. Virk:** None. **R. Patel:** None.

Poster Board Number:

MONDAY-041

Publishing Title:

Antimicrobial Susceptibility of Clinical *Ureaplasma* Isolates

Author Block:

J. Fernandez-Dominguez, X. Wang, M. Karau, K. Greenwood-Quaintance, S. Cunningham, R. Patel; Mayo Clinic, Rochester, MN

Abstract Body:

Background: *Ureaplasma urealyticum* and *Ureaplasma parvum* are involved in urogenital tract infection and associated with adverse pregnancy outcomes and bacteremia alongside complications such as bronchopulmonary dysplasia and meningitis in newborns. Recently, *Ureaplasma* species have been associated with fatal hyperammonemia among lung transplant patients. There are limited reports on the antimicrobial susceptibility of *Ureaplasma* species, especially separated by species. The aim of this study was to determine the susceptibility of contemporary isolates of this genus recovered at Mayo Clinic (including from Mayo Medical Laboratories patients). **Methods:** Over a three month period (October-December 2015), all patient specimens of any origin positive by PCR at Mayo Clinic for *U. parvum* or *U. urealyticum* were cultured in SP4 media with urea. A total of 131 recovered isolates (113 *U. parvum* and 18 *U. urealyticum*) were studied. MICs to doxycycline, azithromycin, ciprofloxacin, tetracycline, erythromycin and levofloxacin were determined by broth microdilution and the last three interpreted according to CLSI guidelines. The *tetM* gene for tetracycline resistance was assessed by PCR. **Results:** All isolates were susceptible to tetracycline and had equal or lower doxycycline MICs; *tetM* was not detected in any isolate. All isolates had azithromycin MICs ≤8µg/mL; however, two *U. parvum* isolates (1.7%) were resistant to erythromycin (MIC >16µg/mL). Resistance to levofloxacin was observed in 3 *U. parvum* isolates (2.6%) and 1 *U. urealyticum* isolate (5.5%). Furthermore, 6 (5.3%) and 3 (16.6%) isolates of *U. parvum* and *U. urealyticum*, respectively, had ciprofloxacin MICs ≥8µg/mL. All isolates had MICs at least one dilution lower for levofloxacin compared to ciprofloxacin. **Conclusion:** We report the antimicrobial susceptibility of contemporary *U. parvum* and *U. urealyticum* isolates from the United States. The resistance rate was low in comparison with previous studies from elsewhere in the world and simultaneous resistance to more than one antimicrobial class was not observed in the same isolate.

Author Disclosure Block:

J. Fernandez-Dominguez: None. **X. Wang:** None. **M. Karau:** None. **K. Greenwood-Quaintance:** None. **S. Cunningham:** None. **R. Patel:** None.

Poster Board Number:

MONDAY-042

Publishing Title:

***Aerococcus urinae* Antimicrobial Susceptibility Testing Methods Comparative Analysis**

Author Block:

S. Grandjean Lapierre[1], F. Brisebois[1], D. Creely[2], D. Pincus[2], S. Dufresne[3], J-M. Leduc[1]; [1]Hosp. Sacré-Coeur de Montréal, Montreal, QC, Canada, [2]BioMérieux, Hazelwood, MO, [3]Hosp. Maisonneuve Rosemont, Montreal, QC, Canada

Abstract Body:

Background: *Aerococcus urinae* is an increasingly recognized urinary pathogen. The objective of this study was to compare Kirby-Bauer (KB) disk diffusion, broth microdilution (BMD) and E-Test™ methods for *A. urinae* antimicrobial susceptibility testing (AST). **Methods:** This study included 47 *A. urinae* isolates from urinary tract clinical samples. Identification was confirmed by ribosomal 16S RNA gene sequencing. Thirteen antibiotics including those recommended by newly introduced Clinical Laboratory Standard Institute (CLSI) M-45 guidelines were tested. KB disk diffusion, BMD and E-Test™ were respectively performed according to CLSI M02-A12, CLSI M-45 and manufacturer's instructions. BMD and E-Test™ interpretation was based on CLSI M-45 susceptibility breakpoints.**Results:** Applying CLSI breakpoints to E-Test™ results, all isolates were susceptible to cefotaxime, meropenem, vancomycin and linezolid. Thirty-six isolates were resistant to trimethoprim-Sulfamethoxazole (TMP-SMX), 2 to ciprofloxacin, 2 to tetracycline and 2 to levofloxacin. KB disk diffusion and E-Test™ MICs correlation was analyzed as E-Test™ MICs and susceptibility categories were stratified for every KB inhibition zones. Isolates with non-susceptible MICs by E-Test™ presented the following KB inhibition zone intervals; penicillin 30-36 mm, ceftriaxone 30 mm, tetracycline 0-12 mm, ciprofloxacin 0-28 mm, levofloxacin 0-15 mm and TMP-SMX 0-20 mm.Concordance between E-Test™ and BMD could only be assessed for a subgroup of 8 isolates. All isolates were uniformly susceptible to penicillin, cefotaxime, ceftriaxone, meropenem and vancomycin. Both methods reported the same unique isolate as resistant to tetracycline. For levofloxacin, TMP-SMX and linezolid respectively 2, 1 and 1 isolates were reported as susceptible by E-Test™ and resistant by BMD. Also, for TMP-SMX, 4 isolates were reported as resistant by E-Test™ and susceptible by BMD.**Conclusions:** It is possible to perform E-test™ for *A. urinae* using a blood-containing medium. However, when considering CLSI M-45 guidelines' BMD as a gold standard, applying the same breakpoints to E-Test™ AST on MHB can lead to major and very major errors regarding TMP-SMX, levofloxacin and linezolid.

Author Disclosure Block:

S. Grandjean Lapierre: None. **F. Brisebois:** None. **D. Creely:** D. Employee; Self; Biomérieux R&D Microbiology. **D. Pincus:** D. Employee; Self; Biomérieux R&D Microbiology. **S. Dufresne:** None. **J. Leduc:** None.

Poster Board Number:

MONDAY-043

Publishing Title:

Instantaneous Antimicrobial Susceptibility Testing (AST) Using Piezoelectric Plate Sensors

Author Block:

X. Xu[1], C. L. Emery[2], B. Sen[1], K. Krevolin[2], S. G. Joshi[1], W-H. Shih[1], W. Y. Shih[1]; [1]Drexel Univ., Philadelphia, PA, [2]Drexel Univ. - Hahnemann Univ. Hosp., Philadelphia, PA

Abstract Body:

Background: MALDI-TOF mass spectrometry has revolutionized the clinical microbiology laboratory by generating definitive bacterial and yeast culture isolate identifications within minutes, however FDA-approved automated phenotypic AST methods still require at least 8 additional hours to generate AST results. We developed a novel patent-pending rapid phenotypic AST method using low cost piezoelectric plate sensors (PEPS) that generates virtually instantaneous AST results.**Methods:** Clinical Laboratory Standards Institute (CLSI)-recommended quality control (QC) strains used for AST (*S. aureus* ATCC 29213, *E. coli* 25922, *E. coli* 35218) and *S. aureus* ATCC BAA-1026 (MRSA) were tested against various antibiotics using PEPS rapid AST. Strain suspensions were standardized to a 0.5 McFarland prior to testing. Minimum inhibitory concentration (MIC) results obtained for each strain/antibiotic combination tested using PEPS rapid AST were compared to the modal MIC result (or breakpoint MIC value range) obtained using the VITEK$^{@}$2 (each strain tested in quintuplicate) and the modal MIC result obtained using the E-test method (each strain tested in triplicate). Broth microdilution testing was used to confirm discrepant results.**Results:** For all three CLSI-recommended QC strains, PEPS rapid AST MIC results correlated well (100% essential agreement) with VITEK modal MICs (or breakpoint MIC ranges), and with E-test MICs (83% essential agreement); 100% interpretive categorical agreement was achieved among PEPS rapid AST results and VITEK and E-test results, and all PEPS rapid AST results fell within published CLSI QC strain MIC ranges. For *S. aureus* ATCC BAA-1026 (MRSA), PEPS rapid AST MIC results correlated well (100% essential agreement) with VITEK modal MICs (or breakpoint MIC ranges), and with E-test MICs (100% essential agreement); 100% interpretive categorical agreement was achieved among all three methods.**Conclusions:** PEPS rapid AST is a novel method that yields virtually instantaneous antibiotic MIC results, and its results correlated well with commonly-used AST methods based on our limited preliminary study. Additional testing of different microbes and antimicrobial combinations is warranted to further assess PEPS rapid AST performance characteristics.

Author Disclosure Block:

X. Xu: None. **C.L. Emery:** None. **B. Sen:** None. **K. Krevolin:** None. **S.G. Joshi:** None. **W. Shih:** None. **W.Y. Shih:** None.

Poster Board Number:

MONDAY-044

Publishing Title:

Comparison of *Clostridium difficile* Antimicrobial Susceptibility Testing by Agar Dilution, Broth Microdilution, and Etest Methods

Author Block:

G. Igawa[1], M. Casey[1], E. Sawabe[2], Y. Nukui[2], S. Okugawa[3], K. Moriya[3], S. Tohda[2], R. Saito[1]; [1]Tokyo Med. and Dental Univ., Tokyo, Japan, [2]Med. Hosp., Tokyo Med. and Dental Univ., Tokyo, Japan, [3]The Univ. of Tokyo Hosp., Tokyo, Japan

Abstract Body:

Background: The Clinical Laboratory Standard Institute (CLSI) recommends agar dilution method (AD) for antimicrobial susceptibility testing of *Clostridium difficile*. However, because automation is difficult for the AD and most *C. difficile* clinical isolates are generally susceptible to metronidazole and vancomycin, antimicrobial susceptibility testing is routinely omitted. This study evaluates the performance of broth microdilution method (BMD) and Etest methods in testing the antimicrobial susceptibility of *C. difficile* to metronidazole and vancomycin, by comparing it with the performance of the CLSI recommended AD. **Methods:** Sixty-four non-duplicate *C. difficile* clinical isolates from the Medical Hospital of Tokyo Medical and Dental University were used in this study. The AD was performed according to the CLSI document M100-S24. The BMD was performed with Brucella broth supplemented with vitamin K_1 (1 μg/mL), haemin (5 μg/mL), and 5% laked sheep blood. Etest was conducted according to the manufacturer's instructions with some modifications.**Results:** All three methods showed that all 64 isolates were susceptible to metronidazole and vancomycin. Based on the quality control ranges for *C. difficile* ATCC 700057 according to CLSI, the MICs of metronidazole and vancomycin showed 100% agreement between the AD and BMD. On the other hand, more than 95% agreement in MICs for metronidazole and vancomycin was noted between the AD and Etest, although 4.7% (3/64) had MICs out of the quality control range for metronidazole.**Conclusions:** Our results show that the BMD and Etest are acceptable for routine antimicrobial susceptibility testing of *C. difficile*, as their performances were comparable with that of the AD. In addition, we noted that metronidazole-or vancomycin-resistant isolates are extremely rare in Japan.

Author Disclosure Block:

G. Igawa: None. **M. Casey:** None. **E. Sawabe:** None. **Y. Nukui:** None. **S. Okugawa:** None. **K. Moriya:** None. **S. Tohda:** None. **R. Saito:** None.

Poster Board Number:

MONDAY-045

Publishing Title:

Kirby-Bauer-Based Automated Antibiotic Susceptibility Panel Testing for Antibiotic Resistance Determination

Author Block:

C. C. Otto, S. E. Kaplan, T. He, K. A. Gilhuley, E. Babady, S. K. Seo, Y-W. Tang; Mem. Sloan Kettering Cancer Ctr., New York, NY

Abstract Body:

Background: Rapid and accurate phenotypic antimicrobial susceptibility testing (AST) has been shown to improve patient care by decreasing length of stay and mortality. The i2a SIRscan 2000 Automatic is a system that performs automated Kirby-Bauer (KB)-based AST on a panel of user-chosen antibiotics. The use of this system has several benefits over broth microdilution including customizable antibiotic panels and the SIRscan allows for enhanced laboratory automation when used in combination with the i2a plate streaker. Here, we validate the performance of the SIRscan as compared to conventional Microscan AST.**Methods:** A total of 309 consecutive clinical isolates, including Gram-negative rods (GNR) and staphylococci, were collected and tested with Microscan as part of the routine clinical practice. The samples were also tested in parallel with the SIRscan. Results from the two platforms were interpreted as concordant or minor (sensitive-intermediate, intermediate-resistant), major (sensitive, reported as resistant), or very major errors (resistant, reported as sensitive) based on Clinical Laboratory Standards Institute criteria.**Results:** A total of 309 consecutive clinical isolates, including Gram-negative rods (GNR) and staphylococci, were collected and tested with Microscan as part of the routine clinical practice. The samples were also tested in parallel with the SIRscan. Results from the two platforms were interpreted as concordant or minor (sensitive-intermediate, intermediate-resistant), major (sensitive, reported as resistant), or very major errors (resistant, reported as sensitive) based on 2015 Clinical Laboratory Standards Institute (CLSI) interpretive criteria.**Conclusions:** The SIRscan is a platform for automated KB-based AST to test a panel of antibiotics in clinical laboratories. Overall, the SIRscan performed equivalently to Microscan for the majority of the drugs tested. The antibiotics tested on the KB panel are customizable, providing the potential for personalized AST.

Author Disclosure Block:

C.C. Otto: None. **S.E. Kaplan:** None. **T. He:** None. **K.A. Gilhuley:** None. **E. Babady:** None. **S.K. Seo:** None. **Y. Tang:** None.

Poster Board Number:

MONDAY-046

Publishing Title:

Expression of Type 1 Fimbriae in Broad-Host-Range *Salmonella* and Host-Adapted *Salmonella* Serovars

Author Block:

H-C. Lin, C-H. Chang, **K-S. Yeh**; Natl. Taiwan Univ., Taipei, Taiwan

Abstract Body:

Fimbriae are hair-like structures present on the outer membrane of bacteria and are implicated in adherence to the host epithelial cells. Type 1 fimbriae with the binding specificity to mannose residue is the most commonly found fimbrial type in *Salmonella*, a food borne pathogen with public health concern. While previous reports indicated that 80% of *Salmonella* isolates possessed type 1 fimbriae, information regarding the correlation between the specific serovars and the capacity to produce such fimbriae remained limited. Here we would like to test the hypothesis that most of the broad-host-range *Salmonella* serovars like Typhimurium or Enteritidis would possess the capacity to produce type 1 fimbriae, which confer *Salmonella* to trigger intestinal inflammatory response, resulting in shedding *Salmonella* from feces to contaminate the environment. On the contrary, most of the host-adapted serovars such as Choleraesuis may be devoid of this fimbrial antigen and thus cause less of an inflammatory response. The non-fimbriated *Salmonella* may evade the host immune attack and easily enter bloodstream by lowering inflammatory response. In the present study, 187 *Salmonella* strains comprising 68 serovars were screened by yeast agglutination test to detect the expression of type 1 fimbriae in vitro. Our findings revealed that Choleraesuis, Pullorum, Paratyphi A and B, Cerro, Dressau, and Arizonae were the only serovars that were fimbriae-negative (Fim^-), while other serovars including Typhimurium, Enteritidis, Derby, and Albany, just to name a few, produced type 1 fimbriae (Fim^+) when incubated either in static broth, solid agar or both culture conditions. Fimbrial structures from representative strains of Fim^+ and Fim^- groups were also examined by transmission electron microscopy using negative stain. Current results are in line with our argument that a higher percentage of broad-host-range *Salmonella* produce type 1 fimbriae, whereas host-adapted serovars like Choleraesuis and Pullorum would inhibit the expression of this fimbriae. Gain or loss of gene function and/or expression in type 1 fimbriae in different *Salmonella* serovars may be a selective advantage for bacteria to sustain during their spread and growth in diverse host milieu.

Author Disclosure Block:

H. Lin: None. **C. Chang:** None. **K. Yeh:** None.

Poster Board Number:

MONDAY-047

Publishing Title:

Emergence of Ceftriaxone and Ciprofloxacin Non-Susceptible Isolates of Non-Typhoidal *Salmonella enterica* from Nebraska

Author Block:

C. N. Murphy[1], R. C. Fowler[1], A. Williams[2], P. C. Iwen[2], P. D. Fey[1]; [1]U of Neb Med Ctr., Omaha, NE, [2]Neb Publ. Hlth.Lab, Omaha, NE

Abstract Body:

Background: Non-typhoidal *Salmonella enterica* enteritis typically does not require antimicrobial treatment except in systemic infections where fluroquinolones (e.g. ciprofloxacin [CIP]) and expanded-spectrum cephalosporins (e.g. ceftriaxone [CRO]) are recommended as treatment options for adult and pediatric patients, respectively. The purpose of this study was to determine the prevalence and genetic mechanisms associated with CRO and CIP resistance among *Salmonella* isolates collected in Nebraska. **Methods:** Antibiotic resistance rates among human isolates of S*almonella* were monitored as part of an ongoing surveillance program. Isolates were subjected to serotyping and pulsed-field gel electrophoresis. The ARM-D® for ß-Lactamase ID (Streck, Inc.) was used to screen for β-lactamase genes. CIP resistance determinants such as *gyrA*, *gyrB, parC*, *parE*, *qnrS, qnrD, oqxA*, and *oqxB* were screened for by PCR and sequencing. **Results:** From 2010-2014 >1,500 *Salmonella* isolates were submitted for susceptibility testing. Only one of these isolates was non-susceptible to both CRO and CIP. However, in 2015, eight isolates that were non-susceptible to CRO and CIP were isolated. These isolates are members of three different serotypes, Typhimurium (3 isolates), Newport (1 isolate), and 4,[5],12:i:- (4 isolates), and have four unique PFGE patterns. Characterization of these isolates demonstrated that resistance to CRO was due to the presence of either $bla_{\text{CTX-M-15}}$ in 4,[5],12,:i:- isolates or $bla_{\text{CMY-2}}$ in the Newport and Typhimurium isolates. In all but the Newport isolate, no mutations in the quinolone resistance determining region or only a single point mutation in *gyrA* was observed. Screening for quinolone resistance genes demonstrated that the 4,[5],12,:i:- isolates contained *qnrS,* a topoisomerase protection gene, and the Typhimurium isolates contained *oqxAB,* quinolone efflux pump genes. **Conclusion:** These data add to a growing body of literature demonstrating a diversity of resistance mechanisms underlying both fluoroquinolone and expanded-spectrum cephalosporin resistance in the United States. In addition, our study demonstrates that these resistance genes are isolated from a variety *Salmonella* serotypes and backgrounds suggesting rapid horizontal gene transfer within the population.

Author Disclosure Block:

C.N. Murphy: None. **R.C. Fowler:** None. **A. Williams:** None. **P.C. Iwen:** None. **P.D. Fey:** None.

Poster Board Number:

MONDAY-048

Publishing Title:

***Campylobacter* Spp., *Brachyspira* Spp. and *Salmonella* Spp. In Western Jackdaw (*Corvus monedula*) Populations In Finland**

Author Block:

T. T. Nieminen[1], S. M. Kovanen[1], M. Pohja-Mykrä[1], M. Raunio-Saarnisto[2], S. Olkkola[1], R. Kivistö[1], M-L. Hänninen[1]; [1]Univ. of Helsinki, Helsinki, Finland, [2]Finnish Food Safety Authority Evira, Helsinki, Finland

Abstract Body:

Background: During this millennium, western jackdaws have become common in some urban and agricultural surroundings in Finland, which has increased the interest of their potential role as reservoir of certain human and production animal pathogens.**Methods:** We sampled fecal droppings of a total of 212 jackdaws and cultured the droppings for *Campylobacter* spp., *Brachyspira* spp. and *Salmonella* spp., all of which are bacterial pathogens associated with wild birds. Samples were collected in 2014-2015 within a period of five months (September-February) from two rural cities in Finland and represented a total of eight flocks.**Results:** *Salmonella enterica* serovars Typhimurium and Kentucky were isolated from a single flock from three out of seven pooled samples each consisting of fecal material of five to six birds. Altogether 43 pooled samples were collected from the eight flocs. *Brachyspira* spp. was isolated from 112/212 fecal samples (53 %) originating from all eight flocks. Ninety-seven of these isolates were identified by partial 16S rRNA gene sequencing. All of the sequences were nearly identical to that of a tentatively described species "*Brachyspira corvi*" and different from the known pathogenic *Brachyspira* species. *Campylobacter* spp. was isolated from 107/212 fecal samples (50 %) originating from all eight flocs. Multi locus sequence types (MLST) were obtained from 91 *C. jejuni* strains. These were assigned to 63 sequence types of which 46 (74%) were novel to the *Campylobacter* PubMLST database. ST-1339, ST-6442, ST-6460 and ST-6589 have been earlier isolated from human patients in Sweden or The Netherlands. Clonalframe analysis showed that most jackdaw isolates had no common microevolution with other *C. jejuni* STs suggesting that these strains have mainly evolved in jackdaws.**Conclusions:** We conclude that the studied populations of jackdaws could be a source for transmission of salmonella to domestic animals or humans. Most *C. jejuni* ST types detected from jackdaws seem to have evolved with the bird species. Similarly, "*Brachyspira corvi"* is a host-associated sp. that has not been associated with pathogenicity.

Author Disclosure Block:

T.T. Nieminen: None. **S.M. Kovanen:** None. **M. Pohja-Mykrä:** None. **M. Raunio-Saarnisto:** None. **S. Olkkola:** None. **R. Kivistö:** None. **M. Hänninen:** None.

Poster Board Number:

MONDAY-049

Publishing Title:

Enteropathogenic *E. coli* Multidrug Resistance (Mdr) in a Human Diarrhea and Swine Colonization Interface Model

Author Block:

C. Alpuche[1], E. Tamayo[1], E. Moreno[1], D. Arellano[1], J. Silva[1], F. Tellez[1], F. Mariscal[2], H. Lopez-Gatell[1], V. Richardson[3], F. Hernández[3], R. Medina[3], LESP Group; [1]Natl. Inst. of Publ. Hlth., Cuernavaca, Mexico, [2]SAGARPA, Cuernavaca, Mexico, [3]Human Hlth.Services, Cuernavaca, Mexico

Abstract Body:

Selection of MDR in bacteria, including *E. coli*, is a major pubic health problem and it has been associated to antimicrobial use in the human-animal interface. We determined the frequency and profile of MDR and pathotype markers in *E. coli* isolates from swines colonization and human diarrhea cases in a close surveillance model. Stool samples of every swine in a semitechnified farm in the state of Morelos Mexico, were obtained (April 2015). A focalized, clustered surveillance system of diarrheal disease was developed in paralleled from June-December 2015 in the community surrounding the farm. Conventional microbiology, API 20E and Kirby Bauer test (CLSI) were used for bacteria and antimicrobial susceptibility detection. Three colonies suggestive of *E.coli* were collected per sample for further characterization. Enteropathogenic *E. coli* was detected by multiplex PCR. Stool cultures of 280 pigs and 186 human diarrhea cases, were obtained; 821 and 510 *E. coli* were isolated from pigs and humans respectively. MDR was identified in 286/821 (34.8%) isolates in the swine group and 11.2% of these were enteropathogenic (23 EPEC, 1 EPEC/EHEC, 1 ETEC and 7 EHEC); 19/280 pigs were colonized with enteropathoghenic MDR *E. coli*. The main MDR profile detected in pigs, were resistance to AMP-SXT-NAL-CIP (75%). In the human isolates, 149/510 (29.2%) were MDR and 27.5% were enteropathogenic (13 EPEC, 13 DAEC, 7 EAEC, 5 ETEC, 2 EHEC and 1 EIEC). Two main profiles of MDR enteropathogenic *E.coli* were detected in the human isolates: AMP-SXT-NAL (78%), and AMP-SXT-NAL-CIP-CAZ (14.6%). MDR enteropathogenic *E. coli* was associated to 28% of diarrhea cases with a stool culture positive for enteric bacteria. These results support that swine are a potential reservoir for MDR enteropathogenic *E. coli*. Antimicrobial resistance such as AMP and SXT are broadly selected either in the *E. coli* colonizing swine or those causing human diarrhea; CIP resistance was higher in the swine isolates but CAZ resistance seems to be selected only in the human isolates. Further genetic characterization will allow to define the spread of same clonal strains between human and swine populations in this study.

Author Disclosure Block:

C. Alpuche: None. **E. Tamayo:** None. **E. Moreno:** None. **D. Arellano:** None. **J. Silva:** None. **F. Tellez:** None. **F. Mariscal:** None. **H. Lopez-Gatell:** None. **V. Richardson:** None. **F. Hernández:** None. **R. Medina:** None.

Poster Board Number:

MONDAY-050

Publishing Title:

Up-Regulation of Immuno-Regulatory Genes by Bcg Vaccine

Author Block:

N. T. Iqbal, K. Ahmed, J. Samad; Aga Khan Univ., Karachi, Pakistan

Abstract Body:

Background: Pakistan has more than 90% coverage of BCG vaccine through Extended Program on Immunization (EPI). As per schedule, BCG is administered in neonates during first month of life. BCG does not confer protection against transmissible form of tuberculosis. However, BCG provides additional benefits to young infants in the form of immune surveillance. This nonspecific immune surveillance has been evidenced in bladder and melanoma cancers where BCG vaccine is used as immunotherapeutics. The immune surveillance mechanism of BCG is thought to be mediated by cell wall components that activate TLRs present on antigen presenting cells, which activates inflammatory cascade and provide maturation signals for immune cells. Among several immunomodulatory genes, BCG up-regulates co-stimulatory genes belong to Butyrophilins (BTN) family which are structurally similar to B7 molecule. The aim of this study was to understand the mechanism of immune surveillance of BCG by activation of innate and adaptive genes in BCG vaccinated subjects. We studied Butyrophilin (BTN3A2), Granzyme and TNFa gene in BCG vaccinated healthy controls (n=9). **Methods:** We stimulated PBMCs with BCG vaccine (MOI 1) at 24 hrs for expression of BTN gene (BTN3A2), TNFα and TLR4 genes using q-RTPCR (SYBR green, Biorad labs, Hercules CA, USA) from healthy donors. Two step q-RT PCR was performed after RNA extraction through Trziole using CDNA and q-RT PCR protocol as per manufacture instructions. **Results:** We found a 3-7 fold higher induction in BTN3A2 and TNFα genes using BCG compared to un-stimulated media control. The fold changes were calculated after normalization of target genes with housekeeping gene. **Conclusions:** Our results show an increase expression of BTN3A2 (2 fold), and TNFα (2-7 fold) gene in healthy controls. The differential expression of BTN3A2 gene will be further confirmed in BCG vaccinated and non-vaccinated healthy donors. **Funding resource:** AKU Seed money project **ERC**: *2885-BBS-ERC-14*1

Author Disclosure Block:

N.T. Iqbal: None. **K. Ahmed:** None. **J. Samad:** None.

Poster Board Number:

MONDAY-051

Publishing Title:

Lipid and Protein Profiles in Tuberculous Patients on Anti-Tuberculosis Therapy

Author Block:

C. N. N. Nwadike; Imo State Univ. Owerri, Imo State Nigeria, Owerri, Nigeria

Abstract Body:

AbstractThe present study assessed the serum lipid and protein profiles of Pulmonary Tuberculosis (TB) patients on Anti-Tuberculosis therapy in Owerri, Imo State ,Nigeria.The subjects were divided into four groups, comprising of 105 apparently healthy adults, 50 newly diagnosed TB patients, 50, three months post treatment TB patients and 50 six months post treatment TB patients, respectively. Serum fasting lipids and protein profiles of these subjects were analyzed, using standard methods.Serum levels of total cholesterol (TC), Triglyceride (TG), HDL and albumin, were significantly reduced in newly diagnosed TB patients than in other group, ($P<0.05$), but showed significant increase after treatment, in exception of TG. Globulin and LDL increased significantly in newly diagnosed patients, as compared to the control and other groups.These findings suggest that hypocholesterolaemia and hypoalbulminaemia are of diagnostic importance in pulmonary tuberculosis and could also assist in assessing the progress of treatment.

Author Disclosure Block:

C.N.N. Nwadike: None.

Poster Board Number:

MONDAY-052

Publishing Title:

Factors Associated with Successful Screening for Latent Tuberculosis Infection with the Interferon Gamma Release Assay in an Urban Hiv Clinic

Author Block:

A. Andrews[1], J. Adams[2], S. Varghese[1], **C. Vinnard**[1]; [1]Drexel Univ. Coll. of Med., Philadelphia, PA, [2]Brown Univ., Providence, RI

Abstract Body:

Background: National guidelines recommend screening for latent tuberculosis infection (LTBI) in all HIV-infected patients, with either tuberculin skin testing (TST) or interferon gamma release assays (IGRAs) as acceptable testing methods. We sought to determine the rates of successful LTBI screening in an urban HIV clinic following the adoption of IGRA-based testing as the preferred screening method in 2012.**Methods:** We conducted a retrospective study of 178 new patients enrolled in the Partnership Comprehensive Care Practice, the largest HIV clinic in the Philadelphia region, between January 1, 2013 and December 31, 2013. We collected data regarding demographics, CD4+ T cell counts, viral load, and receipt of antiretroviral therapy (ART). We hypothesized that patients with lower CD4 T cell counts would be less likely to receive LTBI screening within one year, given the competing needs of the provider to address barriers to ART and then initiate therapy. We performed multivariate logistic regression to examine the association of CD4+ T cell counts with LTBI screening, adjusting for potential confounders.**Results:** Among 178 patients new to the clinic, 93 (53%) had no discussion of LTBI within the first year of follow-up. In an additional 17 (10%) patients, LTBI screening was discussed by the provider but not performed. Among the remaining 68 patients, 12 (7%) had documentation from another clinic demonstrating a negative test for LTBI, and 56 (31%) had an IGRA test performed during the first year in the clinic (all were negative). In an adjusted analysis, we found that patients with high CD4+ T cell counts (>1000 cells/uL) were least likely to have successful LTBI screening performed (aOR 0.12, 95% CI 0.02, 0.54), compared with patients with CD4+ T cell counts under 350 cells/uL.**Conclusion:** Less than half of patients new to our HIV clinic received LTBI screening within the first year of follow-up. Contrary to our hypothesis, patients in the highest tier of CD4+ T cell counts were least likely to have LTBI screening performed during the first year of follow-up. There were no positive IGRA tests out of 56 performed. Future work will compare LTBI screening rates before and after the conversion from TST to IGRA based testing.

Author Disclosure Block:

A. Andrews: None. **J. Adams:** None. **S. Varghese:** None. **C. Vinnard:** None.

Poster Board Number:

MONDAY-053

Publishing Title:

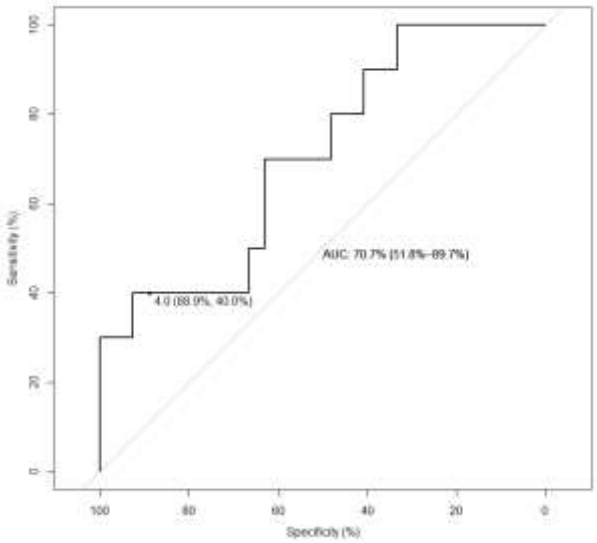
Urine Colorimetry to Detect Low Rifampin Exposure During Tuberculosis Therapy: A Proof-of-concept Study

Author Block:

I. Zentner[1], H. P. Schlecht[1], L. Khensouvann[2], N. Tamuhla[3], M. Kutzler[1], V. Ivaturi[4], J. Pasipanodya[5], T. Gumbo[5], C. A. Peloquin[6], G. P. Bisson[7], **C. Vinnard**[1]; [1]Drexel Univ. Coll. of Med., Philadelphia, PA, [2]Genomind, Inc, King of Prussia, PA, [3]Botswana-Upenn Partnership, Gaborone, Botswana, [4]Univ. of Maryland, Baltimore, Baltimore, MD, [5]Baylor Inst. for Immunology Res., Dallas, TX, [6]Univ. of Florida, Gainesville, FL, [7]Univ. of Pennsylvania, Philadelphia, PA

Abstract Body:

Background: We sought to determine whether urine colorimetry could have a novel application as a form of therapeutic drug monitoring during anti-TB therapy.**Methods:** Among healthy volunteers, we evaluated 3 dose sizes of rifampin (150 mg, 300 mg, and 600 mg), performed intensive pharmacokinetic sampling, and collected a timed urine void at 4 hours post-dosing. The absorbance peak at 475 nm was measured after rifamycin extraction. The optimal cutoff for the colorimetric urine assay was evaluated in a study of 39 HIV/TB patients undergoing TB treatment in Botswana, based on a receiver-operating-characteristic (ROC) curve.**Results:** In the derivation study, a urine colorimetric assay value of 4.0 x 10-2 Abs, using a timed void 4 hours after dosing, demonstrated a sensitivity of 92% and specificity of 60% to detect a peak rifampin concentration (Cmax) under 8 mg/L, with an area under the ROC curve of 0.92. In the validation study among HIV/TB patients in Botswana, this cutoff was specific (100%) but insensitive (28%). We observed similar test characteristics for a target Cmax target of 6.6 mg/L, and a target area under the drug concentration-versus-time curve (AUC0-8) target of 24.1 mg•hour/L (Figure).**Conclusions:** The urine colorimetric assay was specific but insensitive to detect low rifampin serum concentrations among HIV/TB patients.



Author Disclosure Block:

I. Zentner: None. **H.P. Schlecht:** None. **L. Khensouvann:** None. **N. Tamuhla:** None. **M. Kutzler:** None. **V. Ivaturi:** None. **J. Pasipanodya:** None. **T. Gumbo:** None. **C.A. Peloquin:** None. **G.P. Bisson:** None. **C. Vinnard:** None.

Poster Board Number:

MONDAY-054

Publishing Title:

Causes Of Death In Hiv-Infected Patients In The Va Loma Linda Healthcare System

Author Block:

M. McCormick[1], L. Strnad[2], W. Brown[3], J. Cho[1], H. San Agustin[4], G. Aung[4], M. Ing[4]; [1]Loma Linda Univ., Loma Linda, CA, [2]Univ. of California San Francisco, San Francisco, CA, [3]Univ. of Utah, Salt Lake City, UT, [4]Loma Linda VA Med. Ctr., Loma Linda, CA

Abstract Body:

Background: As antiretroviral therapy has improved in efficacy and accessibility, primary causes of death in the HIV population are shifting from AIDS-related complications towards chronic disease-related deaths[1]. The VA medical system treats a unique subset of patients with risk factors differing from the general population. We aimed to identify primary causes of death and note various epidemiological and clinical associations among a population of HIV-positive veterans at a single VA medical center.**Methods:** We conducted a retrospective chart review for 156 HIV-positive patients of the Loma Linda VA Medical Center who died between 2000-2014. The specific cause of death was aggressively pursued including obtaining death certificates when death summaries and inpatient notes were unavailable. Cause of death data were categorized using a standardized case report form from the Coding Causes of Death in HIV (CoDe) project[2]. Other analyzed data included demographics, lab studies, comorbidities, and substance use history.**Results:** AIDS-related deaths accounted for 21.8% of the 156 total deaths. Cardiovascular (19.9%), non-AIDS malignancy (16.0%), and accidental/violent deaths (14.7%) were notable contributors in the remaining 78.2%. AIDS-related deaths per year decreased steadily from 45.5% in 2000 to 0% in 2010-2013 with an increase to 22.2% in 2014. At the time of death, 48% of subjects were actively smoking, 32% had active drug or alcohol abuse, and 55% had documented psychiatric diagnoses. Further statistical analysis is pending.**Conclusions:** The percentage of AIDS-related deaths is within the range reported by prior HIV-mortality studies. The high percentage of accidental and violent deaths may be related to the prevalence of substance abuse and psychiatric disease among this population. Furthermore, a high smoking prevalence may contribute to the rates of malignancy and cardiovascular related deaths. An HIV-positive VA population may require increased access to psychiatric support and addiction treatment, and HIV providers may require additional training in these domains.

Author Disclosure Block:

M. McCormick: None. **L. Strnad:** None. **W. Brown:** None. **J. Cho:** None. **H. San Agustin:** None. **G. Aung:** None. **M. Ing:** None.

Poster Board Number:

MONDAY-055

Publishing Title:

Serological and Molecular Surveillance for Influenza A Virus in Dogs and Their Owners in Oyo State, Nigeria

Author Block:

D. O. Oluwayelu, O. B. Daodu, A. I. Adebiyi; Univ. of Ibadan, Ibadan, Nigeria

Abstract Body:

Background: The close interaction of dogs with humans coupled with their possession of specific receptors for human and avian influenza viruses provide opportunities for cross-species virus transmission, thus posing a threat to human health. In Nigeria, there is an upsurge in the acquisition of exotic and local dogs as pets accompanied by increased bonding between the dogs and their owners. Moreover, Nigerian game hunters traditionally enjoy a close association with their dogs. Therefore, the objective of this study was to detect the presence of antibodies against influenza A virus (IAV) in dogs, as well as the presence of RNA of IAV in nasal swabs of dogs and their owners, and thereby assess the possible transmission of the virus between humans and dogs.**Methods:** Sera from 239 apparently healthy pet and hunting dogs in Oyo state, Nigeria were screened for IAV antibodies using competition ELISA while haemagglutination inhibiting (HI) antibodies were detected using canine influenza virus (CIV) H3N8 antigen. Suspensions prepared from 239 and 39 nasal swabs from dogs and their owners, respectively were tested for presence of IAV RNA by RT-PCR.**Results:** The ELISA revealed that 1.7% (4/239) and 4.6% (11/239) of the dog sera were positive and doubtful for IAV antibodies, respectively while 1.9% and 1.2% seroprevalence was obtained for hunting and pet dogs, respectively. HI test to confirm presence of CIV H3N8 antibodies in the ELISA-positive and doubtful sera showed that a sample each was positive with HI titre of 1:128 and 1:64, respectively. All the nasal swabs assayed by RT-PCR were negative for IAV nucleic acid.**Conclusions:** This study provides serologic evidence of IAV infection in pet and hunting dogs which are major companion animals of humans. Although at a low prevalence, this finding suggests that dogs may serve as source of transmission of novel influenza viruses to humans in Nigeria. Continuous surveillance for IAV among dog populations in Nigeria is recommended to facilitate early detection of emergent IAV strains that could be potentially harmful for humans and or animals.

Author Disclosure Block:

D.O. Oluwayelu: None. **O.B. Daodu:** None. **A.I. Adebiyi:** None.

Poster Board Number:

MONDAY-056

Publishing Title:

Antibacterial Action of Copper Plus Blue Light

Author Block:

J. M. PISCIOTTA, N. Russo; West Chester Univ., West Chester, PA

Abstract Body:

Waterborne illnesses are a major cause of mortality and morbidity and novel, low cost water treatment options are needed. Copper has known antibacterial properties and is relatively low cost. However, the EPA limits the concentration of copper ion permissible in drinking water to 1.3 ppm. Our preliminary testing indicated that 1.3 ppm copper was insufficient to inhibit diverse species of waterborne bacteria and thus would not be an efficient means of low cost wastewater treatment. Accordingly, the goal of this research was to test the hypothesis that blue light, recently found to possess antimicrobial properties, can bolster the efficacy of low dose copper ions present in water at below EPA threshold levels. Visible blue light, from 400-500nm, has recently been found to exert anti-microbial effects against diverse species including *Pseudomonas aeruginosa* and *Staphylococcus aureus* (MRSA). Here, *E. coli, P. aeruginosa, S. typhimurium, S. aureus* and *B. subtilis* were grown on LB agar with or without copper and variable exposures of blue light to ascertain if the dual treatments could additively or synergistically inhibit bacteria at EPA allowable levels of copper in the treated water. All species had reductions of ~20% with copper treatment alone. Consistent with prior published studies, *P. aeruginosa* was the only species to show sensitivity to blue light alone. Synergistic reductions using copper plus blue light were evident after 10 min, 30 min, and 45 min for *P. aeruginosa, E. coli,* and *S. typhimurium* respectively. Gram positive microbes were relatively less susceptible than were the gram negatives tested. These results indicate low-dose copper plus blue light exposure can provide a safe, low cost means of targeting Gram negative pathogens during water treatment.

Author Disclosure Block:

J.M. Pisciotta: None. **N. Russo:** None.

Poster Board Number:

MONDAY-057

Publishing Title:

Epidemiology, Impact, Prevention & Control of Nipah Encephalitis in Bangladesh: A Systematic Review

Author Block:

M. Asaduzzaman, S. Mahmood; Inst. of Epidemiology,Disease Control and Res.(IEDCR), Dhaka, Bangladesh

Abstract Body:

Background: Nipah virus poses an eminent threat in Bangladesh as it encounters almost yearly outbreak since 2001.So,comprehensive understanding of the disease is of paramount importance for public health professionals due to high case fatality as well as person to person transmission. The objective of this systematic review is to summarize the coherent understanding of the disease.**Method:** We searched three different databases- PubMed, Web of Science & Google Scholar using different search term as appropriate for those databases mixing the Boolean operators that yielded 144, 113 & 1530 articles respectively. We screened the titles first for relevance & later abstracts. 47 articles met our criteria & were finally reviewed. A literature matrix was formed in Excel for compilation & analysis.**Results:** Nipah outbreaks in Bangladesh were different compared to initial outbreak in Malaysia in 1999. Unlike Malaysian outbreak, person to person transmission occurred in Bangladesh. Infections were reported in domesticated pigs, dogs, cats in South Asia & serological study in Bangladesh showed 6.5%, 4.3% & 44.2% seroprevalence in cattle, goat & pig respectively. *Pteropus* bat plays key role in Nipah transmission & risk factors include drinking raw date palm sap contaminated with bat urine or faeces, eating half-eaten bat saliva laden fruit & close contact or involvement in burial of cases. As date palm sap is harvested during winter, Nipah outbreaks occurred during this season. Nipah occurred mostly within the “Nipah belt” & had a high case fatality of more than 70%. Avoiding raw date palm sap & drinking sap from trees protected with bamboo skirt or “bana” remain the principle preventive measures along with strict infection control in hospitals for prevention of secondary cases.**Conclusion:** Nipah encephalitis is an emerging zoonosis and extensive knowledge of the disease is the key for prevention and saving lives. Community sensitization and multidisciplinary action through One Health approach can prevent this deadly disease especially in outbreak areas where date palm sap consumption is a delicacy.

Author Disclosure Block:

M. Asaduzzaman: None. **S. Mahmood:** None.

Poster Board Number:

MONDAY-058

Publishing Title:

2-Aminoimidazole Based Small Molecule Compound Potentiates Bactericidal Activity of ß-Lactam Drugs against *Mycobacterium tuberculosis*

Author Block:

A. B. Jeon[1], D. Ackart[1], A. Obregon-Henao[1], B. Podell[1], J. Belardinelli[1], M. Jackson[1], R. Melander[2], C. Melander[2], R. Basaraba[1]; [1]Colorado State Univ., Fort Collins, CO, [2]North Carolina State Univ., Raleigh, NC

Abstract Body:

Controlling the global spread of tuberculosis (TB) continues to be a challenge due in part to the lack of new anti-TB drugs and the emergence of drug-resistant *Mycobacterium tuberculosis* (*Mtb*). Despite the proven efficacy of ß-lactam drugs against bacterial pathogens, these drugs have no practical clinical use in the treatment of TB owing to the expression of inherent resistance by *Mtb*. Previously, our group have shown the ability of 2-aminoimidazole (2-AI) based small molecules to reverse both phenotypic drug tolerance and genotypic drug resistance in a variety of different microorganisms. Based on the mode of action demonstrated in other bacterial species, we hypothesized that 2-AI would reverse inherent resistance of *Mtb* against ß-lactam drugs either by inhibiting ß-lactamase enzyme activity or affecting cell wall integrity. For determination of bactericidal capacity of ß-lactam drugs against *Mtb*, *Mtb* H37Rv was treated with sub-minimum inhibitory concentrations of carbenicillin (4 and 64 µg/µL), amoxicillin (4 and 64 µg/µL), cefotaxime (2 and 32 µg/µL) and meropenem (0.0625 and 1 µg/µL) in the presence or absence of 2-AI compounds (62.5 and 125 µM). All four drugs tested showed improved bactericidal capacity when used in combination with 2-AI compounds as determined by colony forming units (CFUs) of cultures. For assessment of ß-lactamase activity, *Mtb* cultures in mid-log phase growth were treated with 2-AI compounds (62.5 and 125 µM) for 3 and 24 hrs and ß-lactamase activities were measured by colormetric assay using nitrocefin. *Mtb* cultures treated with 2-AI compounds had reduced ß-lactamase activity in both time points (3 and 24 hrs) tested. Finally, we evaluated cell wall integrity by measuring sensitivity to membrane disrupting agent SDS. *Mtb* cultures treated with 2-AI compounds (62.5 and 125 µM) for 24 hrs were exposed to 0, 0.1, and 0.05% SDS for 0, 1, and 4 hr. Percent survivals after exposure were determined by CFUs. Decreased survival rates over the course of the experiment were observed in 2-AI treated cultures compared to non-treated cultures. These data suggest that 2-AI based small molecules may be effective at reversing inherent drug resistance of *Mtb* to ß-lactam drugs.

Author Disclosure Block:

A.B. Jeon: None. **D. Ackart:** None. **A. Obregon-Henao:** None. **B. Podell:** None. **J. Belardinelli:** None. **M. Jackson:** None. **R. Melander:** None. **C. Melander:** None. **R. Basaraba:** None.

Poster Board Number:

MONDAY-059

Publishing Title:

Effect of Drug Resistance and Lineage of *Mycobacterium tuberculosis* on the Outcome of HIV-Positive and HIV-Negative Patients of Tuberculous Meningitis

Author Block:

M. Sharma, K. Sharma, M. Modi, A. Sharma, S. Varma; PGIMER, Chandigarh, India

Abstract Body:

Background: Most common and most fatal presentation of neurotuberculosis in Indian subcontinent is tuberculous meningitis (TBM). Little is known about the effect of HIV, drug resistance and bacterial lineage on patient outcome in TBM patients. Therefore, the present study was undertaken to evaluate the effect of drug resistance and bacterial lineage on the outcome of HIV positive and HIV negative patients of TBM from North-India.**Methods:** Genotypic profiling using 24-loci MIRU-VNTR was conducted on 30 (8 HIV-positive patients, 22-HIV negative patients) cerebrospinal fluid culture isolates of *M. tuberculosis*. Drug susceptibility was performed by MGIT-960. All patients were followed up for minimum three months after completion of ATT.**Results:** Out of 8 HIV + culture isolates, 3/8 (37.5%) were MDR and 5/8 (62.5%) were drug sensitive. Mycobacterial lineages in drug resistant isolates were Beijing type 2/3(66.6%) and CAS type 1/3 (33.3%), respectively. All the MDR cases in HIV positive patients had poor outcome. Among 22 HIV negative culture isolates, 3/22 (13.66%) were MDR and isolated DR was observed in 5/22 (22.77%). Out of 3 MDR HIV negative isolates 2 belonged to CAS type and one was Beijing type. Out of 5 mono-resistant cases, 3 were CAS, 1 was EAI and 1 was Haarlem type. Among the 14 DS HIV negative cases, 6/14 were CAS, 5 were EAI, 2 were Haarlem and one was untypable. Unfavorable outcome was observed in more than 87.5% patients with drug-resistant and in less than 30% patients with drug-susceptible profile. Among resistant strains, outcome was poor in isolates belonging to lineages of CAS and Beijing. The worst and the best outcomes were with CAS and Haarlem lineages, respectively, irrespective of the drug susceptibility profile and HIV status.**Conclusions:** Multidrug resistance, lineage of *M. tuberculosis* and HIV status are important determinants of mortality in patients of TBM. Targeting these factors can contribute to a favorable outcome.

Author Disclosure Block:

M. Sharma: None. **K. Sharma:** None. **M. Modi:** None. **A. Sharma:** None. **S. Varma:** None.

Poster Board Number:

MONDAY-060

Publishing Title:

Sequence Analysis for Detection of Drug Resistance in *Mycobacterium tuberculosis* Complex Isolates from the Central Region of Cameroon

Author Block:

J. ASSAM; Univ. OF DOUALA, DOUALA, Cameroon

Abstract Body:

The potential of genetic testing to rapidly diagnose drug resistance has lead to the development of new diagnostic assays. However, prior to implementation in a given setting, the association of specific mutations with specific drug resistance phenotypes should be evaluated. The purpose of this study was to evaluate molecular markers in predicting drug resistance in the Central Region of Cameroon. From April 2010 and March 2011, 725 smear positive pulmonary tuberculosis patients were enrolled and all positive cultures were tested for drug susceptibility. A total of 63 drug resistant and 100 drug sensitive Mycobacterium tuberculosis complex clinical isolates were screened for genetic mutations in katG, inhA, ahpC, rpoB, rpsL, rrs, gidB and embCAB loci using DNA sequencing. Of the 44 isoniazid resistant (INHR) isolates (24 high level, 1 μg/ml and 20 low level, 0.2 μg/ml), 73% (32/44) carried the katG315 and/or the −15 inhA promoter mutations. Of the 24 high level INHR, 17 (70.8%) harbored katG315 mutation, 1 a point mutation (−15C→T) in the inhA promoter and 6 were (25.0%) wild types. Thus, for INHR high level detection, katG315 mutation had a specificity and a sensitivity of 100% and 70.8% respectively. Of the 20 low level INHR, 10 (50.0%) had a −15C→T mutation in the inhA promoter region, and 1 (2.2%) a −32G→A mutation in the ahpC promoter region. All of the 7 rifampicin resistant (RIFR) isolates carried mutations in the rpoB gene (at codons Ser531Leu (71.4%), His526Asp (14.3%), and Asp516Val (14.3%)). Of the 27 streptomycin resistant (SMR) isolates, 7 carried mutations at the rpsL and the gidB genes. 1 of the 2 ethambutol resistant (EMBR) isolates displayed a mutation in embB gene. This study provided the first molecular investigation assessing the correlation of phenotypic to genotypic characteristics on MTB isolates from the Central Region of Cameroon using DNA sequencing. Mutations on rpoB, katG315 and −15 point mutations in inhA promoter loci could be used as markers for RIF and INH -resistance detection respectively.

Author Disclosure Block:

J. Assam: None.

Poster Board Number:

MONDAY-061

Publishing Title:

Adverse Events of Anti-Tuberculosis Drugs in Hiv Co-Infected Tb Patients; Earlier and Higher Occurrence Than in Hiv-Uninfected Patients

Author Block:

T. Matono, T. Nishijima, K. Teruya, E. Morino, J. Takasaki, H. Gatanaga, Y. Kikuchi, S. Oka; Natl. Ctr. for Global Hlth.and Med., Tokyo, Japan

Abstract Body:

Background: Tuberculosis (TB) drug-related adverse events (AEs) are common in HIV-infected TB patients (HIV/TB). However, the details and timing of AE occurrence are unknown. This study was aimed at evaluating first-line anti-TB drug-associated AEs in HIV/TB patients. **Methods:** This nested case-control study was conducted in Tokyo, Japan, between 2003 and 2015. The cases included HIV-infected culture-proven TB patients who were administered standard first-line anti-TB drugs. Controls were age- and sex-matched HIV-uninfected TB patients (non-HIV/TB) randomly selected from the same years as the patients with one-to-four ratio. Moderate-to-severe AEs of anti-TB drugs were defined as those with grade 2 or more which required drug discontinuation or regimen change. The groups were compared using Chi-square (χ^2) or Fisher test for nominal variables and Mann-Whitney test for continuous variables.**Results:** HIV/TB cases (94) and non-HIV/TB controls (376) were analyzed. For HIV/TB, the median age was 39 (interquartile range [IQR] 32–47) years, 95% were men, and 16% received antiretroviral therapy (ART), with a median CD4 count of 117 (IQR 51–226)/μl; isoniazid (INH) + rifampin (RIF)/rifabutin (RFB) + pyrazinamide (PZA) + ethambutol (EMB) was initiated in 91% cases. Intergroup differences in treatment regimen were insignificant. HIV/TB cases had a higher frequency of extrapulmonary TB (67% vs 28%; $p < 0.001$) and multidrug-resistant (MDR)-TB (4% vs 1%; $p = 0.016$), and a longer total treatment duration (median 12 [IQR 9-12] vs 6 [IQR 6-9] months; $p < 0.001$). AEs of anti-TB drugs were more frequent in HIV/TB (51% vs 11%; $p < 0.001$), mainly, fever (19% vs 1%; $p < 0.001$), rash (11% vs 3%; $p = 0.024$), and neutropenia (12% vs 1%; $p < 0.001$). Moreover, the time to AE from anti-TB drug initiation was shorter in the HIV/TB (median 17 [IQR 12-28] vs 26 [15-58] days; $p = 0.017$). Intergroup differences in all-cause mortality were insignificant (3% vs 2%; $p = 0.442$).**Conclusions:** Moderate-to-severe anti-TB drug AEs, mainly fever, rash, and neutropenia, occurred more commonly and earlier in HIV/TB than in non-HIV/TB patients. This frequent and early AEs of anti-TB drugs in HIV/TB may delay ART initiation.

Author Disclosure Block:

T. Matono: None. **T. Nishijima:** None. **K. Teruya:** None. **E. Morino:** None. **J. Takasaki:** None. **H. Gatanaga:** None. **Y. Kikuchi:** None. **S. Oka:** None.

Poster Board Number:

MONDAY-062

Publishing Title:

Rapid Detection of Rpob Mutations Associated with *Mycobacterium tuberculosis* in Lagos, Nigeria

Author Block:

L. O. Efere; Nigeria Inst. of Med. Res., Lagos, Nigeria

Abstract Body:

Background: Rifampicin (RIF) is a major drug used in the treatment of tuberculosis (TB) disease and is considered a surrogate marker for multi drug resistant Tuberculosis (MDR-TB). The nature and frequency of mutations in the *rpoB* gene which accounts for majority of Rifampicin (RIF) resistance in *Mycobacterium tuberculosis* complex (MTBC) varies considerably between different geographical regions.This study determined the predominant *rpoB* gene mutations associated with RIF resistance in MTBC. **Method:** Mutation on the *rpoB* gene region conferring resistance to RIF was assessed in 164 smear positive sputum specimens using the line probe assay (Genotype MTBDRplus®) technique. The test involved deoxyribonucleic acid(DNA) extraction from the decontaminated sputum specimens, multiplex polymerase chain reaction amplification and reverse hybridization step. Resistance was interpreted based on the absence of at least one of the wild type probes and or the presence of one of the mutant genes of the *rpoB* gene. **Result:** 161(98.2%) Of the 164 specimens were identified as MTB complex while 3(1.8%) were non MTB complex. 119(73.9%) were resistant to rifampicin, which includes multidrug resistance tuberculosus (MDR-TB) while 40(24.8%) were sensitive. Mutant 3 gene (Mut 3) accounted for 90(75.6%) resistant strain, Mutant 1 gene(Mut1) 8(6.7%), Mutant 2A gene(Mut2A) 8 (6.7%), while 13(11.0%) showed no mutations. **Conclusion:** A high percentage of RIF resistance was associated with mutant 3 gene (MUT3). This characterizes the RIF resistance *Mycobacterium tuberculosis* associated with this environment, and can serve as a basis for developing or improving rapid molecular diagnostics for detection of RIF resistance in this setting.

Author Disclosure Block:

L.O. Efere: None.

Poster Board Number:

MONDAY-063

Publishing Title:

Genotypes and Drug Susceptibility Pattern of *Mycobacterium tuberculosis* Isolates in Hiv Seropositive Patients

Author Block:

J. SINGH[1], N. Singh[2], U. Aggarwal[2], S. Singh[1]; [1]All India Inst. of Med. Sci., New Delhi, India, [2]Natl. Inst. of TB and RD, New Delhi, India

Abstract Body:

Objectives: The molecular data obtained from the *M. tuberculosis* so far in India have not addressed the issue of HIV-TB co-infected patients. Therefore, we aimed to investigate the spoligotyping and 24 loci MIRU-VNTR based population structure of *M. tuberculosis* clinical isolates in HIV-TB co-infected patients in a tertiary care setting. **Materials and Methods**: A total of 249 HIV positive patients, suspected of tuberculosis were recruited in this study. All the samples obtained were decontaminated using N-acetyl-L-cystine/ Sodium hydroxide (NALC-NaOH) method and were inoculated in BACTEC MGIT 960 as well as on LJ medium. Drug Sensitivity testing was done by using SIRE kit (Becton & Dickinson, USA). DNA for genotyping from positive cultures was extracted using QIAGEN kit. Spoligotyping was performed using a commercially available kit (M/s Ocimum Biosolutions) while 24 loci MIRU-VNTR was performed by PCR amplification of individual loci using specific primers as described previously (**Supply et al 2006)**. **Results:** From 249 samples, 120(49.3%) strains were isolated. All of them were subjected to genotype and drug susceptibility. On genotyping of HIV co-infected MTB isolates revealed that 120 strains were grouped in 23 numbers of Shared types (ST) representing 9 major clades and 10 sub-clades. CAS (46.7%) clade was most prevalent, followed by Beijing (22.5%), EAI(10.8%), LAM (5.0%), T-clade (5.8%),U (5.8%), X(1.6%), Manu(0.8%) and 0.8% strains were from Cameroon lineage. Among the CAS family, 45(80.3%) isolates of CAS1_DEL sub-family (ST26) was the most predominant. Highly discriminant HGDI was observed in miru10, miru16, miru26, miru31, miru40, Mtub04, Mtub21, Mtub30, QUB26, QUIB4156, ETR-A, ETR-C. Seventy Seven isolates (64.2%) were susceptible to all four drugs; 9(7.5%) were resistant to at least one drug; 30(25%) were Multi drug resistant TB (MDR-TB) strains and 4(3.3%) were dual drug resistant cases. Among the 30 MDR-TB isolates, 15 (50%) isolates were Beijing family strains and remaining 15(50%) were from CAS, EAI, LAM, T and U families which is statistically significant. **Conclusion:** CAS was the most prevalent strain among HIV-TB co-infected patients . Drug resistance rate was high in Beijing family strains.

Author Disclosure Block:

J. Singh: None. **N. Singh:** None. **U. Aggarwal:** None. **S. Singh:** None.

Poster Board Number:

MONDAY-064

Publishing Title:

Phenotypic and Genotypic Determination of Drug Susceptibility and Molecular Characterization of *Mycobacterium tuberculosis* Isolates at Debre Berhan Referral Hospital, Ethiopia: A Cross Sectional Study

Author Block:

D. G. Weldehana[1], S. K. Biru[2], S. Habtemariam[3]; [1]Wollo Univ., Coll. of medicine and Hlth. Sci.s, Dessie, Ethiopia, [2]Addis Ababa Univ., Coll. of medicine and Hlth. Sci.s, Addis Ababa, Ethiopia, [3]Addis Ababa Univ., Aklilu Lemma Inst. of pathobiology, Addis Ababa, Ethiopia

Abstract Body:

Background: Globally, *Mycobacterium tuberculosis* is the leading cause of death from a single infectious disease and it is a major public health problem in Ethiopia. Multidrug-resistant tuberculosis poses a formidable challenge to control tuberculosis due to its complex diagnostic and treatment challenges. The main aim of the study is to investigate the molecular epidemiology of mycobacterial isolates and to characterize drug susceptibility pattern phenotypically and genotypically**Methods:** Sputum samples were collected from smear positive PTB patients from January 2013 to July 2013. The bacteria were isolated on MGIT and DST was determined phenotypically and genotypically. Molecular characterization was performed by deletion and spoligotyping.**Results:** A total of 57 smear positive PTB patients were included, 46 were new cases. Out of 57 *Mycobacterium tuberculosis* isolates drug susceptibility was performed only for 45; of which, 14 were resistant to at least one of the four first line drug. There were 3 MDR. Only *katG* and *rpoB* gene are responsible for isoniazid and rifampicin resistance, respectively. There were 40 shared spoligotype and 17 orphan strains; SIT 53 and 149 were dominant strains. The predominant family was T1.**Conclusion:** the reported rate of first line drug resistance and the existence of various strain type of *MTB* isolate is an indicator for the need of large scale study in the study area.**Key words:** Debre Berhan, drug susceptibility pattern, TB, MGIT, MTBDRplus

Author Disclosure Block:

D.G. Weldehana: None. **S.K. Biru:** None. **S. Habtemariam:** None.

Poster Board Number:

MONDAY-065

Publishing Title:

Multi-drug Resistant *Mycobacterium tuberculosis* and Associated Risk Factors in the Oromia Region of Ethiopia

Author Block:

G. Mulisa, T. Abebe; Adama Hosp. Med. Coll., .Adama, Ethiopia

Abstract Body:

Background: Multi drug resistant tuberculosis (MDR-TB) is prevalent in Ethiopia. The study aim was to determine the risk factors for tuberculosis caused by multi drug resistant MDR-TB in Oromia region, Ethiopia. **Methods:** In a 6 months case control study in 2013-14, sputum samples and standardized questionnaire data (demographics, treatment, TB contact history, underlying disease, history of imprisonment) were collected from suspected MDR-TB cases≥18 years of age. Sputum was processed locally in the Oromia public health laboratory using standard techniques. Data from MDR-TB cases and TB positive controls were compared using logistic regression analysis. For each factors, their association with outcomes variable was estimated by calculating the odd ratio (OR) together with 95% confidence intervals (95% CI).**Results:** Of 439 suspected MDR-TB cases, 265 had confirmed *Mycobacterium tuberculosis* infection, of whom 33% (88) had laboratory confirmed MDR-TB. Over two thirds (65%) were between18 to 39 years of age. On multivariable analysis occupation (farming), known TB contact history, alcohol use, HIV infection, previous known TB history and previous TB treatment outcome were predictors of MDR-TB.**Conclusion:** The rate of MDR- TB was high among suspected cases in the Oromia region of Ethiopia. If TB is suspected, the presence of any of the above factors should alert Oromia region clinicians and public health to be screen for the MDR-TB.

Author Disclosure Block:

G. Mulisa: None. **T. Abebe:** None.

Poster Board Number:

MONDAY-066

Publishing Title:

The Epidemiology of Mdr-Tb at Times of Mass-migration at a Western European Tb Clinical Reference Center

Author Block:

I. D. Olaru, B. Kalsdorf, J. Heyckendorf, E. Terhalle, N. Smitsman, S. Großmann, C. Lange; Res. Ctr. Borstel, Borstel, Germany

Abstract Body:

The objective of this study is to evaluate the change in the epidemiology and characteristics of patients with multidrug-resistant tuberculosis (MDR-TB).**Methods:** Retrospective analysis of patients with microbiologically confirmed MDR-TB with available drug susceptibility test results who were hospitalized at the Research Center Borstel between January 2002 and January 2016. To evaluate the change in the characteristics of patients with MDR-TB we compared the period before and after January 2014, when an increase in population migration and the number of asylum seekers was recorded.**Results:** Of the 82 patients with MDR-TB admitted during the study period, 76 had drug susceptibility results available and were further analyzed. Median patient age was 38.5 years and male to female ratio was 2.2:1. Of the total number of patients 38 (50%) were admitted between January 2002 and December 2013 (Group 1) and 38 (50%) between January 2014 and January 2016 (Group 2). 64 (84.2%) patients were from countries of the WHO European Region, with 52 (68.4%) from countries of the former Soviet Union and 7 (9.2%) from Germany; 5 (6.6%) patients were from the WHO Western Pacific Region and Africa each, and 1 from the South-East Asia and Eastern Mediterranean Regions. There was no difference in the origin of patients according to WHO region with 27 (71%) of patients in Group 1 from former Soviet Union countries vs. 25 (66%) in Group 2. 18 (47.4%) patients in Group 1 and 19 (51.4%) had been previously treated for TB. Of the 14 patients with extensively drug-resistant (XDR)-TB, 7 (50%) were admitted since 2014. There was no difference in the proportion of patients with culture conversion after 6 months of therapy, 82.8% in Group 1 vs. 85.7% in Group 2, respectively. **Conclusions:** Although there was a dramatic increase in the number of patients with M/XDR-TB admitted for treatment since the beginning of 2014, their characteristics were not different. This was probably because the majority of patients were not from countries from where a large number of refugees originated and they were mainly from Eastern European countries.

Author Disclosure Block:

I.D. Olaru: None. **B. Kalsdorf:** None. **J. Heyckendorf:** None. **E. Terhalle:** None. **N. Smitsman:** None. **S. Großmann:** None. **C. Lange:** N. Other; Self; Sponsored an independent lecture at a sponsored symposium by Chiesi, MSD, Gilead, Abbvie outside the submitted work.

Poster Board Number:

MONDAY-067

Publishing Title:

High Rates of Antimicrobial Resistance in *Mycobacterium abscessus* from Patients with Interferon-Gamma Autoantibodies

Author Block:

A. Jitmuang[1], H. Boshoff[1], C. Henderson[1], S. Shallom[1], L. Rosen[1], S. Browne[1], P. Chetchotisakd[2], K. Olivier[1], C. Barry, III[1], S. Holland[1], A. Zelazny[1]; [1]Natl. Inst. of Hlth., Bethesda, MD, [2]Khon Kaen Univ., Muang, Thailand

Abstract Body:

Host control of mycobacterial infections relies on the interleukin-12 and interferon-gamma (IFN-γ) pathway. Patients with IFN-γ autoantibodies are exquisitely susceptible to mycobacteria, particularly *Mycobacterium abscessus* (MAB). Infections are often disseminated, refractory to treatment and tend to relapse. Microbiological data including susceptibility testing of MAB isolates from these patients is warranted for establishing optimal treatment and predicting outcomes. *M. abscessus* isolates (n=37) from US and Thai patients with IFN-γ autoantibodies were characterized phenotypically and identified to subspecies level by genotypic methods. Antimicrobial susceptibility testing was performed to 18 drugs including bedaquiline, clofazimine and newer oxazolidinones. Isolate sources were lymph node (62%), skin (11%), blood (8%), abscess (8%), and other (11%). Isolates displayed rough (52%), smooth (32%), and mixed (16%) colony morphology and were genotypically identified as MAB subsp. *abscessus* (84%), MAB subsp. *massiliense* (13%), and MAB subsp. *bolletii* (3%). All strains were amikacin susceptible and showed low MICs to tigecycline (≤0.015-2 μg/mL). Only 4% of MAB subsp. *abscessus* and 86% of MAB subsp. *massiliense* were susceptible to clarithromycin. A high percentage (25%) of isolates showed high MICs (≥16) to linezolid but increased susceptibility to newer oxazolidinones. Most isolates were intermediate or resistant to quinolones, imipenem, and cefoxitin (97%, 91%, and 81%, respectively), including 4 strains (11%) showing multidrug resistance. Interestingly, all isolates were susceptible to bedaquiline, a new agent for MDR TB. *M. abscessus* isolates (mostly *subsp. abscessus)* from patients with IFN-γ autoantibodies present a therapeutic conundrum. Their intrinsic and presumptively acquired drug resistance makes these strains challenging for testing newer drugs. Tigecycline, newer oxazolidinones, and bedaquiline showed promising results *in vitro*.

Author Disclosure Block:

A. Jitmuang: None. **H. Boshoff:** None. **C. Henderson:** None. **S. Shallom:** None. **L. Rosen:** None. **S. Browne:** None. **P. Chetchotisakd:** None. **K. Olivier:** None. **C. Barry:** None. **S. Holland:** None. **A. Zelazny:** None.

Poster Board Number:

MONDAY-068

Publishing Title:

Geographic Distribution of Nontuberculous Mycobacteria in the United States

Author Block:

N. K. Helstrom, J. L. Marola, K. S. Messina, **M. Salfinger**; Natl. Jewish Hlth., Denver, CO

Abstract Body:

Background: While *Mycobacterium tuberculosis* is a reportable entity in the United States, nontuberculous mycobacteria (NTM) are only a reportable entity in a few states, not nationwide. Although the overall prevalence of pulmonary NTM is increasing, there is no recent nationwide data about the various NTM isolated. National Jewish Health (NJH) receives isolates from all 50 states. The purpose of this analysis is to gain a better understanding of the distribution of NTM isolates across the United States.**Method:** An analysis was conducted of all NTM isolates submitted for identification to NJH within a two year period and screened for duplicate entries; submissions from reference laboratories were excluded. Of a total of 3,096 unique isolates, 79 different species were identified using *rpoB* gene sequencing between July 2013 and July 2015. These isolates were grouped by state and by region, and frequencies were calculated per 100,000 population (pop.) in that state/region.**Results:** Only data for the most commonly identified NTM are reported here. The Southern states (pop. 183,843,053) had 2,186 NTM isolates for a frequency of 1.19/100,000; the Northern states (pop. 132,844,549) had 854 NTM isolates for a frequency of 0.76/100,000. The following differences in frequencies per 100,000 among a selection of taxa were noted between Southern and Northern states: *M. abscessus* group (N=820) 0.33, 0.16; *M. avium* complex (N=843) 0.34, 0.19; *M. gordonae* (N=204) 0.08, 0.05; *M. kansasii* (N=58) 0.03, 0.01; *M. simiae* (N=22) 0.01, 0.01; *M. xenopi* (N=61) 0.005, 0.05, respectively. Among the *M. abscessus* group the frequencies per 100,000 were *M. abscessus* (N=490) 0.18, 0.11; *M. massiliense* (N=274) 0.12, 0.04; and *M. bolleti* (N=56) 0.03, 0.004, respectively. Also, *M. avium* (N=391) 0.14, 0.12; *M. intracellulare* (N=276) 0.13, 0.04, respectively.**Conclusions:** *M. abscessus* group and *M. avium* complex are the most identified taxa. The Southern states sent 2.6 times more isolates for identification than Northern states. There are major differences in frequencies per 100,000 between Southern and Northern states, especially for *M. xenopi, M. massiliense, M. bolletii*, and *M. intracellulare*. For the following species similar frequencies were noted: *M. abscessus, M. avium, M. gordonae, M. kansasii*, and *M. simiae.* These preliminary results warrant a population based investigation.

Author Disclosure Block:

N.K. Helstrom: None. **J.L. Marola:** None. **K.S. Messina:** None. **M. Salfinger:** None.

Poster Board Number:

MONDAY-069

Publishing Title:

Next-Generation Sequencing of Drug Resistance Mutations in Mycobacterium Tuberculosis

Author Block:

A. J. Caulfield, S. P. Buckwalter, N. L. Wengenack; Mayo Clinic, Rochester, MN

Abstract Body:

Mycobacterium tuberculosis is a highly transmissible bacterial pathogen and is the causative agent of tuberculosis, a disease causing significant worldwide morbidity and mortality, especially among HIV-infected patients. Multi-drug resistant strains have emerged which reduce viable treatment options and elicit great economic and quality of life burdens due to costly and time-consuming therapeutic interventions. As a slow-growing organism, current methods for the detection of *M. tuberculosis* drug resistance require weeks to months, while delayed administration of appropriate therapy may be detrimental to patient outcome. In this study, the MiSeq (Illumina) next-generation sequencing (NGS) platform was used to detect mutations within the genome of *M. tuberculosis* that are commonly associated with drug resistance. The entire coding regions of nine genes were retrospectively examined from clinical isolates with demonstrated resistance to first- and second-line drugs as determined by standard phenotypic methods. Of all isolates tested phenotypically in 2014, 23.2% were resistant to at least one first-line drug and 43.2% were resistant to a second-line aminoglycoside or fluoroquinolone. Sequencing of these isolates provided presumptive drug resistance data based on the detection of known resistance mutations. This study supports the use of NGS technology as a more rapid alternative to current drug susceptibility testing methods and addresses a largely unmet diagnostic need that will contribute to improve patient management.

Author Disclosure Block:

A.J. Caulfield: None. **S.P. Buckwalter:** None. **N.L. Wengenack:** None.

Poster Board Number:

MONDAY-070

Publishing Title:

Design and Validation of a Three Color Single Tube Assay for Pyrazinamide (Pza) Resistant *M. tuberculosis*

Author Block:

J. Rice[1], L. Wangh[1], S. Marras[2], B. Kreiswirth[2], M. Whitfield[3], P. D. van Heldan[3], R. M. Warren[3]; [1]Brandeis Univ., Waltham, MA, [2]Publ. Hlth.Res. Inst. Ctr., Newark, NJ, [3]Stellenbosch Univ., Tygerberg, South Africa

Abstract Body:

Background: Pyrazinamide (PZA) is a key first-line antibiotic to treat tuberculosis and it has become a critical drug in new drug regimens. A recent meta-analysis from Whitfield et al. revealed 608 distinct mutations at 397 locations in the *pncA*, the vast majority of which are non-synonymous and correlate with PZA resistance. Importantly, sequencing data also revealed that a wild-type *pncA* gene correlates with PZA susceptibility. We have developed a LATE-PCR assay using Lights-On/Lights-Off probes to distinguish the wild type drug susceptible *pncA* gene sequence from all strains that have non-synonymous genetic alterations. **Methods:** LATE-PCR is used to amplify a 685 base long amplicon encompassing the entire coding region of the *pncA* gene, including the upstream flanking sequence. The reaction mix also contained a reagent that suppressed mis-priming throughout the amplification process. Following amplification, the reaction temperature is lowered to 25^{o}C resulting in the coating of the single-stranded DNA templates with 35 Lights-On/Lights-Off fluorescent probes labeled in three colors. The probes are then melted off of the target and the resulting fluorescent signature reveals the presence or absence of mutations, which are distinguishable from a highly reproducible wild-type profile. Total time per assay 3.5 hrs.**Results:** The assay testing has been carried out at three different locations using > 500 samples of TB DNA purified from cultured cells. The results show 100% sensitivity and >97% specificity. Additional optimization is underway and will be reported at the meeting.**Conclusions:** We have successfully designed and tested a single closed-tube assay that is able to identify and distinguish the wild type *pncA* gene from strains with almost every known genetic alteration. Additional refinements will improve identification of the rare synonymous mutations in *pncA* and the few non-synonymous that were not correctly scored as PZA resistance. Supported by NIH Grant #R21AI106551.

Author Disclosure Block:

J. Rice: None. **L. Wangh:** None. **S. Marras:** None. **B. Kreiswirth:** None. **M. Whitfield:** None. **P.D. van Heldan:** None. **R.M. Warren:** None.

Poster Board Number:

MONDAY-071

Publishing Title:

Molecular Analysis of *pncA* Gene Target of *M. Tuberculosis* Clinical Isolates

Author Block:

V. R. Wabale, 38 years; Grant Government Med. Coll., MUMBAI, India

Abstract Body:

Pyrazinamide (PZA) is an important first line drug for tuberculosis (TB) treatment whose drug susceptibility testing (DST) in vitro is pH dependent. Gold standard for PZA DST is *pncA* Whole Gene Sequencing (WGS). Hence study was conducted to perform PZA DST by WGS and analyse sequence data using CLC Protein Workbench Software. Strain variation among drug resistant population studied by doing phylogenetic analysis. A 300 proven *M. tuberculosis* strains were tested by Wayne's Assay and WGS using Sanger's method. BLASTn and BLASTx performed for nucleotide sequence analysis and identification of amino acid changes in the drug target in comparison with wild-type *M. tuberculosis* H37RV done using CLC protein workbench. A 36.66% (110/300) showed missense & nonsense mutations causing amino acid substitutions in most cases or frame shifts leading to nonsense polypeptide. Overall 226 mutations in 110 PZA resistant strains found dispersed along *pncA* gene indicative of main mechanism for PZA resistance. Most alterations occurred in 561 bp region of open reading frame and in 82 bp region of its putative promoter. WGS analysis of *pncA* using CLC protein workbench is reliable alternative to phenotypic PZA DST technique that usually gives irreproducible results and discordance among findings from different laboratories. High degree of diversity in *pncA* gene mutations among PZA resistance strains is epidemiologically useful in predicting linked cases of TB in our high incidence setting.**Key words:** *pncA* gene mutations, *Mycobacterium tuberculosis*, Pyrazinamide resistance.

Author Disclosure Block:

V.R. Wabale: None.

Poster Board Number:

MONDAY-072

Publishing Title:

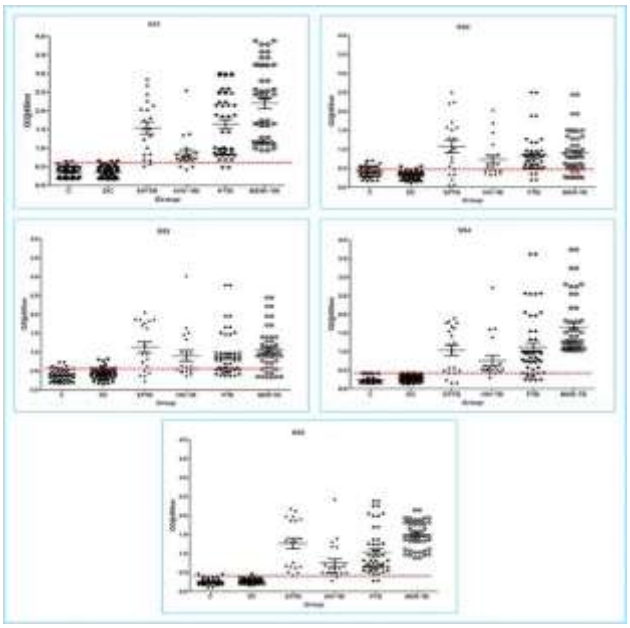
Identification of Novel Point-of-Care Biomarkers: Validation and Evaluation for the Rapid Detection of Tb and Mdr-Tb

Author Block:

A. Singh[1], A. Gupta[1], K. Gopinath[2], S. Singh[1]; [1]All India Inst. of Med. Sci., New Delhi, India, [2]Max Plank Inst. for Infection Biology, Berlin, Germany

Abstract Body:

Background: Accurate and rapid diagnosis of TB is paramount importance in the management of tuberculosis infection. The recommended culture based methods for the diagnosis of tuberculosis are cumbersome and time consuming while molecular methods are costly. The serological methods are easy and cost effective, but after finding currently available serological methods very inferior, these tests were banned in 2011 by WHO and Government of India. In this study, we report five novel protein biomarkers by comparative proteomic analysis (1) **Methods:** The selected genes were cloned, expressed, purified and named these as SS1, SS2, SS3, SS4 and SS5 (***patent filed***). The diagnostic potential of these antigens were validated using well characterized tuberculosis patients using dot-blot, ELISA and Flow through (FT) assay. The sera from PTB (n=111), EPTB (n=29) diseased controls (n=60) and healthy subjects (n=50) were included. **Result:** Our experimental results of dot-blot & FT shows 100% (SS1, SS3, SS4), 96% (SS2) and 86.3%) sensitivity and 100% (SS1), 92% (SS2), 88% (SS3, SS4) and 96% (SS5) specificity. In ELISA the AUC value of selected antigens were ranging 0.98 to 0.88 for antigens. The sensitivity of ELISA was ranging from 97.4% to 73.4% and specificity of 98.2% to 86.4%. However, for MDR-TB prediction shows the sensitivity of 100% (SS1, SS4 and SS5) and 79.2% (SS2, SS3) **(Figure 1)**. The ROC curve shows significant differences of healthy *vs* TB and MDR-TB (p=0.001). SS1, SS4 and SS5 antigens were able to discriminate drug susceptible-TB and MDR-TB (p_{ss1}=0.008, $p_{ss4,\ ss5}$=<0.001). **Conclusion:** Our results indicate that these antigens could be used as novel biomarker for detection of TB and MDR-TB directly from patient sera.



Author Disclosure Block:

A. Singh: None. **A. Gupta:** None. **K. Gopinath:** None. **S. Singh:** None.

Poster Board Number:

MONDAY-074

Publishing Title:

Evaluation of a Molecular Detection Assay for Diagnosis and Antimicrobial Susceptibility Testing of *Mycobacterium tuberculosis* in Pulmonary and Extrapulmonary Clinical Specimens

Author Block:

C. Jaramillo Jaramillo, D. Caceres Contreras, J. Carmona Valencia, **L. Gonzalez Nino**; DINAMICA IPS, Medellin, Colombia

Abstract Body:

Background: In 2014, 9.6 million people fell ill with Tuberculosis (TB) and 1.5 million died. Worldwide an estimated of 480,000 people developed multidrug-resistant TB. In Colombia, around 500,000 new cases of TB were diagnosed, with a mortality rate of 1.5/100,000 population. It is important to continue improving diagnostic methods for TB more accuracy, faster and cheaper. The present study aimed to evaluate the Anyplex II MTB/MDR/XDR Detection assay comparing it against conventional diagnostic tests. **Methods:** A total of 59 samples (19 pulmonary and 40 extrapulmonary) from 32 patients with clinical suspicion of TB were collected prospectively (Dinamica IPS, Medellín, Colombia). Samples were tested using the multiplex real-time PCR Anyplex II MTB/MDR/XDR Detection assay for simultaneous detection of *Mycobacterium tuberculosis* and its resistance to anti-tuberculosis drugs and compared against conventional diagnostic tests (Kinyoun stain, mycobacterial cultures BD MGIT, BD Löwenstein-Jenssen and 7H11 Thin layer agar and drugs phenotypic susceptibility testing: Bactec MGIT 960 and the indirect proportion method). **Results:** Diagnosis of TB was proven according to microbiological criteria in 4 of 32 patients (13%). When Anyplex II MTB was used in the 59 samples, diagnostic accuracy was: sensitivity 85%, specificity 96%. Kappa index was 0.45 compared with Kinyoun stain, 0.79 with mycobacterial cultures and 1.0 with drugs phenotypic susceptibility testing. The mean time to deliver a result in positive samples was 1 day for Kinyoun stain (1 to 2 days), for Anyplex II MTB was 4 days (1 to 11) and 35 days for culture (16 to 59). Regarding negative samples, the mean time to deliver a result was 1 day for Kinyoun stain (1 to 3), 4 days for Anyplex II MTB (1 to 8) and 59 days for culture (52 to 66). The cost of microbiological analysis per sample was 237 USD, compared with 230 USD of the molecular test. **Conclusions:** The Anyplex II MTB/MDR/XDR Detection assay provides results 8 times faster than culture and 55% more accurate than kinyoun stain. This multiplex assay is a rapid, cost effective and highly sensitive and specific method and therefore a viable option for the rapid and safe diagnosis of TB and antimicrobial susceptibility testing in health centers.

Author Disclosure Block:

C. Jaramillo Jaramillo: None. **D. Caceres Contreras:** None. **J. Carmona Valencia:** None. **L. Gonzalez Nino:** None.

Poster Board Number:

MONDAY-075

Publishing Title:

Application of Fluorophages for TB Detection and Screening of Antitubercular Drugs

Author Block:

E. Urdániz[1], L. Rondón[1], M. Martí[1], G. Hatfull[2], M. Piuri[1]; [1]Univ. of Buenos Aires, Buenos Aires, Argentina, [2]Univ. of Pittsburgh, Pittsburgh, PA

Abstract Body:

Tuberculosis (TB) is a major cause of human mortality. The emergence of resistant *Mycobacterium tuberculosis (M.tb)* strains has become a serious public health problem worldwide complicating treatment and control of the disease. Nowadays, there is a need for new and efficient anti-TB drugs. The bottleneck on the TB drug discovery pipeline still remains in the time-consuming activity testing of compounds. Our aim is to develop a novel, rapid and sensitive assay to be used for *in vitro* and *in vivo* drug susceptibility testing (DST) of compounds as part of the solution to the serious TB health problem.The development of fluorophages, mycobacteriophages carrying fluorescent genes, was described as a simple means of revealing the metabolic state of *M. tb* cells, and therefore their response to antibiotics. Recently, we have constructed an improved version of fluorophages with higher sensitivity and shorter time to detection of signal. Red bright *M.tb* cells were observed by fluorescent microscopy after only 5 hours of infection with the new $mCherry_{bomb}\Phi$. We optimized the conditions for automated fluorimetric detection in a multiwell format as a good alternative for *in vitro* DST. Briefly, pure cultures of *M.tb* were infected at a MOI of 100 with $mCherry_{bomb}\Phi$ in the presence of the most common anti-TB drugs. A decrease in the fluorescent signal was observed over time for increasing concentrations of compounds with different targets in the bacteria and MICs were succesfully determined.To test whether our methodology would also be useful for *in vivo* DST, we developed an infection assay in eukaryotic cells. We were able to detect intracellular *M.tb* by confocal microscopy after infection with $mCherry_{bomb}\Phi$ in THP-1 derived macrophages and pulmonary epithelial cell line A549. Basically, cells were incubated with bacteria and after extensive washing, $mCherry_{bomb}\Phi$ was added overnight to reveal the presence of intracellular *M.tb*. Cells were stained with nuclear DAPI and inmunofluorescence was performed using a cytoplasmic antibody anti- LC3 autophagy marker. An automated infection assay in presence of different concentrations of drugs still must be evaluated. However, these promising results show that our phage-technology could be used for both *in vitro* and *in vivo* activity testing of compounds in a full-scale, fast and sensitive assay.

Author Disclosure Block:

E. Urdániz: None. **L. Rondón:** None. **M. Martí:** None. **G. Hatfull:** None. **M. Piuri:** None.

Poster Board Number:

MONDAY-076

Publishing Title:

Relational Sequencing Tb Data Platform (Reseqtb): Development of a Standardized Grading System for Validating Drug-resistance Associated Mutations

Author Block:

P. Miotto[1], E. Tagliani[1], B. Tessema[2], N. Hillery[3], L. Chindelevitch[4], T. Rodwell[2], D. Dolinger[2], M. Schito[5], ReSeqTB Consortium; [1]San Raffaele Hosp., Milano, Italy, [2]Fndn. for Innovative New Diagnostics (FIND), Geneva, Switzerland, [3]Univ. of California San Diego, La Jolla, CA, [4]Simon Fraser Univ., Burnaby, BC, Canada, [5]Critical Path Inst., Tucson, AZ

Abstract Body:

A global public-private partnership has been established to develop a Relational Sequencing TB Data Platform (ReSeqTB) to simplify and standardize whole genome sequencing data analysis for interpretation of antimicrobial drug susceptibility. Here we describe the development of a standardized analytical approach for validating and interpreting *M. tuberculosis* (MTB) drug resistance (DR) associated mutations. To this aim, we merged data from two recently published data sets: the PhyResSE web tool (www.phyresse.org) and the Knowledge Synthesis TBDR DB (TBDR.org). An expert, consensus-driven approach was used to independently define a starting list of "high-confidence" DR-associated mutations in MTB and to develop a standardized approach for validating and scoring DR-associated mutations. The total number of MTB isolates (DR+ drug susceptible (DS)) used for evaluating the relationships between phenotypic and genotypic resistance to each drug ranged from 571 to 9537. Mutation frequencies across DR and DS cases were calculated, together with statistical measures of their performance, as predictors of phenotypic DR. Using statistical thresholds based on likelihood ratios (LR)—a method derived from evidence-based medicine—mutations were categorized as having "high-" (LR ≥10), "moderate-" (5≤ LR <10) or "minimal-" (1≤ LR <5) confidence associations with DR phenotypes, while mutations with LR <1 were categorized as "not-associated" with drug resistance. A list of 44 mutations to 10 drugs with moderate to high confidence associations with DR phenotypes was identified. Extensive systematic literature review for improving this first list is currently ongoing. ReSeqTB provides the first standardized and validated list of DR-associated mutations organized by level of confidence using objective analytical criteria. These methods can be applied not only to data from ReSeqTB, but also other TB DR databases and genotypic/phenotypic resistance data derived from systematic reviews and meta-analyses.

Author Disclosure Block:

P. Miotto: None. **E. Tagliani:** None. **B. Tessema:** None. **N. Hillery:** None. **L. Chindelevitch:** None. **T. Rodwell:** None. **D. Dolinger:** None. **M. Schito:** None.

Poster Board Number:

MONDAY-077

Publishing Title:

In Vitro* Activity of Clofazimine and Dapsone against Multidrug Resistant Strains of *Mycobacterium abscessus, M. Chelonae* and *M. Avium

Author Block:

M. Schwartz, K. Carroll, N. Parrish; The Johns Hopkins Med. Inst.s, Baltimore, MD

Abstract Body:

Background: Treatment of immunocompromised and chronically ill patients infected with multidrug resistant (MDR) strains of non-tuberculous mycobacteria (NTM) is a challenge for physicians. Resistance is emerging to all classes of traditional drugs used to combat these organisms. In the absence of better options, patients are now being treated with alternative drugs which often have higher toxicity. New drugs or alternatives are needed. Clofazimine is a 3rd line antibiotic used to treat tuberculosis; dapsone is used to treat leprosy. We investigated the *in vitro* susceptibility of MDR clinical isolates of *M. abcessus* (ABC), *M. chelonae* (CHE) and the *M. avium* complex (MAC) to clofazimine and dapsone using microbroth dilution.**Methods:** A total of 35 isolates (ABC, n = 17; CHE, n = 4; MAC, n = 14) were selected based on resistance to 3 or more drug classes. Resistance was defined as an MIC above currently established breakpoints for which interpretations exist or those demonstrating confluent growth at the highest concentration tested. Microbroth dilution was used to test clofazimine and dapsone against all isolates with incubation times adjusted by species (3 to 7 days for ABC and CHE; 14 days for MAC). Panels were read using a mirror-box and ambient light.**Results:** All species and strains tested had MICs to clofazimine ≤ 3 μg/ml. Of these, however, 80% (29/35) had MIC's of ≤ 1.5 μg/ml. However, there were differences between species. MAC isolates were consistently more susceptible to clofazimine with 92% (13/14) demonstrating an MIC ≤ 1.5, versus 72% (15/21) for ABC and CHE. In comparison, most of the MAC isolates tested had MICs to dapsone ≥ 50 μg/ml, or the highest concentration tested. Only one MAC strain demonstrated an MIC of 3 μg/ml versus 15/21 (71%) for ABC and CHE. Of the latter, 7/15 (33%) had MICs ≤ 1.5 μg/ml.**Conclusions:** Given the increasing threat of MDR-NTM few options remain for treatment. Clofazimine and dapsone show promising results against these organisms. Further research is needed to determine if synergy can be achieved between these and other drugs to which the organisms are still susceptible.

Author Disclosure Block:

M. Schwartz: None. **K. Carroll:** None. **N. Parrish:** None.

Poster Board Number:

MONDAY-078

Publishing Title:

Mutations in *Pepq* Confer Cross-Resistance to Bedaquiline & Clofazimine in *Mycobacterium tuberculosis*

Author Block:

D. V. Almeida[1], T. Ioerger[2], S. Tyagi1, S Li1, K Mdluli3, J Grosset1, J Sacchettini4 & E Nuermberger1; [1]Johns Hopkins Univ., Baltimore, MD, [2]Texas A&M, College station, TX

Abstract Body:

Background: Bedaquiline (BDQ), a new ATP synthase inhibitor was recently approved for treatment of MDR tuberculosis. A new non-target-based mechanism of cross-resistance to BDQ & clofazimine (CFZ) mediated by mutation of Rv0678 was recently described. For new TB drugs, it is essential to define & catalog resistance-conferring mutations in order to conduct proper follow-up of patients who fail & relapse after treatment, to design diagnostic tests (including rapid molecular tests) & to conduct population level surveillance for changes in susceptibility. Here we present our findings on the selection & characterization of a novel mutations conferring cross-resistance to BDQ and CFZ. **Methods:** Resistant colonies were isolated on BDQ- & CFZ-containing plates from mice receiving BDQ with or without CFZ. MICs were determined on 7H11 agar. Mutation analysis was done by whole genome sequencing & confirmed by PCR. To confirm cross-resistance, BALB/c mice were infected with either the H37Rv parent strain or one resistant mutant (J5) & treated with either isoniazid (10 mg/kg) alone, BDQ (12.5, 25, or 50 mg/kg) alone, CFZ (20 mg/kg) alone, or BDQ 25 mg/kg plus CFZ for 4 wks before determining lung CFU counts. Complementation with the wild type gene was performed & the complemented strain was tested for BDQ & CFZ susceptibility in vitro & in vivo with the parent & mutant strains. The BDQ MIC of parent strain & the mutant was tested in presence of verapamil 40 µg/ml & reserpine 3 µg/ml. **Results:** Mice receiving BDQ alone or BDQ-CFZ harboured mutants. Their MIC for BDQ & CFZ were 0.12-0.25 & 0.5-1 µg/ml, in contrast to the wild type MICs of 0.03 & 0.25 µg/ml respectively. Whole genome sequencing revealed that 4 out of 5 isolates had mutations in *pepQ* (Rv2535c). Efficacy studies in mice with one mutant (J5) showed reduced killing by BDQ & CFZ as compared with wild type H37Rv. Complementation with wild type pepQ restored the susceptibility to BDQ & CFZ in vitro & in vivo. Use of verapamil & reserpine reduced the MIC for the H_{37}Rv parent strains from 0.03 to 0.007 µg/ml & 0.015- 0.03 µg/ml respectively, in addition for the J5 mutant, both the efflux inhibitors reduced the MIC comparable to that of the H37Rv parent strains, indicating a possible role of efflux pumps **Conclusions:** Mutations in *pepQ* gene reduce susceptibility to BDQ & CFZ.

Author Disclosure Block:

D.V. Almeida: None. **T. Ioerger:** None.

Poster Board Number:

MONDAY-079

Publishing Title:

Multi Drug Resistant *Mycobacterium tuberculosis* in Pakistan

Author Block:

R. Khan, S. Khan, U. Mazahir; Dow Univ. of Hlth.Sci., Karachi, Pakistan

Abstract Body:

Introduction: Tuberculosis is the most life threatening air born bacterial infection. According to the WHO about 2 billion people worldwide have been infected by Mycobacterium tuberculosis complex. PCR is recognized as rapid, specific and sensitive diagnostic technique as compared to the conventional methods. TB can be controlled through early diagnosis followed by effective treatment. Drugs Rifampicin and Isoniazid are commonly used for treatment, but their excessive use has led to the emergence of mutations in the genome of MTB. **Objective:** To investigate the Prevalence of mycobacterium tuberculosis and its drug resistance pattern in different areas of Sindh, Pakistan. **Methods:** Total 380 pulmonary and extra pulmonary specimens were collected from different regions of Sindh, Pakistan. Samples were decontaminated using NALC-NaOH protocol followed by DNA extraction. Amplification of extracted MTB DNA was done by real time PCR using primers and fluorescent probes specific for the MTB complex. To check the drug resistant strains, Hybridization of amplified products of positive samples were carried out by MTBDRplus assay. **Results:** The overall prevalence of MTB infection in this study population was 11%. Prevalence of MTB infection among females and males was 10.14% and 12.13% respectively. Highest positive ratio among different clinical specimens was determined in pus (52%) whereas age group 1-20 years was found most susceptible (13.5%). Among positive samples 33.3% were resistant to Rifampicin and 23.3% were resistant to Isoniazid and 6.6% samples were resistant to both the drugs. **Conclusions:** Real-time assay is rapid diagnostic tool, it can be applied to a wide range of clinical specimens including pus which might show negative AFB smear specially in developing countries like Pakistan where the false negative reporting playing crucial role in the spread of TB. In Pakistan prevalence and drug resistance of TB is on rise due to many socio economical and other issues, determination of drug resistance is important for effective treatment particularly in our region where MDR and XDR TB are not very uncommon.

Author Disclosure Block:

R. Khan: None. **S. Khan:** None. **U. Mazahir:** None.

Poster Board Number:

MONDAY-080

Publishing Title:

Drug Resistance Patterns of Mycobacterium Tuberculosis Complex(Mtbc) Among Retreatment Cases Around Jimma Southwest, Ethiopia

Author Block:

K. G. K. W. Abdulsemed; Ji,mma Univ., Jimma, Ethiopia

Abstract Body:

Background: Information on the pattern of drug resistant tuberculosis (TB) among re-treatment cases is crucial to develop appropriate control strategies. Therefore, we conducted this study to assess the drug resistance pattern of M. tuberculosis complex (MTBC) isolates and associated factors among re-treatment cases in Jimma area, Southwest Ethiopia.**Methods:** Health facility-based cross-sectional study was conducted between March 2012 and April 2013 in Jimma area, Southwest Ethiopia. We included 79 re-treatment cases selected conveniently. Socio demographic and clinical data were collected using structured questionnaire. Sputum sample processing, mycobacterial culture, isolation and drug susceptibility testing (DST) were done at Mycobacteriology Research Centre (MRC) of Jimma University. All data were registered and entered in to SPSS version 20. Crude odds ratio (COR) and adjusted odds ratios (AOR) were calculated. P-values less than 0.05 were considered statistically significant.**Results:** Seventy-nine re-treatment cases included in the study; 48 (60.8 %) were males. Forty- seven (59.5 %) study participants were from rural area with the mean age of 31.67 ± 10.02 SD. DST results were available for 70 MTBC isolates. Majority (58.6 % (41/70)) isolates were resistant to at least one of the four first line drugs. The prevalence of multidrug-resistant TB (MDR-TB) was 31.4 % (22/70). Place of residence (AOR = 3.44 (95 % CI: 1.12, 10.60), duration of illness (AOR = 3.00 (95 % CI: 1.17, 10.69) and frequency of prior TB therapy (AOR = 2.99, (95 % CI: 1.01, 8.86) were significant factors for any drug resistance. Moreover, history of treatment failure was found to be associated with MDR-TB (AOR = 3.43 (95 % CI: 1.14, 10.28).**Conclusions:** The overall prevalence of MDR-TB among re-treatment cases around Jimma was high. The rate of MDR-TB was higher in patients with the history of anti-TB treatment failure. Timely identification and referral of patients with the history of treatment failure for culture and DST need to be strengthened.

Author Disclosure Block:

K.G.K.W. Abdulsemed: None.

Poster Board Number:

MONDAY-081

Publishing Title:

A Comparison of Two Multiplex Molecular Methods for Direct Organism Identification from Positive Blood Culture Bottles

Author Block:

J. McCallum, S. Bollinger; Tufts Med. Ctr., Boston, MA

Abstract Body:

Direct molecular testing of positive blood cultures can significantly decrease the time to identification of both the causative organisms of bacteremia and select antimicrobial resistance markers. The rapid availability of this information can lead to more targeted initial antimicrobial treatment and/or more rapid de-escalation of empiric treatment. For the purpose of this study, two nucleic acid-detection based tests were compared: the multiplex PCR Biofire FilmArray BCID assay and the Nanosphere Verigene GP-ID and GN-ID non-amplified assays. 35 gram positive and 30 gram negative clinical samples were tested; results were juried with culture and susceptibility testing utilizing biochemical testing, KB, and the Vitek 2 system. The overall agreement fluctuated by target, but exceeded 96% for all shared targets. Of the samples with comparable results, the Biofire BCID demonstrated increased sensitivity to true positives on 2 occasions. Instances of false positives also occurred on the Biofire, but for targets that were not present on the Nanosphere assay and thus cannot be compared. The Biofire FilmArray BCID provides a shorter run time, requires less hands on time, and is a more comprehensive single panel than the Nanosphere Verigene GP and GN tests, though the Nanosphere Verigene tests are less costly to run, and tests for a significantly greater number of gram-negative resistance mechanisms, and gram-positive species. Overall, both platforms performed similarly in the detection of comparable organisms; differences in panel comprehensiveness and methodologies each have advantages dependent on the lab performing the test.

Author Disclosure Block:

J. McCallum: None. **S. Bollinger:** None.

Poster Board Number:

MONDAY-082

Publishing Title:

Can a Tablet Computer App Ease the Workflow for Advanced Molecular Testing?

Author Block:

L. L. Samson[1], **H. C. Schønheyder**[2], L. B. Pape-Haugaard[1], O. Hejlesen[1]; [1]Aalborg Univ., Aalborg, Denmark, [2]Aalborg Univ. Hosp., Aalborg, Denmark

Abstract Body:

Background: Molecular tests are becoming increasingly important for rapid identification of microbes causing bloodstream infections. MuxBCT is a multiplex FISH test under development aiming to identify multiple microbes at the species or genus level within one hour. The test is performed with a slide comprising 10 separate reaction wells each with up to three significant observations by fluorescence microscopy. We developed a tablet computer app to ease the test workflow, and we evaluated the performance of the prototype and the usability of the app in a clinical laboratory setting.**Methods:** This non-interventional study was performed independently but parallel to the routine work-up of blood culture flasks flagged positive (BACTEC, BD) during the dayshift. Four biotechnicians received 'hands on' training including use of the tablet with the app and fluorescence microscopy. They alternated thereafter for 25 weekdays. A sample was drawn from the first flask flagged positive in each set. The MuxBCT was performed in a manner similar to the commercial *Quick*FISH tests (AdvanDx Inc.). The app guided each step of the reading procedure at the same time recording readings and verifying controls. Results were communicated securely via Wi-Fi from the tablet computer to the laboratory information system (LIS). MuxBCT identification was compared with culture-based identification mainly by use of MALDI-TOF (Bruker).**Results:** 124 positive BCs were included in the study, 109 with one pathogen, 11 with two to four, and 4 with a false positive signal. Median time to read and record the test was 2.8 min; (median turn-around time was 74.2 min). For comparison, the median time for Gram stain results was 54.4 min, which provided less specific isolate information. Concordance of MuxBCT results with culture-based identification was 92.7% (115/124) with a PPV of 98.9% (89/90) and a NPV of 76.5% (26/34).**Conclusions:** The app supported biotechnicians in the performance of the MuxBCT prototype to obtain accurate microbe identification results that were communicated directly from the tablet app to the LIS.

Author Disclosure Block:

L.L. Samson: I. Research Relationship; Self; Innovation Fund Denmark. **H.C. Schønheyder:** I. Research Relationship; Self; Innovation Fund Denmark. **L.B. Pape-Haugaard:** I. Research Relationship; Self; Innovation Fund Denmark. **O. Hejlesen:** I. Research Relationship; Self; Innovation Fund Denmark.

Poster Board Number:

MONDAY-083

Publishing Title:

Evaluation of the Portrait Staph ID-R Blood Culture Panel in Pediatric and Adult Patient Populations

Author Block:

A. Hopper[1], M. V. Powers-Fletcher[2], S. Holt[1], A. Phillips[1], R. Grand-Pre[1], M. Dickie[1], A. Blaschke[3], M. R. Couturier[2], J. A. Daly[1]; [1]Primary Children's Med. Ctr., Salt Lake City, UT, [2]Univ. of Utah, Salt Lake City, UT, [3]U of U / Primary Children's Hosp., Salt Lake City, UT

Abstract Body:

Bloodstream infections (BSI) are associated with significant morbidity and mortality. The most common BSI pathogens are *Staphylococcus* species such as *S. aureus* and *S. epidermidis*, both which can harbor the gene (*mecA*) encoding methicillin resistance. The ability to rapidly identify these organisms is critical for patient care, particularly the choice of antibiotic therapy. The purpose of this study is to determine performance of a novel molecular diagnostic assay in identifying *Staphylococcus* spp., with or without *mecA*, from blood cultures compared to traditional methods.Blood samples collected from adult and pediatric patients were cultured and processed according to standard practice. Blood culture bottles positive for gram positive cocci were then stored up to 72 hours at 2-8°C, after which an aliquot of broth was tested using the Portrait Staph ID/R Blood Culture Panel (Great Basin Diagnostics, Utah). Results were compared to those obtained using either the BD Phoenix™ PID panel or MALDI-TOF MS for identification and cefoxitin disk diffusion for prediction of methicillin resistance.During the study period, 150 blood cultures were tested using the Portrait Staph ID/R panel; 115 of those were positive for staphylococci. Out of that total, 37 were *S. aureus* and 78 were a Coagulase negative *Staphylococcus*. The Staph ID/R panel performed with high sensitivity (99.1%) and specificity (96.3%) compared to BD Phoenix™ or MALDI-TOF MS for identification of *Staphylococcus* spp., with 100% accuracy for the identification of *S. aureus.* The Staph ID/R panel was able to predict methicillin resistance with 95.2% sensitivity and 94.3% specificity compared to cefoxitin disk diffusion for all *Staphylococcus* spp. combined, and 100% sensitivity and specificity for the detection of methicillin-resistant *S. aureus*.The Portrait Staph ID/R panel performs with high sensitivity and specificity for the identification of *Staphylococcus* spp. and detection of *mecA* compared to current laboratory methods. Implementation of this methodology in clinical laboratories could allow for accurate and more rapid identification of BSI caused by methicillin-resistant staphylococci and improve patient care.

Author Disclosure Block:

A. Hopper: None. **M.V. Powers-Fletcher:** None. **S. Holt:** None. **A. Phillips:** None. **R. Grand-Pre:** None. **M. Dickie:** None. **A. Blaschke:** None. **M.R. Couturier:** None. **J.A. Daly:** None.

Poster Board Number:

MONDAY-084

Publishing Title:

Diagnostic Procedures Associated with Making a Diagnosis of *Propionibacterium acnes* Infective Endocarditis

Author Block:

J. M. Banzon, S. J. Rehm, S. M. Gordon, S. B. Hussain, G. B. Pettersson, N. K. Shrestha; Cleveland Clinic, Cleveland, OH

Abstract Body:

Background: *Propionibacterium acnes* is a slow-growing bacterium that is a rare cause of infective endocarditis (IE), and it may be underdiagnosed. It seems likely that diagnostic procedures employed might influence the ability to make a diagnosis of *P. acnes* IE. The aim of this study was to identify diagnostic procedures that are associated with making a diagnosis of *P. acnes* IE. **Methods:** Episodes of definite IE by Duke Criteria from 2007 to 2015 were identified from the Cleveland Clinic Infective Endocarditis Registry. 1084 episodes with an identified causative microorganism other than *P. acnes* were excluded. The remaining episodes were classified as *P. acnes* IE or IE with no identified pathogen. Diagnostic procedures examined were number of blood cultures done, extended incubation of blood cultures (beyond the standard 5 days), valve culture done, and valve microbial sequencing done. A multivariable logistic regression model was constructed, including the four diagnostic procedures, and all other factors associated with *P. acnes* IE at a significance level of 0.2. The model was reduced using stepwise backward elimination until only factors that were significant at a level of 0.05 remained in the model. **Results:** We identified 23 episodes of *P. acnes* IE and 49 episodes of IE with no identified pathogen, each in a unique patient. Almost all patients with *P. acnes* IE were male and almost all had prosthetic valve endocarditis (PVE). Factors associated with *P. acnes* IE were male sex (96% vs. 67%, p=0.008), PVE (96% vs. 71%, p=0.03), invasive disease (78% vs. 49%, p=0.02), extended incubation of blood cultures (30% vs. 10%, p=0.04), and valve sequencing (87% vs. 37%, p<0.001). Number of blood cultures (4 vs. 4 sets, p=0.88), and valve culture (91% vs. 76%, p=0.2) were not associated with a diagnosis of *P. acnes* IE. In the reduced multivariable model, the diagnostic procedures of extended incubation of blood cultures (OR 16.1, 95% CI 2.40-191.01, p=0.01) and valve sequencing (OR 15.8, 95% CI 3.5- 126.5, p=0.002) were independently associated with *P. acnes* IE. **Conclusion:** In patients with possible *P. acnes* IE (i.e. those with IE without another identified pathogen), extended incubation of blood cultures and microbial sequencing of excised heart valves are strongly associated with identifying *P. acnes* as the cause of IE.

Author Disclosure Block:

J.M. Banzon: None. **S.J. Rehm:** None. **S.M. Gordon:** None. **S.B. Hussain:** None. **G.B. Pettersson:** None. **N.K. Shrestha:** None.

Poster Board Number:

MONDAY-085

Publishing Title:

Multicenter Evaluation Of The Miacom Hemofish For Bacterial Identification From Positive Blood Cultures

Author Block:

M. L. Faron[1], C. Coon[1], B. W. Buchan[1], M. Guralnik[2], V. J. LaBombardi[2], N. A. Ledeboer[1]; [1]Med. Coll. of Wisconsin, Milwaukee, WI, [2]New York-Presbyterian Queens, New York, NY

Abstract Body:

Background: Bloodstream infections are a life-threatening condition that can lead to mortality in 25-80% of infected patients. Early identification and administration of appropriate antibiotics can greatly reduce mortality rate as studies have shown that improper treatment is associated with significantly longer hospital stays and a 33% increase in mortality. In this study we evaluated the HemoFISH assay (Miacom Diagnostics, Dusseldorf, Germany), to identify organism from positive blood culture bottles.**Methods:** Positive VersaTREK™ blood culture bottles were collected and enrolled at two different sites. Enrolled positive bloods were Gram stained and analyzed in the HemoFISH assay according to the package insert. The laboratory standard of care (SOC), which included Verigene BC-GN and BC-GP (Nanosphere, Northbrook, IL), MALDI Tof MS, BD Phoenix (Becton Dickinson, NJ) or Vitek2 (bioMerieux, Marcy l'Etoile, France) was collected and used as a comparator assay. Isolates identified by the SOC were compared to the HemoFISH result and concordance for each target was determined.**Results:** In total, 543 monomicrobial blood specimens were enrolled within the study. The most commonly identified organisms were Coagulase Negative Staphylococcus (CoNS) 138 (25%), E. coli, 80 (15%) and S. aureus, 62 (11%). Total agreement for each target was high ranging from 99.3-100%. Positive and negative agreement for each target ranged from 96.8-100% and 99.6-100% respectively. 6 specimens were incorrectly identified by the HemoFISH, 1 correctly identified to the genus level. The remaining 5 discordant results consisted of *M. luteus* called *Staph*, *E.faecalis* called *Streptococcus*, *B. distasonis* called *E. coli*, *Acinetobacter* spp called *Enterobacteriaceae* and an *E. coli* called *Streptococcus* by the HemoFISH assay.**Conclusions:** The HemoFISH assay is an accurate test for identification of bacteria from positive blood culture bottles giving results in 30 minutes. The use of 8 wells containing two unique probes per well allows the assay to detect 14 of the most commonly identified organisms from the blood stream. Early Identification can greatly impact patient outcomes as organism ID coupled with hospitals antibiogram can guide therapy.

Author Disclosure Block:

M.L. Faron: None. **C. Coon:** None. **B.W. Buchan:** None. **M. Guralnik:** None. **V.J. LaBombardi:** None. **N.A. Ledeboer:** None.

Poster Board Number:

MONDAY-086

Publishing Title:

Multi-Center Evaluation of the Portrait Staph Id/R Blood Culture Panel

Author Block:

G. A. Denys[1], S. Young[2], J. Daly[3], M. Couturier[4], B. Buchan[5], N. Ledeboer[5]; [1]Indiana Univ. Sch. of Med., Indianapolis, IN, [2]Univ. of New Mexico/Tricore Labs, Albuquerque, NM, [3]Univ. Of Utah/Primary Children's Med. Ctr., Salt Lake City, UT, [4]Univ. of Utah/ARUP Labs, Salt Lake City, UT, [5]Med. Coll. of Wisconsin, Milwaukee, WI

Abstract Body:

Background: Blood stream infections (BSI) are a leading cause of morbidity and mortality in the United States. For hospitalized patients, MRSA BSI are associated with increased healthcare cost. The Portrait Staph ID/R Blood Culture Panel from Great Basin Scientific (Salt Lake City, UT) is a rapid, automated, DNA multiplex PCR assay performed on the Portrait Dx Analyzer for simultaneous identification (ID) of *Staphylococcus aureus, S epidermidis, S. lugdunensis* and *Staphylococcus* spp. and the detection of *mec*A gene directly from positive blood culture bottle. We evaluated the performance of the Portrait Staph ID/R compared to standard reference methods. **Methods:** A total of 723 positive blood culture bottles (BD BACTEC^{TM}) yielding Gram positive cocci (GPCC) in clusters were analyzed at 3 clinical sites using the Portrait Dx System. Aliquots of positive blood cultures were sent to a reference site for analysis. Reference ID methods included catalase and coagulase tests, BD Phoenix instrument, and MALDI-TOF MS. Detection of methicillin resistance was tested by cefoxitin disk diffusion according to CLSI. Discordant resolution of *Staphylococcus* ID and *mec*A detection was performed on colonies by *rpoB* gene sequencing and *mec*A gene sequencing, respectively. **Results:** Of the 723 positive blood culture bottles with GPCC, 647 had *Staphylococcus* spp. (*S. aureus* 209, *S. epidermidis* 236, *S. lugdunensis* 3, and *Staphylococcus* spp.155) Overall *Staphylococcus* ID agreement was 99.69% PPA and 94.74% NPA. Of the 6 false positive results by Portrait Staph ID/R, 3 were no growth and 3 were positive for *Staphylococcus* by sequencing. One false negative *Staphylococcus* by Portrait Staph ID/R was non-*Staphylococcus* by sequencing. The overall agreement for detection of *mec*A was 94.75% PPA and 94.70% NPA. **Conclusion:** The performance characteristics of the Portrait Staph ID/R Blood Culture panel compared favorably to reference methods. This multiplex amplification assay provides valuable information beyond the initial Gram stain in less than 2 hrs. of testing. Accurate and rapid organism ID and resistance mechanism could have a positive impact on patient management.

Author Disclosure Block:

G.A. Denys: F. Investigator; Self; Great Basin Scientific. **S. Young:** F. Investigator; Self; Great Basin Scientific. **J. Daly:** F. Investigator; Self; Great Basin Scientific. **M. Couturier:** F.

Investigator; Self; Great Basin Scientific. **B. Buchan:** F. Investigator; Self; Great Basin Scientific. **N. Ledeboer:** F. Investigator; Self; Great Basin Scientific.

Poster Board Number:

MONDAY-087

Publishing Title:

Evaluation Of Bactalert Fa Blood Culture Bottles And T2candida Assay For Detection Of *candida* in The Presence Of Antifungals

Author Block:

N. D. Beyda[1], J. Amadio[1], J. R. Rodriguez[2], K. Malinowski[2], K. W. Garey[1], A. Wanger[2], L. Ostrosky-Zeichner[2]; [1]Univ. of Houston Coll. of Pharmacy, Houston, TX, [2]UTHlth., Houston, TX

Abstract Body:

Objectives: Blood culture is the gold standard for diagnosing candidemia. Antifungal therapy has been shown to negatively impact the performance of some blood culture systems and other serodiagnostics. The objective of this study was to determine the effect of fluconazole (FLU) and caspofungin (CAS) on the performance of the T2Candida assay (T2CA) when compared to BacT/Alert FA blood culture bottles (BCX).**Methods:** Simulated candidemia samples were prepared by inoculating human whole blood with clinical bloodstream isolates of *C. albicans, C. glabrata, C. tropicalis, C. parapsilosis,* and *C. krusei* at 50 CFU/mL and 5 CFU/mL. CAS (8 ug/mL), FLU (20 ug/mL), or 0.85% NS (antifungal negative control) where then added at concentrations achieved after therapeutic doses. Samples were inoculated into either BCX bottles and incubated for up to 5 days or into K2 EDTA tubes for testing by T2CA. The proportion of samples detected by BCX and T2CA was compared. The impact of antifungals on the time to positivity (TTP) of BCX was also assessed.**Results:** A total of 240 simulated candidemia samples were tested by each method (n=8 for each species, CFU/mL, and antifungal). In the absence of antifungals, overall detection rate for all species combined was 100% for both methods at 50 CFU/mL. At 5 CFU/mL it was 97.5% and 95% for BCX and T2CA respectively (*C. parapsilosis* not detected in 1 sample for BCX; *C. glabrata* and *C. krusei* not detected in 1 sample each for T2CA). For BCX, presence of FLU reduced overall detection rates to 92.5% and 85% at 50 and 5 CFU/mL respectively, while CAS had no impact. CAS or FLU had no impact on T2CA results at either CFU/mL concentration. Compared to no-antifungal controls, presence of CAS and FLU led to a significant increase in TTP of BCX for most species, particularly for *C. albicans* at 5 CFU/mL (increase of 3.1 ± 1.3 and 66.8 ± 13.4 hrs respectively; $p<0.01$).**Conclusions:** CAS and FLU did not impact the performance of the T2CA. FLU reduced the detection rate of Candida in BCX, and both FLU and CAS prolonged TTP. These results suggest T2CA is reliable and can be used for the detection and management of candidemia in patients already receiving antifungals.

Author Disclosure Block:

N.D. Beyda: E. Grant Investigator; Self; T2 Biosystems. **J. Amadio:** None. **J.R. Rodriguez:** None. **K. Malinowski:** None. **K.W. Garey:** F. Investigator; Self; T2 Biosystems. J. Scientific

Advisor (Review Panel or Advisory Committee); Self; T2 Biosystems. **A. Wanger:** E. Grant Investigator; Self; T2 Biosystems. **L. Ostrosky-Zeichner:** E. Grant Investigator; Self; T2 Biosystems, Immunetics, Merck, Astellas, Pfizer, Scynexis, Meiji. J. Scientific Advisor (Review Panel or Advisory Committee); Self; Astellas, Merck, Scynexis, Cidara. L. Speaker's Bureau; Self; Merck, Pfizer, T2 Biosystems.

Poster Board Number:

MONDAY-088

Publishing Title:

Development of a Rapid Diagnostic Method for *Staphylococcus aureus* and Antimicrobial Resistance in Positive Blood Culture Bottles Using a Pcr-Dna-Chromatography Method

Author Block:

T. Ohshiro[1], C. Miyagi[1], Y. Tamaki[1], T. Ezaki[2]; [1]Naha City Hosp., Naha, Japan, [2]Graduate school of Med. Gifu univ., Gifu, Japan

Abstract Body:

Background: When gram-positive coccoid cells are observed in blood culture bottles, it is important to determine whether the strain is *Staphylococcus aureus* and whether it has resistance genes for antibiotic selection. We developed a rapid and simple PCR-DNA-Chromatography method (PDC) based on the single tag hybridization chromatographic printed array strip method for identifying *S. aureus* and for concurrently detecting the *mecA* and *blaZ* genes in positive blood culture bottles.**Methods:** The principle of PDC is to detect the tagged PCR amplicons bound to the anti-tag printed on the strip. It is possible to detect multiple PCR amplicons simultaneously. We evaluated PDC using 42 clinical materials. Sample preparation method from positive BACTEC FX (BD) blood culture was processed using the centrifugal washing method. Cell wall synthesis gene, *ftsY*, of *S. aureus* and *mecA*, *blaZ* was selected for PCR. After completion of the PCR, treptavidin-coated blue latex was added to the PCR tube and a DNA-strip (Tohoku Bio-Array, Sendai, Japan) was placed in the tube. The results can be read 10 min. These series of operations was completed within 1 h. The results by PDC were compared with those by the conventional methods.**Results:** Of the 33 strains identified as *S. aureus* by the culture method, 32 (97.0%) were identified as *S. aureus* by the PDC. One *S. aureus* strain was misidentified as being negative for *ftsY*, but it was identified as *S. aureus* by the re-examination from the strain. Of the 33 cases identified as *S. aureus* by the culture method, 12 cases were both oxacillin resistant and *mecA* positive. Only one case among 21 MSSA cases was *mecA* positive. It was speculated that no protein was expressed from it. Of the 21 cases identified as MSSA by the culture method, 12 cases were positive for both β-lactamase and the *blaZ* gene. Only one case among the 9 β-lactamase-negative cases was positive for the *blaZ* gene by the PDC. Sensitivity and specificity of PDC for identifying *S. aureus*, *mecA*, *blaZ* were 97.0% and 100%; 100% and 95.2%; 100% and 88.9% respectively.**Conclusions:** The PDC is very rapid and simple, results can be obtained within 1 h from positive blood culture bottles, it may be useful for making rapid diagnoses even in places with a limited testing environment such as community hospitals.

Author Disclosure Block:

T. Ohshiro: None. **C. Miyagi:** None. **Y. Tamaki:** None. **T. Ezaki:** None.

Poster Board Number:

MONDAY-089

Publishing Title:

Evaluation of Rapid Diagnostic Technology and Pharmacist Notification in Gram-negative Bloodstream Infections Using Time Dependent Variables

Author Block:

A. Gibble[1], S. L. Revolinski[1], **A. M. Huang**[1], N. A. Ledeboer[2], M. Graham[2], J. N. Wainaina[2], L. S. Munoz-Price[1]; [1]Froedtert & the Med. Coll. of Wisconsin, Milwaukee, WI, [2]Med. Coll. of Wisconsin, Milwaukee, WI

Abstract Body:

Background: Previous analyses showed associations between Nanosphere Verigene® Gram-Negative Blood-Culture (BC-GN) coupled with pharmacist (RPh) notification and both effective (EFTx) and optimal antimicrobial therapy (OPTx). We now aim to evaluate the impact of time on these associations by using time dependent variables and Cox proportional hazards.**Methods:** This interventional study evaluated patients with Gram-negative bloodstream infections (GN BSI). During the baseline phase (12/01/2012 - 06/30/2013) positive blood cultures were identified using traditional methods and reported directly to physicians. During the intervention phase (12/01/2014 - 06/30/2015) positive cultures were identified by BC-GN and reported to the RPh. OPTx was targeted antimicrobial therapy after de-escalation and discontinuation of unnecessary agents. EFTx was therapy deemed sensitive per culture and susceptibility report. Cox proportional hazards were used in order to evaluate the effect of exposures on time-to-OPTx or time-to-EFtx. Time zero was time of first positive blood culture draw. Patients never receiving OPTx or EFTx were censored. Exposure variables evaluated were: time dependent covariate, phase (coded as binary), age, gender, Charlson Comorbidity Index, comorbidities, and ID consult. The time dependent covariate changed through the time of observation based on both time-to-notification, and either time-to-OPTx or time-to-EFTx.**Results:** 244 GN BSIs were analyzed: 127 (52.3%) in the baseline phase and 116 (47.7%) in the intervention phase. Time-to-OPTx was associated with the time dependent covariate (adjusted hazards ratio [aHR]: 2.334; 95% CI: 1.347-4.044; p=0.003), the intervention phase (aHR: 1.599; 95% CI: 1.174-2.177; p=0.003), age greater than 60 years (aHR: 0.740; 95% CI: 0.551-0.994; p=0.046) and an ID consult (aHR: 1.430; 95% CI: 1.078-1.897; p=0.013). Time-to-EFTx was associated with the intervention phase (aHR: 1.478; 95% CI: 1.116-1.958; p=0.006) and Charlson Comorbidity Index (aHR: 0.931 [per single digit increment]; 95% CI: 0.882-0.983; p=0.010).**Conclusions:** BC-GN technology and RPh notification was found to be associated with time-to-OPTx and time-to-EFtx.

Author Disclosure Block:

A. Gibble: None. **S.L. Revolinski:** N. Other; Self; travel grant from Nanosphere to ECCMID 2015. **A.M. Huang:** None. **N.A. Ledeboer:** C. Consultant; Self; Nanosphere. E. Grant Investigator; Self; Nanosphere. **M. Graham:** None. **J.N. Wainaina:** None. **L.S. Munoz-Price:** None.

Poster Board Number:

MONDAY-090

Publishing Title:

Identification of Beta Haemolytic *Streptococci*, Alpha-Hemolytic *Streptococci* and *Enterococci* Directly From Positive Blood Culture Bottles

Author Block:

M. Kedfors Holm, P. Lüthje, M. Ullberg, **V. Ozenci**; Karolinska, Stockholm, Sweden

Abstract Body:

Background: Bloodstream infections (BSI) caused by streptococci and enterococci are related to high morbidity and mortality. Identification of these microorganisms directly from blood culture (BC) bottles can facilitate early effective treatment of the patients. The aim of this study was to evaluate the performance of agglutination tests in identification of streptococci and enterococci directly from positive BC bottles. Methods: BacT/ALERT-FA and -FN Plus BC bottles were used for blood cultures in the BacT/ALERT 3D (bioMérieux, Durham, NC, USA) system. The agglutination tests for A, B, C, D and G (Phadebact® Streptococcus Tests, Sollentuna, Sweden) were performed on each 1 drop of BC broth from the positive bottle. The results were read after 1 min and analyzed by two laboratory technicians independently. Conventional methods were used as reference. Results: A total of 100 BC bottles that flagged positive with gram-positive bacteria in chains or pairs were studied. The agglutination assays could correctly identify 16/16 group A streptococci (GAS), 17/17 group B streptococci (GBS), 6/6 group C streptococci (GCS), 9/9 group G streptococci (GGS). The agglutination tests were positive for D in 25/32 enterococci. In 2/32 BC bottles with Enterococcus spp. the tests reacted positive for C and negative in the remaining 5 isolates. The agglutination tests were negative for A, C, G, B, and D in 16/18 alpha-hemolytic streptococci and enabled the preliminary identification of these isolates. In 2/18 alpha-hemolytic streptococci the tests were positive for C. In BC bottles with Aerococcus and Lactococcus spp. (one each) the agglutination tests were negative. The test results were available 15 min after blood culture positivity. Conclusions: The present study shows that the agglutinations tests are reliable in preliminary identification of streptococci and enterococci directly from positive BC bottles. The present approach does not require costly instrumentation and can easily be implemented in the clinical routine in large as well as small laboratories. A faster response to clinicians might support earlier administration of appropriate antimicrobial treatment for patients with BSI.

Author Disclosure Block:

M. Kedfors Holm: None. **P. Lüthje:** None. **M. Ullberg:** None. **V. Ozenci:** None.

Poster Board Number:

MONDAY-091

Publishing Title:

A Retrospective Comparison of Blood Culture Positivity Rates Between Blood Culture Systems

Author Block:

B. Williams, A. Sweeney, A. Kerr, P. Gilligan; Univ. of North Carolina Hosp., Chapel Hill, NC

Abstract Body:

Background: In July 2014, the CMIL at UNC Hospitals switched from BacT/ALERT (bioMerieux, Inc., Durham, NC) to the BACTEC FX (BD Diagnostics, Sparks, MD) for its primary blood culture system. The bottles used on BacT/ALERT system were FAN Aerobic, Standard Anaerobic and Pediatric FAN. For the BACTEC FX system, Plus Aerobic/F and Lytic/10 Anaerobic/F bottles were used. After implementing the BACTEC FX system, a higher rate of positive blood cultures was observed. This study is a retrospective data analysis of these positivity rates comparing the BacT/ALERT system with those of the current BACTEC FX system.**Methods:** Blood cultures from February 2013 - June 2014 (BacT/ALERT) and August 2014 - December 2015 (BACTEC FX) were used to determine the positivity rate. This rate was calculated by dividing the number of positive blood cultures by the total number of blood cultures. Both true positive and contamination rates were compared.**Results:** Using the BacT/Alert, 2,935 (6.8%) of 43,321 blood cultures were positive. Using the BACTEC FX, 4,001 (9.1%) of 43,762 blood cultures were positive. These data indicate a significant increase of 33.8% ($P < 0.001$) positive cultures using the BACTEC FX system. Staphylococcus aureus was isolated in 431 (BacT/Alert) and 1049 (BACTEC FX) cultures, an increase of 141% ($p < 0.01$). *Enterobacteriaceae* were isolated in 627 (BacT/Alert) and 814 (BACTEC FX) cultures, an increase of 35.7% ($p < 0.01$). *Enterococcus sp*. were isolated in 212 (BacT/Alert) and 289 (BACTEC FX) cultures, an increase of 40% ($p < 0.01$). Anaerobic organisms were isolated in 109 (BacT/Alert) and 132 (BACTEC FX) cultures ($p=0.08$). Isolation of *Candida sp*. was relatively unchanged, 213 (BacT/Alert) and 218 (BACTEC FX) cultures (p 0.45). *Streptococcus pneumoniae* isolation was also relatively unchanged, with 39 isolates by each system (p 0.48). Organisms indicating contamination were detected in 610 (BacT/ALERT) and 845 (BACTEC FX) cultures, an increase of 37% ($p < 0.01$).**Conclusions:** Conversion to the BACTEC FX system has resulted in increased blood culture positivity rates especially for *S. aureus*, *Enterococcus sp.* and *Enterobacteriaceae*. An increase in contamination rate was also observed with the BACTEC FX system which is likely due to the overall increase in isolation of coagulase negative staphylococci.

Author Disclosure Block:

B. Williams: None. **A. Sweeney:** None. **A. Kerr:** None. **P. Gilligan:** None.

Poster Board Number:

MONDAY-092

Publishing Title:

***Klebsiella pneumoniae* Volatile Metabolites Produced During Growth in Human Blood**

Author Block:

C. A. Rees[1], A. Smolinska[2], J. E. Hill[1]; [1]Dartmouth Coll., Hanover, NH, [2]Maastricht Univ., Maastricht, Netherlands

Abstract Body:

Background: *Klebsiella pneumoniae* bacteremia is an important cause of morbidity and mortality. Present diagnostic modalities require culturing the agent from blood samples, a time-consuming process that lacks sensitivity. A more rapid and sensitive diagnostic could improve patient outcomes by expediting time-to-diagnosis. Here, we show that *K. pneumoniae* produces a suite of volatile metabolites not normally present in sterile blood that may represent putative diagnostic biomarkers that can be sampled directly from blood or, via partitioning, patient breath. **Methods:** *K. pneumoniae* ATCC 13883 was incubated in human blood for 7 and 12 hours, corresponding approximately to mid-exponential and early stationary growth phases. Cultures were transferred to air-tight vials, and volatile metabolites were analyzed using GCxGC-TOFMS. The Mann-Whitney U test with Benjamini-Hochberg correction was used to identify volatiles that differed in relative abundance ($p < 0.05$) between cultures and sterile blood.**Results:** 34 volatile metabolites were identified that differed significantly in abundance between cultures and sterile blood controls. Of these, 22 were not detected in sterile blood, and likely represent the consequence of bacterial metabolism. For example, 3-hydroxy-2-butanone and 2,3-butanedione were detected only in cultures. These are known bacterial metabolites of pyruvate that have not previously been measured in the headspace of healthy blood samples [1]. 11 compounds were identified that have never previously been associated with *K. pneumoniae* metabolism. 3 significant volatiles remained detectable in cultures diluted in saline to a cell density of approximately 1×10^3 CFU/mL.**Conclusion:** Volatile metabolites can reliably differentiate *K. pneumoniae*-inoculated human blood samples from sterile blood. A subset of these metabolites remain detectable after dilution of cultures in saline to a cell density of approximately 1×10^3 CFU/mL. We hypothesize that further method optimization could reduce the limit of detection to bacterial concentrations seen in clinical bacteremia. Volatile biomarkers produced by *K. pneumoniae* in the setting of bacteremia could inform the development of a rapid, minimally-invasive breath- or blood-based diagnostic, potentially reducing time-to-diagnosis, thereby improving outcomes.

Author Disclosure Block:

C.A. Rees: None. **A. Smolinska:** None. **J.E. Hill:** None.

Poster Board Number:

MONDAY-093

Publishing Title:

Clinical Evaluation of a Dna-based Microarray Platform in Identification of Microorganisms from Positive Blood Culture Bottles

Author Block:

M. Ullberg, e. beste, P. Luthje, M. Melaku, A. Ohlsson, **V. Ozenci**; Karolinska, Stockholm, Sweden

Abstract Body:

Background: The Prove-it sepsis assay (Mobidiag, Helsinki, Finland) is a broad-range PCR followed by specific identification on a microarray. The new, improved CE-IVD version of Prove-it assay covers 60 bacteria, 13 fungi and the *mecA* methicillin resistance marker. We evaluated the performance of the Prove-it sepsis assay in identification of bacteria and yeasts from positive BC bottles prospectively. **Methods:** The microorganisms were identified by Prove-it using 0.5 ml broth from positive blood culture bottles. BacT/ALERT-FA and -FN BC bottles and the BacT/ALERT 3D BC system were used in the study. Standard culture-based methods served as reference. **Results:** A total of 213 positive BC samples were tested. Only one blood culture specimen per patient was included. The Prove-it sepsis assay repertoire covered all present microorganisms in 195/213 (91.6%) BC bottles. Among these 195 samples, Prove-it could identify 180/195 (92.3%) microorganisms correctly. In 2/195 (1%) BC bottles microorganisms were misidentified, one *K. pneumonia* as *E. coli* and one *S. aureus* as *S. epidermidis*. In one sample positive for *S. aureus* and 14 samples with coagulase-negative staphylococci, the assay detected the *mecA* gene.[PL1] Among 4 polymicrobial samples, Prove-it could identify all microorganisms in 3/4 BC bottles. In the remaining sample that contained *C. glabrata* and *C. albicans*, the assay could identify only *C. glabrata*. Overall sensitivity of the Prove-it sepsis assay was 93.26% (95% CI: 88.76%-96.37%). [PL2] Overall specificity was not analyzed. When method accuracy was evaluated for subgroups of gram-negative and gram-positive bacteria, sensitivity and specificity values were 92.37% (95% CI: 86,01%-96,45%) and 98.7% (95% CI: 92.98%-99.97%) respectively for gram-positives, and 95.65% (95% CI: 87.82%-99.09%) and 99.21% (95%CI: 95.66%-99.98%) respectively for gram-negatives. **Conclusions:** The total procedure for the Prove-it assay took 4 hours. In contrast, results by standard methods were obtained after 24-48 hours. The present prospective study shows that the Prove-it sepsis assay has high performance in identification of microorganisms directly from BC bottles. [PL1]Was this confrimed by standard techniques? *mecA* gene which was confirmed by standard methods [PL2]Overall specificity? Negative bottles were not tested.

Author Disclosure Block:

M. Ullberg: None. **E. beste:** None. **P. Luthje:** None. **M. Melaku:** None. **A. Ohlsson:** None. **V. Ozenci:** None.

Poster Board Number:

MONDAY-094

Publishing Title:

A Cost-Effective Dual Platform Algorithm For Rapid Molecular Detection Of Organisms In Positive Blood Culture Bottles

Author Block:

S. Collier, P. C. Schreckenberger, R. Golash, V. Rekasius; Loyola Univ. Med. Ctr., Maywood, IL

Abstract Body:

Background: In an effort to reduce the financial burden of rapid pathogen identification in blood culture samples, our laboratory adopted a testing algorithm based on the Gram stain of positive blood culture bottles by utilizing either the Xpert® BC MRSA/SA Panel (Xpert BC)[Cepheid, Sunnyvale, CA] or the FilmArray® Blood Culture Identification Panel (BCID) [Biofire Diagnostics, Salt Lake City, UT].**Methods:** A retrospective study was conducted using 505 positive blood culture samples collected between May 2015 and October 2015 (154 days). Upon signal from blood culture instrument (BACTEC™ FX), positive blood culture bottles were removed and Gram stain performed. Specimens with Gram stains showing gram-positive cocci in clusters were tested using the Xpert BC (n= 229). Specimens with Gram stains indicating yeast, multiple morphologies, or bacteria other than GPC-CL were tested using BCID (n= 276). Results were compared to traditional culture-based ID methods. Results were evaluated for Gram stain and/or instrument errors.**Results:** Of 505 blood cultures evaluated by Gram stain, 276 were reflexed to BCID (54.6%) and 229 were reflexed to Xpert BC (45.3%) for ID. There were 21 MRSA, 31 MSSA, and 177 reports of no targets detected (NTD) with Xpert BC. Of the 177 NTD instances, 173 grew Coag. Neg. *Staph* (*CoNS)* or micrococci only and 4 (1.7%) grew other organisms indicating that the Xpert BC was set up inappropriately (see Table). In 7 instances BCID was set up when only *S. aureus* (1) or CoNS (6) grew in culture (see Table). In 2 instances the Xpert BC was down for repair necessitating the use of BCID, leaving 5 (1.8%) instances where BCID was set up inappropriately. The difference in panel cost for the two systems is $68.33/panel. By reflexing 229 positive blood cultures to Xpert BC (at lower cost) instead of BCID we saved $15,647.57 in reagent cost over 154 days, extrapolated to an annual savings of $37,086.77.**Conclusion:** Based on the low Gram stain reading error rate (< 2%), the dual platform approach provides savings to the laboratory without compromising patient care.

Blood Culture Results		
Gram Stain	No. Cultures with Growth of *S. aureus/Micrococcus*/CoNS only	No. Cultures with Growth of Other Organisms or NTD
GPC-CL Run on Xpert BC (n=229)	225	4

Non GPC-CL Run on BCID (n=276)	7	269

Author Disclosure Block:

S. Collier: None. **P.C. Schreckenberger:** F. Investigator; Self; Accerlerate Dx, Beckman Coulter, BioFire, Cepheid. J. Scientific Advisor (Review Panel or Advisory Committee); Self; Actavis, BioFire, Cempra, Cepheid, GenMark, Quidel, Thermo Fisher. L. Speaker's Bureau; Self; Accelerate Dx, Beckman Coulter, BioFire, Cepheid, Hardy Diagnostics, Merck, Thermo Fisher. **R. Golash:** None. **V. Rekasius:** None.

Poster Board Number:

MONDAY-095

Publishing Title:

Laboratory and Clinical Impact of Rapid Identification of Gram Negative Bacteremia in a Cancer Patient Population Using the Gram-Negative *quick*fish™ bc Kit

Author Block:

D. Cianciminio-Bordelon, A. Quianio, K. Gilhuley, A. Paskovaty, N. Cohen, Y. Tang, S. Seo, N. Babady; Mem. Sloan Kettering Cancer Ctr., New York, NY

Abstract Body:

Background: Bacteremia caused by Gram negative rods (GNR) can be life threatening for immunocompromised, neutropenic cancer patients. Early administration of targeted antibiotic therapy is ideal. Rapid identification is therefore critical to improving patients' outcomes. The Gram-Negative *Quick*FISH™ peptide nucleic acid fluorescence in-situ hybridization (qPNA FISH) allows for identification of *E. coli*, *K. pneumoniae* and *P. aeruginosa* directly from blood culture within 30 minutes after Gram stain. We evaluated the diagnostic performance of qPNA FISH and determined the laboratory and clinical utility of the assay for patients with GNR bacteremia.**Methods:** Prior to implementation, the qPNA FISH performance characteristics were determined using both seeded blood culture bottles (for analytical sensitivity and specificity studies) and 250 consecutive blood cultures positive for GNR collected from 113 patients (for accuracy studies). Post-implementation, the sensitivity, specificity, turn-around time (TAT) to identification from receipt of culture in the laboratory, and the intervention by the antibiotic stewardship team (AST) was monitored for three months. **Results:** The overall sensitivity and specificity was 100% for all seeded bottles. For the 250 patient samples, the overall sensitivity and specificity was 100% and 93%, respectively. Post-implementation, 112 positive GNR blood cultures were tested, the sensitivity was 91% and specificity was 97%. Time to identification decreased by ~28 hours following implementation of qPNA FISH. Post-implementation, 70 patients qPNA FISH result were reported to the ordering physician and the AST simultaneously. Earlier intervention, including de-escalation and escalation of therapy, occurred in 15% and 7.5% of analyzed cases, respectively. **Conclusions:**qPNA FISH offers increased sensitivity, specificity, and faster TAT for the three most common Gram negative pathogens. This, in turn, leads to more rapid evaluation and intervention when necessary by the AST. Further monitoring and additional studies (e.g. cost analysis) will provide additional data on the potential of qPNA FISH to improve clinical outcomes.

Author Disclosure Block:

D. Cianciminio-Bordelon: None. **A. Quianio:** None. **K. Gilhuley:** None. **A. Paskovaty:** None. **N. Cohen:** None. **Y. Tang:** None. **S. Seo:** None. **N. Babady:** I. Research Relationship; Self; AdvanDX supported research.

Poster Board Number:

MONDAY-096

Publishing Title:

Does Blood Culture Bottle Transport Time Before Instrument Entry Impact Instrument Detection Time of Positives?

Author Block:

R. B. Thomson, Jr., B. Link, I. Dusich, Y. Zhou, S. Das; NorthShore Univ. Hlth. Systm., Evanston, IL

Abstract Body:

Background: Positive blood culture results provide critical information for the sickest patients. Data have shown that delayed detection or reporting of results can negatively impact patient care. No data are available to judge the impact of transport times less than 24 hr. This study examines the impact of bottle transport times ranging from 2-12 hr on instrument detection times of positive cultures. **Methods:** NorthShore is a 4 hospital healthcare system with a single microbiology laboratory located at one of the hospitals. Blood for culture is collected at all 4 hospitals and transported by car in a room temp insulated carrier (3 hospitals at 4, 11 and 13 miles distance) or tube system (core hospital) to the laboratory. 451 blood cultures positive for *E. coli* collected between May, 2014 and May, 2015 were included. Blood collection time was recorded by a bar code reader used by the phlebotomist. BACTEC FX entry and positive detection times were recorded by the instrument. Transport time was calculated by subtracting BACTEC entry time from blood collection times. Instrument detection time was calculated by subtracting BACTEC positive from BACTEC entry times. Total time to positive blood culture detection was transport plus instrument detection times.**Results: T**ransport times up to 12 hr did not impact instrument detection times for *E. coli*. In fact, 89% of cultures were detected within 24 h and for this group of 400 specimens there was a statistically significant negative correlation, i.e., as transport times increased from 2-12 hr, instrument detection times decreased. Median transport times among hospitals varied between 2-6 hr with ranges from 30 min-12 hr. Total time to positive blood culture detection was approx 14 hr and was not statistically different regardless of transport time. There were no significant differences in total time to detection among hospitals.**Conclusions:** BACTEC blood culture bottle transport times up to 12 hr did not prolong instrument detection times for *E. coli*. Transport times up to 12 hr did not shorten total time to positive blood culture detection. In spite of transport time differences, total time to detection for all hospitals was similar.

Author Disclosure Block:

R.B. Thomson: J. Scientific Advisor (Review Panel or Advisory Committee); Self; Advisory Committee. L. Speaker's Bureau; Self; Speaker Honoraria. **B. Link:** None. **I. Dusich:** None. **Y. Zhou:** None. **S. Das:** None.

Poster Board Number:

MONDAY-097

Publishing Title:

Genotypic Differences of the *Mycobacterium tuberculosis* Beijing Family Originated in Adjacent Countries Based on Whole Genome Sequencing

Author Block:

S. Ryoo[1], H. Lee[1], J. Lee[2], S. Lee[3]; [1]Korean Inst. of Tuberculosis, Cheongju-si, Korea, Republic of, [2]Natl. Instrumentation Ctr. for Environmental Management, Seouol, Korea, Republic of, [3]Korea Univ. Ansan Hosp., Ansan, Korea, Republic of

Abstract Body:

Background: Tuberculosis (TB) of immigrants or foreigners is increasing in Korea. Determining the molecular epidemiology of *Mycobacterium tuberculosis(M.TB)* isolates with identifying the transmission dynamics between adjacent countries is essential for upgrading the strategy for immigration TB screening. Especially Korea and China's cultural economical interchange is rapidly increasing now. Genotypic differences of the *M.TB* Beijing family originated in adjacent countries based on whole genome sequencing is needed. We've been initiated a project on the molecular and bio-informatics for discriminating TB strain's originality, it includes multi- and extensively drug-resistant tuberculosis (MDR-/XDR-TB) clinical isolates from China and Korea in the period from 2014 to 2015. **Methods:** 1) Preliminary Bio-informatics study: We used *M. TB* genome sequences, annotation refinement and orthology assignment. The complete whole genomes of five *M. TB* strains were downloaded from NCBI, and the plasmid sequences were eliminated. The annotation refinement was carried out through coding sequence (CDS) mapping by BLAST (v2.2.26); thus, the CDSs collected from the thirteen strains were used to map each genome. Clusters of Orthologous Groups (COGs) were used as references to assign orthology, and orthoMCL was employed to generate customized orthology clusters. 2) PacBio: We construct and adopted Genome Topology Network (GTNs).**Results:** We got Mauve alignment for China and Korean *M. TB* strains. **Conclusions:** The comparative genomic analysis of these genome sequences will facilitate the identification of significant distinguishing markers associated with their local origins. Also give us more insights into the genetic and epidemiology variations occurring in MDR/XDR-TB strains circulating in Korea and China. Bio-informatics tool can be allows us comparing closely related TB genomes exist adjacent regions of Korea. We believe that our study methodology will be suitable for the exploration of genomic connections of TB strains, and phylogenetic analysis will provide additional insights into whole genome-based phylogenetic analysis.

Author Disclosure Block:

S. Ryoo: None. **H. Lee:** None. **J. Lee:** None. **S. Lee:** None.

Poster Board Number:

MONDAY-098

Publishing Title:

Comparison of Gene Xpert MTB/RIF Assay with Multiplex PCR for Diagnosis of Tuberculous Meningitis (TBM): Experience from High-Endemic Low-Resource Country

Author Block:

M. Sharma, K. Sharma, M. Modi, A. Sharma, S. Varma; PGIMER, Chandigarh, India

Abstract Body:

Background: Rapid and specific diagnosis of tuberculous meningitis is of paramount importance to decrease morbidity and mortality. Therefore, present study was undertaken to do the comparison of Gene Xpert MTB/RIF (GX) assay and multiplex PCR (MPCR) using three targets (IS6110, MPB64 and protein b) for diagnosing tuberculous meningitis.**Methods:** GX and MPCR were performed on cerebrospinal fluid samples of 60 patients with culture positive TBM, 10 patients with non-TB infectious meningitis and 10 patients with non-infectious neurological disorder. rpoB gene sequencing was done for diagnosing rifampicin resistance in all positive cases.**Results:** GX and MPCR were positive in 50 (83.33%) and 56 (93.33%) patients respectively. Both the tests were negative in all controls. Rif resistance was detected in 8 of 50 (16%) by GX, and in 7 of 56 (12.5%) by MPCR along with rpoB gene sequencing. There was a case of false Rif resistance detected by GX which was Rif sensitive on rpoB gene sequencing. Cost of doing MPCR was less than $1 whereas GX required $10 per isolate.**Conclusions:** MPCR has a higher sensitivity than GX for diagnosing TBM. MPCR is a robust and cost effective method for diagnosis of TBM in low-resource and high-endemic countries.

Author Disclosure Block:

M. Sharma: None. **K. Sharma:** None. **M. Modi:** None. **A. Sharma:** None. **S. Varma:** None.

Poster Board Number:

MONDAY-099

Publishing Title:

Multi Targeted Loop-Mediated Isothermal Amplification (LAMP) for Rapid Diagnosis of Extra-Pulmonary Tuberculosis in 60 Minutes: Experience from North India

Author Block:

K. Sharma, M. Modi, A. Sharma, S. Varma; PGIMER, Chandigarh, India

Abstract Body:

Background: Rapid and accurate diagnosis of extra-pulmonary tuberculosis (EPTB) is imperative for early treatment and better patient outcome. Loop-mediated Isothermal Amplification (LAMP) is a promising nucleic-acid amplification assay. LAMP assay could be carried out in simple water bath under isothermal conditions in 60 minutes, and can be performed in any laboratory even in rural setting in resource poor endemic countries. We evaluated LAMP assay using two different target regions specific for *Mycobacterium tuberculosis* complex for the diagnosis of EPTB.**Methods:** LAMP assay using 6 primers was performed on patients suspected of EPTB on various EPTB samples (CSF, Synovial fluid, Lymph node and tissue biopsies and various other samples). Results of 150 patients clinically suspected of EPTB (50 culture positive, 100 culture negative) and 100 non-TB control subjects were compared with *IS*6110 PCR, culture and ZN smear examination.**Results:** Overall LAMP test (using any of the two targets) had a sensitivity and specificity of 96% and 100%, respectively, for confirmed (50 culture positive) cases and 84% and 100%, respectively for clinically suspected cases. Sensitivity of *IS*6110 LAMP, MPB64 LAMP and *IS*6110 PCR for clinically suspected cases was 78 (78%), 82 (82%) and 70 (70%), respectively. In total 150 EPTB patients, the overall sensitivity of microscopy, culture, *IS*6110 PCR, *IS*6110 LAMP, MPB64 LAMP and the multi-targeted LAMP assay (if any of the two targets were used) were 4%, 33.3%, 74.6%, 82.66%, 86.66% and 92%, respectively. Specificity of all the tests was 100%. There were 6 cases which were missed by *IS*6110 LAMP and 2 cases by MPB64 LAMP.**Conclusions:** LAMP assay using two targets is a promising technique for rapid diagnosis of EPTB in 60 minutes especially in a resource poor setting who are still battling with this deadly disease.

Author Disclosure Block:

K. Sharma: None. **M. Modi:** None. **A. Sharma:** None. **S. Varma:** None.

Poster Board Number:

MONDAY-100

Publishing Title:

Comparison of MTBDR Plus Assay, Gene Xpert MTB/RIF Assay and Multiplex PCR for the Diagnosis of Ocular Tuberculosis

Author Block:

K. Sharma, R. Bansal, A. Sharma, V. Gupta, A. Gupta; PGIMER, Chandigarh, India

Abstract Body:

Background: Ocular tuberculosis (OTB) is an important cause of vision loss in Indian subcontinent. Rapid and timely diagnosis of ocular tuberculosis is of paramount importance to decrease the morbidity and to save these eyes from blindness. Therefore, the present study was undertaken to carry out a comparative evaluation of MTBDR plus (LPA), Gene Xpert MTB/RIF (GX) and Multiplex PCR (MPCR) for the diagnosis of intraocular tuberculosis (IOTB).**Methods:** LPA, GX and MPCR were performed on vitreous fluid (VF) of 75 patients with presumed IOTB, 20 disease controls and 20 non-uveitic controls. rpoB gene sequencing was done for diagnosing Rifampicin resistance in positive cases.**Results:** LPA, GX and MPCR were positive in 27 (36%), 19(25.33%) and 55(73.33. %) patients, respectively. All tests were negative in all controls. Rif resistance was detected in 3 by GX and 6 by LPA. MPCR followed by rpoB gene sequencing detected Rif resistance in 5 cases. False Rif resistance was detected in one patient each by LPA and GX as they were Rif sensitive on sequencing.**Conclusions:** Comparing with other two techniques, MPCR is a cost effective , robust and reliable technique for the diagnosis of paucibacilliary condition like ocular tuberculosis.

Author Disclosure Block:

K. Sharma: None. **R. Bansal:** None. **A. Sharma:** None. **V. Gupta:** None. **A. Gupta:** None.

Poster Board Number:

MONDAY-101

Publishing Title:

Challenges Establishing TB Culture Laboratory in Remote Environments

Author Block:

W. G. Jones[1], T. Commans[2], R. Arpornsilp[1], D. Miriti[1], M. H. Abdi[3], j. Okari[4]; [1]Intl. Organization for Migration, Nairobi, Kenya, [2]Ctr. for Disease Control and Prevention, Atlanta, GA, [3]Intl. Organization for Migration, Dadaab, Kenya, [4]Ministry of Hlth.Kenya, Nairobi, Kenya

Abstract Body:

Problem: To provide tuberculosis diagnostic services for 600,000 Somali Refugees in Dadaab area Northern Kenya. The International Organization for Migration, Centers for Disease Control and Prevention US, Ministry of Health Kenya, United Nations High Commission for Refugee, CARE partnered to provide diagnostics by smear, culture and DST testing to support MDR ward at Ifo Hospital Dadaab, a hostile, remote environment with challenges. Somali refugees travel to access treatment for MDR TB at the clinic. CDC arranges through the Kenyan Ministry of Health, National TB Program for the supply of XpertMTB/rif cassettes and media for culture testing, UNHCR supplies diesel for 24/7 electricity. **Method:** Security: 24/7 guards required at the TB laboratory, police escorts to the laboratory each day. Electricity: 24/7 staff to ensure running of the generators. Water: Water supplied from CARE in Dadaab Issues included; Contamination rates, reduced working hours, dust, sterilization of reagents, operation of equipment Increase decontamination agent in processing step remaining within 2% per volume, adding Penicillin solution to the residue make-up. Transport of specimen from camps to laboratory with security vehicles. Improving the sterilization by replacing autoclaves. Efficiency required, the workday in the laboratory was 3-4 hours due to security. Improved cleaning the laboratory, replacing filters in BSC, air conditioners regularly. Autoclaves repaired, replaced. Logistics for flights, accommodation, escorts for service technicians. Contamination rates were reduced 30% to 9% in MGIT and 27% to 4% in Lowenstein Jensen Media. **Results:** From April 2013 to 2015 diagnosis of 233 cases of MDR TB, 86 are on treatment and 126 cured. The laboratory tested sputa with GenXpert then, if positive (95%), on to smear and culture testing to identification of MTB. The isolates of MTB were shipped to Nairobi for Drug Susceptibility Testing. It was found 99% were MDR TB cases. MDR TB Cases Detected per year. The provision of TB smear and culture testing facilities in refugee camps provide excellent results for the detection of MDR TB, but many challenges must be overcome.

Author Disclosure Block:

W.G. Jones: None. **T. Commans:** None. **R. Arpornsilp:** None. **D. Miriti:** None. **M.H. Abdi:** None. **J. Okari:** None.

Poster Board Number:

MONDAY-102

Publishing Title:

Long Term Evaluation of Xpert Mtb/Rif Pcr Test for Detecting *Mycobacterium tuberculosis* in Respiratory Specimens

Author Block:

J. Aslanzadeh, L. Coppola, P. Hamilton, Y. Maldonado; Hartford Hosp., Hartford, CT

Abstract Body:

Background: *Mycobacterium tuberculosis* (MTB) remains a major global public health problem. Early detection of infectious patients is essential to reduce the death rate and interrupt transmission. Most clinical laboratories rely on acid fast bacilli (AFB) smear for preliminary diagnosis of MTB. The reliability of AFB smear depends on the experience of the medical technologist and on the number of AFB present in the specimen. In addition, AFB smear does not differentiate MTB from mycobacteria other than TB (MOTT). Xpert MTB/RIF PCR (Cepheid, Sunnyvale, CA) is reported to detect MTB and it's multidrug-resistant form with high degree of sensitivity and specificity. **Methods:** In a prospective study, from November, 2011 to October 2015, AFB stain, AFB culture and Xpert MTB/RIF PCR test were performed on 864 specimens collected from 541 patients.**Results:** Severn hundred thirty three samples tested negative for MTB by AFB smear, AFB culture and Xpert MTB/RIF PCR. Forty eight samples grew MTB and 83 samples grew MOTT. Among the 48 MTB culture positive samples, AFB smear and PCR were positive on 38 and 43 samples respectively. Among the 83 samples that were culture positive for MOTT, 2 samples from 2 different patients tested PCR positive for MTB and were considered false positive. In addition, 35 of the 83 samples (42%) were AFB smear positive. Except for one sample that grew *M. avium* and was false positive for MTB by PCR the remaining 863 samples tested PCR negative for the rifampin resistant gene. The overall sensitivity, specificity, positive and negative predictive value of Xpert MTB/RIF PCR was 89.5%, 99.8%, 95.6% and 99.4% respectively.**Conclusions:** We conclude Xpert MTB/RIF PCR test is highly sensitive and specific for detecting MTB in respiratory specimens.

Author Disclosure Block:

J. Aslanzadeh: None. **L. Coppola:** None. **P. Hamilton:** None. **Y. Maldonado:** None.

Poster Board Number:

MONDAY-103

Publishing Title:

Diagnosis of Tuberculosis Meningitis by Real Time Pcr: Comparison of Extraction Protocols and Molecular Targets

Author Block:

F. S. Palomo, M. G. C. Rivero, M. G. Quiles, F. P. Pinto, A. M. O. Machado, A. C. C. Pignatari; Federal Univ. of São Paulo, São Paulo, Brazil

Abstract Body:

Tuberculosis meningitis is a severe form of extrapulmonary tuberculosis, because of its high morbidity and mortality. This study aimed to compare and to validate molecular protocols (PCR) for the diagnosis of tuberculosis meningitis directly from cerebral spinal fluid system (CSF) samples including the mycobacterium DNA extraction techniques and targets for PCR amplification. One hundred CSF samples from 68 patients suspected of tuberculous meningitis attended at Hospital São Paulo, Brazil from June 2011 to June 2014 were studied. Four DNA extraction techniques (Phenol-Chloroform-Thiocyanate guanidine, Silica Thiocyanate guanidine, Resin and Resin with ethanol) were compared and CSF samples were used to determine the best target (*IS6110, MPB64* and *hsp65KDa*) by qPCR for diagnosis of tuberculosis meningitis. For statistical analyzes the CSF samples were classified as true positive and negative after a survey in the site BrazilianTBweb, laboratory CSF data and evaluation of medical records of patients. The extraction protocol using the phenol chloroform-thiocyanate guanidine showed the best results in terms of quantification and sensitivity of PCR amplification, presenting up to 10 times more DNA than the second best (silica guanidine thiocyanate). The targets that showed the best amplification results was the the *IS6110* qPCR with a sensitivity and specificity, respectively, of 100 % and 79 % when compared to culture. The sample analysis for *IS6110* qPCR amplification showed 91% sensitivity, 97% specificity with the clinical diagnosis. When this analysis was grouped by patient, we showed a 100% sensitivity, specificity of 98 % and a very good agreement with the clinical diagnosis. A protocol using extraction with phenol chloroform and guanidine thiocyanate, followed by amplification of the *IS6110* target for real time PCR in house showed to be suitable for molecular diagnosis of tuberculosis meningitis in our clinical setting.

Author Disclosure Block:

F.S. Palomo: None. **M.G.C. Rivero:** None. **M.G. Quiles:** None. **F.P. Pinto:** None. **A.M.O. Machado:** None. **A.C.C. Pignatari:** None.

Poster Board Number:

MONDAY-104

Publishing Title:

Evaluation ofMycotube, a Modified Version of Lowenstein-jensen Medium, for EfficientRecovery of *Mycobacterium tuberculosis*

Author Block:

R. NAMBIAR[1], S. Chatellier[2], N. Bereksi[3], A. van Belkum[2], A. Shetty[4], C. Rodrigues[4]; [1]PD Hinduja Hosp. & Med. Res. Ctr., Mumbai, India, [2]bioMérieux, La Balme-les-Grottes, France, [3]bioMérieux, Craponne, France, [4]PD Hinduja Hosp. & Med. Res. Ctr., Mumbai, Maharashtra, India

Abstract Body:

This study aimed to evaluate the performance of LJ Mycotube, a new variant of Lowenstein-Jensen (LJ) medium, by comparing it with MGIT (gold standard), local LJ medium, and the widely used bioMérieux LJ-T medium for *Mycobacterium tuberculosis* (MTB) cultivation, in terms of recovery rate and time.Pulmonary and extrapulmonary specimens from “anti-TB treatment-naïve clinical suspects” (n = 207) visiting our tertiary care center were included in this study, after obtaining informed consent. NALC-NaOH method was used for decontamination; the decontaminated specimens were used to inoculate MGIT, local LJ, LJ-T, and LJ Mycotube. The results were evaluated using objective (total number of positive tubes and time to growth detection) and subjective (time to tube removal and contamination) parameters.LJ Mycotube yielded 140 positive results, compared to 162, 69, and 141 from MGIT, local LJ, LJ-T, respectively. The mean time to growth detection was 17.43 days for LJ Mycotube, versus 14.53, 28.11, and 16.52 days for MGIT, local LJ, LJ-T, respectively. The mean time to growth detection for local LJ significantly differed from that of MGIT, but this parameter did not show a significant difference in case of LJ-T and LJ Mycotube ($p < 0.05$). The time to tube removal was higher in case of LJ Mycotube because the negative tubes were incubated for a longer period to confirm culture negativity. The contamination rate was 0% for all media. LJ Mycotube had a sensitivity of 85.80% and a specificity of 97.78%, with respect to MGIT.LJ Mycotube is a good solid medium, comparable with MGIT and LJ-T. Although liquid culture tends to be more rapid with higher sensitivity, the newly developed LJ Mycotube, a modification of the traditional solid LJ medium, offers good results at cheaper cost and with basic infrastructure. Moreover, contamination rate generally tends to be lower in case of solid media, giving this new medium an edge over routine liquid culture. This new reliable medium helps with definitive diagnosis of TB, as well as with the isolates required for conventional drug susceptibility testing in underdeveloped and developing countries.

Author Disclosure Block:

R. Nambiar: None. **S. Chatellier:** D. Employee; Self; Director Innovation R&D Microbiology. **N. Bereksi:** D. Employee; Self; Head Research Scientist. **A. van Belkum:** J. Scientific Advisor (Review Panel or Advisory Committee); Self; Corporate Vice President R&D Microbiology. **A. Shetty:** None. **C. Rodrigues:** None.

Poster Board Number:

MONDAY-105

Publishing Title:

An Encapsulating Microfluidics Platform for Rapid Mycobacterial Growth on Alginate-Based Particles

Author Block:

L. López-Cerero[1], M. Ramos-Guelfo[1], L. Delgado-Ramos[2], F. March[3], S. Chávez[2], P. Coll[3]; [1]Univ. Hosp. Virgen Macarena, Seville, Spain, [2]Inst. de Biomedicina de Sevilla, Seville, Spain, [3]Univ. Hosp. Santa Creu i Sant Pau, Barcelona, Spain

Abstract Body:

Background: *Mycobacterium tuberculosis* (MTB) growth is quite heterogeneous and usually involves different phenotypes with an impact in phenotypic resistance. Attempts have been made to quantify mycobacterial growth by encapsulating individual cells using agarose microdrops emulsioned in oil. This method provides limited control over particle diameter, and produces an immiscible co-flowing liquid. The aim of the study is develop a simple and rapid method of MTB culture, based on microencapsulating single bacteria cell by using a microfluidic technology (Cellena®) capable of monodisperse particles of predictable and controllable size.**Methods**: Reference *M. tuberculosis* H37Ra ATCC 25177 was used. A 0,5 McFarland suspension in 7H9 broth of the strain was filtered to remove any clump or aggregation. Single cell suspension was confirmed by using phase contrast microscopy. The different volumes of cell suspension and different concentration of alginate in medium were tried, in order to obtain occupied particles by single cells. The Cellena® system was used to generate alginate particles of a mean diameter of 100 mm. The control of inoculum was carried out by platting 100 ml of cell suspension on 7H10 agar and incubated for 3 weeks and the CFU was determined. After incubation in 7H9 broth for 24, 48 and 72 hours, the samples with encapsulated cells were fixed with 1% formaldehyde. Microcolonies detection was performed by microscopic examination using phase contrast as well as by acid fast staining.**Results**: After incubating 24 h, microcolonies were detected in 8-10% of particles in all experiments, both in unstained and Ziehl-Neelsen and Rhodamine/Auramine stained samples. The size of microcolonies increased during incubation and burst occupied particles were seen after incubating 48 and 72 h. Alginate concentrations below 3,5% were not able to retain mycobacterial growth. The limit on the number of encapsulated cells for a 99% 1-cell occupancy was determined in 1,5 x 10^5CFU/ml.**Conclusion**: The Cellena® system technology can be used to study the growth of individual cells from a mycobacterial population. The microenvironment permits a more rapid detection, measuring microcolony growth in 24h.

Author Disclosure Block:

L. López-Cerero: None. **M. Ramos-Guelfo:** None. **L. Delgado-Ramos:** None. **F. March:** None. **S. Chávez:** None. **P. Coll:** None.

Poster Board Number:

MONDAY-106

Publishing Title:

application Of Protein Microarrays For The Identification Of Sero-Reactive Antigens In *mycobacterium Avium* Subsp. *paratuberculosis*

Author Block:

L. Li[1], J. P. Bannantine[2], C. A. Praul[1], J. Raygoza Garay[1], M. E. Hines II[3], R. Katani[1], V. Kapur[1]; [1]The Peensylvania State Univ., University Park, PA, [2]Natl. Animal Disease Ctr., USDA-ARS, Ames, IA, [3]The Univ. of Georgia, Tifton, GA

Abstract Body:

Mycobacterium avium subspecies *paratuberculosis* (MAP) is the causative agent of Johne's disease (JD), a chronic intestinal disease of domestic animals that is controversially associated with human Crohn's disease. JD has a high prevalence rate and results in considerable adverse impact on animal health, but methods for detection of animals, particularly early in infection are currently lacking. To address this need, we have developed a MAP protein microarray as a tool for sero-diagnostic antigen discovery by studying the dynamics of the humoral immune response in a baby goat model of infection. A protein microarray consisting of ~600 recombinant MAP proteins was constructed and used to identify reactive antigens in serum samples from experimentally MAP-infected (n=10) and negative control (n=10) goats at months 0, 2, 4, 6, 8, 10, and 12 post MAP infection. The analysis resulted in the identification of a total of 53 MAP recombinant proteins that were sero-reactive in infected animals, but not in the control group. In contrast, there was no sero-reactivity noted with 3 *M. tuberculosis*-specific control antigens. Of the 53 candidate MAP antigens, a total of 15 were detected between 2 and 4 months post-infection, which is considerably sooner than the detection of fecal MAP shedding (month 6 or later) by using fecal culture and/or PCR in these same animals. Further, our preliminary analyses suggest that individual MAP recombinant proteins were reactive even in MAP-infected goats that remained serologically negative on commercially available MAP ELISA testing, suggesting that the protein microarray approach may enable the identification of antigens that are sero-reactive even during the early (pre-shedding) stages of infection. Future studies with larger numbers of recombinant proteins and serum samples are planned.

Author Disclosure Block:

L. Li: N. Other; Self; LL is listed as inventor on an issued patent application on mycobacterial diagnostics jointly owned by the University of Minnesota and the USDA and as such may be the beneficiaries of future royalties. **J.P. Bannantine:** N. Other; Self; JB is listed as inventor on an issued patent application on mycobacterial diagnostics jointly owned by the University of Minnesota and the USDA and as such may be the beneficiaries of future royalties. **C.A. Praul:** None. **J. Raygoza Garay:** None. **M.E. Hines II:** None. **R. Katani:** None. **V. Kapur:** N. Other;

Self; VK is listed as inventor on an issued patent application on mycobacterial diagnostics jointly owned by the University of Minnesota and the USDA and as such may be the beneficiaries of future royalties.

Poster Board Number:

MONDAY-107

Publishing Title:

Molecular Detection And Identification Of Acid-Fast Bacteria From Formalin-Fixed Paraffin-Embedded Tissues

Author Block:

J. R. Bao, R. B. Clark, R. N. Master, K. L. Shier, L. L. Eklund; Nichols Inst., Quest Diagnostics, Chantilly, VA

Abstract Body:

Background: Identifying AFB in formalin-fixed paraffin-embedded (FFPE) tissues is challenging, and PCR methods may detect only the *Mycobacterium tuberculosis* complex. We developed a method coupling a polymerase chain reaction with pyrosequencing (PCR-Seq) to detect and identify various AFB organisms from FFPE tissues; and here, evaluate its performances.**Methods:**FFPE tissues from various tissue types were received from laboratories around the country sequentially. Most specimens were ≤6 months old from biopsy. Each block of tissue was sectioned at 6 µm thickness for a total of 6 sections. Tissue was recovered by depleting paraffin using xylene. DNA was extracted, and the 16S rRNA gene fragment was amplified by PCR. Signature sequences produced in the PyroMark® ID system (Qiagen) from the amplified fragment were aligned to an in-house database and the database of Ribosomal Database Project for AFB identification. Additional tissue sections were stained using a Ziehl-Neelsen procedure and examined for AFB cells.**Results:** The PCR-Seq method was first evaluated using paraffin-embedded tissues spiked with 1 of 14 different AFB species (11 *Mycobacterium*, 2 *Nocardia,* and 1 *Rhodococcus equi*). The method was 100% accurate with no cross-reactivity with non-AFB organisms. The detection limit was 100 spiked cells (*M. chelonae*) per reaction. The method was further evaluated using 188 patient FFPE tissues. Of these, 73 (39%) were AFB positive and 54 (74%) were identified, including 12 TB complex, 11 *M. avium*, 9 *M. intracellulare*, 1 *M. leprae*, 1 *M. genevense*, and 3 in the *M. marinum/ulcerans* group, and 27 other AFB organisms. Nineteen positive FFPE tissues were indeterminate due to either inhibited PCR reactions or failed amplifications. The rest were AFB negative and had no identification. Eight of 12 TB positive specimens were later confirmed by AFB staining and/or culturing or other detection methods. One arm tissue specimen from a southern US patient was *M. leprae* and later confirmed by The National Hansen Disease Program in Louisiana. The PCR-Seq method failed to detect a TB-positive brain biopsy specimen. The specimen was negative by TB transcription-mediated amplification and AFB stain, but was TB positive from culture.**Conclusions:** The PCR-Seq method evaluated here can directly detect various AFB species in FFPE tissues.

Author Disclosure Block:

J.R. Bao: None. **R.B. Clark:** None. **R.N. Master:** None. **K.L. Shier:** None. **L.L. Eklund:** None.

Poster Board Number:

MONDAY-108

Publishing Title:

Evaluation of Five Targeted Multiplex Pcr for Rapid Diagnosis of Tuberculous Meningitis in Hiv Positive Patients

Author Block:

A. Sharma, K. Sharma, M. Modi, S. Varma; PGIMER, Chandigarh, India

Abstract Body:

Background: Rapid and specific diagnosis of tubercular meningitis (TBM) is of paramount importance to decrease morbidity and mortality, as the case fatality rate for untreated TBM is almost 100% and the delay in institution of appropriate treatment can lead to permanent neurological sequelae. The aim of the study was to evaluate five targeted multiplex PCR (MPCR) using protein b, MPB 64, IS6110, Dev R and Sda primers directed against M. tuberculosis complex for the diagnosis of tuberculous meningitis (TBM). **Objectives**: To evaluate the five targeted MPCR test using *IS*6110 , MPB64, Protein B, Dev R and Sda targets for *Mycobacterium tuberculosis* complex for rapid diagnosis of TBM. Comparison of results of *Five targeted MPCR* with *IS*6110 PCR, culture and ZN smear examination. **Methods:** MPCR assay using primers (each for *IS*6110, MPB64 , Protein b, Dev R and Sda) specific for *Mycobacterium tuberculosis* complex were performed on cerebrospinal fluid (CSF) of 100 patients (10 confirmed, 90 suspected) of TBM and 100 non-TBM control subjects. **Results:** Overall five targeted MPCR test (using any of the five targets) had sensitivity and specificity of 94% and 100% for confirmed (10 culture positive) TBM cases. In 90 clinically suspected but unconfirmed TBM cases, Five targeted MPCR was positive in 79 out of 90 cases (87.77%). In total 100 TBM patients, the overall sensitivity of microscopy, culture, *IS*6110 PCR, and the MPCR test (if any of the five targets were used) were 4%, 33.3%, 74.6% and 89%, respectively. Specificity of all the tests was 100%. **Conclusion:** MPCR using five targets is a promising, cost effective technique for rapid diagnosis of TBM especially in a resource poor setting where tuberculosis is an important cause of mortality and morbidity.

Author Disclosure Block:

A. Sharma: None. **K. Sharma:** None. **M. Modi:** None. **S. Varma:** None.

Poster Board Number:

MONDAY-109

Publishing Title:

Pre-Analytical Protocol for the Enrichment and Lysis of Mycobacteria in Blood and Tissue Samples for the Direct Analysis with Genexpert® Mtb/Rif

Author Block:

C. Baussmerth[1], J. Becker[2], S. Saremba[2], **M. Linow**[1], C. Disqué[1]; [1]Molzym GmbH & Co.KG, Bremen, Germany, [2]Cepheid GmbH, Frankfurt a. M., Germany

Abstract Body:

Background: The diagnosis of disseminated and extrapulmonary tuberculosis is elusive and time-consuming. Rapid molecular methods may support the diagnosis of MTB infection. However, the WHO procedure recommended for GeneXpert® MTB/RIF involves tissue grinding which bears the risk of sample cross contamination and increased MTB exposure to the staff. A new method is described involving enzymatic tissue digestion and MTB lysis in a tube before loading to the GeneXpert® MTB/RIF cassette for analysis. **Material/methods:** Negative EDTA blood (10ml) was spiked with *M. bovis* BCG culture and extracted until MTB lysis using the MTB-DNA Blood kit (Molzym). Tissue biopsies were dissected and subjected to proteinase K digestion, degradation of human DNA and MTB lysis. Extracts from blood and tissue were mixed with GeneXpert® MTB/RIF sample reagent and loaded to the cartridge for further processing and analysis. For reference, the MTB-DNA Blood protocol was followed until DNA elution and then analysed by supplier X MTB test. **Results:** Blood spiked with *M. bovis* BCG was pre-treated with MTB-DNA Blood kit and then analysed by GeneXpert®. For reference, DNA eluates were analysed by another system (supplier X). BCG was detected 100% at 2.5 cfu/ml by both analytical systems, while at 1cfu/ml GeneXpert® still was 80% positive compared to only 20% using supplier X. Tissue samples from two subjects with clinically confirmed tuberculosis and one non-TB control were analysed by MTB-DNA Blood-GeneXpert®. The test confirmed the presence of MTB in the tissues of patient A and patient B and the absence in the control patient C. The reference test (MTB-DNA Blood-supplier X) was false-negative for patient A which, like with blood, may indicate a higher limit of detection compared to MTB-DNA Blood-GeneXpert®. The tests were completed within 3 hours.**Conclusions:** The MTB-DNA Blood-GeneXpert® test system proved to be rapid (3 h) and at the same time highly sensitive for the detection of MTB in EDTA blood (1cfu/ml) and tissues. The new protocol provides a procedure with a decreased risk of cross contamination.

Author Disclosure Block:

C. Baussmerth: None. **J. Becker:** None. **S. Saremba:** None. **M. Linow:** None. **C. Disqué:** None.

Poster Board Number:

MONDAY-110

Publishing Title:

A Rapid One Step Extraction Method for Identification of Mycobacteria and Nocardiae by Maldi-Tof Ms

Author Block:

J. K. Lemon, A. M. Zelazny; NIH, Bethesda, MD

Abstract Body:

Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) is a quick and accurate method to identify Mycobacteria and Nocardiae in the clinical laboratory. However, extraction protocols for these genera are laborious and require a large amount of organisms (10μL). Current identification of Mycobacteria involves heat killing, ethanol washing, and a two-step extraction method. This study compared the current extraction method with a faster one-step extraction protocol, requiring only 1μL of bacteria, by prospectively analyzing clinical isolates and also frozen strains. For this rapid approach, bacteria were inoculated directly into solutions with varying ratios of formic acid, acetonitrile, and water. Proteins were extracted using a high-powered bead beating homogenizer (PowerLyzer) and the lysates directly spotted onto a target plate for MALDI-TOF MS analysis. Viability studies were conducted before and after the bead-beating step. Preliminary testing included 20 Mycobacteria isolates (*M. tuberculosis* complex, *M. avium* complex, rapid growers) and 9 *Nocardia* species, with testing ongoing to reach 100 isolates. Overall, 64 - 71% of Nocardiae and 75 - 95% of Mycobacteria isolates were identified to the species/complex level by all conditions tested (score cut off ≥1.8). Average scores for Mycobacteria using the current protocol were 2.14 ± 0.22 versus 1.94 ± 0.19 or 1.89 ± 0.21 with the faster one-step extraction, depending on the solution used. Average scores for Nocardiae with the current protocol were 1.99 ± 0.32 versus 1.96 ± 0.28 and 1.99 ± 0.29 with the one-step method (difference was not statistically significant). Notably, scores for the isolates were similar between both procedures, even though the rapid extraction protocol requires 10-fold less bacteria. Viability studies confirmed killing of the organisms prior to the bead-beating step, suggesting that the one-step protocol can be performed outside the BSL-3. Importantly, this rapid method successfully reduced extraction procedure time from over 1 hour to 5 minutes. Our data show that this one-step method substantially reduces both extraction time and quantity of organism required for testing without sacrificing the quality and accuracy of identification of clinical isolates.

Author Disclosure Block:

J.K. Lemon: None. **A.M. Zelazny:** None.

Poster Board Number:

MONDAY-111

Publishing Title:

Detection of Latent Tb Among Health Care Workers Vulnerable to Tb Exposure in Karachi Pakistan

Author Block:

A. Anum, s. Khan; Dow Univ. of Hlth.Sci., Karachi, Pakistan

Abstract Body:

Background: Latent tuberculosis infection is asymptomatic and in-transmissible diseases. According to the World Health Organization (WHO), in 2014 estimated incidence of Tuberculosis (TB) is 181 per 100 000, with 40% of the population infected with TB in Pakistan and approximately one-third of the population is infected worldwide. Laboratory workers dealing with positive Mycobacterium Tuberculosis (MTB) samples are always at risk of infected with TB. In this study we have investigated prevalence of latent TB in health care providers who are at risk of TB using QuantiFERON assay. **Objective:** To screen the health care workers at risk to occupational exposure for latent TB using QuantiFERON Assay **Methodology:** 3ml of whole blood were collected into three specific QFT tubes.(NIL,TB, Mitogen) from 120 health care workers including phlebotomists, Medical technologist, nurses, doctors and faculty members working closely with TB samples or patients. Samples were tested for detection interferon specifically released against TB according to the manufacturer QuantiFERON TB Gold protocol. **Result:** Out of 137 samples 27 samples were found positive, 5 samples showed indeterminate result and 105 were found negative. Out of 27 positive samples 20 were from medical technologists working closely since long time with TB samples or TB patients and 4 were from phlebotomists collecting samples from patients. **Conclusion:** Health care providers usually work with TB infected samples with minimal infection control measures. This study shows the need for effective latent TB infection control measures and emphasizes on the importance to improve over all biosafety precautions during dealing with the TB patients or samples. The study also provides recommendations for routine and regular screening and checkup of the health care workers working with TB to ensure their safety rather safety of all as no one is safe until everyone is safe.

Author Disclosure Block:

A. Anum: None. **S. Khan:** None.

Poster Board Number:

MONDAY-112

Publishing Title:

The Long-Term Mortality of Tuberculosis Meningitis Patients in New York City

Author Block:

C. Vinnard[1], L. King[2], D. Proops[2], A. Crossa[2], K. Iwata[3], J. Pasipanodya[4], L. Trieu[2], S. Munsiff[5], S. Ahuja[2]; [1]Drexel Univ. Coll. of Med., Philadelphia, PA, [2]New York City Dept. of Hlth.and Mental Hygiene, Long Island City, NY, [3]Kobe Univ., Kobe, Japan, [4]Baylor Inst. for Immunology Res., Dallas, TX, [5]Univ. of Rochester, Rochester, NY

Abstract Body:

Background: Tuberculosis meningitis (TBM) is the most devastating clinical presentation of infection with *M. tuberculosis.* We sought to determine the relationship between the initial drug resistance and long-term mortality among TBM patients in New York City (NYC).**Methods:** We performed a retrospective cohort study of TBM patients reported to the NYC TB registry between January 1, 1992 and December 31, 2001. Date of death was ascertained by matching the registry with death certificate data for 1992 through 2012 from the NYC Office of Vital Statistics (OVS), which maintains data collected from death certificates issued in NYC. HIV status was ascertained by medical record review, matching with the NYC HIV Surveillance registry, and review of cause of death. We used multivariate survival analysis to determine the independent association of initial drug resistance with mortality.**Results:** The study population included 324 patients with culture-confirmed TBM, susceptibility results reported for isoniazid (INH) and rifampin (RIF), and initiation of at least two anti-TB drugs. HIV-infection was identified in 63 of 67 TBM patients (94%) with RIF-resistant isolates, including 57 of 61 patients (93%) with multidrug- resistant isolates. Death occurred before the completion of anti-TB therapy in 63 of 67 TBM patients (94%) with RIF-resistant disease. Among the 257 TBM patients without RIF-resistant disease, INH resistance was associated with mortality after the first 60 days of treatment (adjusted HR 2.77, 95% CI 1.28 to 6.01) when controlling for age and HIV co-infection.**Conclusion**: We observed an independent association between INH-resistant TB and death after the first 60 days of therapy for culture-confirmed TBM. While RIF-resistant TB was strongly associated with death, we were unable to estimate this association independent of HIV infection given that all patients with RIF-resistant, INH-sensitive, disease were co-infected with HIV. These findings support the continued evaluation of rapid diagnostic techniques and the empiric addition of second-line drugs for patients with clinically suspected drug-resistant TBM.

Author Disclosure Block:

C. Vinnard: None. **L. King:** None. **D. Proops:** None. **A. Crossa:** None. **K. Iwata:** None. **J. Pasipanodya:** None. **L. Trieu:** None. **S. Munsiff:** None. **S. Ahuja:** None.

Poster Board Number:

MONDAY-113

Publishing Title:

Impact of Whole Genome Sequencing of *Mycobacterium tuberculosis* Isolates in a Public Health Laboratory

Author Block:

J. Shea, T. Halse, P. Lapierre, M. Shudt, P. Van Roey, V. Escuyer, K. A. Musser; Wadsworth Ctr., Albany, NY

Abstract Body:

Background: *Mycobacterium tuberculosis* (Mtb) is a pathogen of global importance, estimated to be infecting a third of the world's population and emerging drug resistance is a growing threat. Growth-based drug susceptibility testing (DST), the gold standard, can take weeks to months to identify resistance due to the growth rate of Mtb. While molecular assays have improved the early detection of resistance, these are limited in scope detecting only a few mutations associated with resistance. Whole genome sequencing (WGS) is an alternative for Mtb diagnostics, capable of providing comprehensive drug resistance profiles more rapidly than current methodologies. **Methods:** A novel DNA extraction method was developed combining lysis using a tissue homogenizer and purification using chelex resins. Sequencing of 230 isolates from both solid and liquid media was performed on the Illumina MiSeq platform, utilizing the Nextera XT protocol for library preparation modified to incorporate a 15-cycle index PCR step. Data were analyzed using an in-house developed bioinformatics pipeline for species identification, resistance profiling, and genotyping. **Results:** WGS outperformed our current molecular assays used for species identification and genotyping and provided far more comprehensive resistance profiles an average of 18 days sooner than conventional DST. Species identification using WGS was 98% accurate, a 5% improvement over the current real-time PCR assay. Concordance between the drug resistance profile generated by WGS and current molecular and conventional methods was greater than 94% for eight drugs, with a resistance-predictive value of 93% and susceptible-predictive value of 95%. **Conclusions:** This single cost-effective WGS assay can replace several molecular assays currently used for species identification, genotyping, and drug resistance. This WGS approach to screen isolates of Mtb generates species identification, genotyping, and comprehensive drug resistance profiling results accurately and reproducibly, making it possible to identify resistance and inform patient treatment decisions sooner than before. It allows the physicians to design and implement an optimized treatment for their patients in a dramatically shortened time frame.

Author Disclosure Block:

J. Shea: None. **T. Halse:** None. **P. Lapierre:** None. **M. Shudt:** None. **P. Van Roey:** None. **V. Escuyer:** None. **K.A. Musser:** None.

Poster Board Number:

MONDAY-114

Publishing Title:

Mycobacterial Inactivation/Extraction/Identification from Positive Mgit Tubes Using Afa-Ultrasonication and MALDI-ToF Ms

Author Block:

S. Fisher, L. Adams, K. Dionne, N. Parrish; The Johns Hopkins Med. Inst.s, Baltimore, MD

Abstract Body:

Background: MALDI-ToF MS (MALDI) has been used for identification of *Mycobacteria*, primarily from growth on solid media. MALDI-based identification from positive MGIT tubes has been problematic. In the current study, we used adaptive focused acoustics (AFA) and ultrasonication (US) to standardize an extraction method for MALDI-based identification from positive MGIT tubes.**Methods:** A total of 99 mycobacterial isolates were used representing 12 species. For all assays, MGIT tubes were spiked with a standard volume of clinical specimen (e.g. sputum, tissue, various sterile fluid, stool, etc.) and supplement containing PANTA. Tubes were spiked with known quantities of each mycobacterial isolate to be tested and placed on the instrument. Once positive, tubes were removed and tested using the optimized AFA protocol with subsequent identification attempted using MALDI. Aliquots (1 ml) were collected from the bottom of MGIT tubes after settling for 1 hr at various timepoints past positive (48-72 hrs) and washed twice with sterile water. AFA-US was done using microTUBES™ containing glass beads and acetonitrile:70% formic acid (1:1) and instrument settings of 40 watts Peak Incident Power and 50% duty factor for 2 min. Completed extracts were spotted on polished steel plates and identification done by MALDI and an existing database. Standard identification was done from solid media using a validated MALDI assay.**Results:** Timepoints at or 24 hrs post positive on the MGIT yielded unreliable results for all species/specimen types tested. However, at 48 hrs post positive, overall agreement for all species/strains considered together was 93% (92/99) using a log confidence score of ≥ 1.7. However, differences were noted between rapid-growing (RG) and slow-growing species (SG) where agreement was 91% and 95%, respectively. Of the specimen types tested stool was the most problematic regardless of species resulting in a failure to identify any peaks in 4/4 spots. Tissues occasionally resulted in log confidence scores of < 1.7 or failure to identify any peaks. Sampling at 72 hrs resulted in 100% (14/14) agreement for the RG species, but only 73% (35/48) for the SG**Conclusions:** This study demonstrates that AFA-US-MALDI-based identification is possible from positive MGIT tubes and can be standardized for most species/strains at 48 hrs.

Author Disclosure Block:

S. Fisher: None. **L. Adams:** None. **K. Dionne:** None. **N. Parrish:** None.

Poster Board Number:

MONDAY-115

Publishing Title:

Performance of the Xpert Mtb/Rif Assay on Formalin Fixed Paraffin-Embedded Tissues in an Oncology Patient Population

Author Block:

T. McMillen[1], J. Chen, A. Gomez, J. Kaplan, Y.W. Tang, M. Kamboj, MSKCC, I. Budvytiene, N. Banaei, Stanford Medical Center, N. Babady[2]; [1]Mem. Sloan-Kettering Cancer Ctr., New York, NY, [2]Mem. Sloan Kettering Cancer Ctr., New York, NY

Abstract Body:

Oncology patients often undergo invasive tissue biopsies as part of their cancer evaluation. Incidental findings of positive acid-fast bacilli (AFB) stain on formalin fixed paraffin-embedded (FFPE) tissue biopsies prompts additional investigation particularly requests for detection of *Mycobacterium* tuberculosis complex (MTBC). A common challenge is non availability of fresh tissue for conventional testing and inability to perform AFB cultures on FFPE samples. The Xpert MTB/RIF is a real-time PCR assay, IVD cleared for the detection of MTBC in expectorated sputum samples. Data on the performance of the assay in FFPE samples is limited. The goal of this study is to evaluate the performance of the Xpert MTB/RIF on FFPE tissue in a tertiary care cancer center with low rates of TB and high prevalence of non-tuberculosis mycobacteria (NTM). A total of 84 FFPE tissue samples were tested by the Xpert MTB/RIF, including 36 samples positive (4 TB positive by culture/PCR) by FITE stain for AFB and 48 samples negative by FITE for AFB. The 84 samples represented 61% pulmonary FFPE tissues and 39 % extrapulmonary FFPE tissues. Additionally, one PCR confirmed MTBC positive FFPE sample was included as a positive control. Each sample was de-paraffinized, digested and pre-treated prior to inoculation into the Xpert cartridge. The Xpert MTB/RIF test was then performed according to manufacturer's recommendations. The agreement between culture result and Xpert MTB PCR was very good at 98.0% (κ=0.87). Sensitivity, specificity, positive and negative predictive value were 100% (5/5, 95% CI: 39.7-100%), 98.8% (80/81, 95% CI: 93.3-99.9%), 80% (95% CI: 28.3-99.5%), and 100% (95.5-100%) respectively. One sample was Xpert positive, FITE stain and culture negative and repeat tissue cultures three months later were positive for MTBC. For an oncology patient population, with low incidence of TB, the specificity and negative predictive value of the assay on FFPE was excellent. With its rapid turn-around time, the Xpert MTB/RIF assay offers a valid option for ruling out TB and minimizing resource utilization

Author Disclosure Block:

T. McMillen: None. **N. Babady:** I. Research Relationship; Self; Cepheid.

Poster Board Number:

MONDAY-117

Publishing Title:

Use of Pleural Fluid Interferon-Gamma Enzyme-Linked Immunospot Assay in the Diagnosis of Pleural Tuberculosis

Author Block:

T. Adilistya, D. Astrawinata; Cipto Mangunkusumo Gen. Hosp., Jakarta Pusat, Indonesia

Abstract Body:

Background: Early and accurate diagnosis of pleural tuberculosis (TB) is difficult as current diagnostic methods are lack of specificity (pleural fluid cell counts and biochemical levels), lack of sensitivity (acid fast bacilli smears), and time-consuming (TB culture). It is suggestive that whilst only a small number of antigen-specific T cells are found in peripheral circulation, highly activated, antigen-specific effector T cells accumulate at disease site and rapidly produce Th-1-type cytokines. We evaluated the diagnostic value of an interferon-gamma release assay (IGRA) with enzyme-linked immunospot (ELISPOT) method, T-SPOT.TB, in the diagnosis of pleural TB using pleural fluid mononuclear cells (PFMC). **Methods:** Forty-eight subjects, presumed to have pleural TB with exudative pleural effusion by Light's criteria, dominated by mononuclear cells, had their pleural fluid specimen tested with T-SPOT.TB, Mycobacterial Growth Indicator Tube (MGIT) culture, and adenosine deaminase (ADA) activity. MGIT culture and ADA activity with the cut-off value of 40 U/L were used as the gold standard for pleural TB. Other causes of pleural effusion such as heart failure, chronic kidney disease, hepatic cirrhosis, and malignancy were excluded.**Results:** Thirty-nine (81.25%) out of 48 patients were considered having pleural TB based on positive MGIT culture and/or ADA activity. All of them (100%) were positive for T-SPOT.TB. Among 9 patients with no pleural TB, there was 1 patient with positive T-SPOT.TB. The sensitivity, specificity, positive predictive value, and negative predictive value of the IGRA ELISPOT assay using PFMC for the diagnosis of pleural TB were 100%, 88.89%, 97.5%, and 100%, respectively.**Conclusions:** These results demonstrate that the IGRA with ELISPOT method performed on PFMC is useful for a rapid and reliable diagnosis of pleural TB in clinical practice, especially in area with high TB burden.

Author Disclosure Block:

T. Adilistya: None. **D. Astrawinata:** None.

Poster Board Number:

MONDAY-118

Publishing Title:

Diagnosis of *Mycobacterium tuberculosis* and Non-tuberculous *Mycobacterium* Infections Using a Novel Plasma-Based Next-Generation Sequencing Assay

Author Block:

D. K. Hong[1], C. Truong[2], N. Banaei[2]; [1]Karius, Inc., Menlo Park, CA, [2]Stanford Univ. Sch. of Med., Palo Alto, CA

Abstract Body:

Background: Mycobacterial infections can be difficult to diagnose by conventional culturing methods. Both *Mycobacterium tuberculosis* and non-tuberculous *Mycobacterium* species often require invasive sampling and long culturing times to make a laboratory diagnosis. Molecular diagnostic techniques have been particularly useful in increasing diagnostic sensitivity and shortening turnaround time. **Methods:** We are developing a minimally invasive next-generation sequencing (NGS)-based infectious diseases assay that takes advantage of circulating cell-free plasma DNA originating from invasive pathogens. After filtering human sequences, remaining sequences are aligned to a pathogen reference sequence database and relative abundance is assigned. **Results:** We report two patients with *Mycobacterium tuberculosis* and one patient with disseminated *Mycobacterium haemophilum* in whom the diagnosis was confirmed by our novel assay. These patients had culture-confirmed *Mycobacterium* infections from invasive biopsies. Plasma samples obtained at the time of diagnostic biopsy had high levels of *Mycobacterium* sequences that could be resolved to *M. tuberculosis* complex and *M. haemophilum*. **Conclusions:** This is the first demonstration of the use of a new NGS-based plasma assay to diagnose invasive *Mycobacterium* disease. Given the high sensitivity and open-ended nature of this NGS assay, it can aid in the diagnosis of deep infections with other fastidious organisms.

Author Disclosure Block:

D.K. Hong: D. Employee; Self; Karius, Inc. **C. Truong:** None. **N. Banaei:** None.

Poster Board Number:

MONDAY-119

Publishing Title:

Detection of Rapid Growing *Mycobacterium* Directly from Clinical Samples Using the Open Mode System of the Bd Max™ Platform

Author Block:

T. T. Rocchetti[1], S. Silbert[2], A. Gostnel[2], C. Kubasek[2], A. C. C. Pignatari[1], R. Widen[2];
[1]UNIFESP, São Paulo, Brazil, [2]Tampa Gen. Hosp., Tampa, FL

Abstract Body:

Background: *Mycobacterium chelonae* (MC), *M. abscessus* Group (MAG) and *M. fortuitum* Complex (MFC) are the most common rapid growing mycobacteria isolated from respiratory infections. The aim of this study was to validate a new laboratory developed multiplex Real-Time PCR (qPCR) test to detect MC, MAG and MFC directly from clinical samples using the open mode system of the BD MAX™ platform.**Methods:** A total of 134 clinical respiratory samples previously submitted for mycobacterial culture, were tested using the new multiplex qPCR test. Before testing, samples were treated with Proteinase K at 60°C for 30 minutes and heated at 95°C for 5 minutes. After this pretreatment step, each sample was inoculated into the BD MAX Sample Preparation Reagent Tube. Extraction and multiplex PCR were performed by the BD MAX™ system, using the BD MAX™ ExK™ TNA-3 extraction kit and BD MAX™ TNA MMK master mix, along with specific in-house designed primers and probes for MC, MAG, MFC and Beta Globin (internal control) detection. One set of common forward and reverse primers and distinct probes for MC and MAG detection were designed using the ITS region. For MFC, 2 forward and 1 reverse primers, as well as 1 probe were designed using the *rpoB* gene. Cycling conditions were: 80°C for 10min and 40 cycles of 95°C for 15s, 60°C for 60s. The Limit of Detection (LoD) of each target was evaluated by testing a serial of seven 10-fold dilutions of 4 different species of mycobacteria (1 MC, 1 MAG and 2 MFC). Specificity was carried out by testing different species of mycobacteria (n=17), aerobic bacteria (n=19) and candida (n=7) commonly found in respiratory infections.**Results:** Out of 134 clinical respiratory samples included, 73 were positive and 61 were negative for mycobacteria by culture. Four samples were identified as MC, 15 for MAG and three for MFC by culture and by the new multiplex PCR test. All 61 samples identified as negative by culture were also identified as negative by the new multiplex PCR test. The LoD was 10^2 for MC, 10^2 for MAG and 10^3 for MFC. The new multiplex was specific and correctly identified the species correspondent to each PCR target.**Conclusions:** The new multiplex qPCR test performed on the BD MAX System proved to be a sensitive and specific test to detect MC, MAG and MFC on an automated sample-in results-out platform.

Author Disclosure Block:

T.T. Rocchetti: None. **S. Silbert:** None. **A. Gostnel:** None. **C. Kubasek:** None. **A.C.C. Pignatari:** None. **R. Widen:** None.

Poster Board Number:

MONDAY-120

Publishing Title:

Performance Evaluation of the Cobas Taqman Mtb Assay According to Its Clinical Application

Author Block:

J. Park, D. Song, H. Huh, C-S. Ki, N. Lee; Samsung Med. Ctr., Seoul, Korea, Republic of

Abstract Body:

Background: The COBAS TaqMan MTB test (COBAS MTB ; Roche Diagnostics, Basel, Switzerland) is used for rapid diagnosis of tuberculosis from clinical specimens. The purpose of this study was to evaluate the performance of the COBAS MTB assay according to its clinical application in a country with intermediate prevalence of tuberculosis.**Methods:** We retrospectively analyzed data from 514 consecutive patients from whom mycobacterial cultures and COBAS MTB assay were requested at the same day. Based on patients' medical records, two different categories of clinical applications of the COBAS MTB assay were identified: (1) clinically high probability of pulmonary tuberculosis according to their clinical and radiological features (n=66); (2) clinically low probability for pulmonary tuberculosis (n=448). Using the mycobacterial culture as the gold standard, the sensitivity, specificity, positive predictive value (PPV), and negative predictive values (NPV) of the COBAS MTB assay in each clinical application were calculated.**Results:** The sensitivity for MTB detection in category 1 was 91.7% (95% confidence interval [CI], 59.8-99.6%) while that in category 2 was 33.3% (1.8-87.5%). The specificities in category 1 and 2 were 94.4% (83.7-98.6%) and 99.3% (97.9-99.8%), respectively. The PPV and NPV were 78.6% (48.8-94.3%) and 98.1% (88.4-99.9%) in categories 1, respectively, and 25.0% (1.3-78.1%) and 99.5% (98.2-99.9%) in category 2, respectively.**Conclusion:** The COBAS MTB assay showed different performance according to its clinical application. Through this study, careful interpretation for the results of COBAS MTB assay would be needed according to its clinical application. We believe that this study would be of helpful for physicians to interpret the COBAS MTB assay.

Author Disclosure Block:

J. Park: None. **D. Song:** None. **H. Huh:** None. **C. Ki:** None. **N. Lee:** None.

Poster Board Number:

MONDAY-121

Publishing Title:

Genus-Specific Screening for Mycobacteria from Hundreds of Samples in 4 Hours

Author Block:

Z. Cheng[1], H. Jiang[2], H. Wang[2], Z. Zheng[1]; [1]Inst. of Basic Med. Sci., Chinese Academy of Med. Sci., beijing, China, [2]Inst. of Dermatology, Chinese Academy of Med. Sci., Nanjing, China

Abstract Body:

Background: The mycobacteria bring about miscellaneous disorders in humans, especially in the lungs. Treating patients early and rendering them non-infectious is essential for control and elimination of these diseases. New diagnostics with improved sensitivity and simplified procedure are still needed. Capture and ligation probe-PCR (CLIP-PCR) is ahigh throughput RNA quantification technology that requires no RNA purification and reverse transcription, which has been used for large scale screening of*Plasmodium* 18S rRNA. In this study, we adopted CLIP-PCR for rapid, large-scale identification of genus *Mycobacterium*. **Methods:** 16S rRNA sequence of seven reference strain *Mycobacterium leprae* (GenBank: X53999.1),*Mycobacterium tuberculosis*(GenBank: X52917.1),*Mycobacterium marinum* (GenBank: X52920.1), *Mycobacterium fortuitum* (GenBank: X65528.1), *Mycobacterium abscessus* (GenBank: AJ536038.1), *Mycobacterium avium*(GenBank: X52918.1)and *Mycobacterium intracellulare*(GenBank: X52927.1)were aligned and the consensus part was selected as target. In CLIP-PCR, targeted RNA is released from blood by lyse, captured onto 96-well plates with probes, where continuously hybridized probes would be ligated, and quantified with qPCR by amplifying the ligated probes. To accelerate the hybridization process, and avoid false-negative caused by genetic variation between clinical isolates, we adopted a multi section strategy, in which more than one set of probes were added to the reaction. These probe sets would either individually or collectively capture, and quantify the target. The assay was tested using clinically collected isolates of the seven strains. **Results:** All samples of the seven strains resulted in a distinct peak in melting curve analysis. With multi section strategy, the turn around time of CLIP-PCR was reduced from about 18 hours down to 4 hours, with little compromise of sensitivity. **Conclusions:** In conclusion, CLIP-PCR is of great potential for fast and large scale screening for genus mycobacteria.

Author Disclosure Block:

Z. Cheng: None. **H. Jiang:** None. **H. Wang:** None. **Z. Zheng:** None.

Poster Board Number:

MONDAY-122

Publishing Title:

Comparison of Quantiferon-TB Gold-Plus to Quantiferon-TB Gold Assay in Health Care Workers at a Low-Incidence Setting

Author Block:

H. Moon[1], R. L. Gaur[1], M. Pai[2], N. Banaei[1]; [1]Stanford Univ. Sch. of Med., Palo Alto, CA, [2]McGill Univ., Montreal, QC, Canada

Abstract Body:

Interferon-gamma release assays (IGRAs) are used by many health programs to diagnose latent *M. tuberulosis* infection (LTBI). We prospectively compared the performance of QuantiFERON-TB Gold-Plus (QFT-plus) to QuantiFERON-TB Gold in-tube (QFT) in low risk health care wokers (HCW) at a U.S. institution. In total, 988 HCWs presenting to Stanford Health Care Occupational Clinic for TB screening were tested with the QFT and the QFT-Plus assays. Tube order for blood draw included: purge, nil, TB antigen 1 (TB1), TB 2, TB antigen, and mitogen. Risk factors for LTBI were obtained. The QFT-Plus assay was interpreted as positive when either TB1 or TB2 result was above the assay cut-off. TB1 and TB2 results were also analyzed separately. The positivity rate of QFT, QFT-plus, QFT-plus TB1, and QFT-plus TB2 were 4.3%, 6.4%, 4.1% and 5.2%, respectively. Agreement of QFT with QFT-plus, QFT-plus TB1, QFT-plus TB2 and between QFT-plus TB1 and QFT-plus TB2 was good at 95.6%, 96.7%, 96.5% and 96.5%, respectively (Kappa, 0.57, 0.59, 0.61 and 0.61, respectively). Most of the discrepant results (44/51, 86.3%) were within the borderline range of 0.2-0.7 IU/mL. Quantitative results obtained with QFT showed high degree of correlation with QFT-plus TB1 and QFT-plus TB2 (Pearson's correlation coefficient (r) = 0.743 and 0.748, respectively). QFT-plus TB1 and QFT-plus TB2 also showed very high correlation with each other (r = 0.902). The specificities of QFT, QFT-plus, QFT-plus TB1 and TB2 in 608 HCWs with no identifiable risk factors were 98.0%, 96.9%, 98.4% and 97.5%, respectively. The specificities were not significantly different between assays (P>0.05). The QFT-plus assay showed fairly good agreement with the standard QFT assay in HCW at a low-incidence setting.

Author Disclosure Block:

H. Moon: None. **R.L. Gaur:** None. **M. Pai:** None. **N. Banaei:** None.

Poster Board Number:

MONDAY-123

Publishing Title:

Effect of Incubation at Collection Site on the Rate of Indeterminate Results of Quantiferon®-Tb Gold Samples

Author Block:

H. Kapoor[1], A. S. Edelman[2], H. J. Batterman[3]; [1]Quest Diagnostics, Chalfont, PA, [2]Quest Diagnostics, Horsham, PA, [3]Focus Diagnostics, San Juan Capistrano, CA

Abstract Body:

QuantiFERON®-TB Gold (QFT®) is an in vitro diagnostic test for *Mycobacterium tuberculosis* infection; it uses specific peptide antigens ESAT-6, CFP-10, and TB7.7 proteins to stimulate cells in heparinized whole blood. The QFT system uses specialized blood collection tubes containing these antigens, mitogen (positive control), and NIL control and should be incubated at 37°C ± 1°C within 16 hours of collection. Indeterminate results (lack of significant response in the mitogen tube) can be seen in immunocompromised patients or be secondary to pre analytic variables, including the time from collection to incubation. To reduce the time from collection to incubation and ensure that it was less than 16 hours, Quest Diagnostics started a program of incubation at the collection site in our Patient Service Centers.Phlebotomy staffs that collected the samples were trained on a standard operating procedure for the incubation at collection site. Locations enrolled for this program were provided an incubator to strictly hold temperature at 37°C ± 1°C. The specimens were placed in the incubator within a few hours of collection and the incubation time was standardized. These pre-incubated samples were sent to the Philadelphia laboratory of Quest Diagnostics for the interferon gamma ELISA. Rate of indeterminate results were monitored for non-incubated and pre-incubated samples.The incubation at collection site initiative for QFT samples was started in May 2014. Prior to this initiative the average rate of indeterminate results for the year 2013 and the first 4 months of 2014 was 10.8% of the non-incubated samples (n=2100). During the period from May 2014 through August 2015 approximately 34% of the QFT samples (n=640) were incubated at collection site and 1240 QFT sample were non-incubated. Rate of indeterminate results of non-incubated samples averaged 8.4% and of pre-incubated samples averaged 4.3% (49% reduction, $P<0.0001$).A significant decrease in the rate of indeterminate results was observed when QTF samples were incubated at the collection site.

Author Disclosure Block:

H. Kapoor: D. Employee; Self; Quest Diagnostics. **A.S. Edelman:** D. Employee; Self; Quest Diagnostics. **H.J. Batterman:** D. Employee; Self; Quest Diagnostics.

Poster Board Number:

MONDAY-124

Publishing Title:

Development of a Point of Care Diagnostic for Early Tb Diagnosis

Author Block:

P. Sule, Yunfeng Cheng , Ying Kong, Ronak Tilvawalaa, HexinXie , Hany Hassounaha, Aneesa Noormohameda,; TAMHSC, Brayn, TX

Abstract Body:

Mycobacterium tuberculosis (Mtb) caused 9.6 million new tuberculosis (TB) cases in 2014 (WHO). In the absence of prompt and effective treatment TB mortality is as high as 50% (TBDI 4th) creating a very grim scenario in resource-limited countries. Detecting TB can be a challenge and sometimes take up to months, posing a huge risk of transmission, dissemination and emergence of new cases. The current diagnostics available are either very time consuming or require training and considerable infrastructure. In this project, we detail the development of a unique point of care (POC) diagnostic assay for early TB detection, in an attempt to overcome what is perceived as a major roadblock in TB prevention. The reported test is affordable, delivers results in less than 10 minutes and is easy to perform requiring minimal setup. The diagnostic assay utilizes BlaC, a unique *Mtb* specific biomarker in a reporter enzyme fluorescence system. 160 clinical specimens (sputum) were tested using our diagnostic assay. The results obtained were compared to previously documented culture and smear results for the specimens. In this study our assay diagnosed clinical samples as being *Mtb* positive with a sensitivity of 89% for smear +/culture + samples, 88% for smear -/ culture + samples and a combined specificity of 82%. We propose the use of our diagnostic assay as the much needed triage test performed as a first line diagnostic assay with a potential to greatly benefit tuberculosis containment strategies across the globe.

Author Disclosure Block:

P. Sule: None.

Poster Board Number:

MONDAY-125

Publishing Title:

The Role of Tb Lamp and Xpert Mtb Rif Assay for the Diagnosis of Smear Negative Pulmonary Tb

Author Block:

M. G. Worku, Z. Dagne, Z. Yaregal, A. H/Mariam, S. Moga, A. Meaza, A. Kebede, Y. Feseha; Ethiopian Publ. Hlth.Inst., Addis Ababa, Ethiopia

Abstract Body:

Background: Smear negative TB remains a diagnostic challenge. In the absence of sensitive diagnostic method clinician often make the diagnosis of smear-negative pulmonary TB with information obtained through the clinical history, physical examination and chest X-ray. This information is not specific which leads to over or under treatment. Thus, sensitive diagnostic methods have a considerable contribution to decide the appropriate treatment. **Methods:** The study was conducted from December, 2014 to June, 2015. All TB presumptive referred to St. Peter TB specialized hospital were enrolled according to the national recruitment criteria. Socio-demographics data, TB symptoms, chest X-ray findings and final decision for anti-TB treatment was collected for each TB presumptive. Spot-Moring-spot sputum was collected for direct ZN examination. The morning sputum was used for culture (LJ and MGIT), TB-LAMP, Xpert MTB/RIF assay and fluorescent microscopy examination. **Results:** This study enrolls 459 smear negative presumptive TB patients. Of which 57% (252/442) were Females and median age 40 IQR (28-55) years. The prevalence of HIV among the study participants were 30%. The overall culture positivity rate was 6.8% (30/439), of which 6.6% (26/391) was by MGIT and 5.3% (23/436) was by LJ method. Direct and concentrated fluorescent microscopy adds in 0.9% and 1.3% for smear negative suspects compared to direct ZN. The overall sensitivity and specificity of TB-LAMP was 61.5% (16/26) and 96.6% respectively. The overall sensitivity and specificity Xpert MTB/RIF was 70.8% (17/24) and 97.2% respectively. **Conclusions:** TB-LAMP and Xpert MTB/RIF assay can provide confirmatory result for at least two third of TB cases.

Author Disclosure Block:

M.G. Worku: None. **Z. Dagne:** None. **Z. Yaregal:** None. **A. H/Mariam:** None. **S. Moga:** None. **A. Meaza:** None. **A. Kebede:** None. **Y. Feseha:** None.

Poster Board Number:

MONDAY-126

Publishing Title:

Verification and Clinical Application of a Laboratory Developed Sequencing Assay for the Identification of Mycobacteria in Pediatric Patients

Author Block:

H. Wang, J. Madison, S. Sims, C. Tomatis, A. Leber, S. Antonara; Nationwide Children's Hosp., Columbus, OH

Abstract Body:

In the United States, rates of tuberculosis in young children are low, leaving the nontuberculous mycobacteria (NTM) as the most common cause of mycobacterial infections in pediatric and cystic fibrosis patients. A conclusive diagnosis of mycobacterial infections relies upon the isolation and identification of the causative agent, which historically was a slow and labor intensive process. Currently, a number of molecular and proteomic methods are available that are both quick and precise for mycobacterial identification.Our laboratory has adopted a partial *sec*A1 sequencing technique as the primary method of identification of mycobacteria. This test targets a 700-bp fragment of the *sec*A1 gene coding for 233 amino acid residues located on the N-terminal region of the protein (Zelazny A. *et. al*., 2005). For assay verification, 84 isolates (7 *M. tuberculosis* complex, 19 *M. abscessus* group, 23 *M. avium* complex and others, a total of 24 species) comprising ATCC strains and patient isolates obtained at our hospital were included. Results were compared with two reference laboratories utilizing either a proteomic or a sequencing method. We achieved 100% agreement in identification at species level with the reference methods used. A curated sequence database representing 70 mycobacterial species (including isolates that were specifically identified in our patient population) was built and a phylogenetic tree with partial *sec*A1 sequences from this database was generated.Limitations of use of partial *sec*A1 sequencing in pediatric patients include; a) a lack of subspeciation of *M. abscessus* group isolates, and b) the lack of differentiation of some members of the *M. fortuitum* group (*M. senegalense* and *M. conceptionense*), leaving the clinical significance of these two species in this patient group unclear.The performance of this assay suggests that this is a rapid and reliable tool that reduces the turnaround time of identification of mycobacterial isolates and improves patient care. The continuous expansion of the partial *sec*A1 database ensures an accurate identification of clinical isolates as well as a source of tracking of commonly seen species in our patient population.

Author Disclosure Block:

H. Wang: None. **J. Madison:** None. **S. Sims:** None. **C. Tomatis:** None. **A. Leber:** None. **S. Antonara:** None.

Poster Board Number:

MONDAY-127

Publishing Title:

Clinical Risk Score for Prediction of Extended-Spectrum β-Lactamase-Producing *Enterobacteriaceae* in Bloodstream Isolates

Author Block:

M. Augustine[1], T. L. Testerman[1], J. Justo[2], P. Bookstaver[2], J. Kohn[3], H. Albrecht[1], **M. Al-Hasan**[1]; [1]Univ. of South Carolina Sch. of Med., Columbia, SC, [2]South Carolina Coll. of Pharmacy, Columbia, SC, [3]Palmetto Hlth.Richland, Columbia, SC

Abstract Body:

Background: Appropriate empiric antimicrobial therapy is associated with improved survival in patients with bloodstream infections (BSI). However, fear of extended-spectrum ß-lactamase-producing *Enterobacteriaceae* (ESBLE) may lead to inappropriate use of carbapenems, further contributing to increased antimicrobial resistance. This study aims to develop a clinical score to predict probability of BSI due to ESBLE. **Methods:** Adult patients with BSI due to *Enterobacteriaceae* hospitalized at the Palmetto Health System in Columbia, South Carolina, from January 2010 to June 2015 were identified. Multivariate logistic regression was used to identify independent risk factors for ESBLE BSI. Point allocation in ESBL prediction score was based on regression coefficients. Model discrimination was assessed by area under receiver operating characteristic curve (AUC).**Results:** Among 910 patients with *Enterobacteriaceae* BSI, 42 (4.6%) had BSI due to ESBLE. Overall, median age was 66 years and 509 (56%) were women. Independent risk factors for ESBLE and point allocation in the ESBL prediction score included outpatient procedures within 30 days of BSI (odds ratio [OR] 8.7, 95% confidence interval [CI]: 3.1-22.9; 1 point), prior colonization with ESBLE (OR 26.8, 95% CI: 7.0-108.2; 4 points), and number of courses of ß-lactams and/or fluoroquinolones used within 90 days prior to BSI: 1 course (OR 6.3, 95% CI: 2.7-14.7; 1 point), ≥ 2 courses (OR 22.0, 95% CI: 8.6-57.1; 3 points). AUC for both final logistic regression and ESBL prediction score models was 0.86. Patients with an ESBL prediction score of 0, 1, 3, and 4 had an estimated probability of ESBLE of 0.7%, 5%, 24% and 44%, respectively.**Conclusions:** ESBL prediction score estimates patient-specific risk of BSI due to ESBLE with high discrimination, which may improve empiric antimicrobial therapy and carbapenem utilization.

Author Disclosure Block:

M. Augustine: None. **T.L. Testerman:** None. **J. Justo:** J. Scientific Advisor (Review Panel or Advisory Committee); Self; Advisory board member for Cempra Pharmaceuticals. **P. Bookstaver:** J. Scientific Advisor (Review Panel or Advisory Committee); Self; Actavis Pharmaceuticals (now Allergan). **J. Kohn:** None. **H. Albrecht:** None. **M. Al-Hasan:** None.

Poster Board Number:

MONDAY-129

Publishing Title:

Current Antibiotic Susceptibility Profile of *Streptococcus pneumoniae* from North America: Data from the Test Program 2010-2015

Author Block:

D. Hoban[1], **D. Biedenbach**[1], D. Sahm[1], H. Leister-Tebbe[2]; [1]IHMA, Inc., Schaumburg, IL, [2]Pfizer, Inc, Collegeville, PA

Abstract Body:

Background: *Streptococcus pneumoniae* (SP) is commonly associated with community-acquired pneumonia (CAP), acute exacerbations of chronic bronchitis (AECB), acute bacterial sinusitis (AS), meningitis, bacteremia and acute otitis media (OM) that can progress to debilitating pneumococcal disease, causing significant morbidity and mortality worldwide. The increase in the rates of resistance of SP to frequently used antibiotics and the rapid global spread of multidrug-resistant strains even in countries with extensive SP immunization programs such as North America requires comprehensive surveillance and monitoring. The Tigecycline Evaluation Surveillance Trial (TEST) is an ongoing global surveillance program. In this study, we evaluate the susceptibility of SP from North America from 2010-2015.**Methods:** 2644 SP isolates from various sources were collected from investigative sites in North America. Minimum inhibitory concentrations (MICs) were determined by broth microdilution panels locally and interpreted according to CLSI guidelines.**Results:** Overall (2010-2015), *in vitro* penicillin susceptibility of SP from North America was 62.5%, and erythromycin susceptibility was 59.9%. Additional overall summary data are shown in the following table:

Drug	**MIC_{50}**	**MIC_{90}**	**%S**	**%I**	**%R**
Levofloxacin	1	1	99.3	0.1	0.6
Tigecycline	0.015	0.03	97.6	0	2.4
Ceftriaxone	≤0.03	1	95.3	4.2	0.5
Meropenem	≤0.12	1	80.9	5.2	13.9
Clindamycin	0.06	> 64	82.5	0.5	16.9
Penicillin	≤0.06	2	62.5	22.4	15.2
Erythromycin	0.06	64	59.9	0.6	39.5

Conclusions: Levofloxacin, ceftriaxone and tigecycline all showed potent *in vitro* activity against SP from North America with %S of >95%. Based on percent susceptibility the *in vitro* activities of erythromycin and penicillin were the lowest among the drugs evaluated. Continued

surveillance of resistance in SP to established antimicrobials is warranted and is essential in countries with extensive adult and pediatric SP immunization programs.

Author Disclosure Block:

D. Hoban: M. Independent Contractor; Self; IHMA, Inc. **D. Biedenbach:** M. Independent Contractor; Self; IHMA, Inc. **D. Sahm:** M. Independent Contractor; Self; IHMA, Inc. **H. Leister-Tebbe:** D. Employee; Self; Pfizer, Inc..

Poster Board Number:

MONDAY-130

Publishing Title:

Identification of Genotypic and Phenotypic Antimicrobial Resistance among *Salmonella* isolated from Humans in the United States, 2014

Author Block:

J. P. Folster[1], D. Campbell[1], J. Chen[2], J. Reynolds[1], H. Carleton[1], J. Whichard[1]; [1]CDC, Atlanta, GA, [2]IHRC, Inc, Atlanta, GA

Abstract Body:

The National Antimicrobial Resistance Monitoring System (NARMS) examines antimicrobial resistance among foodborne pathogens, including *Salmonella*, in the United States. In this study, we performed whole genome sequencing (WGS) on 255 *Salmonella* isolated from humans in 2014 that were resistant to ≥1 of 14 drugs tested for antimicrobial susceptibility testing (AST) by NARMS. Sixty different resistance patterns were observed. We used BioNumerics 7.5 to analyze WGS data and identify acquired resistance genes (ResFinder [cge.cbs.dtu.dk]) and mutational resistance determinants (*in silico* PCR). Genetic predictions correlated with traditional phenotypic AST for all 14 drugs (>96% sensitivity, specificity, positive predictive value, negative predictive value and a kappa coefficient =0.97). Resistance to most drugs was mediated by common enteric resistance determinants, e.g.: ampicillin resistance by *bla*$_{\text{TEM-1b}}$, tetracycline by *tetA/B*, sulfisoxazole by *sul1/2*, chloramphenicol by *floR*. Resistance to 3rd generation cephalosporins, including ceftriaxone, was mostly mediated by *bla*$_{\text{CMY-2}}$, an AmpC-type beta-lactamase, however, we did find several extended spectrum beta-lactamases, including *bla*$_{\text{SHV-12, SHV-30, CTX-M-1, CTX-M-55}}$ and two *bla*$_{\text{CTX-M-65}}$. One isolate had decreased susceptibility to the macrolide azithromycin, and contained *mphA*, a macrolide resistance determinant. Resistance to the quinolone nalidixic acid was mainly mediated by mutations in the quinolone resistance determining region (QRDR). Most ciprofloxacin-resistant isolates had both QRDR mutations and plasmid-mediated quinolone resistance (PMQR) genes. We observed some discordant genotypic/phenotypic results, suggesting that some resistance determinants may not be functional. Notably, only three isolates had phenotypic resistance without genotypic determinants, suggesting there may be novel resistance determinants and highlights the need for continued phenotypic testing. This study shows the continued effectiveness of WGS analysis for resistance prediction in *Salmonella*.

Author Disclosure Block:

J.P. Folster: None. **D. Campbell:** None. **J. Chen:** None. **J. Reynolds:** None. **H. Carleton:** None. **J. Whichard:** None.

Poster Board Number:

MONDAY-131

Publishing Title:

One Health Laboratory Surveillance for Multi-Drug Resistant *Escherichia coli* in the Upper USA Midwest: Preliminary Report

Author Block:

T. R. Fritsche[1], B. J. Olson[2], M. C. Hall[1], S. K. Shukla[2], T. J. Novicki[1], F. M. Moore[1], D. A. Ortiz[2]; [1]Marshfield Clinic, Marshfield, WI, [2]Marshfield Clinic Res. Fndn., Marshfield, WI

Abstract Body:

Background: The 2015 "*National Action Plan for Combating Antibiotic Resistant Bacteria*" calls for strengthening of One Health surveillance efforts. Here we compared fecal colonizing and infection isolates of *Escherichia coli* (EC) recovered from humans, companion and food-producing animals for β-lactamases responsible for resistance to extended-spectrum (ES) agents. Our goal is to characterize and track emergence of multi-drug resistant (MDR) EC in urban and rural areas served by our health care system. **Methods**: Residual human, canine and bovine stool specimens (n=2900) submitted during 2014 were screened on chromogenic agars for EC expressing ES β-lactamases (ESBLs), cephalosporinases (AmpCs), and carbapenemases. Human (n=112) and canine (n=104) infection isolates resistant to ES cephems were included for comparison. Isolates were identified using mass spectrometry and had susceptibility profiles generated using dry-form broth microdilution panels. *bla* gene families were characterized using a commercial micro-array targeting 38 gene families. **Results**: Among fecal specimens screened, EC resistant to ES cephems were present in 5.2% (111/2143), 15.3% (96/627) and 49.2% (64/130) of human, canine and bovine samples, respectively. Of those human colonizing and infection isolates positive by microarray, 36.3% and 52.2%, respectively, were positive for ESBL genes, while 19.3% and 17.7% were positive for AmpC genes. Corresponding figures for canine colonizing and infection isolates, respectively, were 19.7% and 15.9% for ESBLs, and 48.3% and 64.5% for AmpCs. Bovine isolates included 26.2% ESBLs and 33.0% AmpCs. Of 491 MDR isolates tested by microarray, 34 (6.9%) were negative for any gene target. Among 10 gene families detected, CTX-M-15-like and CTX-M9 groups (ESBLs), and CMY-2 (AmpC) genotypes were predominant regardless of host origin. Carbapenem resistant isolates were not detected. **Conclusion**: Characterization and tracking of MDR EC emergence in our 'One Health' service area will allow us to better understand risk factors for exposure of urban dwellers, farm families and farm workers to particular pathogens and resistance profiles.

Author Disclosure Block:

T.R. Fritsche: None. **B.J. Olson:** None. **M.C. Hall:** None. **S.K. Shukla:** None. **T.J. Novicki:** None. **F.M. Moore:** None. **D.A. Ortiz:** None.

Poster Board Number:

MONDAY-132

Publishing Title:

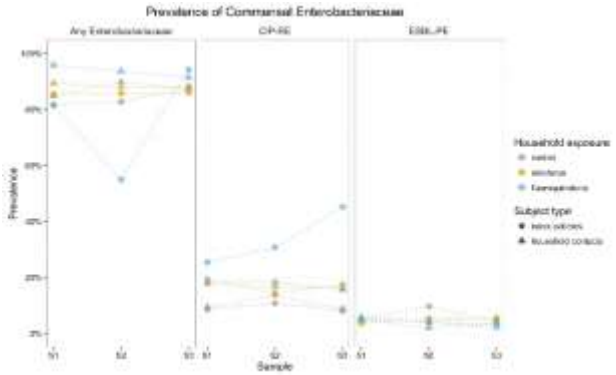
Impact of Outpatient Antibiotics for Urinary Tract Infections (Utis) on Susceptible and Resistant Commensal Enterobacteriaceae

Author Block:

A. J. Stewardson[1], N. Adriaenssens[2], S. Coenen[2], M. Godycki-Cwirko[3], B. Huttner[1], A. Kowalczyk[3], C. Lammens[2], J. Vervoort[2], S. Malhotra-Kumar[2], H. Goossens[2], S. Harbarth[1]; [1]Univ. of Geneva Hosp., Geneva, Switzerland, [2]Univ. Antwerp, Antwerp, Belgium, [3]Med. Univ. of Lodz, Lodz, Poland

Abstract Body:

Aim: To quantify the impact of antibiotics prescribed for UTIs on intestinal Enterobacteriaceae, while accounting for household microbiota clustering. **Methods:** We performed a prospective cohort study from Jan 2011 to Aug 2013 at sites in Belgium, Poland and Switzerland. We recruited outpatients requiring (exposed) and not requiring (non-exposed) antibiotics for suspected UTI, and 1-3 household contacts for each index patient. Subjects provided 3 fecal samples: baseline (S1); end of antibiotics (S2); and 28 days later (S3). We used multivariable, mixed-effects Poisson regression to model the impact of antibiotic class on intestinal colonisation by i) total Enterobacteriaceae, ii) ciprofloxacin-resistant-Enterobacteriaceae (CIP-RE), and iii) extended-spectrum beta-lactamase-producing Enterobacteriaceae (ESBL-PE).**Results:** 303 households (95 non-exposed, 208 exposed) with 722 subjects were included. UTI patients received nitrofurans (88, 42%), quinolones (77, 37%) and other antibiotics. As shown in the figure, exposure to quinolones resulted in transient suppression of Enterobacteriaceae and subsequent 1.9-fold increase in CIP-RE prevalence (adjusted prevalence ratio [aPR], 1.9; 95%CI, 1.1−3.3). Nitrofurans had no impact on CIP-RE (aPR 1.1; 0.7-2.0). Antibiotic exposure was not associated with ESBL-PE colonisation, which was detected infrequently (71 [8%] subjects).



Conclusions: These findings support recommendations to avoid quinolones as first-line UTI therapy given their collateral impact on commensal microbiota.

Author Disclosure Block:

A.J. Stewardson: None. **N. Adriaenssens:** None. **S. Coenen:** None. **M. Godycki-Cwirko:** None. **B. Huttner:** None. **A. Kowalczyk:** None. **C. Lammens:** None. **J. Vervoort:** None. **S. Malhotra-Kumar:** None. **H. Goossens:** None. **S. Harbarth:** None.

Poster Board Number:

MONDAY-133

Publishing Title:

Molecular Characterization Of Colistin-Resistant *acinetobacter Baumannii* Isolated From A University Hospital In Thailand

Author Block:

I. Thaipisuttikul[1], P. Naksena[1], P. Thumnu[1], P. Eiamratchanee[2], S. Prombhul[1], R. K. Ernst[3], C. Tribuddharat[1]; [1]Faculty of Med. Siriraj Hosp., Mahidol Univ., Bangkok, Thailand, [2]Mahidol Univ. Intl. Coll., Nakhon Pathom, Thailand, [3]Univ. of Maryland Sch. of Dentistry, Baltimore, MD

Abstract Body:

Acinetobacter baumannii is currently one of the most concerned bacterial pathogens due to its extensive resistance to almost all antibiotics. In our institution, over 80% of *A. baumannii* isolates already resist to carbapenems and consequently colistin is considered the last line drug against this XDR organism. Although colistin resistance has been found <1%, the emergence of resistance posted a serious concern on the future of antibacterial therapy. We investigated nine colistin resistant isolates collected during the first emergence in 2010-2011, with colistin MIC ranging from 8 to >128 μg/ml, for molecular epidemiology and mechanism of resistance to gain insight into its emergence. All isolates were typed by MLST and examined for resistance mechanism in regard to binding target alteration from constitutive lipid A modification by sequencing and qPCR of *pmrCAB*, MALDI-TOF analysis of lipid A and *in vitro* fitness assays. The results suggested the presence of two separate clonal groups, with 5 and 4 isolates belong to pandemic ST2 clonal complex and ST215, respectively. Both groups exhibited different *pmrCAB* mutational and expression profiles suggesting that they might arise independently. However, only 2 from ST2 and 3 isolates from ST215 were found to have lipid A modification with phosphoethanolamine. Interestingly, ST2 clonal complex harbored fewer *pmrCAB* mutations but having higher MIC and *pmrCAB* overexpression while ST215, with higher number of mutations, was associated with lower MIC and no significant *pmrCAB* overexpression. In contrast to the previous reports that colistin resistance was associated with lower fitness, all isolates demonstrated comparable fitness with reference strain in growth assay, *in vitro* competition assay and membrane permeability assay. In conclusion, colistin resistance emerged independently within two known STs, possibly under strong selection by extensive drug use. Extensive *pmrCAB* mutations and expression modulation coupled with fitness compensation suggested that it might be on the verge of adaption to stabilize the resistance that foreshadows the spread of resistance in the future.

Author Disclosure Block:

I. Thaipisuttikul: None. **P. Naksena:** None. **P. Thumnu:** None. **P. Eiamratchanee:** None. **S. Prombhul:** None. **R.K. Ernst:** None. **C. Tribuddharat:** None.

Poster Board Number:

MONDAY-134

Publishing Title:

Antimicrobial Susceptibility of *Pseudomonas aeruginosa* from Bloodstream and Other Specimens of Same Patients in Japan for 10 Years (2005-2014)

Author Block:

K. Matsuzaki[1], H. Koyama[1], S. Matsumoto[1], A. Kanayama[2], I. Kanesaka[2], I. Kobayashi[2]; [1]LSI Medience Corp., Tokyo, Japan, [2]Faculty of Nursing, Toho Univ., Tokyo, Japan

Abstract Body:

Background: Infection with the multi-drug resistance (MDR) *Pseudomonas aeruginosa* is serious concern in a hospital setting. In this study, we summarized the frequency of *P. aeruginosa* as an etiological agent for bloodstream infection in Japan for 10 years and antimicrobial susceptibility patterns of the isolates from bloodstream were compared with those of the isolates from other specimens in same patients.**Methods:** *P. aeruginosa* isolates were recovered from 303,368 blood specimens collected from hospitals in Japan as well as other specimens of the same patients. Antimicrobial susceptibility testing was carried out according to the CLSI standard. Metallo-β-lactamase genes were detected by the PCR method.**Results:** A total of 0.25% (n=772) of blood culture bottles were positive for *P. aeruginosa*, among them, 65% were males with 91% of these patients being 60 years of age or older. In 36% of the patients (n=278) with positive blood cultures, *P. aeruginosa* were also detected in other specimens, sputum (15%), urine (16%) and central venous catheter (5.1%). The resistance rates to imipenem (IPM), ciprofloxacin (CPFX) and amikacin (AMK) were 30%, 23% and 5.9% in blood culture isolates, and 38%, 24% and 6.5% in other specimen isolates, respectively. Particularly, the antimicrobial susceptibility patterns of 10% of isolates from other specimens were different from those of isolates from bloodstream in same patients. MDR *P. aeruginosa*, resistant to IPM, CPFX and AMK, were also detected in 5.4% (1.5-13% in each year) in blood culture isolates and 5.6% (0-12%) in other specimen isolates, respectively. The metallo-β-lactamase gene, bla_{IMP}, were identified in 87% (53/61) of these MDR *P. aeruginosa*.**Conclusions:** We observed that different antimicrobial susceptibility patterns of *P. aeruginosa* between the isolates from other specimens and bloodstream were observed in the same patient. Antimicrobial therapy for *P. aeruginosa* infection should take into account the differences in antimicrobial susceptibility of isolates from blood cultures and the other specimens in the same patients.

Author Disclosure Block:

K. Matsuzaki: None. **H. Koyama:** None. **S. Matsumoto:** None. **A. Kanayama:** None. **I. Kanesaka:** None. **I. Kobayashi:** None.

Poster Board Number:

MONDAY-135

Publishing Title:

***In Vitro* Activity of Tedizolid and 10 Other Antimicrobial Agents in Gram-Positive Anaerobe Cocci Derived from Clinical Specimens in Austria**

Author Block:

E. Leitner, J. Tribus, E. Ullrich, G. Feierl, A. J. Grisold; Inst. of Hygiene, Microbiolgoy and Environmental Med., Med. Univ. of Graz, Graz, Austria

Abstract Body:

Background: For empiric therapy strategies resistance-surveillance is needed due to an increase of resistance in anaerobic bacteria. Recently, tedizolid was introduced for soft tissue infections. Consequently, the aim of this study was to investigate the in vitro activity of tedizolid and 10 other antimicrobial agents of Gram-positive anaerobe cocci (GPAC) in Austria.**Methods:** In total 83 non-duplicate GPAC isolates including 31 Fingoldia magna, 8 Peptrostreptococcus anaerobius, 23 Peptoniphilus assacharolyticus, 13 Peptoniphilus spp. and 8 Parvimonas micra were analyzed, collected at the Institute of Hygiene from June to December 2014. Etest (bioMérieux) or MIC test strip (Liofilchem) were used to determine the MICs of the following drugs: penicillin, ampicillin/sulbactam, piperacillin/tazobactam, imipenem, meropenem, moxifloxacin, vancomycin, clindamycin, linezolid, tedizolid and metronidazole. Interpretation was performed according to EUCAST or CLSI guidelines. For the antimicrobial agents linezolid and tedizolid with no interpretation criteria available, MIC50 and MIC90 were determined.**Results:** Within the 83 GPAC isolates highest resistance levels were found for clindamycin with 24.1% (20; 12 F. magna, 6 P. assacharolyticus, 2 P. micra) followed by moxifloxacin with 22.9% (19; 12 F. magna, 5 P. anaerobius and 2 Peptoniphilus spp.). Resistance was detected for penicillin; ampicillin/sulbactam and piperacillin/tazobactam in one P. anaerobius isolate and for metronidazole in one F. magna isolate, respectively. No resistance was detected for imipenem, meropenem and vancomycin. The MIC (50/90) were determined for linezolid with 1/2 µg/ml (range; 0.25 to >256) and tedizolid with 0.125/0.5 µg/ml (range; 0.064 to 1).**Conclusions:** The findings of this surveillance study present constantly low resistance levels for GPAC in Austria. Remarkable resistance was only observed for clindamycin and moxifloxacin which underlines the need of periodic resistance surveillance of GPAC. Based on our findings ß-lactam antibiotics and metronidazole remain useful drugs for treatment of anaerobic Gram-positive infections.

Author Disclosure Block:

E. Leitner: None. **J. Tribus:** None. **E. Ullrich:** None. **G. Feierl:** None. **A.J. Grisold:** None.

Poster Board Number:

MONDAY-136

Publishing Title:

Antimicrobial Susceptibility of 39,811 Pathogens Isolated from Patients in Canadian Hospitals: Canward Study 2007-2015

Author Block:

G. G. ZHANEL[1], H. Adam[2], M. Baxter[1], B. Weshnoweski[2], R. Vashisht[1], S. Biju[1], N. Laing[1], K. Nichol[2], A. Denisuik[1], A. Golden[1], P. Lagace-Wiens[2], J. Fuller[3], J. A. Karlowsky[2], A. Walkty[2], M. Gilmour[4], D. Bay[1], F. Schweizer[1], M. R. Mulvey[4], G. R. Golding[4], CARA, D. J. Hoban[2]; [1]Univ. of Manitoba, Winnipeg, MB, Canada, [2]Diagnostic Services Manitoba, Winnipeg, MB, Canada, [3]Alberta Hlth.Services, Edmonton, AB, Canada, [4]Natl. Microbiol. Lab., Winnipeg, MB, Canada

Abstract Body:

Background: CANWARD is a national, annual, Public Health Agency of Canada (PHAC) endorsed surveillance study assessing pathogens causing infections in Canadian hospitals and their antimicrobial resistance patterns.**Methods:** From 2007 to 2015, 39,811 pathogens were collected from tertiary-care hospitals across Canada. Antimicrobial susceptibility testing was performed using CLSI broth microdilution methods with >45 marketed and investigational agents.**Results:** Specimen source composition of the 39,811 isolates was 43.8% blood, 32.7% respiratory, 13.4% urine and 10.2% wound specimens. Patient demographic characteristics were: 54.5/45.5% male/female; 13.1/44.4/42.5% patients aged ≤17/18-64/≥65 years; and 38.1/24.9/18.9/18.1% patients located in medical and surgical wards/emergency rooms/ICUs/clinics. The most common pathogens were: *E. coli* (EC 19.6%), MSSA (16.6%), *P. aeruginosa* (PA 8.9%), *S. pneumoniae* (SPN 6.3%), *K. pneumoniae* (KP 6.1%), *Enterococcus* spp. (5.5%), MRSA (4.7%), and *H. influenzae* (4.1%). Susceptibility rates (SR) for EC were: 99.9% for meropenem (MER) and tigecycline (TGC), 99.7% ertapenem (ERT), 97.6% piperacillin-tazobactam (PTZ), 92.3% ceftriaxone (CTR), 90.4% gentamicin (GEN), 77.1% ciprofloxacin (CIP) and 73.0% TMP-SMX (SXT). SR for PA were: 94.4% colistin, 84.2% PTZ, 82.7% ceftazidime (CAZ), 80.5% MER, 78.0% GEN and 74.3% CIP. SR for MRSA were: 100% for linezolid (LZD) and telavancin (TLV), 99.9% daptomycin (DAP) and vancomycin, 99.1% TGC, and 93.5% SXT. Rates of resistant organisms between 2007-2015 increased significantly for ESBL-producing EC (3.4%-9.2%) as well as VRE (1.8%-4.4%), whereas MRSA rates (26.1%-19.4%) significantly declined.**Conclusions:** EC, MSSA, PA, SPN, KP, and MRSA are the most common pathogens in Canadian hospitals. SR for EC were highest for MER, TGC, ERT and PTZ. SR for PA were highest for colistin, PTZ, CAZ and MER. 99-100% of MRSA were susceptible DAP, LZD, TLV, and vancomycin.

Author Disclosure Block:

G.G. Zhanel: I. Research Relationship; Self; Abbott, Astellas, Galderma, Merck, Pharmascience, Sunovion, Tetraphase, The Medicines Co, Zoetis, Cubist. **H. Adam:** None. **M. Baxter:** None. **B. Weshnoweski:** None. **R. Vashisht:** None. **S. Biju:** None. **N. Laing:** None. **K. Nichol:** None. **A. Denisuik:** None. **A. Golden:** None. **P. Lagace-Wiens:** None. **J. Fuller:** None. **J.A. Karlowsky:** None. **A. Walkty:** None. **M. Gilmour:** None. **D. Bay:** None. **F. Schweizer:** None. **M.R. Mulvey:** None. **G.R. Golding:** None. **D.J. Hoban:** I. Research Relationship; Self; Abbott, Astellas, Cubist, Galderma, Merck, Pharmascience, Sunovion, The Medicines Co, Tetraphase, Zoetis.

Poster Board Number:

MONDAY-137

Publishing Title:

Resistance to Antimicrobial Agents Among *Enterococcus faecalis* and *Enterococcus faecium* Isolates Recovered from Human, Environmental and Food Sources in Brazil

Author Block:

R. Ribeiro[1], C. Melo[2], A. Faria[3], J. Castro[1], A. Gomes[1], T. Pinto[1], A. Regua-Mangia[2], V. Merquior[3], L. Teixeira[1]; [1]Univ.e Federal do Rio de Janeiro, Rio de Janeiro, Brazil, [2]Fundação Oswaldo Cruz, Rio de Janeiro, Brazil, [3]Univ.e do Estado do Rio de Janeiro, Rio de Janeiro, Brazil

Abstract Body:

Enterococci are opportunistic pathogens with a remarkable ability to acquire and exchange mobile genetic elements associated with antimicrobial resistance. Their presence in environmental and food sources may be indicative of human or animal contamination representing a risk to human health. Moreover, the dispersion of resistance-associated genes through a variety of routes has been the subject of increasing attention. We evaluate the susceptibility to antimicrobials among isolates of two major enterococcal species recovered from recreational waters (RW; 139 isolates), chicken carcasses ready to be marketed (CC; 105) and human sources [H; 204: including both colonization (83) and infection (121)], from 2010 to 2014. MALDI-TOF-MS was used for species identification. Antimicrobial susceptibility was evaluated by the disk diffusion method. The presence of genes associated with high-level resistance to aminoglycosides (HLR-A) was evaluated by multiplex PCR. Among 448 isolates, 60.1% were identified as *E. faecium* and 39.9% as *E. faecalis*. The highest rates of non-susceptibility were found to ciprofloxacin (61.1%), norfloxacin (48.4%), nitrofurantoin (42.6%) and levofloxacin (41.7%). Resistance to beta-lactams ranged from 27.3% to 33.7%. Resistance to streptomycin (HLR-S) was observed in 25.4% (15.4% H, 7.8% CC and 2.2% RW) of the isolates, while to gentamicin (HLR-G) was found in 21.2% (18.5% H, 1.6% CC and 1.1% RW). Occurrence of VRE was detected among isolates from H (25.6%), RW (1.3%) and CC (0.3%). Multidrug resistance (MDR) was observed in 310 (69.2%) isolates. The most common genes associated with HLR-A were *ant(6')Ia* (31.8% H, 16.6% CC, 5.3% RW), *aph(3')IIIa* (35.6% H, 3.0% CC and RW each), and *aac(6')-le-aph(2")-la* (34.8% H, 4.5% CC, 1,5% RW). Enterococcal isolates presenting MDR were recovered from the different sources investigated. Considering that these strains may represent a public health risk, these findings indicate the importance for continuous monitoring and improvements on antimicrobial usage and on the quality of environmental and food sources.

Author Disclosure Block:

R. Ribeiro: None. **C. Melo:** None. **A. Faria:** None. **J. Castro:** None. **A. Gomes:** None. **T. Pinto:** None. **A. Regua-Mangia:** None. **V. Merquior:** None. **L. Teixeira:** None.

Poster Board Number:

MONDAY-138

Publishing Title:

Antibiotic Resistance in Sputum Isolates of *Pseudomonas aeruginosa* from Children Receiving Mechanical Ventilation: Impact of Antibiotic Exposure and Mic Heterogeneity

Author Block:

K. B. Flett[1], H. Chung[2], M. Schaefers[1], G. P. Priebe[1]; [1]Boston Children's Hosp., Boston, MA, [2]Harvard Med. Sch., Boston, MA

Abstract Body:

Background: *P. aeruginosa* causes significant morbidity in ventilated patients and frequently develops antibiotic resistance. Few pediatric studies have evaluated the timing of this resistance and impact of antibiotic exposure.**Methods:** We conducted a prospective cohort study of 32 pediatric patients receiving mechanical ventilation and with $\geq$ 2 respiratory cultures growing *P. aeruginosa*. We evaluated change in MIC ratio compared to previous culture, with MICs performed using VITEK. Antibiotic days of therapy during the 30 days prior to culture collection was collected and impact of antibiotic exposure on MIC ratio was assessed by Wilcoxon rank sum test. Multilocus sequence typing (MLST) was performed on samples with MIC change $\geq$ 2-fold to confirm the presence of the same strain within a patient. Additional MIC testing using a high-throughput method was performed on 24 colonies for two paired samples.**Results:** 130 respiratory cultures were evaluated, with 9 subjects having $\geq$ 5 cultures. MIC changes $\geq$ 2-fold often occurred within 1-4 days of the previous culture, sometimes with no exposure to the antibiotic tested. For cefepime and ceftazidime, MIC changes were not significantly associated with cephalosporin or beta-lactam days. For meropenem, the number of carbapenem days approached significance (p=0.06), but the number of beta-lactam days was not significant (p=0.43). For ciprofloxacin, fluoroquinolone days also approached significance (p=0.08). Eleven pairs of samples had MLST analysis performed, with each pair having congruent sequence type. MIC testing of multiple colonies for two paired samples revealed both MIC heterogeneity within each sample and changes in MIC diversity over time.**Conclusions:** MIC changes can occur rapidly and are not robustly associated with exposure to the corresponding antibiotic, suggesting more complex mechanisms. Given the heterogeneity of MIC results for individual colonies in a single sample, insufficient representation of resistance likely contributes to observed changes in MIC.

Author Disclosure Block:

K.B. Flett: None. **H. Chung:** None. **M. Schaefers:** None. **G.P. Priebe:** None.

Poster Board Number:

MONDAY-139

Publishing Title:

Resistance to Ciprofloxacin and Ceftriaxone in Human Non-Typhoidal <*salmonella*> Isolates from Cambodia

Author Block:

W. Lurchachaiwong; Armed Forces Res. Inst. of Med. Sci., Bangkok, Thailand

Abstract Body:

Non-typhoidal *Salmonella* (NTS) causes around 94 million cases of acute gastroenteritis each year. NTS resistance to fluoroquinolones, first line treatment for diarrhea, and decreased susceptibility to third generation cephalosporins, is an emerging problem. This study reports antimicrobial resistance of NTS in Cambodia. A total of 566 stool samples collected from 283 diarrhea cases and 283 non-diarrhea controls during July 2014 - October 2015 were investigated. NTS was identified and serotyped by standard microbiology techniques and antimicrobial susceptibility testing to ampicillin (AMP), azithromycin (AZM), ceftriaxone (CRO), ciprofloxacin (CIP) and co-trimoxazole (SXT) was performed by the disc diffusion assay. NTS infections accounted for 22% (63/283) of cases and 26% (74/283) of controls. Resistance to AMP, SXT, CIP, CRO and AZM was detected in 57%, 86%, 3%, 5%, and 0% of NTS isolates from cases and 51%, 38%, 22%, 5% and 2% of isolates from controls, respectively. Serotyping was performed on isolates that was resistant to CIP and CRO. Among 16 CIP-resistant isolates, *Salmonella* Kentucky (group C) was predominant (52.4%; 9/16), followed by *Salmonella* Give (group E) (37.5%; 6/16). Four of six (67%) of the CRO-resistant NTS were *Salmonella* enterica subsp. enterica serotype 4, 5, 12: i: - (group B) which also showed intermediated susceptibility to CIP. One *Salmonella* Rissen (group C) and one *Salmonella* Corvallis (group C) showed resistant to CRO and the latter was also resistant to CIP. In conclusion, this study demonstrates resistance to CIP and CRO among NTS isolates from the indigenous population in Cambodia. Resistance to CIP was mainly found in *Salmonella* group C while the resistance to CRO was found in *Salmonella* group B. Surveillance for NTS antibiotic resistance is needed particularly in areas where resistance is prevalent and likely underestimated such as Southeast Asia.

Author Disclosure Block:

W. Lurchachaiwong: None.

Poster Board Number:

MONDAY-140

Publishing Title:

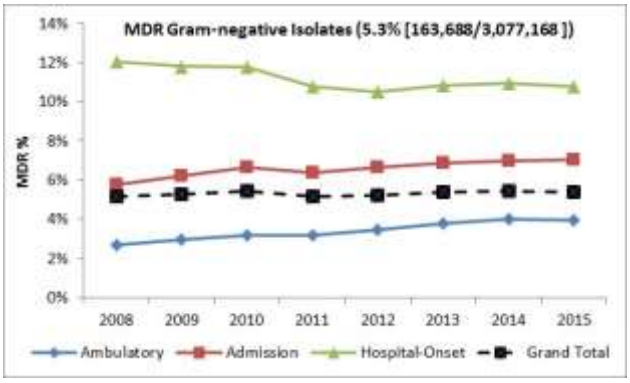
Multidrug Resistance Gram-Negative (Mdr-Gn) Organisms in the Inpatient and Outpatient Settings in the US: 2008-2015

Author Block:

H. Hoffman-Roberts[1], P. Scoble[1], X. Sun[2], J. Mohr[1], Y. Tabak[2], R. Johannes[2], V. Gupta[2];
[1]Tetraphase Pharmaceuticals, Watertown, MA, [2]Becton Dickinson & Co, Franklin Lakes, NJ

Abstract Body:

Background: MDR-GN organisms are increasing and may vary by clinical setting, source, and pathogen. We evaluated trends in MDR-GN organisms over 8 years. **Methods:** The Becton Dickinson electronic research database from 154 US hospitals (2008 - 15) was evaluated. Non-duplicate isolates (first isolate of a species per 30 day period) from respiratory, blood, urine, skin, intraabdominal, and other sources were identified as MDR per NHSN definitions. Isolates were categorized into 3 settings by the specimen collection time: 1) Admission: < 3 days of an inpatient admission and no previous admission ≤ 14 days; 2) Hospital-onset: 3 days post-admission or ≤ 14 days of discharge; 3) Ambulatory. Pearson correlation was used to evaluate trend, and t-tests to compare between sources. **Results:** Overall, 3,077,168 isolates were tested; 5.3% (163,688) were MDR. MDR rates differed by settings: 3.4% (69,599/2,020,015, ambulatory), 6.6% (34,202/522,010, admission), and 11.2% (59,887/535,143, hospital onset) (all p<0.01). Top 5 pathogens accounted for 92.1% of MDR isolates: *E. coli* (38.7%), *P. aeruginosa* (29.5%), *K. pneumoniae* (13.2%), *A. baumannii* (5.5%), and *Enterobacter spp.* (5.3%). Sources with the highest MDR rates were: respiratory (15.9%), blood (7.5%), and skin (7.3%). The overall MDR rate remained stable (p=0.09) with rates decreasing in hospital-onset (p<0.01) setting but increasing in ambulatory and admission settings (both p<0.01). **Conclusion:** MDR-GN rates remained the highest in the hospital onset setting, and in the respiratory source. MDR trends were downward in the inpatient, but upward in ambulatory and admission settings.



Author Disclosure Block:

H. Hoffman-Roberts: D. Employee; Self; Tetraphase Pharmaceuticals. K. Shareholder (excluding diversitied mutual funds); Self; Tetraphase Pharmaceuticals. **P. Scoble:** D. Employee; Self; Tetraphase Pharmaceuticals. K. Shareholder (excluding diversitied mutual funds); Self; Tetraphase Pharmaceuticals. **X. Sun:** D. Employee; Self; Becton Dickinson & Co. K. Shareholder (excluding diversitied mutual funds); Self; Becton Dickinson & Co. **J. Mohr:** D. Employee; Self; Tetraphase Pharmaceuticals. K. Shareholder (excluding diversitied mutual funds); Self; Tetraphase Pharmaceuticals. **Y. Tabak:** D. Employee; Self; Becton Dickinson & Co. K. Shareholder (excluding diversitied mutual funds); Self; Becton Dickinson & Co. **R. Johannes:** D. Employee; Self; Becton Dickinson & Co. K. Shareholder (excluding diversitied mutual funds); Self; Becton Dickinson & Co. **V. Gupta:** D. Employee; Self; Becton Dickinson & Co. K. Shareholder (excluding diversitied mutual funds); Self; Becton Dickinson & Co.

Poster Board Number:

MONDAY-141

Publishing Title:

Antibiotic Susceptibility Profiles of Clinical *Clostridium difficile* Isolates in a Pediatric Population

Author Block:

L. K. Kociolek[1], D. N. Gerding[2], J. R. Osmolski[3], S. J. Patel[1], R. Patel[1], D. W. Hecht[3]; [1]Ann & Robert H. Lurie Children's Hosp. of Chicago, Chicago, IL, [2]Edward Hines, Jr. VA Hosp., Hines, IL, [3]Loyola Univ. Med. Ctr., Maywood, IL

Abstract Body:

Background: The rising incidence of *C. difficile* infections (CDIs) in adults is partly related to the global spread of fluoroquinolone-resistant strains, namely BI/NAP1/027. Although CDIs are also increasingly diagnosed in children, BI/NAP1/027 is relatively uncommon in children. Little is known about antibiotic susceptibility of pediatric CDI isolates.**Methods:** *C. difficile* was cultured from *tcdB*-positive stools collected from children diagnosed with CDI between Mar. 2011 and Dec. 2013 at an academic children's hospital. CDI isolates were grouped by restriction endonuclease analysis (REA). Mean inhibitory concentrations (MICs) were measured by agar dilution method for the 7 antibiotics listed in the Table. Subsequent isolates from the same patient that were identified as the same REA group as the previous CDI (i.e., CDI relapses) were excluded. Susceptibility breakpoints were based on CLSI and/or EUCAST guidelines.**Results:** Antibiotic susceptibilities of the 115 isolates meeting inclusion criteria are summarized in the Table. Only one BI isolate was identified. The 3 moxifloxacin-resistant isolates were REA groups BI, CF, and non-specific. The 2 rifaximin-resistant isolates were REA groups DH and Y. The 21 clindamycin-resistant isolates were distributed among 9 different REA groups (A, CF, DH, G, L, M, Y and 2 unique non-specific REA groups). Resistance to metronidazole or vancomycin, or elevated MICs for fidaxomicin or surotomycin, was not identified among these isolates.**Conclusions:** Antibiotic resistance is uncommon in the diverse group of *C. difficile* isolates collected from children in this single-center cohort.**Table: Summary of antibiotic susceptibility testing of 115 *C. difficile* isolates**

Antibiotic	**Min MIC**	**Max MIC**	**Mean geometric MIC**	**MIC50**	**MIC90**	**% Sen**	**% Int**	**% Res**
Metronidazole	<=0.06	0.5	0.21	0.25	0.25	100%	0%	0%
Vancomycin	0.25	2	0.52	0.5	1	100%	*	0%
Rifaximin	<=0.002	>32	0.0033	0.002	0.0039	98%	*	2%
Fidaxomicin	0.015	0.25	0.071	0.06	0.125	*	*	*

Surotomycin	0.06	2	0.25	0.25	0.5	*	*	*
Clindamycin	0.5	>64	3.26	4	8	35%	47%	18%
Moxifloxacin	1	16	1.99	2	2	97%	0%	3%

Min: minimum; Max: maximum; Sen: Sensitive; Int: Intermediate; Res: Resistant* No breakpoint available

Author Disclosure Block:

L.K. Kociolek: E. Grant Investigator; Self; Merck. I. Research Relationship; Self; Alere/Techlab. J. Scientific Advisor (Review Panel or Advisory Committee); Self; Actelion. **D.N. Gerding:** C. Consultant; Self; ViroPharma/Shire, MedImmune, Sanofi Pasteur, Cubist, Optimer, DaVolterra, and Pfizer. J. Scientific Advisor (Review Panel or Advisory Committee); Self; Merck, Rebiotix, Summit, and Actelion. N. Other; Self; D.N.G. holds patents for the prevention of Clostridium difficile infection licensed to ViroPharma/Shire. **J.R. Osmolski:** None. **S.J. Patel:** E. Grant Investigator; Self; Merck. **R. Patel:** None. **D.W. Hecht:** None.

Poster Board Number:

MONDAY-142

Publishing Title:

Implementation of Antibiotic Susceptibility Testing (AST) for Resistance Tracking Using Clinical and Laboratory Standards Institute (CLSI) M100 and M02 in Cambodian Microbiology Laboratories

Author Block:

S. Oeng, J. Letchford; Diagnostic Microbiol. Dev. Program, Phnom Penh, Cambodia

Abstract Body:

Background: DMDP collaborates with the Cambodian Ministry of Health (CMoH) to build microbiology diagnostic capacity as part of the National Strategic Plan for Medical Laboratory Services. We introduced CLSI M100 and M02 disc diffusion methods to 6 Cambodian laboratories to achieve quality AST results to report to hospital clinicians and create surveillance data for the Ministry of Health.**Methods:** DMDP used a multi-strategy approach in 2014-15 which included 9 manager meetings, the CLSI annual AST update webinar and used M100 (S23, S24, S25) and M02 (A11, A12) documents to prepare an AST guideline adapted to fit Cambodian practices. 3 International (16 months) and 3 Cambodian mentors (24 months) provided regular bench microbiology diagnostic training at all 6 sites. In 2015, DMDP and CMoH organized a 2-day AST workshop of 21 participants and 8 facilitators with a practical exercise book (cases and questions) and pre/post tests to improve and evaluate participant capacity. We created an electronic tool for weekly monitoring AST Internal Quality Control (IQC) performance and provided corrective action advice when problems arose. Monthly activity reports and clinician reports were monitored for adherence to the guideline. Also, practical AST exercises were regular activities in DMDP supported microbiology manager meetings during 2014 and 2015.**Results:** 5 of 6 DMDP supported laboratories have implemented AST according to CLSI M100 and M02. In 2015, 5 of 6 laboratories performed weekly AST IQC. Problems diminished from weekly to none over 12 months. Laboratory assessments in 2015 showed the 'AST' component to be in the top 2 of 9 components assessed at 5 sites. All 6 laboratories achieved good scores in the Pacific Paramedical Training External Quality Assessment (EQA) program. Average scores in 2015 ranged from 85% to 95%. Workshop participant test scores showed 14% improvement.**Conclusions:** The multi-strategy approach strengthened Cambodian laboratory capacity for AST. Regular on site mentoring and support were essential. Repetitive practice, encouragement and cultural awareness were key components. Implementation and standardization of AST have provided opportunity for Cambodia CDC to collect important AMR surveillance data.

Author Disclosure Block:

S. Oeng: None. **J. Letchford:** None.

Poster Board Number:

MONDAY-143

Publishing Title:

Big Data Vs. Bad Bugs: Fighting Eskape Pathogens Electronically

Author Block:

U. Chukwuma[1], T. Coster[2], M. LaCour[2], K. McAuliffe[1], R. Thelus[1], C. Neumann[1], M. Hinkle[3], R. Clifford[3], M. Julius[3], P. Waterman[4], **E. Lesho**[3]; [1]NMCPHC, Portsmouth, VA, [2]PVDAS, Falls Church, VA, [3]WRAIR, Silver Spring, MD, [4]AFHSC, Silver Spring, MD

Abstract Body:

Background: Size and complexity are two components of 'big data' (BD). Organizations increasingly exploit BD to identify opportunity, associations, and patterns, or predict threats or outcomes. We sought to demonstrate how BD can be leveraged to support patient safety and public health. **Methods:** BD is created and analyzed by: 1) leveraging public health and infection control surveillance mandates; 2) using a high-throughput organism identification, susceptibility testing, and whole genome sequencing pipeline; 3) establishing specialized datamarts. The two datamarts are the Military Health System (MHS) Health Level 7 (HL7) repository of the U.S. Navy and the Pharmacovigilance Defense Application System (PVDAS) of the US Army. MHS HL7 receives laboratory, radiology and pharmacy data. The PVDAS receives data for medical claims, eligibility and enrollment, death files, and pharmacy transactions. MHS HL7 uses SQL algorithms to identify ESKAPE pathogens and to generate specific surveillance products. Java, SAS and SQL queries against PVDAS perform pharmacovigilance by monitoring prescribing and adverse drug reactions and safety alerts. 36,000 multidrug resistant ESKAPE pathogens have been centrally characterized, all of which will be fully sequenced by 2017 using the pipeline. **Results:** Analysis of 14.5 million cultures revealed carbapenem resistance (CR) 73 fold higher in non-fermenters compared to Enterobacteriaceae. Bloodstream infections were associated with higher relative risks of CR ($p < 0.001$). The incidence of *S. aureus* with clinically problematic vancomycin minimum inhibitory concentrations decreased or remained constant, and is predicted to be stable from 2016-2018. 311 patients received potentially problematic prescriptions or formulations of an antimicrobial. There were >13 million visits for acute respiratory infections. In 2006, 2011, and 2014, 49%, 54% and 48% respectively, of these visits had an antibiotic prescribed. Antibiotics were more likely to be prescribed to females, retirees and dependents of retirees, and persons 45 years and older. **Conclusion:** The above are just a few highlights of the results, outcomes, and usefulness of our approach, which informed stewardship and quality improvement efforts.

Author Disclosure Block:

U. Chukwuma: None. **T. Coster:** None. **M. LaCour:** None. **K. McAuliffe:** None. **R. Thelus:** None. **C. Neumann:** None. **M. Hinkle:** None. **R. Clifford:** None. **M. Julius:** None. **P. Waterman:** None. **E. Lesho:** None.

Poster Board Number:

MONDAY-144

Publishing Title:

A Hierarchical Classification, Naming, and Annotation System for Acquired Antimicrobial Resistance Protein Sequences

Author Block:

D. H. Haft, V. V. Brover, M. Feldgarden, A. B. Prasad, W. Klimke; Natl. Library of Med., Bethesda, MD

Abstract Body:

Antimicrobial resistance (AMR) proteins have been named variably according to their biochemical activity, the resistance they confer or contribute to, the homology family to which they belong, and even the relative order of their discovery. For beta-lactamases in particular, function may differ if a single amino acid differs and names may be assigned to individual alleles. NCBI has taken over responsibility for providing new allele designations upon submission for beta-lactamases in families whose nomenclature previously was handled by researchers at the Lahey Clinic (www.lahey.org/studies). We have compiled and curated a broadly comprehensive database of bacterial AMR proteins from multiple sources, including Lahey Clinic, Resfinder, the Comprehensive Antibiotic Resistance Database, curated data contributed by outside experts, and our own surveys of primary and review articles. In our hierarchical treatment, AMR proteins are grouped first by type (e. g. beta-lactamases, whose members are not all related by homology), then by broad homology family (e.g. all class D beta-lactamases), then specific family (e. g. OXA-60 family carbapenem-hydrolyzing class D beta-lactamases), then allele. For every node in the hierarchy, we assign a descriptive protein product name that is suitable for use in submissions of sequence data to GenBank. We have developed a collection of over 450 profile hidden Markov models (HMMs) as AMR classifiers, and assigned 3688 distinct complete sequences to a hierarchy with 3419 families, including 2289 alleles. Our classification improves functional predictions for many AMR proteins, especially class D (OXA) beta-lactamases. Our toolkit includes a hierarchical representation of AMR protein families, a matching library of corresponding HMMs with cutoff scores, and a comprehensive curated set of AMR proteins with consistent and informative protein names, for distribution but also replicated as RefSeq records. These resources assist in annotating pathogen genomes, comparing sets of AMR proteins across strains, and predicting resistance phenotypes from whole genome shotgun sequencing data.

Author Disclosure Block:

D.H. Haft: None.

Poster Board Number:

MONDAY-145

Publishing Title:

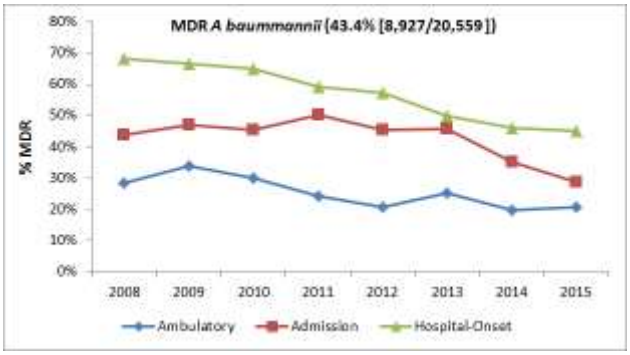
Multidrug-resistance *Acinetobacter baumannii* (Mdr Ab) Trends from All Sources in the Inpatient and Outpatient Setting in US Hospitals: 2008-2015

Author Block:

H. Hoffman-Roberts[1], H. Patel[1], X. Sun[2], Y. Tabak[2], J. Mohr[1], R. Johannes[2], V. Gupta[2];
[1]Tetraphase Pharmaceuticals, Watertown, MA, [2]Becton Dickinson & Co, Franklin Lakes, NJ

Abstract Body:

Background: MDR AB is traditionally a nosocomial pathogen, but started emerging more in the outpatient setting. We evaluated trends in susceptibility of MDR AB over 8 years. **Methods:** The Becton Dickinson electronic data research database from 154 hospitals (2008 - 15) was analyzed. Non-duplicate isolates (first of a species per 30 day period) from respiratory, blood, urine, skin, intra-abdominal, and other sources were identified as MDR per NHSN definitions. Isolates were categorized into three settings by the specimen collection time: 1) Admission: within 3 days of an inpatient admission and no previous admission within 14 days; 2) Hospital-onset: 3 days post-admission or within 14 days of discharge; 3) Ambulatory. Pearson correlation was used to evaluate the trend of MDR rates over time, and t-tests to compare between sources. **Results:** Overall 20,559 isolates were tested; 43.4% (8,927) were MDR AB. MDR rates differed by setting: 25.2% (1,868/7,417, ambulatory), 43.6% (1,992/4,568, admission), and 59.1% (5,067/8,574, hospital onset) (all $p<0.01$). There were downward trends in MDR AB rates for three settings (all $p < 0.05$) and for all sources (all $p < 0.01$). MDR rates remained highest in respiratory isolates across three settings (49.4% to 63.3%). The overall MDR rate for respiratory isolates (60.5%) was higher than each of other sources (ranged from 24.5% to 43.4%; all $p < 0.01$). **Conclusion:** In this collection of isolates, rates of MDR in ACB decreased over time. MDR AB was highest in the inpatient setting. Respiratory isolates showed the highest MDR AB rates in all settings.



Author Disclosure Block:

H. Hoffman-Roberts: D. Employee; Self; Tetraphase Pharmaceuticals. K. Shareholder (excluding diversitied mutual funds); Self; Tetraphase Pharmaceuticals. **H. Patel:** D. Employee; Self; Tetraphase Pharmaceuticals. K. Shareholder (excluding diversitied mutual funds); Self; Tetraphase Pharmaceuticals. **X. Sun:** D. Employee; Self; Becton Dickinson & Co. K. Shareholder (excluding diversitied mutual funds); Self; Becton Dickinson & Co. **Y. Tabak:** D. Employee; Self; Becton Dickinson & Co. K. Shareholder (excluding diversitied mutual funds); Self; Becton Dickinson & Co. **J. Mohr:** D. Employee; Self; Tetraphase Pharmaceuticals. K. Shareholder (excluding diversitied mutual funds); Self; Tetraphase Pharmaceuticals. **R. Johannes:** D. Employee; Self; Becton Dickinson & Co. K. Shareholder (excluding diversitied mutual funds); Self; Becton Dickinson & Co. **V. Gupta:** D. Employee; Self; Becton Dickinson & Co. K. Shareholder (excluding diversitied mutual funds); Self; Becton Dickinson & Co.

Poster Board Number:

MONDAY-146

Publishing Title:

Characterization of Increased Macrolide Resistance in *Campylobacter coli*

Author Block:

C. T. Bennett[1], D. Campbell[2], C. Fitzgerald[2], J. Reynolds[2], J. Chen[1], J. Folster[2]; [1]IHRC, Inc., Atlanta, GA, [2]CDC, Atlanta, GA

Abstract Body:

Campylobacter causes an estimated 1.3 million infections, 13,000 hospitalizations, and 120 deaths in the United States each year. The Centers for Disease Control and Prevention (CDC) estimates that 310,000 *Campylobacter* infections are antimicrobial-resistant annually in the U.S. Primary treatment options for campylobacteriosis are fluoroquinolone or macrolide drugs; however, macrolides such as erythromycin and azithromycin are frequently the sole treatment option due to prevalent resistance to fluoroquinolones. CDC's National Antimicrobial Resistance Monitoring System (NARMS) has screened *Campylobacter* isolates from FoodNet sites since 1997, and has observed an increase in resistance to macrolides among *C. coli* in recent years. NARMS performs antimicrobial susceptibility testing (AST) using the broth microdilution method with nine drugs, and confirms *Campylobacter* genus and species using conventional PCR methods. In 2013, NARMS performed AST on 142 surveillance *C. coli* isolates from humans who sought clinical care for illness, and found that 17% (n = 24) were macrolide-resistant (azithromycin MIC greater than 0.5 and erythromycin MIC greater than 8). In 2012, 9% of 134 *C. coli* isolates tested in NARMS were macrolide-resistant. We performed whole genome sequencing (WGS) on twelve macrolide-resistant isolates from 2013 using the Illumina platform. We identified several acquired resistance determinants conferring resistance to aminoglycosides, β-lactams, and tetracycline; however, we did not find any acquired macrolide-resistance determinants. With WGS analysis we identified common mutations in the 23S ribosomal gene (including A2074G, which is predicted to confer macrolide resistance). Comparative genome analysis indicated that the 12 *C. coli* were not closely related, suggesting that macrolide resistance is not emerging because of clonal expansion of resistant strains.

Author Disclosure Block:

C.T. Bennett: None. **D. Campbell:** None. **C. Fitzgerald:** None. **J. Reynolds:** None. **J. Chen:** None. **J. Folster:** None.

Poster Board Number:

MONDAY-147

Publishing Title:

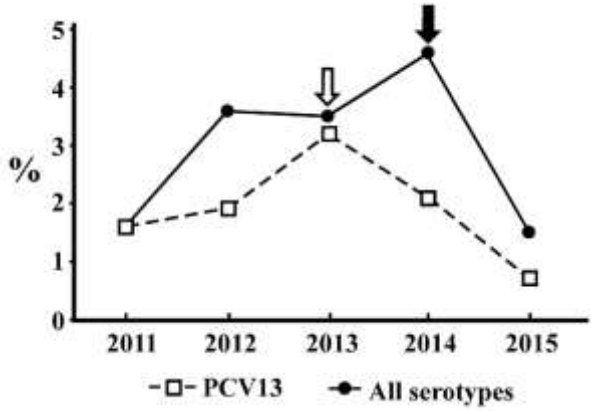
Fluoroquinolone-Nonsusceptible *Streptococcus pneumoniae* Isolates from a Medical Center in Pneumococcal Conjugate Vaccine Era

Author Block:

H-H. Chen, H-C. Li, L-H. Su, C-H. Chiu; Chang Gung Mem. Hosp., Taoyuan City, Taiwan

Abstract Body:

Background: *Streptococcus pneumoniae* is one of the most common pathogens to cause mucosal and invasive infection in humans. Resistance to fluoroquinolones (FQ) was associated with clinical treatment failure when treating pneumococcal diseases.[1] The induction of pneumococcal conjugate vaccine (PCV) was reported to be associated with the reduction of ciprofloxacin resistance in previous observational study.[2] **Methods:** We collected clinical isolates of *Streptococcus pneumoniae* from Jan. 2011 to Jul. 2015 at Chang Gung Memorial Hospital. The levofloxacin or moxifloxacin-nonsusceptible (FQ-R) pneumococcal isolates were analyzed by serotyping, multilocus sequence typing (MLST), and sequencing of the quinolone resistance-determining regions (QRDR) of the *gyrA, gyrB, parC,* and *parE*. **Results:** During the study period, 42 FQ-nonsusceptible pneumococcal isolates were identified. The rate increased from 1.6% of total pneumococcal isolates (2 of 127) in 2011 to 4.6% (13 of 283) in 2014, then decreased to 1.5% (3 of 202) in the first half-year of 2015. These isolates belonged to13 serotypes, and serotype 14 (12 of 42, 33.3%) was the most prevalent. Most of the isolates belonged to international clones or their variants. After QRDR analysis, there were 19 isolates in five clusters that shared both the same sequence type and QRDR mutation. **Conclusions:**FQ resistance emerged in either vaccine or non-vaccine serotypes. Majority of the isolates were international clones or related variants, suggesting the resistance was disseminated through clonal spread. The wide use of PCV since 2013 appears to reduce the spread of FQ-R pneumococci.



White arrow: nation-wide PCV13 catch-up program; black arrow: nation-wide PCV13 program with 3-dose schedule.

Author Disclosure Block:

H. Chen: None. **H. Li:** None. **L. Su:** None. **C. Chiu:** None.

Poster Board Number:

MONDAY-148

Publishing Title:

Resistance Profiles of *K. pneumoniae* Urinary Tract Isolates from European and North American Intensive Care Units (2012 - 2014)

Author Block:

S. Lob, K. Kazmierczak, D. Sahm, D. Biedenbach, **R. Badal**; IHMA, Inc., Schaumburg, IL

Abstract Body:

Background: Intensive care units (ICUs) are often described as hotbeds of antimicrobial resistance, with high rates of extended-spectrum β-lactamase (ESBL)-producing and multidrug-resistant (MDR) *Enterobacteriaceae*, especially among *Klebsiella pneumoniae*. This report from the Study for Monitoring Antimicrobial Resistance Trends (SMART) describes susceptibility patterns of *K. pneumoniae* from urinary tract infections (UTI) in 2012-2014 in Europe (EU) and North America (NA), comparing ICU and non-ICU wards.**Methods**: 82 sites in 20 countries in EU and NA collected up to 50 consecutive gram-negative pathogens from UTI per year. Susceptibility was determined by broth microdilution per CLSI guidelines for 1,377 *K. pneumoniae* isolates. MDR was defined as resistance to ≥3 drug classes (aminoglycosides, β-lactam/β-lactamase inhibitors, cephems, carbapenems, quinolones).**Results**: Susceptibility to selected agents and MDR rates are shown below.

Region/ward type (n)	% Susceptible[A,B]								% MDR[C]
	ETP	IPM	FEP	CRO	CAZ	TZP	CIP	AMK	
Europe									
ICU (170)	67.1	77.7	37.7	37.7	40.6	51.2	39.4	80.6	55.3
Non-ICU (725)	88.3	**94.2**	61.8	59.2	62.9	73.5	60.7	**92.4**	31.3
North America									
ICU (69)	**95.7**	**94.2**	87.0	84.1	84.1	92.8	89.9	**95.7**	8.7
Non-ICU (413)	**97.1**	**96.9**	**92.3**	**91.5**	**92.5**	**93.0**	89.6	**97.1**	6.3

[A] Susceptibility values ≥90% are bolded.
[B] Significant differences between ward types are shaded (Fisher's exact test, p<0.05).
ETP=ertapenem, IPM=imipenem, FEP=cefepime, CRO=ceftriaxone, CAZ=ceftazidime, CIP=ciprofloxacin, TZP=piperacillin-tazobactam, AMK=amikacin

Among both ICU and non-ICU isolates, susceptibility was significantly lower and MDR rates higher in EU than NA (p<0.05). The most common ESBL in both regions was CTX-M-15. Carbapenemases detected in EU included KPC, OXA, NDM, and VIM, whereas in NA only KPC were found.**Conclusions:**•Resistance in *K. pneumoniae* from UTI was very high in EU ICUs with significantly lower susceptibility and higher MDR rates than in non-ICU wards.•In contrast, differences between ward types were small in NA. Susceptibility was significantly higher in NA than in EU, especially in ICUs.•Investigation of the reasons for the differences in resistance between EU and NA ICUs may be useful for the development of effective strategies to reduce antimicrobial resistance in ICUs.

Author Disclosure Block:

S. Lob: M. Independent Contractor; Self; IHMA, Inc. **K. Kazmierczak:** M. Independent Contractor; Self; IHMA, Inc. **D. Sahm:** M. Independent Contractor; Self; IHMA, Inc. **D. Biedenbach:** M. Independent Contractor; Self; IHMA, Inc. **R. Badal:** M. Independent Contractor; Self; IHMA, Inc..

Poster Board Number:

MONDAY-149

Publishing Title:

High Prevalence of *qnrb* and Other Plasmid-mediated Quinolone Resistance Determinants Among *Enterobacteriaceae* Isolated from Urine Samples in Sierra Leone

Author Block:

T. A. Leski[1], M. G. Stockelman[2], U. Bangura[3], D. Chae[4], R. Ansumana[3], D. A. Stenger[1], G. J. Vora[1], C. R. Taitt[1]; [1]Naval Res. Lab., Washington, DC, [2]Naval Med. Res. Ctr., Silver Spring, MD, [3]Mercy Hosp. Res. Lab., Bo, Sierra Leone, [4]Thomas Jefferson High Sch., Alexandria, VA

Abstract Body:

Background: Fluoroquinolones are a family of broad-spectrum bactericidal antibiotics used for the treatment of infections caused by both Gram-positive and Gram-negative bacteria. Their widespread and sometimes indiscriminate use has exacerbated the spread of genes that can confer fluoroquinolone resistance. In this study, we analyzed a set of urine *Enterobacteriaceae* isolates from Sierra Leone for the presence and prevalence of plasmid-mediated quinolone resistance genes (PMQR) and found that 86% harbored PMQRs and 47% were highly resistant to ciprofloxacin.**Methods and Results:** We analyzed a total of 70 *Enterobacteriaceae* isolates that were obtained from the urine of subjects with symptoms of urinary tract infection. These isolates belonged to six species (*Citrobacter freundii*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Leclercia* sp./*Enterobacter* sp., *Escherichia hermannii* and *Escherichia coli*) and included both ciprofloxacin-resistant and -sensitive strains. Each isolate was screened for the presence of 10 PMQR genes (*qnrA*, *qnrB*, *qnrC*, *qnrD*, *qnrS*, *qnrVC*, *aac(6')-Ib-cr*, *qepA*, *oqxA* and *oqxB*) by polymerase chain reaction. The identity of the detected genes was confirmed by DNA sequencing. The results demonstrated a high carriage rate of three *qnr* family genes: *qnrA*, *qnrB*, *qnrS* (66%) and *aac(6')-Ib-cr* (60%). They were found in all of the tested species except *E. coli*. The most frequently encountered PMQR gene was *qnrB*, especially among *C. freundii* isolates which harbored at least four alleles of *qnrB* as well as truncated *qnrB* pseudogenes. The *oqxA* and *oqxB* genes were found in 23% and the *qepA* gene in 4% of the isolates.**Conclusions:** Ciprofloxacin is recommended for travelers visiting Sierra Leone and is widely used in this part of West Africa. The results of this study indicate that continued efficacy of this antimicrobial in the region might be threatened by uncontrolled spread of transferrable fluoroquinolone resistance determinants. The ubiquitous presence of multiple *qnrB* alleles and pseudogenes in *C. freundii* support previous evidence suggesting that this species might be the original source of this gene.

Author Disclosure Block:

T.A. Leski: None. **M.G. Stockelman:** None. **U. Bangura:** None. **D. Chae:** None. **R. Ansumana:** None. **D.A. Stenger:** None. **G.J. Vora:** None. **C.R. Taitt:** None.

Poster Board Number:

MONDAY-150

Publishing Title:

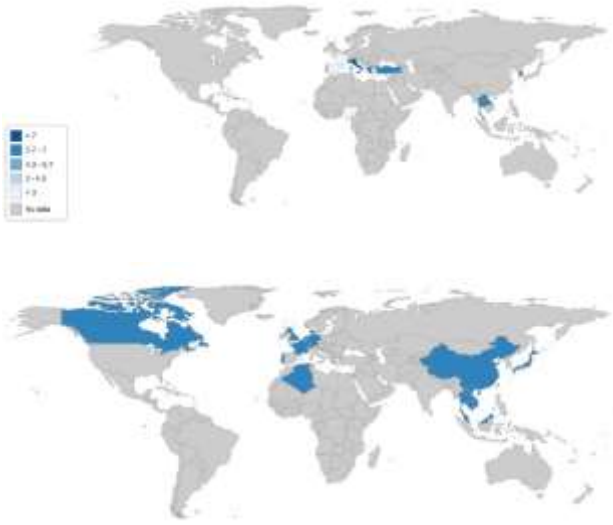
Emerging Global Colistin Resistance

Author Block:

L. S. Gucer, S. Hızal, O. Yilmaz, H. Nuhoglu, F. Can, **o. ergonul**; Koc Univ., Sch. of Med., Istanbul, Turkey

Abstract Body:

Colistin resistance among K.pneumoniae has been increasing in recent years. There are several reports regarding colistin resistance rates and mechanisms of the resistance in veterinary and clinical isolates of K.pneumoniae. This meta-analyses was performed to identify the incidence of colistin resistance in K.pneumonia and to identify the mechanisms of colistin resistance and to assess the risk of spread of the resistance.A systematic search of PubMed database from 1 January 2000 until 10 January 2016 identified 10 original studies. Articles that were included in the systematic review were from 10 different countries. Density of resistance to colistin among human clinical Klebsiella isolates (Figure 1a) and mcr1 plasmid detected countries were presented (Figure 1b).Figure 1. a) Colistin resistance rates of human clinical Klebsiella isolates. b) Global mcr1 positivity.Conclusion: Emergence and spread of colistin resistance and related genotypes should be screened and followed up. Our study group will update emerging data on monthly basis.



Author Disclosure Block:

L.S. Gucer: None. **S. Hızal:** None. **O. Yilmaz:** None. **H. Nuhoglu:** None. **F. Can:** None. **O. ergonul:** None.

Poster Board Number:

MONDAY-151

Publishing Title:

Dissemination of *cfr* by Clonal Spread of St475 and St16 *Enterococcus faecalis* Strains Harboring a Pcppf5-like Plasmid

Author Block:

x-p. liao, j. sun, y-h. liu; South China Agricultural Univ., guangzhou, China

Abstract Body:

Objectives: The *cfr* multiresistance gene of *Enterococcus faecalis* has been associated with transfer of linezolid resistance but little is known about its prevalence and mode of transmission due to only sporadic reports. In this study, the prevalence of the *cfr* gene among *E. faecalis* origin from swine was investigated. **Methods:** A total of 91 *E. faecalis* isolates, obtained from swine faecal swabs from 30 farms in Guangdong Province of China during 2012, was conducted to screen the *cfr* gene. Southern blotting, conjugation and transformation analyses were conducted to confirm the plasmid location and transferability of *cfr* gene. The genetic environment of *cfr* was determined by sequence analysis.**Results:** A total of 11 isolates were *cfr*-positive. All the *cfr*-positive *E. faecalis* strains presented a multi-resistance phenotype but all were still susceptible to vancomycin and linezolid. Molecular typing of the 11 *cfr*-positive isolates indicated clonal spread of ST475 and ST16 *E. faecalis* from different swine farms. S1-PFGE and Southern blotting showed that all the *cfr* genes were located on plasmids. A12 kb *cfr*-positive plasmid (pE30) was identified in most (9/11) isolates. Sequence analysis and comparison showed that pE30 was a pCPPF5-like plasmid and the region surrounding the *cfr* gene was the same with the *cfr*-carrying IS*Enfa5*-composite element of *Streptococcus* plasmid pStrcfr with 4 bp direct repeat (GTAT) on both sides. This suggests that the *cfr* gene on plasmid pE30 may have originated from the *Streptococcus* plasmid pStrcfr.**Conclusions:** In conclusion, the clonal spread of ST475 and ST16 *E. faecalis* strains and the horizontal transfer of the pCPPF5-like plasmid have contributed to the dissemination of the *cfr* gene in *E. faecalis*.

Author Disclosure Block:

X. liao: None. **J. sun:** None. **Y. liu:** None.

Poster Board Number:

MONDAY-152

Publishing Title:

Clinical Sensitivity of a Real-Time PCR Assay Targeting *gyrA* Gene for Prediction of Ciprofloxacin Resistance in *Neisseria gonorrhoeae*

Author Block:

P. Hemarajata, S. Yang, J. D. Klausner, R. M. Humphries; UCLA, Los Angeles, CA

Abstract Body:

Background: *Neisseria gonorrhoeae* (NG) has become an urgent threat to public health due to high prevalence of antimicrobial resistance. Prevalence of fluoroquinolone-resistant gonococci has is currently at 19.2%, suggesting that ciprofloxacin (CIP) may still be a viable option for treatment in the majority of isolates. We previously evaluated accuracy of a *gyrA* RT-PCR with melt genotyping to predict CIP susceptibility based on the presence of Ser91 mutation on *gyrA* gene, for urine specimens positive for NG. This study evaluates clinical sensitivity of *gyrA* RT-PCR on other specimen types.**Methods:** Specimens were processed on COBAS® 4800 CT/NG assay (Roche Diagnostics). Residual DNA from deep-well plates was collected from specimens positive for NG and used for *gyrA* RT-PCR. PCR results were used to evaluate overall and specimen-specific clinical sensitivity of PCR.**Results:** *gyrA* RT-PCR was performed on 46 clinical specimens positive for NG (Table 1). Among these, 31 (67.4%) could be genotyped by RT-PCR, as wild-type (23, 74.2%) or mutant (8, 25.8%), but 15 (32.6%) failed to produce *gyrA* signal. Among specimens tested, 15/16 urines (93.8%) but only 4/18 throat swabs (22.2%) produced a gyrA signal that could be genotyped as either wild type or mutant. The low clinical sensitivity in throat specimens seems to be independent of crossing point (Cp) on COBAS® assay, since all specimens except throat with Cp above the previously established limit of detection of 28 were genotyped successfully, while none of throat specimens with similar Cp was. For 3 patients, both throat and rectal specimens were positive for NG. Among these, 0/3 throat specimens yielded a *gyrA* RT-PCR signal as compared to 3/3 rectal swabs.**Conclusions:** The overall clinical sensitivity of the *gyrA* PCR was 67.4%. Excluding throat specimens, the sensitivity increased to 96.4%. We hypothesize poor performance on throat specimens may be related to the presence of PCR inhibitors unique to this specimen. Further investigation and optimization of PCR to improve sensitivity on throat swabs is underway.

Table 1: PCR results for clinical specimens tested

Specimen type	*gyrA* genotype		
	Wild-type	Mutant	Indeterminate
Genital swabs (3)	2	1	0
Rectal swabs (8)	5	3	0
Throat swabs (18)	3	1	14

ThinPrep® (1)	0	1	0
Urines (16)	13	2	1
Total	23	8	15

Author Disclosure Block:

P. Hemarajata: None. **S. Yang:** None. **J.D. Klausner:** None. **R.M. Humphries:** None.

Poster Board Number:

MONDAY-154

Publishing Title:

High Prevalence of Plasmid-mediated Colistin-resistance Gene *Mcr-1* in Fecal Metagenomes of Dutch Travelers

Author Block:

C. J. H. von Wintersdorff, **P. F. G. Wolffs**, P. H. M. Savelkoul, J. Penders; Maastricht Univ. Med. Ctr.+, Maastricht, Netherlands

Abstract Body:

Background: Colistin is increasingly used as a last resort antibiotic for the treatment of infections with carbapenem resistant bacteria. Recently, the first plasmid-mediated colistin resistance gene (*mcr-1*) was reported in China. Studies thus far have focused on detecting *mcr-1* in existing collections of cultured isolates, or by mining metagenomic sequencing data. As these existing collections were mostly restricted to specific bacteria or resistance profiles (e. g. ESBLs), and sensitivity of sequence-based metagenomics is relatively low, we hypothesize that the prevalence of *mcr-1* reported in these studies might be an underestimation of the carriage rates. Here, we applied a PCR-based targeted metagenomic approach to detect *mcr-1* in the fecal metagenomes of Dutch travelers between 2010 and 2012. **Methods:** Pre- and post-travel stool samples of 122 healthy Dutch travelers were collected and submitted to metagenomic DNA extraction. Detection of *mcr-1* and CTX-M genes was performed by qPCR. All *mcr-1* positive samples were confirmed by sequencing.**Results:** Screening of the fecal metagenomes of 122 travelers before and after travel, yielded 7 samples positive for *mcr-1*, reflecting a post-travel prevalence of 4.9% (95% CI 0.021-0.105) and acquisition rate of 4.1% (95% CI 0.015-0.096). Travelers with *mcr-1* acquisition visited destinations in South(east) Asia or southern Africa. In 4 out of 6 *mcr-1*-positive post-travel metagenomes, CTX-M genes were detected. Moreover, CTX-M harboring *E. coli* were previously cultured from fecal samples of 5 out of 6 *mcr-1*-positive individuals. However, none of these ESBL-producing *E. coli* were positive for *mcr-1*, indicating that the detected *mcr-1* genes originate from other bacteria.**Conclusion:** We report here the highest *mcr-1* prevalence in human microbiomes thus far, highlighting the potential of PCR-based targeted metagenomics as an unbiased and sensitive method to screen for the carriage of *mcr-1*. The high acquisition rates show that travel contributes to the dissemination of *mcr-1*. Furthermore, that one participant was positive for *mcr-1* before travel indicates that *mcr-1* may already be present in the microbiomes of Dutch residents, warranting further investigation of its prevalence in the general population and possible sources.

Author Disclosure Block:

C.J.H. von Wintersdorff: None. **P.F.G. Wolffs:** None. **P.H.M. Savelkoul:** None. **J. Penders:** None.

Poster Board Number:

MONDAY-156

Publishing Title:

Rapid, Accurate, and Actionable Next Generation Sequencing Based Identification of Pathogenic Agents in Heart Valve Tissue and Cerebrospinal Fluid

Author Block:

N. A. Hasan[1], M. Vondracek[2], B. Yen-Lieberman[3], D. A. Wilson[3], R. P. Isom[4], P. Subramanian[4], S. Choi[4], H. Li[4], T. Allander[2], G. W. Procop[5], R. R. Colwell[1]; [1]Univ. of Maryland, Coll. Park, College Park, MD, [2]Karolinska Inst., Stockholm, Sweden, [3]Lerner Coll. of Med., Cleveland, OH, [4]CosmosID, Inc., Rockville, MD, [5]Lerner Coll. of Med., Cleveland, MD

Abstract Body:

Reduced cost, rapid turnaround time, and deep sequencing capability of next generation sequencing (NGS) make it an important component of medical diagnostics and clinical applications. However, bioinformatics analysis remains an impediment to universal adoption. MetaGenID has been developed for rapid (within minutes), accurate (>98%), and actionable microbial identification and was used to identify microbial agents in heart valve tissue and cerebrospinal fluid (CSF). Two sets of tricuspid, aortic, and mitral valve tissues from patients undergoing heart surgery were analyzed by extraction of total nucleic acid, whole genome sequencing (Illumina HiSeq), and sequence analysis employing the MetaGenID system. Results were obtained within minutes and compared with results from culture, PCR, and 16S sequencing. Four heart valve tissue and four CSF samples from Karolinska Institute and 12 heart valve tissue samples from Cleveland Clinic were included, showing pathogens identified by culture and confirmed by PCR were also identified by MetaGenID. In addition, MetaGenID provided antibiotic resistance, pathogenicity factors, and proportional content within samples. *Staphylococcus aureus, Neisseria meningitides*, *Listeria monocytogenes*, and *Enterobacter* were identified in the samples. MetaGenID identified *Candida* species in one sample negative by culture and PCR. In one case, MetaGenID identified *Enterococcus faecium and Enterobacter cloacae* whereas 16S could identify only *Enterobacter* sp. Only two cases in each of the dual sample sets yielded discrepancies whereby either an agent was identified by NGS/ MetaGenID but not by culture and PCR or vice versa. Both sets of tissue samples had been stored for extended periods (in the case of the Cleveland Clinic samples, since 2011 at -80C), thus demonstrating sensitivity and accuracy of NGS/ MetaGenID. The system is operated using a laptop and server.

Author Disclosure Block:

N.A. Hasan: D. Employee; Self; CosmosID, Inc.. **M. Vondracek:** None. **B. Yen-Lieberman:** None. **D.A. Wilson:** None. **R.P. Isom:** D. Employee; Self; CosmosID, Inc. **P. Subramanian:** D. Employee; Self; CosmosID, Inc. **S. Choi:** D. Employee; Self; CosmosID, Inc. **H. Li:** D.

Employee; Self; CosmosID, Inc.. **T. Allander:** None. **G.W. Procop:** None. **R.R. Colwell:** A. Board Member; Self; CosmosID, Inc.. D. Employee; Self; CosmosID, Inc..

Poster Board Number:

MONDAY-157

Publishing Title:

Validation of easyMAG DNA Extractor for Bacterial Isolates for Use in a Clinical Laboratory

Author Block:

E. Powell, **J. Mortensen**; Cincinnati Children's Hosp., Cincinnati, OH

Abstract Body:

Background: Since molecular testing plays an increasingly large role in clinical microbiology laboratories, traditional research methods are being incorporated and thus need to be validated under more stringent guidelines. One such technology is DNA extraction from bacterial isolates, a necessary first step before test methods such as16S rRNA sequencing and whole genome sequencing. The purpose of this study was to evaluate the BioMerieux NucliSENS easyMAG total nucleic acid extractor for extracting DNA from bacterial isolates in the clinical microbiology laboratory. **Methods:** Forty-eight reference isolates from the American Type Culture Collection were selected to represent the variety of isolates typically encountered in the clinical laboratory: Gram-positive cocci (9 isolates), Gram-positive bacilli (10 isolates), *Enterobacteriaceae* (14 isolates), and non- *Enterobacteriaceae* Gram-negative organisms (15 isolates), DNA was extracted from 100 µL of 1.0 McFarland solution of each isolate using the Biomerieux NucliSENS easyMAG. Extracted DNA was quantified using an Eppendorf Biophotometer and confirmed with a NanoDrop spectrophotometer. The two samples with the lowest DNA concentration from each group were submitted for 16S rRNA sequencing.**Results:** For DNA concentrations and qualities, see table below. DNA concentration as measured by the Eppendorf Biophotometer corresponded to concentration as measured by the NanoDrop (r^2=0.9781). For the eight isolates tested by 16s sequencing, there was sufficient DNA for testing and all identifications were in agreement: *Enterobacter cloacae, Klebsiella oxytoca, Staphylococcus aureus* group, *Enterococcus faecalis, Exiguobacterium mexicanum/ aurantiacum, Clostridium septicum, Campylobacter jejuni/coli*, and *Prevotella melaninogenica*.**Conclusion:** The Biomerieux NucliSENS easyMAG total nucleic acid extractor is appropriate for DNA extraction from clinical bacterial isolates and will be incorporated in the clinical microbiology laboratory.

	DNA Concentration (ng/ µL)		A_{260}/A_{280}	
	Median	Range	Median	Range
Gram-positive cocci	8.5	7.4-14.5	1.35	1.05-1.70
Gram-positive bacilli	9.1	8.0-37.8	1.47	1.09-1.80
Enterobacteriaceae	15.9	12.1-20.4	1.63	1.27-1.94

non- *Enterobacteriaceae*	17.4	7.8-22.0	1.67	1.02-1.82

Author Disclosure Block:

E. Powell: None. **J. Mortensen:** None.

Poster Board Number:

MONDAY-158

Publishing Title:

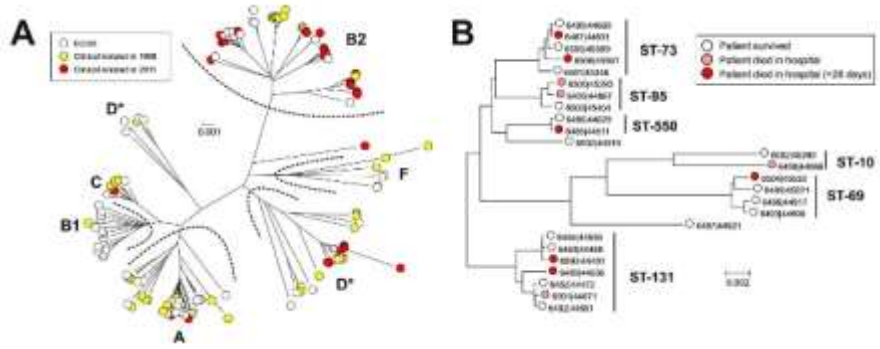
Clinical and Epidemiological Correlation of *E. coli* Bacteraemia Isolates by Whole Genome Sequencing (WGS): A Single UK Centre Experience 25 Years Apart

Author Block:

M. Albur[1], B. Pascoe[2], G. Meric[2], S. K. Sheppard[2], A. P. MacGowan[1]; [1]North Bristol NHS Trust, Bristol, United Kingdom, [2]Swansea Univ., Swansea, United Kingdom

Abstract Body:

Background: *E. coli* is the most common cause of bacteraemia in the UK. ST131 has emerged as a successful pathogenic *E. coli* lineage amongst the extra-intestinal *E. coli* isolates worldwide in the last decade. Whole genome sequencing of consecutive *E. coli* bacteraemia isolates from a UK tertiary centre collected 23 years apart, to study the local epidemiology and correlate different sequence types with clinical outcomes.**Methods:** In total 89 *E. coli* bacteraemia isolates from 1988 (42) and 2011 (47) were sequenced by Illumina MiSeq. Genetic phylogenies of both collections were compared to each other and the published ECOR collection. Isolate lineages were evaluated in context with 28-day and in-hospital mortality. Resistance and virulence genes were also identified and *in silico* antimicrobial susceptibility prediction compared to laboratory assay.**Results:** Phylogenetic tree of all isolates is shown in **Figure 1**. **A)** Annotated Neighbour-joining tree comparing 1988 isolates with 2011 isolates plus the published ECOR collection **B)** Neighbour-joining tree of 2011 isolates including patient outcome. Significant proportion of 2011 isolates belonged to the phylogenetic group B2 lineage as compared to 1988 isolates (p=0.001). ST131 isolates from 2011 were associated with a poor clinical outcome as compared to other sequence types (p=0.002).**Conclusions:** Our findings are in concordance with the global epidemiologic trend of emergence of a successful ST131 clone. Whole genome sequencing in routine clinical microbiology laboratories will improve understanding of this emerging, clinically-relevant epidemiological trend.



Author Disclosure Block:

M. Albur: None. **B. Pascoe:** None. **G. Meric:** None. **S.K. Sheppard:** None. **A.P. MacGowan:** C. Consultant; Self; Cantab Pharma. E. Grant Investigator; Self; The Medicines Company, Bayer Healthcare, Cubist/Merk, Clinigen, AiCuris,, Achaogen, Tetraphase Grant Investigator Tetraphase Grant Investigator. L. Speaker's Bureau; Self; Astellas.

Poster Board Number:

MONDAY-159

Publishing Title:

Characterization of Isolates of *Eisenbergiella tayi* Derived from Blood Cultures Using Whole Genome Sequencing Analysis

Author Block:

K. A. Bernard[1], T. Burdz[1], D. Wiebe[1], C. Huynh[1], A. M. Bernier[2]; [1]Natl. Microbiol. Lab., Winnipeg, MB, Canada, [2]Univ. of Saint Boniface, Winnipeg, MB, Canada

Abstract Body:

Background: *Eisenbergiella tayi* was first described for a strictly anaerobic, curved bacillus derived from a human blood culture [2014 Amir et al, IJSEM 64:907-914, Israeli/French collaboration]. That paper remains to date the sole publication associated with this pathogen. That genus best fit, but was not at the time assigned to, the family *Lachnospiraceae*, part of the Clostridiales 'Clostridium cluster XIVa' group. We describe here a study of 8 Canadian isolates of this agent. **Methods:** Eight blood culture isolates recovered from 2005-2015 and consistent with this genus and species from 7 patients located in 2 Canadian provinces were characterized by whole genome sequencing (WGS), by 16S, biochemically, by MALDI-TOF (Bruker) and cellular fatty acids (CFAs). Some underwent AST. WGS was performed on an Illumina MiSeq and genomes were assembled using Spades (version 3.5.1). Average nucleotide identity values (ANIb and ANIm) were calculated by JSpeciesWS. **Results:** Canadian isolates consistent with *E. tayi* by 16S were found to be similar to each other by WGS, CFAs and biochemically. Whole-genome sequence comparisons found ANIb and ANIm values between all strains was above 96%. Average genome size was large, at 7.5MB with a G+C content of 47%. Isolates were unidentifiable by MALDI-TOF when tested against the Bruker Biotyper library but gave rise to 2.2-2.7 scores if tested against a locally-created entry. These bacteria were curved or 'wavy', staining Gram variable (consistent with the description by Amir as Gram-stain negative but Gram positive structurally). Bacteria were fastidious growing and saccharolytic; some strains when plated on BBE had raised darkened colonies like those of *Bilophila.* AST indicated that strains were susceptible to nearly all drug classes tested. **Conclusions:** Clinicians should be aware of the potential for this strictly anaerobic bacterium to cause disease in humans; as this agent is not included in commercial ID systems, definitive identification must be done using a molecular approach.

Author Disclosure Block:

K.A. Bernard: None. **T. Burdz:** None. **D. Wiebe:** None. **C. Huynh:** None. **A.M. Bernier:** None.

Poster Board Number:

MONDAY-160

Publishing Title:

Superior Detection of *Legionella pneumophila* by Multiplex Real-Time PCR During the 2015 New York City Outbreak

Author Block:

A. Chandrasekran, S. Juretschko, Ph.D.; Northwell Hlth.Lab., Lake Success, NY

Abstract Body:

Background: Legionellosis is a form of pneumonia caused by the bacterium Legionella. *L. pneumophila* is the predominant species accountable for approximately 90% of the reported human cases. The recent 2015 New York City Legionella outbreak resulted in 133 infected individuals and 16 deaths. Conventional culture is still considered the gold standard for identification of Legionella, although showing poor sensitivity and requiring long incubation. We have developed and validated a multiplex real time PCR (MRT-PCR) assay for *L. pneumophila* (*mip* gene) and *L. pneumophila* serogroup-1 (LSg1, *wzm* gene) and compared its performance with culture. **Methods:** Northwell Health Laboratories, serving as reference laboratory during the NYC Legionella outbreak, used 109 outbreak related sputum samples collected from July to August 2015 to validate the Legionella MRT-PCR assay using previously published primers and probes. LOD of the assay was determined using serial dilutions of an ATCC^{T} *L. pneumophila* culture isolate. Culture results were compared with both, MRT-PCR results and the Luminex Respiratory Pathogen Panel (RPP) assay using Luminex MAGPIX system.**Results:** Of the 109 samples tested, only 6 (5.5%) were positive for *L. pneumophila* by culture, whereas 22 (20%) resulted positive by MRT-PCR, 3 of these 22 with the LSg1 target only. A subset of 40 sputum samples (20 positives and 20 negatives by MRT-PCR) was also tested with the Luminex RPP assay. 17 of the 20 samples were positive by RPP, but the same 3 LSg1 positive only samples were missed. The overall correlation between MRT-PCR and RPP was 92.5%. LOD of the MRT-PCR was determined as 10 CFU/mL with a combined detection of 100% using both targets and 80% with individual targets. The overall sensitivity of LSg1 PCR was higher than the *L. pneumophila* species PCR and Luminex RPP. **Conclusions:** The MRT-PCR assay developed and validated for the detection of *Legionella pneumophila* and *Legionella* serogroup-1 in sputum samples is simple, rapid and more sensitive than culture and Luminex RPP. This assay can be used as a fast screening tool to identify Legionella infections for future outbreak situations. Positive PCR samples could subsequently be cultured for epidemiology purposes.

Author Disclosure Block:

A. Chandrasekran: None. **S. Juretschko:** None.

Poster Board Number:

MONDAY-161

Publishing Title:

Molecular Epidemiologic Analysis of *Streptococcus pyogenes* Using the *emm* Cluster System in Korean Children

Author Block:

H. Lee[1], J. Park[2], J. Lee[2], K. Lee[2], K. Yun[2], E. Choi[2], H. Lee[2]; [1]Seoul Natl. Univ. Bundang Hosp., Seongnam, Korea, Republic of, [2]Seoul Natl. Univ. Children's Hosp., Seoul, Korea, Republic of

Abstract Body:

Streptococcus pyogenes (Group A streptococcus, GAS) is responsible for a various spectrum of diseases, including acute pharyngitis, skin and soft tissue infections (SSTI), invasive diseases and also postinfectious complications, which makes this bacteria a major cause of mortality and morbidity in children throughout the world. The *emm* typing method is a molecular typing method widely used in classification of GAS and more than 233 types have been reported. Recently a new classification has been developed based on functional and structional properties of M proteins. In this study, isolates collected from 1992 to 2012 among children under 19 years of age in Korea were analyzed by the *emm* cluster typing system. Among 134 isolates, invasive disease (19, 14.2%), SSTI (34, 25.4%), scarlet fever (37, 27.6%) and acute pharyngitis (44, 32.8%) were included. *emm*-clusters found among these isolates included A-C3, A-C4, A-C5, E1, E3, E4, E6 and clade Y. A-C4 (20.9%), E1 (20.9%), E4 (20.1%) and A-C3 (19.4%) were the most prevalent and accounted for 81.3% of all isolates. *emm*-cluster A-C3 and A-C5 were associated with invasive diseases, whereas E1 and A-C4 were associated with non-invasive infections. A-C3 was the most common cluster in invasive diseases by 47.4% and E1 accounted for 48.6% of all scarlet fever cases. There was no predominance for SSTI and pharyngitis, however A-C4 and E4 were the most common clusters in both cases. This study reports epidemiological information of GAS by the new functional classification system among children in Korea.

Author Disclosure Block:

H. Lee: None. **J. Park:** None. **J. Lee:** None. **K. Lee:** None. **K. Yun:** None. **E. Choi:** None. **H. Lee:** None.

Poster Board Number:

MONDAY-162

Publishing Title:

A Retrospective Analysis of Bacterial Dna Identified from Clinical Isolates Using 16s Rrna Gene Pcr and Sequence Analysis: 2009-2015

Author Block:

M. Dickinson, T. Passaretti, L. Thompson, D. Wroblewski, T. Quinlan, L. Mingle, K. Mitchell, A. Kidney, T. Halse, E. Nazarian, K. Musser; Wadsworth Ctr., New York State Dept. of Hlth., Albany, NY

Abstract Body:

The 16S rRNA gene is essential for the survival of all bacteria and is highly conserved. The characterization of the 16S rRNA gene is accepted as a standard method for the identification of families, genera, and species of bacteria. As a reference laboratory for New York State, we routinely perform 16S rDNA sequence analysis to identify bacterial isolates that are difficult to classify by phenotypic methods alone. Our laboratory has been approved by the Clinical Laboratory Evaluation Program to report 16S rDNA PCR and sequencing results for isolates since 2009. To assess the ability of this method to provide a definitive identification, a retrospective analysis was performed on 3438 isolates that were tested and analyzed from 2009-2015. The range of isolates tested per year was 333-670, with an average of 491. As per our protocol, 16S rDNA sequences are analyzed using the two separate databases. Based on the percent similarity of sequences from clinical isolates compared to these databases, identifications are placed into one of five categories: species level identification (>99% similarity), most closely resembles a species (98-99% similarity), most closely resembles a genus (95-98% similarity), genus level (>99% to multiple species-unable to differentiate), or unable to be identified (<95%). Of the 3438 isolates tested from 2009-2015, 1886 (55%) were identified to the species level, 929 (27%) genus level-unable to differentiate, 272 (8%) most closely resembled a genus, 192 (6%) most closely resembled a species, and 150 (4%) were unable to be identified. Nine samples are pending. The majority of sequences provided identification to one species, followed by a genus level identification. The most frequently identified species were *Staphylococcus epidermidis* and *Streptococcus intermedius* (35 each), followed by *Moraxella osloensis, Neisseria elongata,* and *Nocardia farcinica farcincia* (32 each). Of the 929 sequences that are were >99% similar to multiple organisms, the majority aligned to multiple species within the *Achromobacter* genus (91), followed by *Streptococcus* (60), and *Bacillus* (48). Overall, 16S rDNA sequence analysis is an invaluable tool that our laboratory has used for many years to identify bacterial isolates.

Author Disclosure Block:

M. Dickinson: None. **T. Passaretti:** None. **L. Thompson:** None. **D. Wroblewski:** None. **T. Quinlan:** None. **L. Mingle:** None. **K. Mitchell:** None. **A. Kidney:** None. **T. Halse:** None. **E. Nazarian:** None. **K. Musser:** None.

Poster Board Number:

MONDAY-163

Publishing Title:

Mixed Batch Testing on the Cobas® 4800 System For *Staphylococcus aureus*, MRSA, C*lostridium difficile*, and Herpes Simplex Virus 1 And 2

Author Block:

M. Espy[1], M. Binnicker[1], M. England[2], W. Greene[2], K. Marcy[3], R. Kuhne[3], E. Holdman[3], J. Thompson[3], M. Lewinski[4], O. Liesenfeld[4], **J. C. Osiecki**[4]; [1]Mayo Clinic, Rochester, MN, [2]The Pennsylvania State Univ. Coll. of Med., Hershey, PA, [3]Sanford Hlth., Fargo, ND, [4]Roche Molecuar Systems, Pleasanton, CA

Abstract Body:

Background: Mixed batch testing allows users to mix sample types and tests being included in a single run on the same instrument. We evaluated the performance of the **cobas®** 4800 system using contrived samples for *Clostridium difficile* (Cdiff), Methicillin-resistant *Staphylococcus aureus* (MRSA), *Staphylococcus aureus* (SA), and Herpes simplex virus (HSV) 1 and 2 tested at three US centers. Methods: Contrived samples were diluted at predetermined concentrations with MRSA, SA, HSV-1 and HSV-2 in MSwab media (Copan Diagnostics, Murrieta, CA) or with Cdiff in cobas PCR Media (Roche, Pleasanton, CA). Contrived positive samples were interspersed with negative samples, creating blinded panels for testing. Each participating site performed mixed batch testing with the **cobas®** MRSA/SA Test, **cobas** Cdiff Test, and **cobas** HSV 1 and 2 Test on two batches of specimens per day. Each batch was comprised of 40 reactions, and following three days of testing, provided 240 results per site. Results: All positive samples containing Cdiff, MRSA, SA, HSV-1 or HSV-2 were detected on the **cobas** 4800 system by each of the three testing sites regardless of concentration, and all negatives were "Not detected". The Ct values showed consistent results across testing sites, targets, and dilutions. Conclusions: The cobas 4800 system demonstrated high accuracy and precision among three US testing sites. Further evaluation using a large number of clinical samples is warranted.

			Results Across Test Sites		Ct Values		
Target	Expected Concentration	Number of Replicates (N)	Percent Agreement (n/N)	95% Exact CI	Mean	SD	Range
CDIFF	Negative	54	100.0 (54/54)	(93.4, 100.0)	n/a	n/a	n/a
	10 x LOD	54	100.0 (54/54)	(93.4, 100.0)	38.5	0.69	37.6 - 41.5
	25 x LOD	54	100.0 (54/54)	(93.4, 100.0)	37.3	0.62	36.2 - 39.7
HSV-1	Negative	54	100.0 (54/54)	(93.4, 100.0)	n/a	n/a	n/a
	10 x LOD	54	100.0 (54/54)	(93.4, 100.0)	38.8	0.72	37.1 - 40.8
	25 x LOD	54	100.0 (54/54)	(93.4, 100.0)	37.7	0.54	36.7 - 39.6
HSV-2	Negative	54	100.0 (54/54)	(93.4, 100.0)	n/a	n/a	n/a
	10 x LOD	54	100.0 (54/54)	(93.4, 100.0)	39.2	0.61	38.0 - 41.7
	25 x LOD	54	100.0 (54/54)	(93.4, 100.0)	37.6	0.35	37.1 - 38.5
MRSA	Negative	54	100.0 (54/54)	(93.4, 100.0)	n/a	n/a	n/a
	5 x LOD	54	100.0 (54/54)	(93.4, 100.0)	35.7	0.49	34.8 - 36.8
	10 x LOD	54	100.0 (54/54)	(93.4, 100.0)	34.7	0.51	33.7 - 35.8
SA	Negative	54	100.0 (54/54)	(93.4, 100.0)	n/a	n/a	n/a
	5 x LOD	54	100.0 (54/54)	(93.4, 100.0)	33.0	0.41	32.0 - 33.8
	10 x LOD	54	100.0 (54/54)	(93.4, 100.0)	31.9	0.40	30.8 - 32.7

LOD = limit of detection, CI = two-sided confidence interval, Ct = cycle threshold, SD = standard deviation, n/a = not applicable.

Author Disclosure Block:

M. Espy: None. **M. Binnicker:** None. **M. England:** None. **W. Greene:** None. **K. Marcy:** None. **R. Kuhne:** None. **E. Holdman:** None. **J. Thompson:** None. **M. Lewinski:** D. Employee; Self; Roche Molecular Systems. **O. Liesenfeld:** D. Employee; Self; Roche Molecular Systems. **J.C. Osiecki:** D. Employee; Self; Roche Molecular Systems.

Poster Board Number:

MONDAY-164

Publishing Title:

Performance Analysis of the Bd Max Enteric Bacterial Pathogens Panel in a Regional Clinical Laboratory

Author Block:

S. M. Melanson[1], K. D. Culbreath[2]; [1]TriCore Reference Lab., Albuquerque, NM, [2]Univ. of New Mexico, Albuquerque, NM

Abstract Body:

Background: We evaluated the BD MAX Enteric Bacterial Pathogens Panel (EBPP) (BD, Sparks, MD) to replace routine stool culture as it detects 4 of the most commonly cultured microorganisms *Campylobacter* spp., *Shigella* spp., *Salmonella* spp., and Shiga-toxin 1/2. **Methods:** Fresh and preserved (Cary-Blair) stool samples were run on the EBPP and the results compared to standard lab procedures of culture (*Salmonella, Shigella, Campylobacter)* and immunoassay (Shiga-toxin 1/2). One hundred and twenty five previously run samples were examined.**Results:** Agreement was found for 117 out of 125 samples (93.6%). The 89 positive samples were comprised of *Salmonella* (27), *Campylobacter* (23), *Shigella* (13) and Shiga-toxin (24). There were 2 specimens culture positive for *Salmonella* that were negative on EBPP. Both were detected upon repeat of the EBPP. The remaining 6 discrepant samples were all detected by EBPP and missed by culture or immunoassay (2 C*ampylobacter*, 1 *Salmonella*, 3 Shiga-toxin). Discrepant analysis with an alternative molecular assay demonstrated that the 6 samples were all true positive results. One culture was positive for *Pleisiomonas* which can be a cause of gastroenteritis, but is not detected in this assay. Internal confirmation of the limit of detection, using spiked stool samples in stool were in the range in accordance with the manufacturer claims. Assay results are available within 2.5-3 hours, compared to 48-72 hours for routine methods. The average laboratory hands-on-time is 2.7 minutes for EBPP compared to 22.3 min for culture. **Conclusions:** We have found that the BD MAX EBPP, in our hands, meets the stated performance claims and outperforms our current methods for pathogen detection. The assay has an improved turn-around-time for detection of stool pathogens and decreased hands-on time in the laboratory.

Author Disclosure Block:

S.M. Melanson: None. **K.D. Culbreath:** None.

Poster Board Number:

MONDAY-165

Publishing Title:

Direct Identification of Bacteria in Positive Blood Cultures by Matrix Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) Using a Novel Freeze-thaw Method

Author Block:

D. Nikolic[1], D. Donnell[2], G. L. Woods[3]; [1]Univ. of Arkansas for Med. Sci. (UAMS), Arkansas Children's Hosp. (ACH), Little Rock, AR, [2]ACH, Little Rock, AR, [3]UAMS and ACH, Little Rock, AR

Abstract Body:

Background: Rapid identification of the etiologic agents of bloodstream infections is critical to the timely initiation of the most appropriate antimicrobial therapy. Here we report an optimized in-house method that is cheap, simple, fast and highly reproducible for identification of bacteria in positive blood cultures by MALDI-TOF MS (Bruker Daltonics).**Methods:** When a bottle (Pediatric, Aerobic, Anaerobic, MycoF Lytic) flagged positive (BACTEC 9240, BD), Gram stain and subcultures were done. Then 1 ml of broth was transferred to a 15 ml polystyrene tube, which was placed at -20C for 30 min, rapidly thawed under running hot water for 45 sec, and centrifuged at 150 x *g* for 5 min. The supernatant containing organisms was transferred to a 1.5 ml tube and centrifuged at 13,000 rpm for 2 min. The pellet was washed twice with 1 ml of deionized water and tested by MALDI according to the manufacturer's instructions for bacterial/yeast colonies. Results obtained by testing pellets and colonies were compared.**Results:** Fifty-one broth samples were tested by our freeze-thaw method of lysing red blood cells. Using a score of >1.7 as an acceptable identification with the lysis method, agreement between testing pellets and colonies was 84% (21 of 25) for gram-positive bacteria and 77% (9 of 13) for gram-negative bacteria. There were no incorrect identifications with the lysis method; the result either was correct or no identification was provided. Agreement in identification between testing pellets and colonies by bottle type was: Pediatric, 81% (25 of 31); Aerobic, 50% (2 of 4); and Anaerobic, 100% (3 of 3). The lysis method did not identify yeasts (n=10) in any bottle type, polymicrobial infections (n=3), or any organism in a MycoF Lytic bottle (n= 8).**Conclusions:** Our results indicate that with the freeze-thaw method described here, MALDI can accurately identify the causative agent of bacteremia with the hands-on time < 1 hour. Testing to confirm using a score of >1.7 is ongoing. Further research is needed to validate this method with other blood culture systems and MALDI-TOF MS instruments.

Author Disclosure Block:

D. Nikolic: None. **D. Donnell:** None. **G.L. Woods:** None.

Poster Board Number:

MONDAY-166

Publishing Title:

Whole Sequences of Three Plasmids Carrying the *bla*$_{\text{ndm-5}}$ of *Klebsiella pneumoniae* Isolates Transmitted by Cross-Border Transfer from Uae to South Korea and Their Stability

Author Block:

J. Shin, K. S. Ko; Sungkyunkwan Univ., Su-won, Gyeonggi-do, Korea, Republic of

Abstract Body:

Objectives: Three NDM-5-producing *Klebsiella pneumoniae* isolates were identified in Korea. The first isolate was collected from a patient who was transferred from UAE to Korea, and the second and third isolates were collected from Korean patients after four months. We sequenced the three whole plasmids carrying *bla*$_{\text{NDM-5}}$ and characterized them. **Methods:** Three plasmids pCC1409-1, pCC1410-1 and pCC1410-2 were extracted and analyzed from *K. pneumoniae*. The *K. pneumoniae* ST147 isolates have been determined to produce NDM-5 enzymes to be resistant to most antimicrobial agents. The whole sequences of plasmids pCC1409-1, pCC1410-1 and pCC1401-2, which showed IncFII-type, were determined and analyzed using a MiSeq sequencer system and aligned against existing GenBank data. We also determined their stabilities in *Escharichia coli* J53 transconjugants.**Results:** The pCC1409-1, pCC1410-1 and pCC1410-2 plasmids encode various resistance genes to diverse antibiotics including macrolides, aminoglycosides, sulfonamides, trimethoprim, quaternary ammonium compounds and carbapenems. Two addiction systems (*mok*/*hok* and *pemKI*) are identified in three plasmids, ensuring stabilization of the plasmids in host strains. The plasmids share high similarity in backbone and resistance region with those of pGUE-NDM from Indian *E.coli* isolate and pMC-NDM from Polish *E. coli* isolate. *E. coli* J53 transconjugants lost the third plasmid, pCC1410-2, relatively fast.**Conclusion:** The plasmids pCC1409-1, pCC1410-1 and pCC1410-2 may originate from IncFII plasmids carrying the highly conserved NDM structure adding recombination events of other resistance genes. However, their stabilities in host were different each other.

Author Disclosure Block:

J. Shin: None. **K.S. Ko:** None.

Poster Board Number:

MONDAY-167

Publishing Title:

Role of Specific PCR in the Diagnosis of Blood Culture-Negative Endocarditis

Author Block:

P-E. Fournier, F. Gouriet, J-P. Casalta, H. Lepidi, G. Habib, D. Raoult; Aix-Marseille Univ., Marseille, France

Abstract Body:

Background: Blood culture-negative endocarditis (BCNE) may account for up to 70% of all cases of endocarditis depending on countries.**Methods:** In a prospective study from January 2010 to December 2015, we used systematized sampling and testing of blood and/or valvular biopsy specimens to investigate the aetiologies of 235 patients suspected as having BCNE in Marseille, France.**Results:** We identified an aetiology in 65 patients (28 % of cases). Of these, two (one allergy to pork and marantic endocarditis) were noninfective (0.8%). In the other 63 patients, PCR from valvular biopsies identified a causative agent in 26 patients (11.5%), serology in 19 (8%) and PCR from blood in 17 (7.2%). Streptococci were identified in 35% of BCNE cases, followed by zoonotic agents (29%) and staphylococci (14%). Proportionally, staphylococci, but not streptococci, were less frequent among BCNE agents than in BCPE patients ($p < 10^{-2}$ and 0.06, respectively).**Conclusions:** By using a syndrome-based serological and PCR testing from blood and/or valves, we identified streptococci, zoonotic agents and staphylococci as the main agents of BCNE in Marseille. Specific PCR assays reduced the ratio of IE without identified diagnosis by 28%, including 8% of patients in whom a specific antibiotic therapy was required.

Author Disclosure Block:

P. Fournier: None. **F. Gouriet:** None. **J. Casalta:** None. **H. Lepidi:** None. **G. Habib:** None. **D. Raoult:** None.

Poster Board Number:

MONDAY-168

Publishing Title:

Comparison of Molecular Identification of Gram-Positive and Gram-Negative Bacteria with Phenotypic Identification During Routine Use in a Large Clinical Laboratory

Author Block:

M. Almaayteh[1], R. Chaudhry[2], E. Abdulfatah[2], B. Alosh[2], R. Mitchell[3], M. Fairfax[4], H. Salimnia[4], P. Lephart[3]; [1]Royal Jordanian Med. Services/Detroit Med. Ctr. Univ. Lab., Amman, Jordan, [2]Wayne State Univ., Detroit, MI, [3]Detroit Med. Ctr. Univ. Lab., Detroit, MI, [4]Wayne State Univ. Sch. of Med. & DMC Univ. Lab., Detroit, MI

Abstract Body:

Background: Rapid identification and treatment of etiologic agents of blood stream infections significantly reduces mortality and morbidity. DMC University Laboratories serves eight hospitals (2000 beds) and many clinics. During the four months after implementation of the Verigene System Gram-negative (GN; 9 organism targets) and Gram- positive (GP; 13 target organisms) blood culture tests (Nanosphere, Northbrook, IL), we compared the results of the molecular organism identification (available in about 2.5 hr) to those of our laboratory's phenotypic assays (1-3 days).**Methods**: When a blood culture bottle was flagged as positive by the Bactec FX instrument (BD, Sparks, MD), fluid from the bottle was Gram stained. Based on Gram-stain results, additional aliquots were subjected to Verigene. Other aliquots were streaked on standard laboratory medium; bacterial growth and antibiotic sensitivities was obtained from isolated colonies using the BD Phoenix. Results of organism identification by both systems were compared.**Results**: Of the 1,748 Verigene tests, 1312 were GP and 436 GN. Relative to culture, one Verigene false positive was detected (*Acinetobacter baumannii*; 29 detected by culture). Six Verigene false negatives included: 4/65 *Klebsiella pneumoniae*, 1/156 *Escherichia coli*, 1/44 *Enterobacter*, and 1/68 *Enterococcus fecalis*. Organisms not included in the panel were present in almost 5% of the cultures**Conclusions**: The Verigene GN and GP blood culture identification tests were sensitive, specific, rapid and reliable, providing results in about 2.5 hours after the blood cultures flagged as positive. Verigene gave one FP result. Sensitivities for all organisms were 100% except as noted above.

Author Disclosure Block:

M. Almaayteh: None. **R. Chaudhry:** None. **E. Abdulfatah:** None. **B. Alosh:** None. **R. Mitchell:** None. **M. Fairfax:** None. **H. Salimnia:** None. **P. Lephart:** None.

Poster Board Number:

MONDAY-169

Publishing Title:

Development of TaqMan Real-Time PCR Assays for Typing of *Mycoplasma Pneumoniae* Based on Type-Specific Indels Identified Through Whole Genome Sequencing

Author Block:

B. Wolff, A. Benitez, D. Heta, S. Morrison, M. Diaz, J. Winchell; CDC, Atlanta, GA

Abstract Body:

Background: *Mycoplasma pneumoniae* is a common cause of community-acquired pneumonia. Historically, *M. pneumoniae* has been classified into two subtypes based upon sequence variation within the gene encoding the major adhesion molecule P1. Variation in the global circulation of these type strains is thought to contribute to a cyclic pattern of *M. pneumoniae* epidemics caused by alternating predominance of the two main types. Although a variety of molecular methods have been developed for P1 typing, no one-step real-time PCR assay currently exists.**Methods**: We performed whole genome sequencing on 67 *M. pneumoniae* isolates from various geographical origins, dates, and patient outcomes. Using whole genome comparisons with PacBio and Illumina data, we identified three type-specific indels (13-15 bp in length); two regions were present only in type 1 isolates and one region was found only in type 2 isolates. Based on bioinformatics analysis, we designed two TaqMan real-time PCR assays to rapidly type *M. pneumoniae* based upon the presence or absence of amplification of two of these sequences. We screened 64 *M. pneumoniae* isolates (n=34 type 1 and n=30 type 2), including the type strain M129 and FH, using the newly-developed assays.**Results**: 100% of *M. pneumoniae* isolates were correctly identified as type 1 or 2 based upon comparison to the type identified using a validated real-time PCR assay with high-resolution melt. Additionally, we confirmed the presence of the type-specific sequences in 40 *M. pneumoniae* genomes available in GenBank. Using this approach we confirmed the P1 type of *M. pneumoniae* in nucleic acid extracts and corresponding recovered isolates from 20 clinical specimens.**Conclusion**: Recent whole genome sequence analyses support the separation of *M. pneumoniae* strains into two distinct lineages based on P1. The real-time PCR assays described here for identifying strain type are more robust, rapid, and simpler to interpret compared to traditional P1 typing methods. Monitoring the circulation of *M. pneumoniae* strain types may be useful for epidemiological investigations and for predicting temporal and geographic variation in disease burden.

Author Disclosure Block:

B. Wolff: None. **A. Benitez:** None. **D. Heta:** None. **S. Morrison:** None. **M. Diaz:** None. **J. Winchell:** None.

Poster Board Number:

MONDAY-170

Publishing Title:

Frequency of Aminoglycoside Resistance Genes in Carbapenem-Resistant *E. coli* Isolates

Author Block:

B. Shrestha[1], T. Tada[2], H. Ohara[2], T. Kirikae[2], B. P. Rijal[1], J. B. Sherchand[1], B. M. Pokhrel[1]; [1]Inst. of Med., Kathmandu, Nepal, [2]Res. Inst., Tokyo, Japan

Abstract Body:

Background: Multidrug-resistant (MDR) *Escherichia coli(E.coli*), has increased and spread worldwide. In MDR *E. coli*, resistance to carbapenems and aminoglycosides has become a major concern[1]. The evaluation of cross-resistance and identification of resistance mechanism of MDR *E. coli* is needed.**Methods:** A total of 174 MDR *E. coli* isolates were obtained from single hospitalized patients during December 2013 to December 2014 in two tertiary care centers of Kathmandu, Nepal. Cross-resistance to carbapenems and aminoglycosides was examined in 135 carbapenem-susceptible MDR *E. coli* and 39 carbapenem-resistant MDR *E. coli*. Disk diffusion method was used for screening of MDR *E. coli*. MICs were determined by using the microdilution method. PCR was performed to identify the aminoglycoside resistance genes i.e. 16S rRNA methylase genes and carbapenem-resistant genes in carbapenem-resistant *E. coli* isolates.**Results:** Gentamicin and Amikacin resistance were more common in carbapenem-resistant isolates (35/39 strains; 89.7%) than in carbapenem-susceptible isolates (56/135 strains; 41.5%). The main aminoglycoside resistance gene was *armA* (18/39; 46.1%) followed by *rmtB* (13/39; 33.3%). *rmtC* was detected in 4 isolates. The commonest carbapenemase gene detected was bla_{NDM-1}(16/39; 41.0%). Most of the carbapenem-resistant *E. coli* carried any one of the 16S rRNA methylase genes.**Conclusions:** Our findings strongly suggested that carbapenem-resistant *E. coli* isolates with genes encoding both NDM-type Metallo-β-lactamases and 16S rRNA methylases disseminated in medical settings of Nepal. Thus, the emergence of such carbapenem-resistant *E. coli* strains should be undertaken as a serious public health threat.

Author Disclosure Block:

B. Shrestha: None. **T. Tada:** None. **H. Ohara:** None. **T. Kirikae:** None. **B.P. Rijal:** None. **J.B. Sherchand:** None. **B.M. Pokhrel:** None.

Poster Board Number:

MONDAY-171

Publishing Title:

Focus Diagnostics Simplexa™ Bordetella Direct Assay Verification Study

Author Block:

J. Chen, H. Mai, Y. Xie, M. Tabb; Focus Diagnostics, Cypress, CA

Abstract Body:

Background: Whooping cough is an infection of the respiratory system caused mainly by *Bordetella pertussis* and, less frequently, by *Bordetella parapertussis* and *Bordetella holmesii*. Differentiating Bordetella species can provide epidemiologic value as both *B. parapertussis* and *B. holmesii* can cause *B. pertussis*-like symptoms but vaccines against these 2 species are not available. In addition, patients can receive optimized antimicrobial treatment when the infected Bordetella species is identified. The Simplexa™ Bordetella Direct assay is in development to detect and differentiate *B. pertussis*, *B. parapertussis*, and *B. holmesii* from nasopharyngeal swab specimens. To run the Simplexa Bordetella Direct assay (Simplexa assay), nasopharyngeal swab specimens collected in universal transport media are loaded directly onto a Direct Amplification Disc without up-front nucleic acid extraction or other specimen preparation; real time PCR and detection follow. The goal of this study was to evaluate the clinical performance of the Simplexa assay versus DNA sequencing assays using nasopharyngeal swab specimens. **Methods:** A panel of 284 (249 de-identified nasopharyngeal swab and 35 contrived) specimens were tested with the Simplexa assay and IS481 (*B. pertussis)*, IS1001 (*B. parapertussis*), and hIS1001 (*B. holmesii*) bi-directional DNA sequencing assays. Cross reactivity was evaluated using a panel of bacteria and viruses. **Results:** Positive and negative agreement of the Simplexa assay with DNA sequencing were 97.1% (99/102) and 95.6% (174/182) for *B. pertussis*, 100% (37/37) and 98.8% (244/247) for *B. parapertussis* and 100% (37/37) and 99.6% (246/247) for *B. holmesii*, respectively. No cross reactivity with tested bacteria and viruses was observed. **Conclusions:** The Simplexa Bordetella Direct assay was capable of rapidly (~80 minutes) and directly detecting *B. pertussis*, *B. parapertussis*, and *B. holmesii* from nasopharyngeal swab specimens. The Simplexa assay can identify positive and negative specimens at rates comparable to bi-directional DNA sequencing assays. The Simplexa Bordetella Direct assay is in development; it is not currently available for sale and is not FDA cleared.

Author Disclosure Block:

J. Chen: None. **H. Mai:** None. **Y. Xie:** None. **M. Tabb:** None.

Poster Board Number:

MONDAY-172

Publishing Title:

Rapid, Reagent-Free Identification of MRSA and VRE by Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy

Author Block:

P. Lebel[1], H. Kim[2], L. Lam[2], A. Choudhuri[2], M. Langella[2], J. Sedman[2], S. Levesque[3], A. Ismail[2]; [1]McGill Univ. Hlth.Ctr., Montreal, QC, Canada, [2]McGill Univ., Ste-Anne-de-Bellevue, QC, Canada, [3]Laboratoire de santé publique du Québec, Ste-Anne-de-Bellevue, QC, Canada

Abstract Body:

The effectiveness of active screening and isolation programs in controlling the spread of MRSA and VRE in acute-care settings relies on the availability of rapid, accurate, and cost-effective diagnostic tests for these pathogens. FTIR spectroscopy is a reagent-free technique for bacteria identification and classification with subspecies-level discriminatory capabilities. Advantages of recently developed compact, portable ATR-FTIR spectrometers include the simplicity and enhanced reliability of FTIR bacteria identification in the ATR mode by comparison with the conventional transmission mode. In this study we evaluated the discriminatory power of ATR-FTIR spectroscopy as a potential technique for the rapid identification of MRSA and VRE.ATR-FTIR spectra of 800 Gram-positive clinical isolates were acquired by transferring isolated colonies from blood agar plates onto the sampling surface of a portable ATR-FTIR spectrometer. The spectral acquisition time was ~1 min. Four spectra were collected for each isolate from different colonies on the same culture plate. For a randomly selected subset of 200 clinical isolates, spectra were also acquired on a second portable ATR-FTIR spectrometer. Spectral data analysis was performed by hierarchical cluster analysis (HCA) and principal component analysis (PCA) in conjunction with the use of a feature selection algorithm.HCA of the ATR-FTIR spectra showed clustering of all replicate spectra and demonstrated excellent instrument-to-instrument spectral reproducibility as required for transferability of spectral databases. Discrimination between MRSA and MSSA was achieved by PCA of the *S. aureus* spectra and resulted in 95% concordance with PCR testing. PCA of the spectra of the enterococcal isolates resulted in successful discrimination between VRE and VSE (100% correct classification). The capability of ATR-FTIR spectroscopy to discriminate between antibiotic-resistant and susceptible strains in the absence of antibiotic provides a new rapid, inexpensive and reagent-free technique that can contribute to the control of antibiotic-resistant nosocomial pathogens.

Author Disclosure Block:

P. Lebel: None. **H. Kim:** None. **L. Lam:** None. **A. Choudhuri:** None. **M. Langella:** None. **J. Sedman:** None. **S. Levesque:** None. **A. Ismail:** None.

Poster Board Number:

MONDAY-173

Publishing Title:

Copan Fecalswab^{tm} for the Detection of Enteric Pathogens Using the Nimbus and Allplex^{tm} Gi-Bacterial (I) Assay

Author Block:

A. Giambra[1], S. Razeti[2], M. Renzulli[2], S. Castriciano[1]; [1]Copan Italia, Brescia, Italy, [2]ARROW DIAGNOSTICS SRL, Genova, Italy

Abstract Body:

Background: Proper diagnosis of diarrheal diseases can be complex due to the variety of pathogens that can cause gastro enteric infections. A specimen collection device that allows efficient nucleic acid extraction, Multiplex Real-Time PCR systems and culture is essential. Copan FecalSwab^{TM} (FS) is an LBM device, consisting of a flocked swab and a tube with 2ml Cary-Blair medium, compatible with manual and WASP^{TM} automated plating for culture, and antigen and toxin detection and nucleic acids (NA) amplification assays. Nimbus (Hamilton) is an instrument for nucleic acid extraction and PCR set-up. Allplex^{TM} GI-Bacteria (I) (Seegene) is a multiplex Real-Time PCR that detects 7 pathogens from stool. The objective of this study was to compare original stools to stool samples in FecalSwab, extracted with the Nimbus and tested with Allplex^{TM} GI-Bacterial (I) assay for the detection of enteric pathogens.**Material/methods:** Forty three stools, tested in duplicate, were used for this validation. Two equal aliquots of each stool were weighted: one was added to a FS and another to a microtube with 1 ml of ASL buffer (Qiagen). The FS tube was loaded on Nimbus, while the fresh stool required other preparation steps: 10m incubation at RT, 2m centrifugation prior DNA extraction and PCR reaction set up on the Nimbus and run on the CFX96 Real Time PCR (Bio-Rad). Stool preparation time for NA extraction was also calculated.**Results:** Concordant results were obtained with all the negative samples with both nucleic acids extractions (24/24), 21/21 positive were detected with the FS samples and 20/21 positive with 1 discordant with the samples in ALS buffer, which was previously positive by TOX A/B QUIK CHECK (Alere) . Among positives, 4 Salmonella spp., 2 C. diff toxin B and 3 campylobacter spp. were detected. The Cts analysis confirms that 17/21cases, positive samples in FS amplified as average 3 Cts before original stools, in 2/21 the difference than stool but not higher than 1 Ct. The samples preparation time was compared: FS preparation time 5 seconds versus 15 minutes for stools.**Conclusions:** The results obtained in this study demonstrated that the Copan FecalSwab^{TM} in combination with Nimbus extraction system and Allplex^{TM} GI-Bacterial (I) assay improved both stool positivity rate of gastroenterical pathogens, and preparation time.

Author Disclosure Block:

A. Giambra: None. **S. Razeti:** None. **M. Renzulli:** None. **S. Castriciano:** None.

Poster Board Number:

MONDAY-174

Publishing Title:

Evaluation of Surveillance Cultures and Molecular Methods for Identification of Multidrug Resistance Bacteria in Chronic Renal Failure Patients

Author Block:

T. Rezende[1], **A. Dói**[2], M. Quiles[1], A. Pignatari[1], S. Manfredi[1], C. Groethe[1], M. Taminato[1], L. Fehlberg[1], D. Barbosa[1]; [1]UNIFESP, São Paulo, Brazil, [2]UNIFESP, São Bernardo, Brazil

Abstract Body:

Introduction: Multidrug resistant bacteria (MDR) colonization is a serious problem among chronic renal failure patients (CRFP),because the risk for infection is higher. Surveillance culture is recommended as a component of infection control programs (ICP). **Objective:** Compare the performance of phenotypic methods for surveillance of MDR bacteria with real time PCR (qPCR) in a population of CRFP. **Methods:** We included a total of 546 CRFP from a tertiary center in São Paulo, Brazil, specialized in chronic renal failure care. These patients were divided in 3 groups: conservative treatment (129), dialysis (217) and transplanted (200). We collected nasal swabs for MRSA detection and rectal swabs for carbapenemase and VRE detection, in two different moments; total of 1092 samples (ESwab^{TM}). For phenotypic screening we used CHROMagar for KPC, MRSA and VRE. For the molecular analysis we used the qPCR for detection of the genes: *bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{SPM}$, *bla*$_{GES}$, *bla*$_{VIM}$, *bla*$_{OXA48}$, *van*A, *mec*A and *aur (S. aureus* identification). Conventional PCR were performed if necessary. **Results:** Among the 1,092 samples we found 13.8% of KPC positives according to chromogenic agar (CA). Only 26 were confirmed as KPC positive according conventional PCR. According to qPCR direct from swab 31 (2,8%) were positives to KPC, 39 (3,6%) for GES and 3 (0,3%) for SPM with a kappa of 0,256. For VRE the CA was positive in 16 (1,5%) of the patients and the qPCR positive in 20 (1,8%), kappa of 0,135. For MRSA we tested only 298 samples, and we observed 4 (1,3%) of positivity with CA and 15 (5%) according to qPCR. **Conclusions:** We observed for carbapenemase, VRE and MRSA detection an increased rate of false positives results using CA. For VRE we observed a low sensitivity using the qPCR. The molecular method can provide results faster than cultures allowing the early implementation of ICP to reduce the dissemination of MDR.

Author Disclosure Block:

T. Rezende: None. **A. Dói:** None. **M. Quiles:** None. **A. Pignatari:** None. **S. Manfredi:** None. **C. Groethe:** None. **M. Taminato:** None. **L. Fehlberg:** None. **D. Barbosa:** None.

Poster Board Number:

MONDAY-175

Publishing Title:

Impact of Environment and Attitude on Burden of Intestinal Parasites Among Primary School Children Living Around the Creeks of Calabar, Nigeria

Author Block:

A-A. Eyo, R. R. Solomon; Univ. of Calabar, Calabar, Nigeria

Abstract Body:

Background: Intestinal parasitic infections are among the most common infections worldwide. They are regarded as major health problems in tropical and subtropical countries and still remain potent causes of morbidity and mortality thereby constituting important obstacles to the development of these nations. The WHO estimates that about 576-740 million people are infected with intestinal parasites globally, majority of them children. Intestinal helminthes, especially hookworm, are implicated in anemia in children. Poor sanitation and low socio-economic status promote the survival and transmission of these parasites. This study was designed to assess the impact of environment and attitude on burden of intestinal parasites among primary school children in the Creek Area of Calabar South.**Method:** A total of 200 stool samples were collected from pupils aged 5-13 years randomly selected from four primary schools in the study area, whereas 50 samples collected from children in a primary school located within a University served as control. All stool samples were analyzed for intestinal parasites using methods of direct smear and formal-acetone concentration technique. Questionnaires were used to collect demographics, personal hygiene habits and environmental determinants from subjects/guardians.**Results:** The overall prevalence of intestinal parasite infection was 27.6%(69/250); test and control subjects had 32.5%(65/200) and 8.0%(4/50) respectively. The rate of infection was highest among children aged 5-7 years. There was a statistically significant effect of age on infection rates ($\chi^2 = 18.6$, $p = 0.01$). Hookworm and *Ascaris lumbricoides* had the highest occurrence rates of 35.4%;32.5% and 50.0%;50.0% in test and control subjects respectively. Co-infection of both parasites was also observed. Male children were more infected, 38.8%(40/103) compared to females with 19.7%(29/147) while 56.0%(112/200) and 47.5%(95/200) of test subjects did not have pipe-borne water and toilet facilities respectively in their homes.**Conclusion:** The deplorable state of environmental and personal hygiene, inadequate water supply and improper faecal disposal in the study area are to a great extent responsible for the high prevalence reported.

Author Disclosure Block:

A. Eyo: None. **R.R. Solomon:** None.

Poster Board Number:

MONDAY-176

Publishing Title:

Pneumococcal Carriage and Antimicrobial Resistance among Children after Four Years of Routine Conjugate Vaccines Use in Brazil

Author Block:

F. P. G. Neves[1], N. T. Cardoso[2], D. R. Silva[2], L. A. Motta[2], R. E. Snyder[1], M. A. Marlow[1], C. A. Cardoso[2], L. M. Teixeira[3], L. W. Riley[1]; [1]Univ. of California, Berkeley, CA, [2]Univ.e Federal Fluminense, Niterói, Brazil, [3]Univ.e Federal do Rio de Janeiro, Rio de Janeiro, Brazil

Abstract Body:

Background: Pneumococcal disease is a major cause of morbidity and mortality in children. In 2010, the 10-valent pneumococcal conjugate vaccine (PCV10) was included in the Brazilian immunization program and the 13-valent vaccine (PCV13) was offered in private clinics. To assess changes in pneumococcal carriage after four years of PCV10 and PCV13 use, we investigated the carriage rate, risk factors for colonization, and antimicrobial resistance among pneumococci obtained from the nasopharynges of Brazilian children.**Methods:** We conducted a cross-sectional study recruiting 573 children aged < 6 years in a public (287) and two private (286) clinics in Niterói city, RJ, Brazil from September to December, 2014. Isolates were identified by conventional methods. Antimicrobial susceptibility was assessed by the disk diffusion method. Bivariate analyses (χ^2) was carried out to assess the relationship between overall or resistant isolates carriage and risk factors.**Results:** The overall pneumococcal carriage rate was 23% (132; 20.3% in private clinics vs. 25.8% in public clinic) and it was associated with age ≥ 2 years, day care center or school attendance, presence of fever, acute respiratory symptoms, rhinitis, asthma/bronchitis, household residents ≥ 5, and slum residence ($p<0.05$). All isolates were susceptible to levofloxacin, rifampicin and vancomycin. The highest rate of non-susceptibility was observed for trimethoprim/sulfamethoxazole (53; 40.2%). We detected 50 (37.9%) penicillin non-susceptible pneumococci (PNSP). Erythromycin (ERY) resistance was observed in 36 (27.3%) isolates, which displayed the $cMLS_B$ (25; 69.4%) or M (11; 30.6%) phenotypes, and carried the *erm*(B) (25, 69.4%) and/or *mef*(A/E) (13, 36.1%) genes. Previous antibiotic use was the only risk factor associated with both PNSP and ERY-resistant (ERY-R) isolates carriage ($p<0.05$).**Conclusions:** Since introduction of PCV10 and PCV13 for use in the area investigated, the overall colonization rate decreased by 16.5%, while PNSP and ERY-R isolates carriage frequencies increased by 13.9% and 25.7%, respectively.

Author Disclosure Block:

F.P.G. Neves: None. **N.T. Cardoso:** None. **D.R. Silva:** None. **L.A. Motta:** None. **R.E. Snyder:** None. **M.A. Marlow:** None. **C.A. Cardoso:** None. **L.M. Teixeira:** None. **L.W. Riley:** None.

Poster Board Number:

MONDAY-177

Publishing Title:

Characteristics of *Staphylocccus aureus* Nasal Carriage among United States Industrial Beefpacking Workers

Author Block:

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Abstract Body:

Background: Occupational contact with swine and poultry is associated with human exposure to livestock-associated *Staphylococcus aureus*. While *S. aureus* is known to infect cattle, characteristics of *S. aureus* carriage among industrial cattle workers in the United States are unstudied.**Methods:** We recruited a cross-sectional sample of beef slaughterhouse workers at an industrial facility in Nebraska. Workers were interviewed in English and Spanish and nasal swabs were collected. Culture methods were used to identify presumptive *S. aureus* isolates, which were screened for mecA, SCCmec and staphylococcal protein A genes. Confirmed *S. aureus* isolates were evaluated for susceptibility to 12 antimicrobials and the presence of the *scn* gene, a biomarker of human adaption in *S. aureus*. *Spa* typing and real-time PCR for Panton-Valentin leukocidin (PVL) genes *lukS*-PV and *lukF*-PV were also conducted.**Results:** In total, 137 workers were enrolled. Prevalence of *S. aureus* nasal carriage was 27%, with five workers (3.6% of all participants; 13.5% of *S. aureus* carriers) carrying *scn*-negative methicillin-sensitive *S. aureus* (MSSA) isolates indicating putative association with livestock. Among *scn*-negative isolates, four distinct *spa* types were identified, t338, t748, t1476 and t2379. These *spa* types have not been previously identified as associated with livestock. Prevalence of MRSA nasal carriage was 3.6%, and all MRSA isolates were human adapted. MRSA isolates included *spa* types t002, t008 and t024, representing community-acquired strains. Eighty percent of MRSA isolates were PVL positive.**Conclusion:** Nasal carriage of livestock-associated MSSA was recovered from beefpacking workers in the United States, with *spa* types not previously identified in livestock. *Spa* type t338 and t1476, identified in two scn-negative isolates, are associated with human epidemic clones of MSSA (ST30 and ST8 respectively), which may suggest adaptation of these *S. aureus* strains to livestock and subsequent transmission back to humans. Elevated nasal carriage of PVL-positive MRSA among these beefpacking workers may indicate heightened risk of clinical MRSA infection in this workforce.

Author Disclosure Block:

J.H. Leibler: None. **J.A. Jordan:** None.

Poster Board Number:

MONDAY-178

Publishing Title:

Taking Evidence-Based Microbiology to the Community Level in Lower Nyakach, Kenya, to Eliminate Waterborne Diseases

Author Block:

R. H. Metcalf[1], D. Chienjo[2]; [1]California State Univ., Sacramento, Sacramento, CA, [2]Friends of the Old Dev. Group, Kisumu, Kenya

Abstract Body:

Motivation: WHO estimates that waterborne diseases among a billion people living in extreme poverty EVERY DAY leads to >4,600,000 people suffering from diarrhea and >2,000 deaths. Most of these people must use contaminated unimproved drinking water sources and have no expectation that they will be connected to a safe water supply in the foreseeable future. We have developed and implemented a successful and replicable strategy with the goal of eliminating waterborne diseases in the Friends of the Old (FOTO) project in Lower Nyakach, Kisumu County, Kenya, where 70,000 people have no access to safe drinking water sources and use heavily contaminated rivers, streams, ponds and shallow wells. **Methods:** The first step was for the FOTO staff to take evidence-based microbiology to communities to demystify microbiology and have people understand the relationship between contaminated drinking water and disease. Two tests for *Escherichia coli* from the water and food industries were used: the Colilert® 10 ml presence/absence test (IDEXX, Westbrook, ME) and the *E. coli*/Coliform Count Petrifilm™ (3M, St. Paul, MN), a quantitative test for 1 ml. These tests were inoculated directly, incubated overnight, and provided easily interpreted results the next day that correlated with WHO disease risk categories: low, moderate, high, or very high. The second step was to provide effective household water treatment options. All 14,000 households were given a bottle of commercially available 1.2% sodium hypochlorite every other month, and all 42 schools received chlorine during the academic year. **Results:** From February-May, 2015, two districts adjacent to Lower Nyakach, Migori and Homa Bay, had serious cholera outbreaks: 915 cases and 12 deaths in Migori, 377 cases and 5 deaths in Homa Bay. In Lower Nyakach, where education and use of chlorine was universal, there were no cases of cholera and steadily decreasing rates of diarrhea. **Conclusion:** Evidence-based microbiology was critical in educating communities that their drinking water sources were contaminated and must always be treated. Providing free chlorine to all households and schools enabled all to treat water and avoid disease. Replicating FOTO's approach could bring relief to millions who suffer daily from preventable waterborne diseases.

Author Disclosure Block:

R.H. Metcalf: None. **D. Chienjo:** None.

Poster Board Number:

MONDAY-179

Publishing Title:

Cervicovaginal Microbiome of Isolated Amerindian Populations in Amazonian Brazil

Author Block:

C. P. Zolnik[1], A. J. Fonseca[2], A. E. Miranda[3], L. C. Ferreira[2], Z. Chen[4], R. D. Burk[1]; [1]Albert Einstein Coll. of Med., Bronx, NY, [2]Univ.e do Estado do Amazonas, Manaus, Amazonas, Brazil, [3]Univ.e Federal do Espírito Santo, Vitória, Brazil, [4]The Chinese Univ. of Hong Kong, Shatin, Hong Kong

Abstract Body:

Indigenous women in the Brazilian Amazon have a life-style adapted to the harsh environment and the isolated nature of their existence. They have a high prevalence of cervical HPV, HPV genotypic diversity and cervical cancer. However, comprehensive studies on reproductive health in these groups are limited. Although the composition of the cervicovaginal microbiome has been shown to affect various health outcomes including susceptibility, prevalence, and transmission of STIs, little is currently known regarding the cervicovaginal bacterial communities of these indigenous Amerindian populations.The goal of this study was to characterize the cervicovaginal microbiome of isolated populations from northern Amazonian Brazil. Cervical samples were collected in 2013 from Yanomami women (n=307), geographically and culturally isolated groups living in the forest, and from women in the Eastern Indigenous District (n=360), comprised of isolated populations adapted to living standards of western society. Extracted DNA was amplified for the V4 region of the bacterial 16S rRNA gene and PCR products were sequenced on a MiSeq Illumina platform (300 bp PE). Sequence reads were processed for quality and analyzed using in-house scripts in combination with pplacer, uparse, and QIIME.Overall, 35% of the samples were dominated with *Lactobacillus* spp., (28.7% Yanomami and 40.5% Eastern population), and 9% were dominated by *Gardnerella vaginalis* (13% Yanomami and 6% Eastern population). This contrasts recent studies of North American women where >60% of samples were dominated by bacterial species within these two genera. Our results suggest a more diverse cervical microbiome in these populations, a finding that contributes to a recent study that reported high diversity of fecal and skin microbial communities within isolated Yanomami groups in Venezuela. Variation in lifestyle (hunter-gatherer), social structure, sexual behavior, and isolation may have contributed to the cervical bacterial diversity observed in this study. Future work will examine the cervicovaginal microbiome of these groups in relation to infection with HPV, cervical dysplasia, and progression to cervical cancer.

Author Disclosure Block:

C.P. Zolnik: None. **A.J. Fonseca:** None. **A.E. Miranda:** None. **L.C. Ferreira:** None. **Z. Chen:** None. **R.D. Burk:** None.

Poster Board Number:

MONDAY-180

Publishing Title:

Investigation on Household Contamination of *Vibrio cholerae* in Bangladesh

Author Block:

Z. Hossain[1], I. Farhana[1], S. Tulsiani[2], R. Sultana[1], P. Jensen[2], A. Begum[1]; [1]Univ. of Dhaka, Bangladesh, Dhaka, Bangladesh, [2]Univ. of Copenhagen, Copenhagen, Denmark

Abstract Body:

The role of in-house transmission on the incidence of *Vibrio cholerae*, the deadly waterborne pathogen, is still not developed. The aim of the current study was to investigate possible contamination routes in household domain for effective cholera control in Bangladesh.To examine the prevalence of *V. cholerae*, routine swabs from four hotspots (cutting knife, latrine door knob, drinking water pot and food plate surface) and leftover food samples were collected. The cohort of 22 low income households has been studied for every 6 weeks between November 2014 and December 2015, from an urban area of northwest Dhaka, Bangladesh. Molecular genotypic and phenotypic traits of *V. cholerae* strains were assessed.Total 660 hotspot samples were analyzed by *V. cholerae* species-specific *ompW* gene PCR. Among them most predominant was food plate swabs with 37 samples positive out of 163 (22.69%), followed by water pot (17 of 163, 10.43%), knife (6 of 167, 3.6%), latrine door knob (5 of 167, 2.9%). In total 137 food samples were analyzed, only 10 found positive in PCR. Further characterization of *ompW* positive hotspot total DNA for virulence genotype revealed the presence of *rfb O1, rfb O139, tcp, cep* genes but lacked the major cholera toxin genes *ctxA* and *ctxB*. Only one food tDNA sample was positive for *rfb O1* gene.Of the 12 hotspot and 2 food *V. cholerae* strains isolated from PCR positive samples, 8 (66.6%) were harvested during time period of May-June. One hotspot strain was confirmed as *V. cholerae* O1 and others as non-O1/ O139 through PCR based analysis. The O1 strain lacked cholera toxin genes but possessed other regulatory and virulent genes like *toxR, rtxC, hly, msh, HA/protease*. Genes for type three secretion systems were detected in two non-O1/O139 isolates. All the strains including clinical *V. cholerae* El Tor strain N16961, showed hemolysis and proteolysis activity but none of them exhibited any hemagglutinin activity on human erythrocytes.The study findings indicate that *V. cholerae* contamination is mostly originated in and around kitchen area rather than latrine area. Contaminated food and water supply may be the reason behind this relatively high presence of virulence factors in food plates and water pots. Direct exposure routes of disease transmission should be a major consideration in cholera prevention policies.

Author Disclosure Block:

Z. Hossain: None. **I. Farhana:** None. **S. Tulsiani:** None. **R. Sultana:** None. **P. Jensen:** None. **A. Begum:** None.

Poster Board Number:

MONDAY-181

Publishing Title:

Extended-spectrum Cephalosporin Resistance and Pmqr in *Salmonella* Heidelberg Isolated from Poultry Farms in Argentina

Author Block:

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Abstract Body:

Background: *Salmonella* infections are important zoonoses worldwide. Historically, *S.* Typhimurium and *S.* Enteritidis have been the most prevalent serovars in both humans and poultry but in the last years, in Argentina *S.* Heildelberg (SH) has been found to be the more common serovar isolated from broilers and thus more studies are needed in order to evaluate implications that this emerging serovar. Resistance to third generation cephalosporins (TGC) and fluoroquinolone has been described in SH isolates but very little is known on the associated mechanisms. The aim of this study was to characterize them mechanisms, especially those encoded in plasmids, in SH isolated from broiler farms, in Argentina, between years 2010 and 2012. **Methods:** Susceptibility profile was analyzed in a selected group of 42 strains of SH by disk diffusion according to CLSI recommendations. Screening for extended spectrum β-lactamases (ESBL) or plasmid AmpC β-lactamases (AmpCp) were conducted by double disk synergy tests. ESBL and PMQR determinants were characterized by PCR using specific primers followed by sequencing. **Results:**Twenty-five isolates (59.5%) were found to be resistant to TGC and 13/25 of these strains were also resistant to FEP but remained susceptible to FOX. Furthermore, ESBL confirmatory test was positive for all of them. The ESBLs were characterized as members of CTX-M-14 (n = 8) and CTX-M-2 (n= 5). The remaining strains (12/25) were resistant to FOX but susceptible to FEP. Synergy was observed between PBA and both CAZ and CTX disks, thus suggesting the presence of an AmpCp. Finally, CMY-2 was identified in these 12 isolates. The most prevalent PMQR determinant detected among ESBL producers was *qnr*B (22/42), no association between the presence PMQR and CTX-M enzyme was found. Twelve isolates (28.5%) harbored *qnrB* genes, but all were susceptible to TGC. **Conclusions:** This is the first report of the presence of both CTX-M-14, CTX-M-2 and *qnrB* isolates of *S.* Heidelberg isolates from broiler farms in Argentina. Active monitoring programs of ATM resistance in SH strains should be considered in order to minimize the impact in both poultry and Public Health

Author Disclosure Block:

J.E. Dominguez: None. **P. Chacana:** None. **M. Herrera:** None. **G. Gutkind:** None. **E. Mercado:** None. **J. Di Conza:** None.

Poster Board Number:

MONDAY-182

Publishing Title:

Hepatitis-C Viral Infection among Children Visiting a Tertiary Care Hospital in Lahore, Pakistan

Author Block:

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Abstract Body:

Background: Hepatitis C viral (HCV) infection is basically a blood borne viral infection, ultimately causing malignant disease of liver if untreated. Risk factors play major role in transmission of HCV including blood transfusion, dental and surgical procedures, sharing of razor, blades, toothbrushes, nail clippers and manicure pliers. Hepatitis C infection is an emerging global health problem with a worldwide prevalence of about 3%. Hence a study was designed to investigate the prevalence of Hepatitis C viral infection among the children visiting a tertiary care hospital in Lahore, and also to assess the associated risk factors contributing towards the disease. **Methods:** This prospective and observational. Study was carried out at a children department of a tertiary care public hospital in Lahore Pakistan. The study was conducted during the month of March-July 2012. The children visiting Oncology ward, Emergency and ICUs were also included during the entire study period. A total of 200 Blood samples were collected with the informed consent and were processed to detect the antibodies against HCV by using third generation ELISA kits. **Results:** The percentage prevalence of HCV infection was found as 8%. The prevalence of anti-HCV among children was significantly associated ($P<0.05$) with age group (9-12 years). History of blood transfusion, surgery, visit to dentist, circumcision by barber were found to be important risk factors associated with hepatitis C infection among children. **Conclusion:** A high prevalence of HCV infection has been observed and certain risk factors also identified. A well-streamlined surveillance and reporting system is required to developed strategy at national level for the rapid identification, treatment and infection prevention measures for infected children.

Author Disclosure Block:

T. Ijaz: None. **S. Raja:** None. **S. Ahmad:** None.

Poster Board Number:

MONDAY-183

Publishing Title:

The Prevalence of Lifestyle-Related Diseases in Vietnamese Adult Hiv-Infected Patients on Antiretroviral Therapy

Author Block:

D. Mizushima[1], S. Matsumoto[1], N. T. Dung[2], N. H. Dung[2], N. V. Trung[2], N. V. Kinh[2], S. Oka[1]; [1]Natl. Ctr. for Global Hlth.and Med., Tokyo, Japan, [2]Natl. Hosp. of Tropical Diseases, Hanoi, Viet Nam

Abstract Body:

Background: Lifestyle diseases are becoming common in some developing countries with the westernization of food life. However, little is known about the diseases in Vietnamese HIV-infected patients. Thus, we evaluated the prevalence of the diseases and its risk factors in this population. **Methods:** A cross-sectional study was done in a cohort of Vietnamese HIV-infected patients on ART at the National Hospital of Tropical Diseases in Hanoi, Vietnam. Lifestyle diseases included hypertension (HT), hyperglycemia (HG) and dyslipidemia (DL). Blood pressure, fasting blood sugar and lipid profile were evaluated for every patient who visited the hospital on October and November 2015. As for statistical analysis, to determine factors associated with the diseases, logistic regression was used in uni and multivariate analysis. **Results:** Of 1371 study patients, females were 40%. Age, body mass index (BMI), CD4+ T cell count and the duration of HIV infection were averagely 38.5 years, 21.2 kg/m^2, 456 cells/μl and 6.1 years, respectively. 97.4% of the patients achieved viral suppression. As a key drug, 89.5% of the patients used non-nucleoside reverse transcriptase inhibitors (nevirapine 23.3% and efavirenz 66.2%) and 9.8% of the patients used the second line, lopinavir boosted with ritonavir (LPVr). As backbone drugs, 77% of the patients used tenofovir. The prevalence of HT, HG and DL were 18.7%, 4.2% and 53.5%, respectively. Age, female sex and BMI were significantly associated with HT in multivariate analysis (OR: 1.085, CI:1.067-1.103, OR:0.439, CI:0.313-0.614 and OR:1.161, CI:1.096-1.231, respectively). Age, sex and duration of HIV infection were associated with HG (OR: 1.078, CI:1.050-1.106, OR:0.463, CI:0.240-0.894 and OR:1.084, CI:1.018-1.154, respectively). Age, sex, BMI and use of LPV were associated with DL (OR: 1.032, CI:1.017-1.047, OR:0.417, CI:0.329-0.529, OR:1.212, CI:1.153-1.275 and OR:5.230, CI:3.173-8.620, respectively). **Conclusions:** The prevalence of dyslipidemia was disproportionally higher compared to the other lifestyle diseases and the use of LPVr was statistically associated in addition to known risk factors. A further study is required to evaluate the link between these findings and cardiovascular diseases.

Author Disclosure Block:

D. Mizushima: None. **S. Matsumoto:** None. **N.T. Dung:** None. **N.H. Dung:** None. **N.V. Trung:** None. **N.V. Kinh:** None. **S. Oka:** None.

Poster Board Number:

MONDAY-184

Publishing Title:

Prevalence Of Bacterial Etiologies Of Gastrointestinal Disease In Children Living In The Peruvian Amazon River Basin

Author Block:

A. Huyler[1], H. Montilla-Guedez[1], L. Nguyen[1], A. Rogers[1], M. Lotfipour[1], W. Beachy[1], R. Perez[1], D. Nesterova[1], O. Munizza[1], I. Marji[1], R. Burga[2], R. Abadie[2], C. Rocha[2], H. Tilley[2], D. W. Craft[1]; [1]Milton S. Hershey Med. Ctr., Hershey, PA, [2]NAMRU-6, Lima, Peru

Abstract Body:

Background: In the Peruvian Amazon River Basin, diarrhea has been shown to affect many aspects of children's health and is responsible for 7% of deaths under the age of 5. However the prevalence of bacterial pathogens in fecal samples from children in this area has not been previously reported. This study assesses the prevalence of bacterial etiologies of infectious diarrhea in children in the impoverished Amazon River Basin community of Belen, Peru. **Methods:** 232 stool samples were collected from children under the age of five in Belen from May to October 2014. Fresh stool was cultured using conventional culture media and pathogens identified using standardized techniques. Stool was also directly analyzed for the presence verotoxin- and shiga toxin-producing *E. coli* (VTEC/STEC) using QUIK CHEK (TechLab, Blacksburg, VA). **Results:** The overall prevalence of enteric bacterial pathogens was 21.1% (49/232). 44 of 49 (97.8%) cultures yielded one bacterial pathogen and 5 (2.2%) cultures yielded 2 pathogens. The most prevalent was *Salmonella* spp. (14/232), which was twice that of *Shigella* spp. (7/232). For *Campylobacter* spp., the prevalence of *C. coli* (11/232) was twice that of *C. jejuni* (6/232), the latter being essentially equivalent in prevalence to *Aeromonas* spp. (6/232) and *Plesiomonas* spp. (5/232). There was no evidence of VTEC/STEC based on direct detection of toxin in the stool samples.

Pathogen	**Prevalence**
Salmonella spp.	6.0%
Shigella spp.	3.0%
C. coli	4.7%
C. jejuni	2.6%
Aeromonas spp.	2.6%
Plesiomonas spp.	2.2%
VTEC/STEC	0.0%

Conclusions: Of the 232 stool samples analyzed, 21.1% yielded at least one enteric pathogen, a slightly higher percentage than previously reported for worldwide incidence (vs. prevalence)(10-20%) of bacterial diarrhea in children. Water quality is considered a risk factor in this community. Ongoing longitudinal studies are assessing prevalence against the demographics of Belen households as well as investigation of other etiologies of pediatric and adult diarrhea in the Amazon River Basin of Peru.

Author Disclosure Block:

A. Huyler: None. **H. Montilla-Guedez:** None. **L. Nguyen:** None. **A. Rogers:** None. **M. Lotfipour:** None. **W. Beachy:** None. **R. Perez:** None. **D. Nesterova:** None. **O. Munizza:** None. **I. Marji:** None. **R. Burga:** None. **R. Abadie:** None. **C. Rocha:** None. **H. Tilley:** None. **D.W. Craft:** None.

Poster Board Number:

MONDAY-185

Publishing Title:

Human Brucellosis Cases in Georgia in 2015

Author Block:

M. Makharadze, R. Burdilaze, N. Naveriani, T. Zaqaraia; Res. Inst. of Med. Parasitology and Tropical Med., Tbilisi, Georgia

Abstract Body:

Brucellosis is one of the most widespread and debilitating zoonotic diseases in the world. The causative agent (genus *Brucella*) remains endemic in Georgia, and causes substantial human morbidity and significant agriculture economic loss. In addition, *Brucella* species are considered potential biological threat agents by the United States government. To assess epidemiology, clinical course, laboratory findings, and treatment outcomes of brucellosis cases a study was conducted at the Institute of Parasitology and Tropical Medicine (IPTM) of Georgia, the reference clinic for brucellosis where patients are diagnosed and treated.For detecting *Brucella* antibodies, we used the Wright-Huddleston reaction. All suspected brucellosis cases are tested using ELISA (IgG + IgM). All positive blood samples are sent to National Center for Disease Control for further investigation. All patients were treated with a two-drug combination of antibiotics Doxycycline + Streptomycin/Gentamycin or Doxycycline + Rifampin for six week.A total of 153 brucellosis cases were diagnosed in Georgia from January 2015-October2015, a 6.7% increase in the incidence compared to the same period in 2014. The majority of cases were found in eastern Georgia (93%). In the 2015 study period, the incidence rate of brucellosis was highest in the Kakheti region (2.0 cases per 10000 population). Men (65%) were more likely to be infected compared to women (35%); the most affected age group was patients 30-59 years old. The most frequent clinical symptoms were sweats (90%), joint pain (84%), aches (85%), malaise (90%), fatigue (75%), and rigors (86%). Lymphadenopathy (92%) and hepatosplenomegaly (90%) occurred as well. Neuropsychiatric symptoms such as depression, difficulty concentrating, and sleep disturbance were observed less frequently. Most symptoms resolved after 10-14 days of treatment. In some cases, arthralgia remained for two weeks or longer.The most important risk factor was occupation. The highest incidence was among farmers and shepherds, whose reported source of infection was contact with infected animals and consumption of unpasteurized milk and dairy products. In 2015, the prevalence of brucellosis is still high. Currently there are more cases of brucellosis in eastern Georgia. Treatment of brucellosis is effective, however, preventive measures against the disease is of significant importance.

Author Disclosure Block:

M. Makharadze: None. **R. Burdilaze:** None. **N. Naveriani:** None. **T. Zaqaraia:** None.

Poster Board Number:

MONDAY-186

Publishing Title:

The Critical Factors for a Nationwide Major Reduction of Antimicrobial Use in Animals in the Netherlands with a Parallel Reduction in Antimicrobial Resistance

Author Block:

J. Wagenaar[1], I. Van Geijlswijk[1], J. Mouton[2], D. Mevius[3], K. Veldman[3], J. Jacobs[1], D. Heederik[1]; [1]Faculty of Vet. Med., Utrecht, Netherlands, [2]Erasmus Univ., Rotterdam, Netherlands, [3]Central Vet. Inst., Lelystad, Netherlands

Abstract Body:

Introduction: After a doubling of antimicrobial use (AMU) in Dutch livestock between 1990 and 2007, measures were initiated by the Dutch Government, livestock sectors and the Royal Dutch Veterinary Association (KNMvD) to reduce the use of antimicrobials. A major trigger for action was the emergence of multi-resistant bacteria (LA-MRSA and ESBLs) in livestock. **Methods:** The Netherlands Veterinary Medicines Authority (SDa) was formed as an independent body to i) collect the AMU data of Dutch livestock (approximately 42,000 units); ii) define benchmark targets for AMU for pigs, poultry, veal calves and dairy; iii) report annual trends in AMU; iv) identify frequent- or mis-users/prescribers. Reduction targets were set by the government at 20%, 50% and 70% reduction in 2010, 2013 and 2015, respectively, with reference to 2009. Several other actions were performed at different levels; e.g. report of the Dutch Health Council, the development of treatment guidelines. Parallel to these actions, continuous monitoring of resistance in commensal *E. coli* had already been set up in livestock from 1998 onwards, enabling measurement of trends in resistance. **Results:** The total reduction of AMU between 2009 and 2014 was 58.1%. Compared to 2007, the year with the highest veterinary usage, there was a reduction of 65%. The use of antimicrobials defined as ‘’critically important for human health’’ (fluoroquinolones and 3rd/ 4th generation cephalosporins) in livestock were reduced to almost zero. The Dutch farmers and their veterinarians achieved this through improved infection and health control measures, which resulted in overall less, and more individual treatments instead of group treatments. As a result of the enforced 1-to-1 relationship of farmers and veterinarians, it was possible to develop the Veterinary Benchmark Indicator allowing to compare prescription levels between veterinarians. All farms were benchmarked (action, signaling and target level). **Conclusions:** A major reduction in AMU could be achieved and parallel to reduction of AMU there was a reduction of AMR in livestock observed.

Author Disclosure Block:

J. Wagenaar: None. **I. Van Geijlswijk:** None. **J. Mouton:** None. **D. Mevius:** None. **K. Veldman:** None. **J. Jacobs:** None. **D. Heederik:** None.

Poster Board Number:

MONDAY-187

Publishing Title:

Health and Healthcare Determinants of Antibiotic Resistance in Oecd Countries

Author Block:

S. Karahan, P. Duzgun, E. D. Aksu, D. C. Karaaslan, B. AK, F. Can, **O. Ergonul**; Koc Univ., Sch. of Med., Istanbul, Turkey

Abstract Body:

Background: Emerging antibacterial resistance is one of the leading global public health problems; however, healthcare determinants were not explored in depth. We aimed to identify the health and health care determinants of antibacterial resistance between countries. **Methods:** We reviewed the relevant parameters that could be related with antibacterial resistance. Bacterial resistance was defined as fluoroquinolone resistance to *E. coli*. We retrieved healthcare determinants data from open sources of OECD and resistance data from WHO. By using linear regression, we tested the effect of each health and healthcare parameter on antibiotic resistance (STATA, USA, 12v, $p<0.05$). **Results:** Five parameters were found to have an effect on antibacterial resistance, and three of them were significant (Table 1). 1) In OECD countries, As the ratio of the nurses to doctors increases, antibiotic resistance decreases, 2) As the share of health expenditure in GDP increases, antibacterial resistance decreases, 3) A positive effect of vegetable consumption, but a negative effect of fruit consumption on antibiotic resistance was detected. Fluoroquinolones are stable and have long half-lives compared to other antibiotics that are used in agriculture, 4) Among educational indicators, direct public expenditure on educational institutions plus public subsidies as a percentage of GDP had a negative effect on antibiotic resistance (Table 1). **Conclusions:** In order to decrease resistance to antibiotics, not only should the health system be developed, but also making alterations in the educational expenditures and enhancements in educational systems should be targeted.Table 1: Univariate linear regression of health and healthcare indicators for the rate of fluoroquinolone resistance against E.coli.

Indicator	coefficent	95% Confidence Interval	p
nurses/physicians ratio	-1.8133	-3.3-0.3263	0.019
Educational expenditures	-8.3975	-12.5721-4.2229	<0.001
Share of health expenditure in GDP	-2.7051	-5.4088-0.014	0.041
Vegetable Consumption kg per capita	0.1207	-0.0083-0.2496	0.065

Systemic anti-infectives DDD per thousand inhabitants	0.6657	-0.3352-1.3649	0.061

Author Disclosure Block:

S. Karahan: None. **P. Duzgun:** None. **E.D. Aksu:** None. **D.C. Karaaslan:** None. **B. Ak:** None. **F. Can:** None. **O. Ergonul:** None.

Poster Board Number:

MONDAY-188

Publishing Title:

Acute Dentoalveolar Infections, Microbiology and Susceptibility Patterns Over a 27 Year Period in Glasgow, UK

Author Block:

A. J. Smith[1], G. Morgan[2], L. Smith[3]; [1]Univ. of Glasgow, Glasgow, United Kingdom, [2]Bacteriology Dept, Glasgow, United Kingdom, [3]Dental Practice, Glasgow, United Kingdom

Abstract Body:

Relatively little surveillance data is undertaken on the antimicrobial susceptibility of isolates from acute dento-alveolar infections. We present data collated from routine diagnostic microbiology specimens submitted over a 27 year period in Glasgow, UK. Specimens from acute dento-alveolar infections are routinely processed for predominant facultative and anaerobic isolates. Over the study period isolates were identified using biochemical properties (API system), VITEK 2 and MALDI-TOF. Antimicrobial susceptibility tests were undertaken using combinations of Stokes method and/or E-tests. Baseline data used was that reported from Glasgow by Lewis et al., (JAC 23,69-77 1989) and compared to isolates from 1998 -2015. Data were available from 621 specimens. Predominant facultative groups isolated during the study period comprised Anginosis (n=196) and Mitis group Streptococci (n=448) . Strict anaerobes frequently isolated included anaerobic streptococci (n=116), Fusobacteria (n=121) and Prevotella (n=140) species. With regards to antimicrobial susceptibility the majority of isolates remain susceptible to Penicillin, Macrolides and Clindamycin. Relatively small numbers of annual specimens and isolates confounds data interpretation trends over time, however, no discernable increase in resistance patterns over the 27 year time period of analysis was observed. Conclusion, practitioners should be encouraged to submit samples from acute dento-alveolar infections to provide systematic surveillance data to inform empiric antimicrobial prescribing.

Author Disclosure Block:

A.J. Smith: None. **G. Morgan:** None. **L. Smith:** None.

Poster Board Number:

MONDAY-189

Publishing Title:

Recurrent Melioidosis in a Tertiary Referral Centre in Singapore

Author Block:

E. Y. Lim, J. M. F. Chien; Singapore Gen. Hosp., Singapore, Singapore

Abstract Body:

Background: *Burkholderia pseudomallei* infections are notoriously difficult to eradicate. Reported rates of recurrent infections ranged from 5.2% to 9.3% but data from Singapore has yet to be published. We report our 14-year clinical experience of recurrent melioidosis. **Methods:** All patients with a positive culture for *B. pseudomallei* from 1 Jan 2001 to 31 Dec 2014 were retrospectively identified and their case records reviewed. An episode of recurrence was defined in a patient on treatment or who had completed treatment and presented with new clinical features or microbiological results suggestive of re-infection. **Results:** Two hundred patients had culture-proven melioidosis, of which 36 (18%) had at least 1 episode of recurrence. Twenty-four were culture-positive recurrences, while twelve were clinical recurrences. 50% of recurrences occurred during maintenance phase of treatment. Recurrent cases were more likely to have skin and soft tissue infection (41.7% vs 20.7%, p=0.008) and involvement of the musculoskeletal system (25% vs 12.2%, p=0.048). Recurrent patients also tended towards a longer duration of bacteremia, 9.48 vs 7 days (p=0.094), and having >1 organ involvement (63.9% vs 51.2%, p=0.168). Susceptibility to co-trimoxazole was significantly less among isolates from the recurrent group (41.7% vs 64%, p=0.013). Mortality rate of recurrent cases was similar to non-recurrent cases. Choice of induction antibiotic across both groups was similar with ceftazidime being the commonest. Mean duration of induction was 34 days among recurrent cases vs 31.8 days in non-recurrent group (p=0.533). Co-trimoxazole and doxycycline combination was the commonest choice of maintenance therapy, with 5mg/kg as the average dose of trimethoprim component. Amongst the recurrent group, doxycycline monotherapy was more commonly used during maintenance (11.7% vs 3.4%, p=0.085). Mean duration of maintenance therapy was also shorter (16.2 weeks vs 21.2 weeks, p=0.025).**Conclusions:** Most of our recurrent cases were a result of incomplete prior therapy and it will be interesting to investigate if the low dose co-trimoxazole and doxycycline maintenance combination in addition to decreased susceptibility to co-trimoxazole had contributed to our high recurrence rates. In agreement with published studies, the duration of bacteremia and type of oral maintenance therapy is associated with risk of recurrence.

Author Disclosure Block:

E.Y. Lim: None. **J.M.F. Chien:** None.

Poster Board Number:

MONDAY-190

Publishing Title:

Ctx-M-15-Producing Multidrug Resistant *Klebsiella pneumoniae* High-risk Lineages Cause Urinary Tract Infection in Companion Animals

Author Block:

C. Marques[1], J. Menezes[1], G. Trigueiro[2], A. Belas[1], L. Telo da Gama[1], C. Pomba[1]; [1]CIISA, Faculty of Vet. Med.-UL, Lisbon, Portugal, [2]Laboratório Joaquim Chaves, Lisbon, Portugal

Abstract Body:

The increase of multidrug-resistant (MDR) *Klebsiella pneumoniae* is a great concern. Companion animals with urinary tract infection (UTI) caused by MDR *K. pneumoniae* may have a critical role as reservoirs to humans. This study aimed to determine clonal lineages, antimicrobial resistance and resistance mechanisms of *Klebsiella* spp. isolated from humans and CA with UTI.Susceptibility testing against 28 antimicrobials was conducted by disk diffusion in 37 *Klebsiella* spp. from CA (2002-2015) and 61 from humans (2014) with UTI. The following resistance genes were screened by PCR: ESBL (TEM, SHV, CTX-M), pAMPc (CMY, DHA, MOX), *aac*(6')-Ib, *aph*AI-IAB, *qnr (*A, B, C, D, S), *sul*1, *sul*2, t*et*A and *tet*B. *K. pneumoniae* from CA and third-generation cephalosporin (3GC) resistant human isolates were typed by MLST.CA *Klebsiella* showed high frequencies of resistance to cefotaxime (54.0%), ciprofloxacin (67.6%), trimethoprim/sulphamethoxazole (61.1%) and tetracycline (62.2%). Only *qnr*B was detected in ciprofloxacin-resistant isolates. *tet*A (55.6%, n=10/18) and *tet*B (38.9%, n=7/18) were found in CA tetracycline-resistant *Klebsiella*. Most CA and human (90.6%, n=29/31) trimethoprim/sulphamethoxazole resistant *Klebsiella* harbored *sul*1 and/or *sul*2. All CA 3GC-resistant *K. pneumoniae* (56.2%, n=18/32) were MDR. Both in CA and human *Klebsiella*, CTX-M-15 was the most frequent (66.7%, n=12/18), followed by CMY-2 (16.1%, n=5/31) and DHA-1 (9.7%, n=3/31). ST15 (n=11/15) was the main 3GC-resistant *K. pneumoniae* lineage found in CA. Yet, one ST11, one ST37, one ST147 and one ST348 were also detected. *K. pneumoniae* ST15 clonal lineage was also found among CA 3GC-susceptible isolates (n=4/10). Eleven human community-acquired 3GC-resistant *Klebsiella* STs were found including ST15 (n=2/13) and ST348 (n=1/13).ESBL CTX-M-15 is known to be worldwide distributed in companion animals and humans. This study shows a wide dissemination of the MDR zoonotic ST15 CTX-M-15-producing lineage in CA with UTI. Furthermore, the detection of other human high-risk lineages such as ST11 is worrisome. Since CA with UTI are generally treated in ambulatory by the owners, measures should be taken to minimize the spread of MDR *K. pneumoniae* to humans and the environment.

Author Disclosure Block:

C. Marques: None. **J. Menezes:** None. **G. Trigueiro:** None. **A. Belas:** None. **L. Telo da Gama:** None. **C. Pomba:** None.

Poster Board Number:

MONDAY-191

Publishing Title:

Evaluation of Copan Fecalswab^{tm} for Molecular Detection of Pathogens Using the Bd Max^{tm} Enteric Bacterial Panel

Author Block:

V. P. Prakash, L. LeBlanc, K. Chapin; Rhode Island Hosp., Providence, RI

Abstract Body:

Background: The Copan FecalSwab^{TM} (FS) is an FDA-cleared collection device consisting of a flocked swab in modified Cary-Blair (CB) medium for transporting and preserving enteric stool pathogens for culture. Collection of a rectal swab specimen at the time the patient presents addresses the unmet clinical need of consistently obtaining a specimen for analysis. No GI molecular assays are FDA-cleared for use with the FS. The objective of this study was to evaluate and validate the FS for detection of GI pathogens using the BD MAX^{TM} Enteric Bacterial Panel (EBP).**Methods:** Optimal specimen input volume, limit of detection (LoD) and clinical performance for the FS with the EBP assay were assessed. Input volume for EBP was determined by testing volumes between 50-500 ul in triplicate of pooled negative stool in the FS medium and analytical sensitivity (LoD) by spiking negative FS medium matrix with concentrations (1.5×10^6 - 1.5×10^1 CFU/mL, 4 replicates/dilution) of ATCC strains bearing the assay targets. The LoD was set as the lowest concentration that was positive for 3/4 replicates with matching corresponding bacterial concentration in CFU/ml. Clinical performance was determined by FS EBP results compared to FS culture results. Discrepant analysis included EBP and culture from CB stool and alternate PCR.**Results:** Input volume of 500 ul FS medium was the highest volume found to be non-inhibitory for EBP based on Ct value of the Sample Processing Control (SPC) compared to the control specimens. The LoD values were 571 CFU/mL Sample Buffer Tube (SBT) for *Salmonella*, 100 CFU/mL SBT for *Shigella*, 1261 CFU/mL SBT for STEC and 20 CFU/mL SBT for *Campylobacter*. Clinical performance among 55 FS samples (to date), showed five samples positive (9%) for 6 analytes: *Salmonella* (3), *Shigella* (1), *Campylobacter* (1) and STEC (1). Paired CB stool tested simultaneously for 38/55 samples were concordant with FS results by EBP and culture.**Conclusions:** Detection of enteric pathogens using the FS collection system and EBP assay was equivalent to culture and EBP results from stool collected in CB. LoD values were similar to those reported in the EBP package insert. The FecalSwab™ provides convenient collection, preservation and testing by multiple methods, including culture and the BD MAX^{TM} Enteric Bacterial Panel.

Author Disclosure Block:

V.P. Prakash: None. **L. LeBlanc:** None. **K. Chapin:** B. Collaborator; Self; BD, BioFire Diagnostics. I. Research Relationship; Self; BD, BioFire Diagnostics.

Poster Board Number:

MONDAY-192

Publishing Title:

Pathogen Distribution in Infectious Diarrhea in Beijing Area Determined with a Comprehensive Detection Method

Author Block:

X. Lu, W. Feng, X. Gu, S. Zhang, M. Wang, Y. Huang, X. Li, X. Li, M. Zhang, L. Yuan, Y. Sun, J. Wu; Beijing Tongren Hosp., Capital Med. Univ., Beijing, China

Abstract Body:

Background: Infectious diarrhea (ID) is the second leading cause of death worldwide, so understanding its regional epidemiology should improve preventive practices. **Methods:** In this study, 592 stool samples were collected from outpatients diagnosed with ID between October, 2013, and September, 2014, at Beijing Tongren Hospital, Capital Medical University. A new method, xTAG® GPP multiple PCR and traditional methods (culture, rapid immunochromatography, microscopic examination, and real-time PCR) were used to identify the causative organisms.**Results:** Pathogens were detected in 340 samples (57.5%) with xTAG® GPP multiple PCR and in 254 samples (42.9%) with traditional methods. Viruses were detected in 107 (18.1%) samples. *Rotavirus A* was the most prevalent virus (8.8%),followed by *Norovirus* (8.4%) and adenovirus (0.84%). A variety of bacteria was detected in 263 (44.4%) samples. *Vibrio parahaemolyticus* was the most frequently detected bacterial pathogen (13.5%),followed by enterotoxigenic *E. coli* (8.4%), *Campylobacter* (7.7%), *Salmonella* (7.0%), *Clostridium difficile* A/B (3.5%), *Shigella* (3.3%), *E. coli* O157 (3.3%), and others(4.2%). In 10 samples, the following parasites were found: *Cryptosporidium* (5), *Entamoeba histolytica* (3), and *Giardia* (2).**Conclusions:** Our data show the specific epidemiological characteristics of the ID pathogens detected in our hospital in different populations. We also demonstrated that xTAG® GPP multiple PCR combined with traditional methods effectively increases the detection rate for ID pathogens.**Table Samples results detected from 592 samples compared between xTAG® GPP and the traditional method**

Pathogens	**xTAG® GPP**	Results of Traditional methods		**Traditional methods**	**P value**
		P	**N**		
RotV A	**P**	50	2	Rapid Gold Standard Method+PCR	0.617
	N	2	538		

NorV GI/GII	**P**	44	6	PCR	0.040
	N	0	542		
ETEC-LT/ST	**P**	42	8	PCR+ Culture + serum agglutination	0.010
	N	0	542		
Camp	**P**	37	8	PCR+ Culture	0.010
	N	0	547		

Note:**P**= Positive; **N**= Negative

Author Disclosure Block:

X. Lu: None. **W. Feng:** None. **X. Gu:** None. **S. Zhang:** None. **M. Wang:** None. **Y. Huang:** None. **X. Li:** None. **X. Li:** None. **M. Zhang:** None. **L. Yuan:** None. **Y. Sun:** None. **J. Wu:** None.

Poster Board Number:

MONDAY-193

Publishing Title:

Utilizing BD MAX Enteric Bacterial Panel to Detect Stool Pathogens from Rectal Swabs

Author Block:

B. DeBurger, S. Hanna, C. Ventrola, E. Powell, J. Mortensen; Cincinnati Children's Hosp., Cincinnati, OH

Abstract Body:

Background: The BD MAX™ Enteric Bacterial Panel (EBP) is designed to detect *Salmonella, Shigella, Campylobacter,* and Shiga toxin from stool samples. However rectal swabs, which are not FDA-cleared for clinical testing with the BD MAX EBP, are common specimens received from pediatric patients for enteric pathogen testing. The purpose of this study was to evaluate the ability of the BD MAX EBP to detect stool pathogens from rectal swabs. **Methods:** Routine cultures, Shiga toxin testing, and molecular testing with BD MAX EBP were performed on 272 sequential rectal swabs collected from August 2015 to December 2015. 36 challenge samples (3 *Shigella* spp., 13 *Salmonella* spp., 10 *Campylobacter* spp., and 10 Shiga toxin positive *E. coli*) were tested using reference strains (American Type Culture Collection and previous patient isolates diluted to10^3-10^4 cfu/ml in saline then adding to Sample Buffer Tube (SBT) with negative stool matrix. Limit of detection testing was performed by serial 10 fold dilutions in saline then adding to SBT with negative stool matrix. Discrepant test results were resolved using Verigene® Enteric Pathogens Nucleic Acid Test.**Results:** See table for the patient results. All discrepant results were BD MAX EBP positive and culture negative. 8.8% of the patient samples did not initially yield a result on the BD MAX System. Upon repeat, 4.4% resolved. All organisms in the challenge samples were detected. Limits of detection for BD MAX EBP testing of rectal swabs were as follows (in CFU/mL in SBT): *Shigella*-5.10 x 10^0; Shiga Toxin-1.13 x10^3; *Salmonella*-1.44 x 10^2 ; and *Campylobacter*- 1.51 x 10^1.**Conclusion:** Rectal swabs are acceptable samples for detecting *Salmonella, Shigella, Campylobacter,* and Shiga toxin using BD MAX™ EBP.

Target	Number Positive	Positive Percent Agreement	Negative Percent Agreement
Shigella	79	100%	95.3%
Shiga Toxin	2	100%	100%
Campylobacter	4	100%	99.6%
Salmonella	4	100%	100%

Author Disclosure Block:

B. DeBurger: None. **S. Hanna:** None. **C. Ventrola:** None. **E. Powell:** None. **J. Mortensen:** E. Grant Investigator; Self; Becton Dickinson. J. Scientific Advisor (Review Panel or Advisory Committee); Self; Becton Dickinson.

Poster Board Number:

MONDAY-194

Publishing Title:

Validation of Bd Max Enteric Parasite Pathogen (Epp) Panel with Alternate Transport Systems

Author Block:

B. DeBurger, S. Hanna, C. Ventrola, E. Powell, J. Mortensen; Cincinnati Children's Hosp., Cincinnati, OH

Abstract Body:

Background: The BD MAX™ EPP is FDA-cleared for the detection of *Giardia lamblia*, *Cryptosporidium* spp., and *Entamoeba histolytica* from unpreserved stool or 10% formalin-fixed stool. In an effort to eliminate the use of 10% formalin from the laboratory due to its hazardous nature and increase efficiency with a single vial for both culture and parasitic examination, four different transport systems were compared: 10% formalin, Total-Fix™, Cary-Blair medium, and the Para-Pak® SVT. **Methods:** Each transport system was inoculated with 5 ml of stool, previously determined to be negative for the three targets. Reference stocks of the target organisms were serially diluted 10 fold. Sample Buffer Tubes (SBT) were inoculated in quadruplicate with 10 µl of the appropriate organism dilution and 10 µl of the fixed negative stool matrix. The BD MAX EPP was performed on each SBT using the BD MAX™ System. The limit of detection (LOD) was defined to be the concentration of organism in SBT where all four replicates were positive.**Results:** The challenge samples show equivalent LOD for each transport type for *G. lamblia* and *E. histolytica*. The alternate transport systems may have superior analytical sensitivity for *Cryptosporidium parvum* detection as compared to 10% formalin.**Conclusion:** In these initial laboratory studies, Total-Fix, Cary-Blair, and Para-Pak SVT have equal or superior limits of detection for all pathogens tested as compared to 10% formalin. Validation of these various transport systems for use with the BD MAX™ EPP with patient specimens is ongoing.

Target	Limits of Detection (organisms/ml in SBT)			
	10% formalin	Total Fix	Cary-Blair	SVT
Giardia lamblia	$1.6x10^1$	$1.6x10^1$	$1.6x10^1$	$1.6x10^1$
Cryptosporidium parvum	$1.6x10^3$	$1.6x10^1$	$1.6x10^2$	$1.6x10^2$
Entamoeba histolytica	$5.3x10^1$	$5.3x10^1$	$5.3x10^1$	$5.3x10^1$

Author Disclosure Block:

B. DeBurger: None. **S. Hanna:** None. **C. Ventrola:** None. **E. Powell:** None. **J. Mortensen:** E. Grant Investigator; Self; Becton Dickinson. J. Scientific Advisor (Review Panel or Advisory Committee); Self; Becton Dickinson. L. Speaker's Bureau; Self; Becton Dickinson.

Poster Board Number:

MONDAY-195

Publishing Title:

Molecular Detection of Gi Pathogens Offers Greater Diagnostic Yield versus Conventional Ordering Practices for Diarrheal Illness in a Large Academic Hospital System

Author Block:

B. Couturier[1], M. Powers-Fletcher[2], W. Hymas[1], K. Kalp[1], M. R. Couturier[2]; [1]ARUP Lab., Salt Lake City, UT, [2]Univ. of Utah, Salt Lake City, UT

Abstract Body:

Background: Bacteria, parasites, and viruses cause diarrheal illness with any number of non-specific symptoms such as diarrhea, vomiting, abdominal pain, and fever. Testing for gastrointestinal (GI) pathogens causing acute diarrhea typically includes stool cultures, ova & parasite microscopic examination, and enzyme immunoassays. Our study examined the analytical yield of a laboratory-developed molecular GI panel compared to conventional testing using stool cultures +/- adjunct conventional stool testing.**Methods:** Stool samples sent for routine stool culture from the University of Utah were collected between June 2012 to February 2013 (n=583). Nucleic acid was extracted and tested in a real-time PCR panel that targets bacterial, viral, and parasitic pathogens (*Salmonella* sp [Salm], *Shigella* sp., *Campylobacter jejuni/C. coli*, *C. upsaliensis*, *C.lari/ureolyticus/hyointestinalis*, shiga-like toxin 1 and 2, *Cryptosporidium* sp [Crypto], *Giardia* sp., *Dientamoeba fragilis*, *Cyclospora cayetanensis*, *Entamoeba histolytica*, astrovirus, norovirus group 1 & 2, rotavirus, and adenovirus 40/41). An internal control was used to monitor inhibition. Charts were reviewed for all PCR or culture positive specimens to assess adjunct testing.**Results:** A total of 104/583 (17.8%) stool samples were positive by PCR. Fourteen patients were positive for 2 targets yielding a total of 118 positive results. Of the 118, 55.9% contained a bacteria, 28.0% a virus, or 16.1% a parasite; 13 results correlated with stool culture, or antigen testing. The most frequently detected targets were Salm. (n=34), Norovirus (n=26), *C. jejuni/C. coli* (n=14), and Crypto (n=12). Five *C. jejuni/C. coli* PCR positives and 30 Salm PCR positives were not detected by culture. One *C. jejuni* culture positive sample was negative by real-time PCR. Three samples PCR positive for Crypto were tested by antigen, but only 1 was detected by Crypto antigen.**Conclusions:** The use of molecular methods for stool samples from suspected GI infections has increased analytical sensitivity and specificity compared to traditional stool culture and antigen testing methods, and reveals significant deficiencies with the performance of conventional methods used in routine ordering practices.

Author Disclosure Block:

B. Couturier: None. **M. Powers-Fletcher:** None. **W. Hymas:** None. **K. Kalp:** None. **M.R. Couturier:** None.

Poster Board Number:

MONDAY-196

Publishing Title:

Evaluation of the Bd Max™ System for Detection of Bacterial and Parasitological Infectious Gastroenteritis

Author Block:

M. England, C. Lowe, D. Myers, D. Craft; Penn State Hershey Med. Ctr., Hershey, PA

Abstract Body:

Introduction: Infectious gastroenteritis due to bacteria and parasites is a leading cause of morbidity and mortality worldwide. Routine identification is labor-intensive, slow, and considered insensitive compared to molecular assays. We compared sensitivity and turnaround time of the BD MAX™ Enteric Bacterial Panel (EBP) and BD MAX™ Enteric Parasite Panel (EPP) performed on the BD MAX™ System to routine culture, shiga-toxin EIA, and parasite microscopy/antigen detection (EIA). **Methods:** Over a 4 month period, 409 bacterial culture (unpreserved & Cary-Blair) and 151 parasite (10% formalin) specimens were concurrently tested via conventional stool culture or microscopy/EIA and BD MAX™ EBP and EPP. Stool culture specimens were inoculated onto routine media and GN broth. Broths were analyzed for shiga-toxin producing *E. coli* (STEC) by ImmunoCard STAT!® EHEC (EIA; Meridian). Parasite specimens were sent to a reference laboratory for ova and parasite (O&P) identification. Discrepancies were adjudicated by FilmArray™ Gastrointestinal Panel (BioFire).**Results:** The BD MAX™ EBP reported 29 organisms in 28 positive specimens (6.8%), with 1 false positive *Campylobacter* spp.; whereas, routine culture correctly identified 17 organisms (4.2%). Notably, EIA failed to identify all 4 STEC-positive specimens, all of which were identified by BD MAX™ and confirmed by FilmArray™ or PA DOH. The BD MAX™ EBP improved time for reportable results by 12-20 hours. The BD MAX™ EPP matched microscopy/EIA in sensitivity (3/151 positive) and improved report time by 5 days.**Conclusions:** The BD MAX™ EPP correlated with EIA and O&P microscopy, though the positivity rate was low (n=3). The BD MAX™ EBP increased sensitivity by 50% over routine stool culture and shiga-toxin EIA and decreased overall turnaround time by 12 hours. Decreased time to result may lead to faster appropriate management of outpatients and discharge of inpatients with suspected gastroenteritis. Importantly, we noted a significant improvement in sensitivity of BD MAX™ EBP over EIA for STEC. While further validation is needed to confirm the BD MAX™ EPP, the decreased turnaround time is significant if parasite testing is sent to reference labs. A limitation of these multiplex assays is the inability to distinguish between active and resolving infection and lack of bacterial isolates for epidemiological analysis.

Author Disclosure Block:

M. England: I. Research Relationship; Self; Becton Dickinson. **C. Lowe:** None. **D. Myers:** None. **D. Craft:** I. Research Relationship; Self; Becton Dickinson.

Poster Board Number:

MONDAY-197

Publishing Title:

Evaluation of the Great Basin Shiga Toxin Direct Molecular Assay for the Detection of Shiga-Like Toxin Producing *Escherichia coli* (Stec) in Diarrheal Stool Specimens

Author Block:

M. L. Faron[1], N. A. Ledeboer[1], J. Connolly[1], P. A. Granato[2], J. Dien Bard[3], J. A. Daly[4], S. Young[5], **B. W. Buchan**[1]; [1]Med. Coll. of Wisconsin, Milwaukee, WI, [2]Lab Alliance of Central New York, Syracuse, NY, [3]Children's Hosp. Los Angeles, Los Angeles, CA, [4]Univ. of Utah, Salt Lake City, UT, [5]Univ. of New Mexico, Albuquerque, NM

Abstract Body:

Background: *E. coli* harboring Shiga Toxin genes *stx1* or *stx2* (STEC) cause acute gastroenteritis and HUS. Serotype O157 is commonly associated with STEC, however, > 100 other *E. coli* serotypes may carry *stx1* or *2*. Detection of STEC using EIAs or culture requires a minimum of 24 h and is 29-80% sensitive compared to NAAT. The Shiga Toxin Direct Test (ST Direct) is a 2 h sample to result test that uses NAAT to identify *stx1*, *stx2*, and *E. coli* serotype O:157 in stool specimens.**Methods:** Stool specimens (n=1,087) were prospectively collected at 5 clinical centers and tested using ST Direct and culture. Each specimen was inoculated to SMAC agar and MacConkey broth and incubated 18-24 h. Colorless colonies on SMAC were tested with latex agglutination to confirm serotype O:157. Positive broths were tested with a STEC EIA. Retrospectively collected specimens including 36 STEC positive (17 O:157 positive) and 24 STEC negative stools were used to augment results from the prospective study set.**Results:** Results represent 1,082 prospective samples, 5 specimens were unresolved by ST Direct and were excluded. Twelve specimens tested positive for *stx1* or *stx2* and two tested positive for O:157 by ST Direct. No samples were EIA positive and ST Direct negative. Four of the 12 (33%) ST Direct positive samples were Shiga Toxin positive by EIA. No specimens were O:157 positive by culture. All specimens testing positive for STEC or O:157 by ST Direct were analyzed using bi-directional sequencing and an FDA-cleared NAAT capable of detecting *stx1*, *stx2*, and serotype O:157. Both comparator methods supported with the positive ST Direct results. Among retrospectively collected specimens, ST Direct identified 36/36 STEC positive and 17/17 O:157 positive stools.**Conclusions:** In this study ST Direct was 100% sensitive for detection of STEC, including serotype O:157, compared to culture and EIA. ST Direct identified an additional 8 specimens, including 2 serotype O:157, that were missed by culture. Compared to ST Direct, EIA is only 33% sensitive for detection of STEC. Culture is insensitive for the detection of O:157.

Author Disclosure Block:

M.L. Faron: None. **N.A. Ledeboer:** H. Research Contractor; Self; Great Basin. **J. Connolly:** None. **P.A. Granato:** H. Research Contractor; Self; Great Basin. **J. Dien Bard:** H. Research Contractor; Self; Great Basin. **J.A. Daly:** H. Research Contractor; Self; Great Basin. **S. Young:** H. Research Contractor; Self; Great Basin. **B.W. Buchan:** F. Investigator; Self; Great Basin.

Poster Board Number:

MONDAY-198

Publishing Title:

Multiplex Real-Time PCR Dection for Enteropathogenic Microsporidia

Author Block:

B. Couturier[1], M. Powers-Fletcher[2], **W. Hymas**[1], P. D. Khot[1], K. Kalp[1], M. R. Couturier[2];
[1]ARUP Lab, Salt Lake City, UT, [2]Univ. of Utah, Salt Lake City, UT

Abstract Body:

Background: Microsporidia are opportunistic fungal pathogens primarily causing gastroenteritis. Stool ova/parasite exams do not detect microsporidia and the modified trichrome stain is under-utilized and lacks sensitivity/specificity. The purpose of this study was to develop a molecular detection assay for microsporidia in stool specimens to improve diagnostic accuracy and patient care.**Methods:** A real-time PCR assay was developed for the detection of the enteropathogenic microsporidia Enterocytozooon bieneusi (Eb) and Encephalitozoon spp. (Es) (E. cuniculi, E. hellem, and E. intestinalis). Nucleic acid extraction was optimized for fungal spores by incorporating bead-beating prior to automated extraction. Analytical performance was determined using stool specimens spiked with spores of Es/Eb or plasmids containing the Eb target. Clinical specimens sent for routine stool culture between June 2012 and February 2013 were tested to assess the clinical utility of the assay, with correlative chart review.**Results:** The analytical sensitivity was 100% for the detection of Eb and 97% for Es. No cross-reactivity was observed for 27 tested organisms. The limit of detection for Eb and Es was 1.6×10^4 copies/ml and 4.4×10^3 copies/ml, respectively. Of the 583 clinical specimens tested, 3 tested positive for microsporidia by PCR and had documented persistent diarrhea. Two of the patients were immunocompromised, while the third had evidence of disrupted intestinal flora post-cholecystectomy. For all cases, no microsporidia testing was ordered, no infectious etiology was detected, and gastrointestinal symptoms were ultimately attributed to underlying conditions or abnormal bacterial growth in the intestine.**Conclusions:** Molecular detection of enteropathogenic microsporidia provides high analytical specificity and sensitivity. In patients with symptoms sufficient to warrant stool culture, microsporidia were detected in very few samples. This suggests lack of carriage in symptomatic patients and the potential for this assay to provide clinically relevant information when applied judiciously to an immunocompromised patient population or those with persistent/chronic diarrhea without an alternative diagnosis.

Author Disclosure Block:

B. Couturier: None. **M. Powers-Fletcher:** None. **W. Hymas:** None. **P.D. Khot:** None. **K. Kalp:** None. **M.R. Couturier:** None.

Poster Board Number:

MONDAY-199

Publishing Title:

Evaluation of a Multiplex PCR Assay for the Detection of Gastrointestinal Pathogens

Author Block:

J. Karichu, D. Wilson, S. M. Harrington, G. W. Procop, **S. S. Richter**; Cleveland Clinic, Cleveland, OH

Abstract Body:

Background: Molecular assays detecting multiple gastrointestinal (GI) pathogens directly from stool are available to evaluate patients with diarrhea. We compared the performance of the FilmArray (FA) GI Panel (BioFire Diagnostics) to current methods used for the diagnosis of gastroenteritis at the Cleveland Clinic. **Methods:** Stool samples in Cary Blair medium from patients with positive results for a bacterial, viral or parasite using routine methods (culture, EIA, microcopy) were selected for testing by the multiplex PCR FA panel within 4 days of specimen collection date. Frozen archived positive stool samples were also selected for testing. For each positive sample selected for FA testing, a negative sample was also tested. Discrepant results were arbitrated by comparator PCR/sequencing testing for targets that differ from those in the FA.**Results:** The age distribution of patients with samples tested was 12% ≤5 years, 14% 6-20 years, 54% 22-64 years, and 20% ≥65 years. FA detected at least one target in 145 (72%) of the 200 samples tested. One, two, three, four and five targets were detected in 98 (68%), 37 (26%), 5 (3.4%), 4 (2.8%), and 1 (0.7%) of the 145 positive samples, respectively. Only 49 of the 96 samples negative by routine test ordered were also negative by FA. The predominant targets detected by FA were *Salmonella* (n=33), *Clostridium difficile* toxin A/B (n=30), *Campylobacter* (n=26), Norovirus (n=20), Enteroinvasive *E. coli* [EIEC] (n=13), Enteroaggregative *Escherichia coli* [EAEC] (n=12), Sapovirus (n=12), Rotavirus (n=10), Astrovirus (n=9), Enteropathogenic *E. coli* [EPEC] (n=9), and *Shigella* spp. (n=9). The FA sensitivity for the 18 different targets detected was 100%. The highest rates of co-infections (77-100%) occurred for samples with EIEC, EAEC, EPEC, Enterotoxigenic *E. coli* [ETEC], and *Plesiomonas shigelloides*. FA specificity ranged from 96.9-100% with 20 FA results that could not be confirmed by comparator molecular testing (6 Sapovirus, 4 *C. difficile* toxin, 3 Norovirus, 2 *Giardia lamblia*, 1 *Cryptosporidium*, 1 *Campylobacter*, 1 *Salmonella*, 1 EIEC, 1 ETEC, 1 Astrovirus).**Conclusion:** Use of the FA GI panel in our patient population would identify etiologic agents of infectious diarrhea that are currently unrecognized.

Author Disclosure Block:

J. Karichu: None. **D. Wilson:** None. **S.M. Harrington:** None. **G.W. Procop:** None. **S.S. Richter:** I. Research Relationship; Self; bioMerieux, BioFire, OpGen, Roche, Pocared, BD Diagnostics, Nanosphere.

Poster Board Number:

MONDAY-200

Publishing Title:

GI Panel Testing of Inpatients Detects Organisms Missed by Routine Stool Testing

Author Block:

C. Kwong, P. Schreckenberger; Loyola Univ. Med Ctr, Maywood, IL

Abstract Body:

Background: In a hospital setting, determining infectious causes of acute diarrhea in a timely manner is important when a pathogen warrants contact precautions (e.g. C. difficile). The detection of pathogens by either traditional culture methods or by target specific molecular methods is limited because it often results in no pathogens found. The goal of this study is to determine if stools that tested negative for C. difficile by PCR contain a pathogen that was not detected. **Methods**: Stool samples with negative C. difficile PCR results were stored at -20C. The electronic health record was examined for patient inclusion criteria (inpatient status and qualitative and/or quantitative stool descriptors) and exclusion criteria (no mention of diarrhea in the chart, therapeutics that cause diarrhea, diarrhea associated with certain disease states). Thirty stool samples were tested by Biofire FilmArray Gastrointestinal (GI) panel, a multiplex PCR array that detects 23 common bacterial, viral and protozoal GI pathogens. Cost comparison between current laboratory methods versus the GI panel was examined. **Results**: 8 samples out of 30 (26.7%) tested positive for one or more of the pathogens on the panel. The pathogens detected included: astrovirus, norovirus GI/GII, sapovirus (2 patients), giardia (2 patients), enteroaggregative and enterotoxigenic E. coli (both positive in 1 patient), and enteropathogenic E. coli. A total of 66 stool related laboratory tests were performed on these patients at a total cost of $2780.94. 30 GI panels cost $4231.80. The cost difference is $1450.86 or an additional $48.36 per patient to perform the GI panel only. **Conclusion**: The FilmArray GI panel identified 26.7% more pathogens that otherwise would have been missed by physician ordered tests at an increased cost of $48.36 per patient. With a quick turn around time at a relatively low increase in cost per patient, the use of the GI panel will lead to appropriate isolation precautions and earlier diagnosis and thus earlier initiation of appropriate therapy, shorter duration of symptoms, shorter length of stay and increased patient satisfaction.

Test	Unit price ($)	Total tests	Total cost ($)
C. difficile PCR	52.70	30	1581.00
Culture	40.67	18	732.06
Giardia Ag	8.00	7	56.00
Cryptosporidium DFA	59.05	6	354.30
Ova and parasite Smear	5.86	3	17.48

Shiga toxin EIA	20.00	2	40.00
Total		66	2780.94

Author Disclosure Block:

C. Kwong: None. **P. Schreckenberger:** A. Board Member; Self; Biofire. E. Grant Investigator; Self; Biofire. J. Scientific Advisor (Review Panel or Advisory Committee); Self; Biofire.

Poster Board Number:

MONDAY-201

Publishing Title:

Comparison Of Verigene® Enteric Pathogens Test, Biofire Filmarray™ Gastrointestinal Panel And Luminex Xtag® Gastrointestinal Pathogen Panel For Identification Of Enteric Pathogens

Author Block:

C. JOHNSON[1], R. Huang[1], R. Hepler[2], J. Dunn[2]; [1]Baylor Coll. of Med., Houston, TX, [2]Texas Children's Hosp., Houston, TX

Abstract Body:

Introduction: Stool culture has traditionally been considered the gold standard for identifying common bacterial pathogens, but the turnaround time can be 72 hours or longer. Rapid identification of stool pathogens would facilitate more timely treatment and/or infection control decisions. Several rapid, multiplex molecular assays are currently available for simultaneous detection and identification of common pathogenic enteric bacteria and viruses. This study compared the performance characteristics of three FDA-approved molecular assays.**Methods:**One hundred thirty-five stool specimens (40 prospective, 95 retrospective) in Cary-Blair media were tested according to the manufacturer's instructions with the three platforms: Verigene Enteric Pathogens Test, Biofire FilmArray Gastrointestinal Panel and Luminex xTAG® Gastrointestinal Pathogen Panel. The assays were assessed only for the targets common among all three; namely, *Campylobacter*, *Salmonella*, *Shigella*, Shiga toxin-producing *E. coli* (STEC), Norovirus and Rotavirus. Conventional methods including culture and EIA were used for comparison.**Results:**The sensitivities (%) and specificities (%) of the three assays for each target were: *Campylobacter*, Biofire (100, 100), Verigene (100, 99.2), Luminex (29, 100); *Salmonella*, Biofire (94, 100), Verigene (76, 100), Luminex (71, 100); *Shigella*, Biofire (98,100), Verigene (100, 100), Luminex (100, 100); STEC, Biofire (100, 100), Verigene (91, 100), Luminex (73, 99.2); Norovirus, Biofire (100,100), Verigene (98,100), Luminex (88,100). Two Rotavirus-positive samples were detected by Biofire and Luminex while Verigene detected only one.**Conclusions:** Overall, Biofire exhibited the best performance followed by Verigene and then by Luminex. The decrease in turnaround time afforded by the Verigene EP, Biofire GI and Luminex GPP assays compared to traditional culture provides a substantial benefit to laboratories with medium to high volume testing.

Author Disclosure Block:

C. Johnson: None. **R. Huang:** None. **R. Hepler:** None. **J. Dunn:** J. Scientific Advisor (Review Panel or Advisory Committee); Self; Nanosphere, Inc..

Poster Board Number:

MONDAY-202

Publishing Title:

Comparison of Commercial Clinical Diagnostic Kits for Shiga Toxin-producing *Escherichia coli*

Author Block:

E. L. Brown, M. Stark, R. Boyer, R. Williams; Virginia Tech, Blacksburg, VA

Abstract Body:

Shiga toxin-producing *Escherichia coli* (STEC) are pathogens that cause bloody diarrhea, vomiting, and, in severe cases, hemolytic uremic syndrome. Immunological diagnostic kits are able to differentiate illnesses caused by STEC by detecting the presence of Shiga toxin antigens. The objective of this study was to compare the performance of four diagnostic kits: Premier® EHEC and Immuno*Card* STAT!® EHEC (Meridian Bioscience, Inc.) and *SHIGA TOXIN CHEK*™ and *SHIGA TOXIN QUIK CHEK*™ (TECHLAB®, Inc.) Fecal samples (n=189) were tested using the Premier® EHEC, *SHIGA TOXIN CHEK*™, and *SHIGA TOXIN QUIK CHEK*™ according to each kit's directions for direct fecal testing. The Immuno*Card* STAT!® EHEC is not intended for direct fecal samples. Standard curves created with purified Shiga toxin toxoids diluted in negative fecal broth culture were tested with each kit to determine the limit of detection (LOD; ng/mL) in order to compare the experimental result with the package insert. Additionally, correlation of toxin production with concentration of cells in broth culture was examined using 12 STEC strains isolated from foodborne illness. Results from direct fecal testing were compared to PCR detection of *stx1* and *stx2* (38 fecal samples positive). Sensitivity/specificity (calculated using PCR) for the Premier® EHEC = 81.6%/ 97.3%; *SHIGA TOXIN CHEK*™ = 86.8%/98.6%; *SHIGA TOXIN QUIK CHEK*™ = 94.7%/99.3%. Between the rapid tests, the *SHIGA TOXIN QUIK CHEK*™ detected lower levels of toxin compared to the Meridian Immuno*Card* STAT!® EHEC in purified toxin tests (Table 1). Between the ELISA tests, the Meridian Premier® EHEC detected lower levels of toxin than *SHIGA TOXIN CHEK*™ (Table 1).

Table 1. Limit of detection (LOD; ng/mL) of Shiga toxin toxoid in kits when diluted in broth culture

Kit	**Stx1**	**Stx2**
Premier EHEC	0.59	1.60
SHIGA TOXIN CHEK	0.89	2.37
Immuno*Card* STAT EHEC	0.78	3.13
SHIGA TOXIN QUIK CHEK	0.15	0.11

No consistent LOD (CFU/mL) was determined in broth cultures because toxin production varied between the strains; however, the LOD (CFU/mL) was 10 times more sensitive for the Meridian Premier® EHEC, *SHIGA TOXIN CHEK*™, and *SHIGA TOXIN QUIK CHEK*™ in comparison to the Immuno*Card*® STAT!. Due to toxin production discrepancy, the concentration of bacterium in broth is not accurate determination for LOD.

Author Disclosure Block:

E.L. Brown: None. **M. Stark:** None. **R. Boyer:** None. **R. Williams:** None.

Poster Board Number:

MONDAY-203

Publishing Title:

Development of a Real-time Pcr Assay for Simultaneous Identification of Shiga Toxin-producing *Escherichia coli* (Stec) Subtypes *stx*$_{2e}$ and *stx*$_{2f}$ from Clinical Isolates

Author Block:

P. Smith[1], D. Fasulo[2], D. Stripling[1], H. Martin[1], L. M. Gladney[1], N. Strockbine[1], R. L. Lindsey[1]; [1]CDC, Atlanta, GA, [2]Pattern Genomics, LLC, Madison, CT

Abstract Body:

Background: Shiga toxin-producing *Escherichia coli* (STEC) are common foodborne pathogens that may cause mild to severe illness in humans. STEC are characterized by the presence of Shiga toxin (*stx*$_1$ or *stx*$_2$) genes. The *stx*$_2$ virulence gene has seven subtypes (*stx*$_{2a-f}$); in this study we address *stx*$_{2e}$ and *stx*$_{2f}$. The *stx*$_{2f}$ subtypes is increasingly associated with Hemolytic Uremic Syndrome. Commercial molecular diagnostic panels and immunodiagnostic tests do not detect the presence of *stx*$_{2f}$. A conventional PCR assay exists to detect *stx*$_{2e}$ and *stx*$_{2f}$. We developed and optimized a real-time PCR assay targeting these subtypes in a single reaction for a rapid and higher throughput method than the conventional PCR method.**Materials/Methods:** The Daydreamer™ (Pattern Genomics, USA) software platform was used to analyze the whole genome sequence of a total of 342 isolates, 104 positive for Shiga toxin genes (STEC) and 238 negative for Shiga toxin genes. A region was identified as unique to *stx*$_{2e}$ and *stx*$_{2f}$, and TaqMan primers and probe were generated. The primers and probe were validated by both in silico and RT PCR. The primers and probe were tested in the laboratory on a set of 71 STEC isolates using a LightCycler® 96 (Roche, Switzerland).**Results:** When tested with *in-silico* PCR the primers and probe combination were 100% accurate in identifying *stx*$_{2e}$ and *stx*$_{2f}$ strains. Laboratory testing of the primers and probe correctly identified all isolates: *stx*$_{2e}$ positive strains (6), *stx*$_{2f}$ positive strains (33), and *stx*$_{2e/f}$ negative STEC strains (32) all tested in duplicate. To test assay robustness, the concentration of primer and probe mixes were varied and made a minimal difference in detection of target positive and negative strains.**Conclusions:** We developed a singleplex real-time PCR assay that accurately detects *stx*$_{2e}$ and *stx*$_{2f}$ isolates in a rapid, cost-effective and high-throughput manner. This assay should help reference and diagnostic laboratories more efficiently identify these two Shiga toxin subtypes.

Author Disclosure Block:

P. Smith: None. **D. Fasulo:** None. **D. Stripling:** None. **H. Martin:** None. **L.M. Gladney:** None. **N. Strockbine:** None. **R.L. Lindsey:** None.

Poster Board Number:

MONDAY-204

Publishing Title:

Detection of Shiga-toxin Producing *Escherichia coli* (Stec) in the Era of Molecular Diagnostics: A Retrospective Study

Author Block:

M. J. Lee, K. H. Jerke, R. B. Mirasol, R. M. Humphries; David Geffen Sch. of Med. at UCLA, Los Angeles, CA

Abstract Body:

Background: Our laboratory recently replaced routine testing for Shiga-toxigenic Escherichia coli (STEC) from an enzyme immunoassay (EIA) method, to a multiplex stool pathogen PCR that detects STEC (BD Max Bacterial Enteric Panel). Since implementation, we identified a significant increase in the detection of STEC, from 5 over a 30-month period (pre-PCR) to 20 in the first 4 months of testing by the BD MAX. We sought to validate the STEC results by a second method, to confirm performance of this target among our patient population. **Methods:** Twenty remnant, frozen stool specimens that tested positive for STEC by the BD MAX were re-processed using the same method. In parallel, a second PCR was performed to detect and differentiate Stx-1 and Stx-2. Primer/probe used for the second PCR were as described elsewhere (Grys et al, JCM 2009;47(7):2008-2012), and PCR was performed on an LC 480 (Roche Diagnostics) thermocycler. An EIA was performed per manufacturer's instructions (ImmunoCard STAT EHEC, Meridian Biosciences, Inc.). UCLA institutional review board approval was obtained to conduct review of patient charts. Charts for 2 patients were not available for review.**Results:** Upon repeat testing, 16/20 STEC specimens were confirmed as STEC positive by both PCR assays (BD MAX and RT-PCR), but only one was positive by EIA (6.25% agreement). Four samples tested negative by all methods, possibly due to freeze/thaw loss of target. Among the STEC PCR-positive patients, 33% (6/18) had watery diarrhea, 39% (7/18) exhibited bloody diarrhea, 45% (8/18) traveled abroad, and 28% (5/18) had chronic gastrointestinal disorders (CGID). In contrast, among the 5 patients positive for STEC prior to implementation of the PCR, 60% (3/5) exhibited bloody diarrhea, 20% (1/5) traveled abroad, and 60% (3/5) had indications for CGID.**Conclusion:** Collectively, our data suggest the increase in STEC positivity is due to: 1) increase in sensitivity associated with PCR-based testing, and 2) routine screening for STEC among all patients with stool submitted for bacterial pathogen testing. Additional studies and clinical data are needed to further evaluate STEC colonization and the clinical value of PCR testing.

Author Disclosure Block:

M.J. Lee: None. **K.H. Jerke:** None. **R.B. Mirasol:** None. **R.M. Humphries:** None.

Poster Board Number:

MONDAY-205

Publishing Title:

Improvements in Sample Collection, Nucleic Acid Extraction and Target Quantification to Optimize Enteropathogen Detection

Author Block:

J. Liu[1], C. Amour[2], R. Nshama[2], A. Maro[3], J. Gratz[1], J. Platts-Mills[1], E. Houpt[1]; [1]Univ. of Virginia, Charlottesville, VA, [2]Haydom Global Hlth.Inst., Haydom, Tanzania, United Republic of, [3]Kilimanjaro Clinical Res. Inst., Moshi, Tanzania, United Republic of

Abstract Body:

Background: Diarrhea remains the second leading cause of death for children under five worldwide. Detection of enteropathogens from stool may be technically challenging in several aspects, including stool sample collection and transport, nucleic acid extraction, detection of a broad panel of enteropathogens, and target quantification. **Methods:** 173 diarrheal specimens from Tanzanian children were collected with both flocked rectal swab and stool, and pathogen detection was compared. Nucleic acid extracted from a single procedure (TNA) using QIAamp Stool DNA mini kit was compared with combined extracts from separate DNA and RNA extractions (DNA+RNA) on 41 stool samples. Both comparisons were performed with qPCR on TaqMan Array Card interrogating 32 enteropathogens. Standard curves were generated with combined positive controls on TAC and utilized to derive target copy numbers from quantification cycle. Stool spiked with known number of targets were used to demonstrate the principle of quantification.**Results:** *Pathogen detection on swabs.* Comparing to qPCR results on stool, swab yielded higher Cqs (29.7±3.5 vs. 25.3±2.9 on stool, $P<0.001$). >90% of the detection with Cq<25 on stool were also positive by swab.*Total nucleic acid extraction.* 88% (318/361) of the positive results from TNA and DNA+RNA were concordant between the two. There was no significant difference in Cqs between TNA and DNA+RNA ($\Delta Cq_{(DNA+RNA-TNA)} = -0.01 \pm 1.17$, $P = 0.972$, N = 318).*Target quantification.* All the assays exhibited good linearity with 90% - 110% PCR efficiency and 0.990 to 1 Pearson coefficient. After normalization to external controls, the derived target copy numbers were within 0.5 fold of the starting materials (59.3±31.9%).**Conclusion:** Swab can be used as an alternative for specimen collection, especially where flexibility is needed; A single nucleic acid extraction procedure using QIAamp Stool DNA mini kit was sufficient to recover total nucleic acid; A comprehensive panel of enteropathogens were interrogated with TAC; A quantification scheme with normalization to external controls is established, which enables reporting adjusted target copy numbers.

Author Disclosure Block:

J. Liu: None. **C. Amour:** None. **R. Nshama:** None. **A. Maro:** None. **J. Gratz:** None. **J. Platts-Mills:** None. **E. Houpt:** None.

Poster Board Number:

MONDAY-206

Publishing Title:

Impact of Filmarray Gi Panel on *Shigella* Outbreak Management

Author Block:

N. KANWAR, J. Jackson, R. Selvarangan; Children's Mercy Hosp. and Clinics, kansas City, MO

Abstract Body:

Background: Shigellosis is the third most common enteric disease in the United States with highest incidence in children less than five years. Fecal culture for isolation and identification of *Shigella* may take days. The FilmArray Gastrointestinal (GI) Panel is a PCR based assay that detects 22 different enteric pathogens including *Shigella* in an hour. The aim of this study is to evaluate the impact of GI Panel detection of *Shigella* in an emergency department (ED) during an outbreak.**Methods:** Children with acute gastroenteritis were prospectively enrolled and stool specimens were tested by GI Panel. Test results were either withheld in pre-intervention (PRE) or reported to clinicians/families in post–intervention (POST) period during the current *Shigella* outbreak in Kansas City area. The impact of the GI Panel testing on patient management and outcomes was measured.**Results:** To date, 188 subjects (139 PRE and 49 POST) have been enrolled in the study. *Shigella* was identified by GI Panel in the PRE (N=30) and POST (N=11) phase. Diarrhea was the most common symptom in subjects (median age of 46 (6-168) and 70 (16-175) months). During PRE phase, *Shigella* was isolated in 8/10 stool cultures requested as standard of care. In the POST phase, *Shigella* was isolated in 9/11 stool cultures set up per study protocol. Azithromycin therapy was prescribed for 3 (10%) subjects in the PRE phase and 5 (45.5%) subjects in the POST phase (*P*=0.02). Decision to treat *Shigella* infection with Azithromycin therapy seem to be influenced by severity of infection as measured by number of diarrheal episodes (treated: 8(4-20) stools *vs.* not treated: 2.5(1-13) stools) and onset of illness (treated:1(1-6)days *vs.* not treated: 3(1-4)days). Following initial visit to ED, six subjects in PRE phase visited additional providers compared with none in the POST phase (*P*=0.17). More parents missed work days in PRE phase (43%) as compared with POST phase (36%) (*P*=0.7). Similarly, more subjects missed school/daycare in PRE phase (73%) compared with POST phase (55%) (*P*=0.3).**Conclusion:** Prompt diagnosis of shigellosis with the FilmArray GI Panel may provide opportunity for prompt antimicrobial therapy and avoid additional visits to providers due to early definitive diagnosis. Laboratory diagnosis of *Shigella* at ED visit has the potential to optimize patient management, and reduce spread of disease.

Author Disclosure Block:

N. Kanwar: None. **J. Jackson:** None. **R. Selvarangan:** None.

Poster Board Number:

MONDAY-207

Publishing Title:

Compatibility of Alcorfix, for *Giardia* and *Cryptosporidium* Antigen Testing

Author Block:

B. Couturier[1], **M. R. Couturier**[2]; [1]ARUP Lab., Salt Lake City, UT, [2]Univ. of Utah, Salt Lake City, UT

Abstract Body:

Background: Our laboratory recently converted to a single-vial stool fixative (Alcorfix) for ova & parasite testing; eliminating formalin from our laboratory and simplifying specimen processing. A limitation with this change is requiring an additional frozen stool aliquot for parasitic antigen testing. Alcorfix compatibility with antigen detection ELISAs has not been evaluated and it is unknown whether the low concentration of polyvinyl alcohol (PVA) is inhibitory. We assessed and validated Alcorfix for detection of *Giardia* and *Cryptosporidium* [Crypto] antigens and whether concentrated stool sediments were also compatible.**Methods:** Unpreserved stools previously tested by antigen detection ELISA for Crypto or *Giardia* were collected. Aliquots of the stool were preserved in Alcorfix at a 1:3 ratio and spiked with various concentrations of Crypto oocysts (n=40) or *Giardia* cysts (n=40). Spiked specimens of each organism were also concentrated using a Parasep concentrator tube. The pellet and the supernatant were tested for the presence of Crypto or *Giardia* antigen by ELISA. The pellet was tested like a fresh stool specimen (1:4 in diluent). The supernatant was directly tested without dilution. Antigen stability for the Crypto and *Giardia* in Alcorfix was also assessed.**Results:** The analytical sensitivity was 100% (40/40) for the detection of *Giardia* and 92.5% (37/40) for Crypto. The majority of *Giardia* antigen was reactive in the pellet rather than the supernatant. Crypto antigen was also concentrated in the pellet though there was still significant reactivity in the supernatant. The stability of the antigens in Alcorfix was limited to 7 days with Crypto (vs 14 days frozen unpreserved) and 14 days for Giardia (same as unpreserved).**Conclusions:** Alcorfix, despite containing PVA, is compatible with *Giardia* and Crypto antigen testing by ELISA. A pellet from concentrated stool is suboptimal for detecting Crypto, as low parasite burden specimens may not be detected. Furthermore, the supernatant from the concentrated stool specimen is not acceptable for antigen testing. Using a Parasep tube with Alcorfix is a compatible combination for Giardia and Crypto antigen detection only when the entire sample is filtered, pelleted, and then resuspended before testing. The remaining suspension can then be pelleted again for further microscopic examination as needed.

Author Disclosure Block:

B. Couturier: I. Research Relationship; Self; Apacor Ltd. **M.R. Couturier:** I. Research Relationship; Self; Apacor Ltd..

Poster Board Number:

MONDAY-208

Publishing Title:

Cost and Turnaround Time Analysis Comparing Routine Stool Culture to Bd Max Enteric Bacterial Panel for Routine Identification Bacterial Stool Pathogens

Author Block:

K. A. Bryant, V. Hoover; Orlando Helath, Orlando, FL

Abstract Body:

Background: Routine stool culture is the most commonly used method in the clinical microbiology laboratory to identify bacterial pathogens causing infectious diarrhea. Laboratories must provide cost effective methods with the shortest turnaround times (TAT) to provide the best patient care. A retrospective study was performed comparing cost and TAT of the BD Max Enteric Bacterial Panel (EBP) to routine stool culture. **Methods:** Routine stool culture and the EBP detect *Salmonella* spp., *Campylobacter jejuni/coli*, *Shigella* spp. and Shiga-toxin producing *E. coli*. Costs comparison of routine stool culture to EBP was performed using July-October 2015 volumes, expense reports, and estimated technologist time for each method. TAT analysis was performed comparing the average time to final result using EBP (July-October 2015) to the same period the previous year (July-October 2014). **Results:** In the period between July and October 2015, a total of 1387 EBP's were performed with the following results: 1272 no organism detected, 27 *Shigella*, 65 *Salmonella*, 20 *Campylobacter* and 3 Shiga Toxin positive *E. coli* were detected. Cost comparison between EBP and stool culture show EBP was 21% ($9,803.64) more expensive than stool culture. The EBP had significant hands on technologist time savings 231 hours compared 836 hours for stool culture. TAT analysis comparing July-October showed a significant difference between EBP and stool culture reducing TAT by 81% (49 hours). **Conclusion:** The enteric bacterial panel allows significant reductions in TAT and technologist hands on time with a 21% increase in cost.

Author Disclosure Block:

K.A. Bryant: None. **V. Hoover:** None.

Poster Board Number:

MONDAY-209

Publishing Title:

Implementation of Culture-independent Testing of Gastrointestinal Pathogens in the Clinical Laboratory

Author Block:

R. C. Fowler[1], C. N. Murphy[1], P. C. Iwen[2], P. D. Fey[1]; [1]Univ. of Nebraska Med. Ctr., Omaha, NE, [2]Nebraska Publ. Hlth.Lab., Omaha, NE

Abstract Body:

Background: Culture-independent testing such as the Biofire FilmArray™ GI Panel (FGIP) has improved sensitivity for the identification of infectious causes of gastroenteritis. The FGIP was implemented at our institution in January 2015 to replace traditional methods for the detection of gastrointestinal (GI) pathogens. The purpose of this study was to evaluate the detection rates of GI pathogens with the FGIP and the incidence of GI pathogens detected by the FGIP in 2015 compared to traditional methods in 2014. **Methods:** Stools submitted for FGIP testing from January to December 2015 were evaluated. Stools with *Clostridium difficile* detected were also tested by EIA. The incidence of GI pathogens detected by the FGIP in 2015 was compared to those reported by traditional methods in 2014. Sapovirus, Astrovirus, diarrheagenic *Escherichia coli* (EAEC, EPEC, ETEC), and *C. difficile* were excluded from comparative analyses. **Results:** A total of 2255 stools were tested by the FGIP with ≥ 1 pathogen detected in 910 (40.4%). Among the 910, coinfections were detected in 176 (19.3%) with 2 and ≥ 3 pathogens detected in 145 (15.9%) and 30 (3.3%) of the positive specimens, respectively. The highest rates of detection with the FGIP were observed for *C. difficile* (336 at 14.9%), EPEC/EAEC (234 at 10.4%), Norovirus (196 at 8.7%), *Campylobacter* spp (51 at 2.3%), Sapovirus (44 at 2.0%), and Rotavirus A (36 at 1.6%). Each of the remaining FGIP pathogens had a detection rate of ≤ 1.5%. Of the 336 *C. difficile* detected by the FGIP, only 87 (25.9%) were toxin positive by EIA. Most GI pathogens showed an increased incidence from 2014 to 2015, respectively: *Campylobacter* spp. (18 and 51), *Salmonella* (16 and 30), *Shigella*/EIEC (3 and 15), shiga toxin producing *E. coli* (12 and 32), *Plesiomonas shigelloides* (1 and 11), *Vibrio* spp (0 and 4), *Yersinia enterocolitica* (0 and 10), Norovirus (115 and 196), Rotavirus (8 and 36), *Giardia lamblia* (7 and 17), and *Cryptosporidium* (5 and 28). **Conclusions:** Implementation of the FGIP increased the cases of infectious gastroenteritis detected and provided increased awareness of coinfections. These data suggest that the FGIP can be used to monitor trends in disease incidence and aid in clinical decision-making. Ongoing studies are being done to assess the impact the FGIP has on public health practices and patient outcomes.

Author Disclosure Block:

R.C. Fowler: None. **C.N. Murphy:** None. **P.C. Iwen:** None. **P.D. Fey:** E. Grant Investigator; Self; Biofire Diagnostics, Merck & CO., Inc..

Poster Board Number:

MONDAY-210

Publishing Title:

Performance of the Artus *Clostridium difficile* Qs-rgo Kit for the Detection of Toxigenic *Clostridium difficile*

Author Block:

H. Kim[1], H-W. Moon[1], M. Hur[1], H. Kim[2], Y-M. Yun[1]; [1]Konkuk Univ. Sch. of Med., Seoul, Korea, Republic of, [2]Yonsei Univ. Coll. of Med., Seoul, Korea, Republic of

Abstract Body:

Background: *Clostridium difficile* is the most common cause of healthcare-associated diarrhea, and nucleic acid amplification testing for the direct detection of toxigenic *C. difficile* is commonly used. We evaluated the diagnostic performance of newly launched, artus *C. difficile* QS-RGO Kit (artus *C. difficile*, QIAGEN, Hilden, Germany), in comparison with toxigenic culture and Xpert C. difficile (Cepheid, Sunnyvale, CA, USA).**Methods:** In prospectively collected, 261 diarrheal specimens, the artus *C. difficile* and the Xpert *C. difficile* assays were performed. Toxigenic culture using chromogenic agar (chromID CD agar, bioMerieux, Marcy-l'Etoile, Frace) was used a reference method.**Results:** Based on toxigenic culture, the sensitivity and specificity of the artus C. difficile were 98.2% and 93.6%, respectively, and those of the Xpert C. difficile were 94.6% and 94.6%, respectively; there was no statistical difference. Te agreement between the artus *C. difficile* and the Xpert *C. difficile* was almost perfect (weighted kappa = 0.918).**Conclusions:** The performance of the artus *C. difficile* is excellent compared with toxigenic culture and is comparable to that of the Xpert *C. difficile*. The artus *C. difficile* could be a useful diagnostic option for the direct detection of toxigenic *C. difficile* in clinical laboratories.

Author Disclosure Block:

H. Kim: None. **H. Moon:** None. **M. Hur:** None. **H. Kim:** None. **Y. Yun:** None.

Poster Board Number:

MONDAY-211

Publishing Title:

Potential of the Intergenic Spacer Profiling (Is-Pro) Technique for Simultaneous Assessment of the Intestinal Microbiome and *Clostridium difficile* Ribotyping in Patients with *Clostridium difficile* Infection

Author Block:

J. van Prehn, C. M. J. E. Vandenbroucke-Grauls, Y. H. van Beurden, A. E. Budding; VU Univ. Med. center, Amsterdam, Netherlands

Abstract Body:

Background: *Clostridium difficile* infection (CDI) often arises after antibiotic therapy and is thought to be caused by disruption of the intestinal microbiome, which facilitates outgrowth of toxigenic *C. difficile.* Strain ribotyping is important for hospital epidemiology, as some ribotypes appear to be more virulent than others. We aimed to assess the potential of the high-throughput intergenic spacer profiling (IS-pro) technique for *C. difficile* ribotyping and intestinal microbiome analysis in CDI patients.**Methods:**The intergenic spacer profiling (IS-pro) technique is based on length polymorphisms of the 16S-23S rDNA interspace region which is used for instant species identification by phylum-specific fluorescently labelled PCR primers. The *Firmicutes* primer was used to generate intergenic spacer (IS) profiles of 37 *C. difficile* isolates with known ribotypes: 027 (n=8), 014 (n=8), 001 (n=6), 002 (n=5), 078 (n=5), 015 (n=4) and 106 (n=1). In addition, a pilot study was performed in which the microbiota richness and diversity on the day of CDI diagnosis of was assessed in 7 patients who developed recurrent CDI and in 14 patients who did not developed a recurrence.**Results:**The IS profiles of the isolates clustered as expected by their known ribotype: all strains of each ribotype showed identical profiles, except for one 001 strain that showed a profile identical to the 027 strains, and one 015 strain that showed a unique profile. The peak profiles were concordant with profiles of a recently published international standardized protocol for capillary gel-based electrophoresis PCR-ribotyping.[1] The pilot microbiota study showed a decrease in diversity of *Firmicutes* in patients with recurrent CDI, compared to patients without.**Conclusions:**IS-pro is a useful tool for both *C. difficile* ribotyping and intestinal microbiome analysis. The predictive value of these analyses for CDI outcome should be evaluated in a prospective clinical trial.

Author Disclosure Block:

J. van Prehn: None. **C.M.J.E. Vandenbroucke-Grauls:** None. **Y.H. van Beurden:** None. **A.E. Budding:** N. Other; Self; AEB has proprietary rights on the IS-pro technique and is co-owner of the spin-off company IS-Diagnostics..

Poster Board Number:

MONDAY-212

Publishing Title:

Comparison of Diagnostic Algorithms for Detection of Toxigenic *Clostridium difficile* in Routine Practices at a Tertiary Referral Hospital in Korea

Author Block:

H. Kim[1], H-W. Moon[1], M. Hur[1], H. Kim[2], Y-M. Yun[1]; [1]Konkuk Univ. Sch. of Med., Seoul, Korea, Republic of, [2]Yonsei Univ. Coll. of Med., Seoul, Korea, Republic of

Abstract Body:

Background: Since every single test has some limitations for the diagnosis of *Clostridium difficile* infection (CDI), multistep algorithms are recommended. This study aimed to compare the currently representative diagnostic algorithms and assays for the detection of CDI, using VIDAS *C. difficile* toxin A&B (toxin ELFA) and VIDAS *C. difficile* GDH (GDH ELFA, bioMerieux, Marcy-l'Etoile, France), and Xpert *C. difficile* (Cepheid, Sunnyvale, California, USA).**Methods:** Using 271 consecutive, remnant diarrheal stool specimens, toxigenic culture, toxin ELFA, GDH ELFA, and Xpert *C. difficile* were performed. We simulated two algorithms including screening by GDH ELFA and confirmation by Xpert *C. difficile* (GDH + Xpert) and combined algorithm of toxin ELFA, GDH ELFA, and Xpert *C. difficile* (GDH + Toxin + Xpert). The performances of each assay and algorithms were assessed.**Results:** The agreement of toxin ELFA and GDH ELFA with toxigenic culture were weak and moderate (Kappa, 0.564 and 0.678, respectively). The agreement of Xpert *C. difficile* and two algorithms (GDH + Xpert and GDH + Toxin + Xpert) with toxigenic culture were strong (Kappa, 0.848, 0.857 and 0.868, respectively). The Xpert *C. difficile* demonstrated sensitivity, specificity, PPV and NPV of 94.6%, 94.9%, 82.5%, and 98.6%, respectively. Those of algorithms (GDH + Xpert and GDH + Toxin + Xpert) were 96.7%, 95.8%, 85.0%, 98.1%, and 94.5%, 95.8%, 82.3%, 98.5%, respectively. There were no significant differences between Xpert *C. difficile*, GDH + Xpert, and GDH + Toxin + Xpert in sensitivity, a specificity, PPV and NPV (all $P>0.05$).**Conclusions:** Both algorithms showed comparable performances to that of Xpert *C. difficile*. This data would be helpful to choose the diagnostic algorithms for detection of CDI in clinical laboratories. Each laboratory may optimize the workflow depending on costs, test volume, and clinical needs.

Author Disclosure Block:

H. Kim: None. **H. Moon:** None. **M. Hur:** None. **H. Kim:** None. **Y. Yun:** None.

Poster Board Number:

MONDAY-213

Publishing Title:

Validation Of The Fecalswab As A Transport Medium For Detection Of *c. Difficile* using The Xpert *c. Difficile* assay

Author Block:

M. Faron[1], E. Beck[2], B. W. Buchan[1], **N. A. Ledeboer**[1]; [1]Med. Coll. of Wisconsin, Milwaukee, WI, [2]Wisconsin Diagnostic Lab., Milwaukee, WI

Abstract Body:

Background: Liquid based microbiology (LBM) eases transport of specimens and improves specimen yield compared to cotton swabs; however, many FDA-cleared molecular assays were not cleared using LBM specimens. For this reason it is unclear how the differences in matrix and specimen loading with these devices will affect test performance. The purpose of this study was to determine the amount of FecalSwab medium required (Copan Diagnostics, Inc. Murrieta, CA) to obtain equivalent results to cotton swabs with the Cepheid Xpert *C. difficile* assay (Cepheid, Sunnyvale, CA).**Methods:** *C. difficile* strain BAA-1875 was spiked into *C. difficile* negative stool to create surrogate specimens containing 10 times the limit of detection (LOD) of the assay. FecalSwab collection devices were inoculated with at least 200mg of spiked specimen ensuring the max fill line was not exceeded and varying amounts of the FecalSwab liquid medium ranging from 200µL to 1mL were added to the Xpert sample vial, vortexed, and loaded into the cartridge. A cotton swab loaded with the spiked stool specimen was also tested in parallel following the FDA-cleared package insert protocol to compare CT values. Analytical sensitivity was determined by spiking stool specimens at 10^6, 10^5 and 10^4 CFU/mL in triplicate using the optimal volume of FecalSwab medium obtained in the previous experiment.**Results:** When FecalSwab collection devices were inoculated with stool specimens containing *C. difficile* near the limit of detection, toxin B nucleic acid could be detected in all volumes tested except a single 200µL test. Analytical sensitivity and Ct values were equivalent between cotton swabs and FecalSwabs when using 400µL of FecalSwab medium. No inhibition of the internal control was detected with any amount of FecalSwab medium. All stool specimens with both transport devices at 10^6 CFU/mL were positive for *C. difficile*. At 10^5 CFU/mL each collection device was positive in 1of 3 tests. No positive specimens were detected at 10^4 CFU/mL.**Conclusions:** These data demonstrate that 400µL of FecalSwab medium provides equivalent analytical results compared to the FDA-cleared protocol for the Xpert *C. difficile* assay. Future studies evaluating the clinical performance of FecalSwab collection devices compared to the FDA-cleared collection will also be discussed.

Author Disclosure Block:

M. Faron: None. **E. Beck:** None. **B.W. Buchan:** None. **N.A. Ledeboer:** C. Consultant; Self; Copan Diagnostics Inc..

Poster Board Number:

MONDAY-214

Publishing Title:

Prevalence of *C. diffile* Toxin in Patients Positive by Polymerase Chain Reaction (Pcr)

Author Block:

C. Bittencourt[1], J. Ciurca[2], F. Lee[1], **R. Hollaway**[1]; [1]UT Southwestern Med. Ctr., Dallas, TX, [2]William P. Clements Jr. Univ. Hosp., Dallas, TX

Abstract Body:

Clostridium difficile infection (CDI) is the major infective cause of nosocomial diarrhea in adults. *C difficile* exerts its pathologic effects through the action of toxin A (TcdA) and toxin B (TcdB). Conventional laboratory methods for diagnosis of CDI (cell culture cytoxicity, neutralization assay, toxigenic culture and toxin immunoassay) can be time consuming, labor intensive and/or insensitive. Molecular amplification tests are now often used for detection of *C. difficile* toxin B gene sequences.We compared the performance of *C DIFF* QUIK CHEK COMPLETE® (Alere North America, Orlando, Florida) to Xpert® *C. difficile* assay (Cepheid, Sunnyvale, California) to determine the presence of toxin in patients who had a positive PCR result. Xpert® *C. difficile* PCR detects the gene for *C. difficile* toxin B in stool samples. Alere is a membrane enzyme immunoassay for the detection of *C. difficile* glutamate dehydrogenase antigen (GDH) and toxin A and B.From October 2015 to January 2016, stool samples were submitted to the microbiology laboratory for *C. difficile* testing by Xpert®. Specimens were subsequently tested by Alere to determine the presence of toxin. Thirty percent (24/79) were tested within 72h of collection and 70% (55/79) were frozen samples. *C. difficile* culture with reflex to cytotoxin cell assay is underway to evaluate the 40 discrepant samples.A total of 79 diarrheal stool samples were analyzed by Xpert® and Alere. Eighty-seven percent (69/79) were PCR positive and 13% (10/79) were PCR negative. Of the PCR positive samples, 43% (30/69) were GDH and toxin positive, 54% (37/69) were only GDH positive and 3% (2) were GDH and toxin negative. Positive agreement for the presence of *C. difficile* was 97% (67/69), negative agreement was 90% (9/10) and overall agreement was 96% (76/79).Between the two assays, we found excellent overall agreement for C. *difficile* detection. However, detectable toxin in PCR positive samples was found in only 43% of the cases. Given the sensitivity limitations of current toxin assays and inability to distinguish disease from colonization using PCR, the diagnoses of CDI is still a challenge. Appropriate patient selection and an algorithmic testing approach might improve diagnostic accuracy for CDI. We are seeking IRB approval for chart review of cases to develop an appropriate testing strategy.

Author Disclosure Block:

C. Bittencourt: None. **J. Ciurca:** None. **F. Lee:** None. **R. Hollaway:** None.

Poster Board Number:

MONDAY-215

Publishing Title:

Fecal Calprotectin as an Surrogate Marker for Clinical Diagnosis of *Clostridium difficile* infection in Cancer Patients

Author Block:

T. He[1], S. Kaplan[1], L. Gomez[1], X. Lu[2], L. V. Ramanathan[1], M. Kamboj[1], Y-W. Tang[1]; [1]Mem. Sloan Kettering Cancer Ctr., New York, NY, [2]Futian Hosp., Guangdong Med. Coll., Shenzhen, China

Abstract Body:

Background: Fecal calprotectin (fCPT) has been used as a surrogate marker for inflammation in several gastrointestinal disorders. We explore the utility of fCPT values as a diagnostic aid to distinguish between *C. difficile* infection (CDI) and *C. difficile* colonization (CDC) in cancer patients. **Methods:** From January 2012 to December 2013, 232 stool specimens from 156 patients were randomly selected from patients with diarrhea where stool was submitted for the GeneXpert *C. difficile* PCR and additional testing at MSKCC. The 232 samples included 80 patients with single specimens (PCR-negative, n=39; PCR-positive, n=41) and 76 patients with paired specimens with one *C. difficile* PCR result - negative to positive (n=38) and from positive to negative (n=38), respectively. All specimens were tested for (i) fCPT by the Phical Test (Calpro AS, Oslo, Norway) and (ii) *C. difficile* glutamate dehydrogenase (GDH) and toxin A/B antigens (TechLab, Blacksburg VA). Clinical severity of CDI cases was determined blindly by the IDSA/SHEA severity scoring criteria. **Results:** The Phical Test quantitatively detected 343.3±548.8 (mean±S.D.) µg/g stool of fCPT in 232 stool specimens. There were no significant differences of fCPT values between CDI (n=110; 354.8±384.0) and non-CDI (n=122; 336.8±443.8) groups ($p>0.05$). However, fCPT values were significantly higher in CDI than those without CDI in patients with single (375.4±361.4 vs. 237.0±319.2, $p=0.035$) specimens. Similar results were revealed in severity score determination. Although there were no significant differences in fCPT values between patients with mild-moderate (n=190) and with severe-complicated (n=42) scores ($p>0.05$), fCPT values were significantly higher in severe-complicated than those in mild-moderate scores in patients with single (412.4±479.6 vs. 270.9±286.1, $p=0.039$) and positive to negative paired (729.3±509.0 vs. 437.0±661.2, $p=0.045$) specimens. **Conclusion:** The fCPT values were significantly higher in patients with CDI and severer clinical manifestations than non-CDI and milder clinical manifestations in patients with single specimens collected. The fCPT values provide a potential adjunctive tool to differentiate between CDI and CDC in cancer patients with diarrhea.

Author Disclosure Block:

T. He: None. **S. Kaplan:** None. **L. Gomez:** None. **X. Lu:** None. **L.V. Ramanathan:** None. **M. Kamboj:** None. **Y. Tang:** None.

Poster Board Number:

MONDAY-216

Publishing Title:

Comparison of a Combination Assay for *Clostridium difficile* Gdh Antigen and Toxins A and B Plus the *C. difficile* Lamp Assay with Physicians' Clinical Evaluation of the Patient's Medical Record to Determine True *C. difficile* Disease

Author Block:

E. Francis, T. Crook, M. Ali, W. Greene; Penn State Coll. of Med. M.S. Hershey Med. Ctr., Hershey, PA

Abstract Body:

Background: *C. difficile* may cause serious nosocomial infections. Toxin detection assays lack sensitivity to detect all infections, however molecular assays that detect the toxin genes lack clinical specificity to differentiate patients with disease from those that are carriers.**Methods:** We evaluated 250 unformed fecal specimens using the *C. DIFF QUIK CHEK COMPLETE*, that detects GDH antigen and toxins A/B in a single assay. All specimens were also tested using the *illumigene C. difficile* LAMP assay to detect the presence of the toxin A gene. Three infectious disease physicians performed retrospective chart reviews on 24 patients that were found to be positive by any of the assays. The reviewers were blinded to which 12 specimens were positive by all assays and which 12 were positive by the GDH and molecular assays but negative by the toxin assay. All 3 reviewers used a defined list of clinical criteria for the evaluations.**Results:** Fecal samples (N=250) submitted for *C. difficile* molecular assay were included in this study. One hundred ninety-seven specimens were negative for all 3 targets (GDH, toxin and toxin gene) and 24 were positive for all 3. Seventeen were positive for the GDH antigen only and were not considered to contain toxigenic *C. difficile*. All specimens that were positive by the molecular assay were also positive by the GDH assay. Twelve patients with specimens that were positive for all 3 targets and 12 with specimens that were positive for GDH antigen and the toxin gene but were negative for the toxins were selected for chart review. The reviewers determined that 9/12 of the specimens that were positive for all 3 targets came from patients that met the criteria for *C. difficile* disease. They determined that 8/12 that were positive for GDH and the toxin gene but negative for the antigen toxin also met the criteria for *C. difficile* disease.**Conclusions:** This study has shown that a negative GDH result can be used to identify negative specimens and a positive toxin antigen test can be used to identify positive specimens. The presence or absence of toxin antigens and toxin genes can not be used alone to differentiate patients with *C. difficile* disease from those that are carriers.

Author Disclosure Block:

E. Francis: None. **T. Crook:** None. **M. Ali:** None. **W. Greene:** None.

Poster Board Number:

MONDAY-217

Publishing Title:

Characterizing the *Clostridium difficile* Volatilome

Author Block:

C. A. Rees[1], M. Nasir[1], T. R. Mellors[1], A. Shen[2], J. E. Hill[1]; [1]Dartmouth Coll., Hanover, NH, [2]Univ. of Vermont, Burlington, VT

Abstract Body:

Background: *Clostridium difficile* is a nosocomial pathogen capable of causing pseudomembranous colitis (PMC), a life-threatening disease of the large intestine. Current diagnostic techniques for PMC are limited by low sensitivity, long assay times, and the inability to differentiate between *C. difficile* infection and colonization. Here, we report on a suite of volatile molecules produced by *C. difficile* that could inform the development of a novel, rapid, and highly-accurate diagnostic for PMC.**Methods:** *C. difficile* strain R20291 (isolated from a PMC outbreak) was grown in brain-heart infusion supplemented (BHIS) broth for 4 and 7 hours, corresponding approximately to mid-exponential and stationary growth phases, respectively. Culture supernatants were transferred to air-tight vials, and volatile metabolites were analyzed using GCxGC-TOFMS. The Mann-Whitney U test with Benjamini-Hochberg correction was used to identify volatiles that differed in relative abundance ($p < 0.05$) between mid-exponential and stationary phase cultures. **Results:** GCxGC-TOFMS detected 1079 unique volatile molecules in the headspace of *C. difficile* samples. After eliminating volatile molecules that were also present in media controls, 331 unique volatiles remained that likely represent the consequence of bacterial metabolism. 155 of these differed significantly in relative abundance between cultures at mid-exponential and early stationary phases of growth. Amongst those volatiles that could be assigned putative identifications, the most abundant classes of compounds included sulfur-containing molecules (42.8%), carboxylic acids (8.2%), aldehydes (8.2%), ketones (7.5%), and esters (7.5%). Derivatives of heterocyclic molecules accounted for the majority of observed sulfur-containing compounds, including several that are not previously reported.**Conclusion:** *C. difficile* produces heterocyclic sulfur-containing metabolites when grown in BHIS, which have potential diagnostic value. Volatile biomarkers produced by *C. difficile* in the setting of PMC could inform the development of a rapid, minimally-invasive diagnostic, potentially reducing time-to-diagnosis and improving outcomes.

Author Disclosure Block:

C.A. Rees: None. **M. Nasir:** None. **T.R. Mellors:** None. **A. Shen:** None. **J.E. Hill:** None.

Poster Board Number:

MONDAY-218

Publishing Title:

Comparison of Collection Methods for Oral and Fecal Samples in Microbiome Studies

Author Block:

E. Vogtmann[1], J. Chen[2], A. Amir[3], J. Shi[1], C. Abnet[1], H. Nelson[2], R. Knight[3], N. Chia[2], R. Sinha[1]; [1]Natl. Cancer Inst., Bethesda, MD, [2]Mayo Clinic, Rochester, MN, [3]Univ. of California San Diego, La Jolla, CA

Abstract Body:

Background: Large, prospective cohort studies are needed to evaluate the temporal relationship between the microbiome and health and it is important to be able to collect samples at home. We evaluated the technical reproducibility, stability at ambient temperature, and accuracy of five fecal collection methods (no additive, 95% ethanol, RNA*later*, fecal occult blood test (FOBT), and the fecal immunochemical test (FIT)) and two oral collection methods (OMNIgene DISCOVER and Scope mouthwash). **Methods:** Fifty-three healthy volunteers were recruited from the Mayo Clinic. Oral and fecal samples were collected. One set of each oral and fecal sample collection type was frozen immediately and a second set was left at room temperature for 96 hours and then frozen. DNA was extracted using the MO-BIO PowerSoil kit and the V4 region of the 16S rRNA gene was amplified. Intraclass correlation coefficients (ICC) were calculated for the relative abundance of three phyla, two alpha and four beta diversity metrics. **Results:** For technical reproducibility, the ICCs for all of the duplicate fecal samples frozen immediately were high. For stability, the ICCs were high for all collection types, although the methods tended to have lower ICCs for the relative abundance of the three phyla and weighted UniFrac. When compared to fecal samples frozen immediately, the FOBT cards had the highest ICCs. For the oral samples, Scope mouthwash samples were fairly stable, but accuracy was low compared to the OMNIgene kit frozen immediately. **Conclusions:** The methods for fecal sample collection appeared to be relatively reproducible, stable, and accurate. Scope mouthwash samples were relatively stable, although appeared to differ from oral samples collected using the OMNIgene kit. Future studies could utilize these collection methods to assess the impact of the oral and gut microbiome on health.

Author Disclosure Block:

E. Vogtmann: None. **J. Chen:** None. **A. Amir:** None. **J. Shi:** None. **C. Abnet:** None. **H. Nelson:** None. **R. Knight:** D. Employee; Self; Chief Science Officer and employee of Biota Technology. J. Scientific Advisor (Review Panel or Advisory Committee); Self; Member of the Scientific Advisory Panel at Temasek Life Sciences Laboratory. **N. Chia:** None. **R. Sinha:** None.

Poster Board Number:

MONDAY-219

Publishing Title:

Utility of Perirectal Swabs for Analysis of Intestinal Bacterial and Fungal Biomes of Geriatric Long-term Care Facility Residents

Author Block:

M. E. Tomas[1], M. A. Retuerto[2], B. M. Wilson[1], S. Kundrapu[2], P. K. Mukherjee[2], M. A. Ghannoum[2], C. J. Donskey[1]; [1]Cleveland VA Med. Ctr., Cleveland, OH, [2]Case Western Reserve Univ., Cleveland, OH

Abstract Body:

Background: Characterization of the intestinal biome has led to better understanding of the interplay of organisms in healthy and diseased states. Fecal specimens are most often used for analysis. However, collection of fecal specimens can be challenging in real-world settings such as hospitals and long-term care facilities (LTCFs). In particular, it is often not possible to collect baseline specimens prior to antibiotic therapy or other interventions. Therefore, we evaluated the utility of perirectal swabs for analysis of the intestinal bacterial and fungal biomes of LTCF residents.**Methods:** We collected paired fecal and perirectal swab specimens from LTCF residents. Fecal specimens were cultured for *Clostridium difficile* and fluoroquinolone-resistant gram-negative bacilli. 16S RNA next generation sequencing was used to analyze the bacterial and fungal biomes and results for fecal and perirectal swab specimens were compared.**Results:** 18 paired fecal and perirectal swab specimens were collected from 12 LTCF residents (1-3 specimens per patient). The mean age of the residents was 75 (range, 65-87). Four (33%) residents had received antibiotics within the past 90 days. All 4 antibiotic-exposed LTCF residents were colonized with *C. difficile* and/or fluoroquinolone-resistant gram-negative bacilli, whereas none of the residents with no recent antibiotic exposure were colonized. For both fecal and perirectal swabs, the predominant bacterial phyla were Firmicutes and Bacteroides, while the predominant fungal phyla were Ascomycota, Basidiomycota, and unculturable fungi. All bacterial and fungal genera identified in fecal samples were also identified by perirectal swabs. There was no significant difference in bacterial or fungal diversity based on Shannon's diversity index.**Conclusions:** For analysis of intestinal bacterial and fungal biomes of LTCF residents, perirectal swab and fecal specimens correlated well. Perirectal swabs may provide a relatively non-invasive and convenient method to collect specimens for characterization of the intestinal bacterial and fungal biomes.

Author Disclosure Block:

M.E. Tomas: None. **M.A. Retuerto:** None. **B.M. Wilson:** None. **S. Kundrapu:** None. **P.K. Mukherjee:** None. **M.A. Ghannoum:** None. **C.J. Donskey:** None.

Poster Board Number:

MONDAY-220

Publishing Title:

Theoretical Impact of a Multiplex Gastrointestinal Pcr Panel on Patient Outcomes

Author Block:

G. J. Berry, L. Suazo, G. Nair, A. C. White, M. Loeffelholz; Univ. of Texas Med. Branch, Galveston, TX

Abstract Body:

Diarrhea is among the most common infectious disease syndromes with numerous causes that are often not clinically distinguishable. Current diagnostic testing strategies have significant limitations and are not prompt, leading to inappropriate empiric treatment. Multiplexed molecular testing panels have led to an ability to test more pathogens simultaneously, increase testing sensitivity, and dramatically shortening turn-around time. We evaluated current testing trends in our lab and evaluated how implementation of the FilmArray Gastrointestinal (GI) panel could theoretically improve testing and patient outcomes. 98 stool specimens were tested from September to December 2014 from inpatients (hospitalized within 3 days) and out-patients. A combination of a convenience sample set (positive by routine methods) and a prospective sample set (routine stool cultures), were tested. Demographics, clinical presentation, additional tests, all laboratory results, empirical treatment, and number of health care provider revisits were recorded.The FilmArray GI panel detected twice the number of pathogens as compared to current testing methods. Viral causes of diarrhea were most often missed, followed by cases of Diarrheagenic *E. coli*. Multiple *C. difficile* cases were also missed by routine testing. In addition, traditional stool culture missed 2/8 and 2/2 cases of *Campylobacter* and *Plesiomonas*, respectively. Chart review demonstrated both overtreatment (e.g. viral infections given antibiotics) and undertreatment (*C. difficlie, Campylobacter, E. coli*, and *Plesiomonas* not given antibiotics). Multiple episodes had 2 or more duplicated tests ordered, with 1/3 of patients having 3 or more tests. Several outpatient revisits, Emergency Department visits and hospitalizations for the same episode of diarrhea were also recorded, emphasizing the need for accurate diagnoses during the initial patient visit.In summary, current testing paradigms for diarrhea-causing pathogens are limited by limited sensitivity and the requirement for multiple tests. This leads to costly improper therapy and additional use of inpatient and outpatient facilities. Implementation of a multiplex molecular assay for these pathogens as a diagnostic tool for diarrheal illnesses would potentially aid diagnosis and treatment, leading to better patient outcomes.

Author Disclosure Block:

G.J. Berry: None. **L. Suazo:** None. **G. Nair:** None. **A.C. White:** None. **M. Loeffelholz:** C. Consultant; Self; BioFire Diagnostics, LLC..

Poster Board Number:

MONDAY-221

Publishing Title:

Adaptive Focused Acoustics Processing to Aid Recovery of Fungi from Cf Patient Sputum Samples

Author Block:

H. B. Miller, S. Lewis, A. Gluck, S. Fisher, S. X. Zhang; Johns Hopkins Med. Inst.s, Baltimore, MD

Abstract Body:

Background: Cystic fibrosis (CF) is a genetic disorder that afflicts over 70,000 people worldwide. It is characterized by viscous mucus that accumulates in the lungs creating challenges for microbiology labs processing sputa to isolate slower growing fungal species. Our laboratory attempted to liquefy and homogenize mucoid sputum samples by exposure to Adaptive Focused Acoustics (AFA) in order to increase recovery of fungal pathogens. **Methods:** Twenty sputum samples were processed by AFA using Covaris S220x Focused Ultrasonicator (Covaris Inc., Woburn, MA). Variables considered included power input, time of exposure, sample volume and mechanical disruption. Once optimized, four fungi commonly isolated from CF patients (*Trichosporon mycotoxinivorans*, *Scedosporium apiospermum*, *Exophiala dermatitidis*, *Aspergillus fumigatus*) were selected to determine AFA's potential effect on viability. They were first processed in sterile saline with subsequent emulsions plated in parallel with unprocessed stock. Next, non-CF patient mucoid sputum was pooled and spiked with fungal isolates above; samples were diluted 1:1 with sterile saline. CF sputum was diluted but not spiked. Prior to AFA, samples were inoculated to blood agar plates (BAP) and Sabouraud Dextrose (SAB) plates. Samples were processed by AFA until homogenized and liquefied, then plated to BAP and SAB. BAP incubated for 72 hrs at 37^0C and were read at 24 / 72 hrs; SAB plates were incubated at 25^0C and read over 14 days. **Results:** Dilution (1:1) and AFA was successful in the homogenization and liquefaction of mucoid sputa, including those from our CF clinic. Optimal conditions: 100 Peak-Incident-Power / 50 Duty Factor / 200 Cycles per Burst for 15 sec. Processing by AFA did not affect the viability of fungal organisms either in solution or spiked into sputa. No effects were observed on fungal viability pre-and-post-AFA in CF sputa. Two CF sputum samples exhibited biofilm production on direct- BAP but resulted in isolated colonies on post-AFA BAP. **Conclusion:** AFA paired with sample dilution offers an optimal process for the homogenization / liquefaction of viscous sputa while maintaining fungal pathogen viability. Additionally, it may disrupt biofilm formation, assisting in fungal recovery. Potential application of AFA to enhance fungal culture in CF patients warrants future studies.

Author Disclosure Block:

H.B. Miller: None. **S. Lewis:** None. **A. Gluck:** None. **S. Fisher:** None. **S.X. Zhang:** None.

Poster Board Number:

MONDAY-222

Publishing Title:

Sensitivity Of Calcofluor White Staining For The Detection Of Fungal Organisms In Multiple Specimen Types

Author Block:

E. Washburn, C. Miller, D. Myers, D. Craft; Penn State Hershey Med. Ctr., Hershey, PA

Abstract Body:

Background: Early detection of fungal elements in specimens sent for fungal culture can inform the clinical management of a patient. Calcofluor white (CW) is a fluorescent dye that is commonly used for the rapid detection of yeast and mold fungal elements in patient specimens. We assessed the sensitivity of CW smears in specimens submitted for fungal culture that yielded a fungal isolate(s). Additionally, we compared the sensitivity of CW to Gram stain (GS) for detection of fungal elements in vaginal specimens. **Methods:** A total of 1,924 specimens submitted for fungal culture from Oct-Dec 2014 yielded 422 (21.9%) positive fungal cultures. Laboratory and hospital information systems were queried for data to determine initial smear and final culture results. Vaginal specimen GS for Nugent Score was also reviewed for fungal element comments and compared to CW smear results. **Results:** CW staining identified fungal elements in 73 of 422 (17.3%) positive fungal cultures. CW was most sensitive in vaginal (28.9%), skin/nail/wound (16.3%), respiratory (13.8%), and urine (12.5%) specimens; and least sensitive in bronchoscopy/BAL (5.2%) and sterile site (7.3%) specimens. *Candida* spp. was the most frequent isolate in all specimen types (range 44.9% to 100%). 142 of 688 (20.6%) vaginal specimens were culture positive for fungi, 41 (28.9%) were detected by CW, 53 (38.4%) were detected by GS, with 18 GS + / CW - and 6 GS - / CW + .

Specimens (n=)	**Fungal Culture Positive**	**CW Positive**	**CW Sensitivity**
Total (1924)	**422**	**73**	**17.3 %**
Vaginal (688)	142	41	28.9%
Respiratory secretions *(+ BAL = 350 total)*	109	15	13.8%
Bronchoscopy/BAL *(+ Resp = 350 total)*	57	3	5.3%
Sterile Sites (612)	41	3	7.3%
Skin/Nail/Wound (200)	49	8	16.3%
Urine (74)	24	3	12.5%

Conclusion: These data suggest CW is a relatively insensitive method to detect fungal elements, particularly for bronchoscopy/BAL and sterile site specimens. For vaginal cultures, GS is more sensitive than CW in specimens that yield positive fungal culture results and could replace CW for this specimen type. Most CW smear positive and/or culture positive results yielded *Candida* spp. The clinical and therapeutic relevance of smear results in fungal specimens obtained across mucosal membranes should be discussed with the clinical staff when considering direct smear / staining protocols and result reporting for specimens submitted for fungal culture.

Author Disclosure Block:

E. Washburn: None. **C. Miller:** None. **D. Myers:** None. **D. Craft:** None.

Poster Board Number:

MONDAY-223

Publishing Title:

Eswab™ for the Collection of Fungal Surveillance Culture from Immunocompromised Patients

Author Block:

C. Fontana, M. Favaro, M. Del Principe, M. Bossa, S. Minelli, A. Altieri, C. Favalli; Dep. of Med. ,Hematology and Microbiol. Tor Vergata Univ., Roma, Italy

Abstract Body:

Background: Fungal infections are an important cause of morbidity and mortality in immunocompromised patients especially when undergoing chemotherapy for cancer treatment or immune suppression for bone-marrow and solid organs transplantation. *Candida* and *Aspergillus* are the most common fungal pathogens responsible for invasive fungal infection (IFI) and its diagnosis is difficult and late to confirm. Throat, nasal and rectal swabs are collected for fungal surveillance cultures (SCs) for IFIs. Copan ESwab™ (ES) is a Liquid Based Microbiology device that is used for collecting all clinical specimens for the diagnosis of infectious diseases including fungal. The objective of this study was to demonstrate the performance of ES for the collection of clinical specimens for the detection of fungal and yeast in SCs. **Methods:** The study was carried out on 23148 samples received by the Microbiology laboratory of Tor Vergata university from 05, 2009 to 12 2015. All samples were collected in ES from immunocompromised patients (oncohematology, and stem cell transplantation). SCs for the detection of yeasts and filamentous fungi were performed on swabs collected in ES, (nasal 12,499 (54%), throat 8796 (38%) and rectal 1852 (8%). All ES specimens were loaded on the WASP™, plated on appropriate agar, incubated at 37°C for 24h, and 15d at 28°C and read daily. Positive isolates were identified by Vitek 2 System (bioMérieux) for yeasts and by microscopic exam as well as MALDI-TOF (Bruker) for filamentous fungi (FI). FI were sequenced, with a partial 18S rDNA gene (Applera) and aligned to NCBI data bank.**Results:** Two hundred and sixty (1.12%) positive were isolated (80.1% throat, 8.1% rectal and 11.8% nasal) including (90.7%) *Candida* spp. like *C. albicans*, (59.5%) *C. tropicalis* (15.8%), *C. glabrata* (9.7%) and *C. parapsilosis* (5.1%). *Penicillium* was isolated in 2.2% specimens (3.2% *Aspergillus* (*niger*, *fumigatus* and *nidulans)* 0.8%) other filamentous fungi). SCs detected a fatal case of *Scopulariopsis acremonium* in a bone marrow transplant recipient and 3 clinical cases of *G. clavatum*.**Conclusions:** Both *Candida* and FI were easily detected (after 24-48h for yeast and 48h-5d for fungi) from all clinical specimens submitted in Eswab™, demonstrating that is a good device for preserving yeasts and fungi viability.

Author Disclosure Block:

C. Fontana: None. **M. Favaro:** None. **M. Del Principe:** None. **M. Bossa:** None. **S. Minelli:** None. **A. Altieri:** None. **C. Favalli:** None.

Poster Board Number:

MONDAY-224

Publishing Title:

Performance Evaluation of the T2*candida* Panel in the Icu Setting - Data from an Ongoing Study

Author Block:

M. C. Arendrup[1], J. Schierbeck[2], N. Reiter[3], K. B. Krarup[2], J. S. Andersen[3]; [1]Statens Serum Inst., Copenhagen, Denmark, [2]Odense Univ. Hosp., Odense, Denmark, [3]RigsHosp.et, Copenhagen, Denmark

Abstract Body:

Background: Diagnosis of invasive candidiasis (IC) is suboptimal due to low sensitivity and extended time to result of blood culture (BC). This ongoing study compares T2Candida (T2C), which detects and speciates *Candida* in 3-5 hours directly from whole blood, to BC and Mannan antigen (MAg) testing for diagnosis of IC in critical care patients from two large tertiary hospitals.**Methods:** 102 patients with 261 paired samples have been enrolled. Inclusion criteria are initiation of prophylactic, empiric or pre-emptive antifungal treatment (unless specifically on aspergillus indication), colonisation index ≥0.5 or at least 2 recognised risk factors in patients with fever despite 3-days broad spectrum antibiotics. Blood was taken for BC (BACTEC and BacT/ALERT), MAg (Platelia, Bio-Rad) and T2*Candida* test (T2Biosystems). Microbiology test results were reviewed for each patient. Cases were classified as proven IC according to the EORTC/MSG criteria, likely IC when BC negative but T2 test positive and mannan Ag >500 mg/L and/or multiple non-superficial sites colonised with the same species or no infection (remaining cases). The MAg test was evaluated using the standard (>125 pg/mL) as well as a more stringent cut off (>250 pg/mL) for positivity.**Results:** 11 cases (6 proven and 3 likely) of IC were diagnosed involving 6 *C. albicans*, 1 *C. glabrata*, 1 *C. tropicalis*, 1 *C. krusei*, 1 *C. kefyr* and 1 *C. tropicalis + C. glabrata*. Performance characteristics are shown in the table for each test individually and in combination.

Performance characteristics For T2Candida, BC and Mannan Antigen detection

	Sensitivity	Specificity	PPV	NPV
BC	54.5% (6/11)	100% (91/91)	100% (6/6)	94.8% (91/96)
T2C	81.8% (9/11	96.7% (88/91)	75% (9/12)	97.7% (88/90)
T2C for the target	90% (9/10)	96.7 (89/92)	75.2% (9/12)	98.9% (89/90)
MAg	54.5% (6/11)	95.6% (87/91)	60% (6/10)	94.6% (87/92)
MAg using stringent Cut off	54.5% (6/11)	96.7% (88/91)	100% (6/6)	94.8% (91/96)
T2C+MAg	100% (11/11)	93.4% (85/91)	64.7% (11/17)	100% (85/85)
T2C+MAg w stringent cut off	100% (11/11)	96.7% (88/91)	78.6% (11/14)	100% (88/88)

Conclusions: T2Candida was superior to mannan antigen and BC and may be a valuable tool to initiate appropriate therapy earlier in positive patients and de-escalate empiric antifungal therapy in negative patients. When combined with MAg detection using a stringent cut off the sensitivity was 100% without compromising specificity.

Author Disclosure Block:

M.C. Arendrup: E. Grant Investigator; Self; Gilead, Basilea, Astellas. I. Research Relationship; Self; T2Biosystems. L. Speaker's Bureau; Self; Astellas, Merck, Basilea, Pfizer, Gilead, T2Biosystems. **J. Schierbeck:** L. Speaker's Bureau; Self; Merck. **N. Reiter:** None. **K.B. Krarup:** None. **J.S. Andersen:** L. Speaker's Bureau; Self; Merck.

Poster Board Number:

MONDAY-225

Publishing Title:

Verification of T2candida Panel for the Identification of *Candida* Species in Edta-preserved Whole Blood

Author Block:

A. Styer, K. Rygalski, L. Scicchitano, D. M. Wolk, D. R. Hernandez; Geisinger Hlth.System, Danville, PA

Abstract Body:

Background: Candidiasis in high risk patient populations leads to longer hospital stays, higher cost, and elevated morbidity and mortality. It is estimated that 50% of fungal infections are never identified due to their fastidious growth in blood culture. The lack of highly accurate and sensitive diagnostic tools leads to both overuse of empiric therapy and exposure to needless toxicity for high risk groups, as well as underuse of antifungal therapy when candidemia is not suspected.**Methods:** T2Candida (T2 Biosystems) is an FDA-cleared rapid diagnostic method that uses T2 Magnetic Resonance to detect 5 species of *Candida* from whole blood samples, in 3 analytical channels: *C. albicans/C. tropicalis* (A/T), *C. parapsilosis* (P), and *C. krusei* /*C. glabrata* (K/G). The verification of the T2Candida was conducted under conditions of Good Clinical Practices and according to CLIA'88 regulations.**Results:** An accuracy challenge panel consisting of 16 *Candida* and 3 bacterial species (N=119 spiked whole blood samples) was evaluated. 20 day QC was performed at 100% accuracy. The overall analytical sensitivity of T2Candida was 100%; analytical specificity was 97.7%, substratified by channel, 99.0%, 98.1%, and 97% A/T, P and K/G channel respectively. The limit of detection (LOD) was tested at 10-20X the reported LOD. The T2Candida product insert reports cross reactions with 3 bacterial strains (*E. coli*, *E. faecium* and *A. lwoffii*); however, no cross reactions were identified when we tested these strains or other *Candida* spp. at 100 CFU/ml. Therefore consider local specificity testing prior to implementation and use best molecular diagnostics practice, including strict contamination control policies during testing.**Conclusions:** T2Candida is a rapid and highly accurate test for detection of the most common *Candida* species. While spiked samples may be required for method verification, it will be important to rigorously validate and monitor clinical test performance including review of concurrent focal infections to better understand the clinical utility of this powerful method to initiate appropriate and reduce unnecessary antifungal therapy.

Author Disclosure Block:

A. Styer: None. **K. Rygalski:** None. **L. Scicchitano:** None. **D.M. Wolk:** J. Scientific Advisor (Review Panel or Advisory Committee); Self; T2 Biosystems. **D.R. Hernandez:** None.

Poster Board Number:

MONDAY-226

Publishing Title:

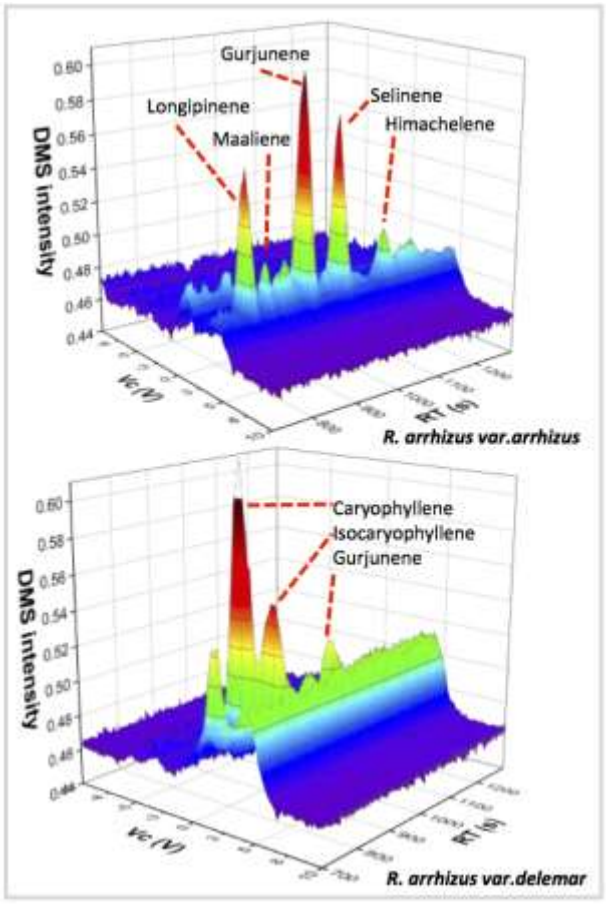
***In Vitro* Volatile Metabolite Signatures of Common Pathogenic Mucorales**

Author Block:

X. Yu[1], M. Al-Kateb[1], O. Aloum[1], L. R. Baden[1], F. M. Marty[1], S. Z. Manolakos[2], N. P. Wiederhold[3], A. Chowdhary[4], **S. Koo**[1]; [1]Brigham and Women's Hosp., Harvard Med. Sch., Boston, MA, [2]Draper Lab., Cambridge, MA, [3]Univ. of Texas Hlth.Sci. Ctr., San Antonio, TX, [4]Vallabhbhai Patel Chest Inst., Univ. of Delhi, Delhi, India

Abstract Body:

Background: Members of the order Mucorales cause life-threatening, rapidly progressive infections. The diagnosis of mucormycosis is challenging.**Methods**: We characterized the *in vitro* volatile metabolite profiles of *Rhizopus arrhizus* var. *arrhizus* (n=8) and var. *delemar* (n=8), *Rhizopus microsporus* (n=4), and *Mucor circinelloides* (n=4), the most common species causing mucormycosis, using thermal desorption-gas chromatography (GC)/tandem mass spectrometry and, in parallel, GC-differential mobility spectrometry (DMS), a rapid, portable method of identifying specific volatile metabolite signatures in complex gas mixtures.**Results**: Each of these 4 species consistently emitted a distinctive profile of volatile sesquiterpene secondary metabolites, all distinct from volatile sesquiterpenes released by *Aspergillus*: longipinene, maaliene, gurjunene, selinene, and himachalene in *R. arrhizus* var. *arrhizus*; caryophyllene, isocaryophyllene, and gurjunene in *R. arrhizus* var. *delemar* (**Figure**: corresponding DMS metabolite plots); caryophyllene and cubebol in *R. microsporus*; and caryophyllene, cubebol, and guaiene in *M. circinelloides.***Conclusion**: Volatile sesquiterpene secondary metabolite signatures of Mucorales are highly distinct from secondary metabolites released by *Aspergillus* spp. These distinctive metabolic features may be useful in the breath-based diagnosis of mucormycosis, particularly in helping distinguish mucormycosis from aspergillosis. DMS appears to be a useful technique for the rapid identification of these sesquiterpene metabolites.



Author Disclosure Block:

X. Yu: None. **M. Al-Kateb:** None. **O. Aloum:** None. **L.R. Baden:** None. **F.M. Marty:** E. Grant Investigator; Self; Astellas. J. Scientific Advisor (Review Panel or Advisory Committee); Self; Astellas. **S.Z. Manolakos:** None. **N.P. Wiederhold:** None. **A. Chowdhary:** None. **S. Koo:** I. Research Relationship; Self; Wako Diagnostics.

Poster Board Number:

MONDAY-228

Publishing Title:

A Rapid and Sensitive (1→3)-β-D-Glucan Microfluidic Assay for Early Detection of Invasive Aspergillosis Infections in a Murine Model

Author Block:

R. Kapoor[1], N. Wiederhold[2], L. K. Najvar[2], T. F. Patterson[2], W. P. Chang[1]; [1]Wako Life Sci., Mountain Vew, CA, [2]UT Hlth.Sci. Ctr., San Antonio, TX

Abstract Body:

Background: Incidence of invasive aspergillosis (IA) is rising in recent decades with poor morbidity and mortality in neutropenic patients. Early detection and treatment are key to improved clinical outcomes. Timely diagnosis is suboptimal using existing tools. A fungal cell wall component, (1→3)-β-D-glucan (BDG), has been used as a surrogate marker for fungal infection. We report here the results from an automated liquid phase binding assay for the early detection of serum BDG in infected mice.**Method:** Immunosuppressed ICR mice were inoculated with 10^5 inhaled *A. fumigatus* conidia (Sheppard et al *Antimicrob Agents Chemother* 2004; 48: 1908). Serum was collected daily for 7 days post infection (5 mice for each day); kidney fungal burden and BDG levels were measured. We measured BDG level by a liquid phase binding assay (Kawabata et al Electrophoresis, 2008; 29:1399) using microfluidic electrophoresis. Here, BDG is recognized by a recombinant protein (BGRP); detection is achieved through BGRP-conjugated fluorescent dye. The BDG level is correlated to peak area of the immunocomplex (BDG-BGRP).**Results:** The median BDG level (10.3, integrated peak area of immunocomplex) of 5 uninfected mice is used as a cutoff. All 5 infected animals from day 1 (median = 34.8; range = 32.0 - 43.7) were positively identified. Mice from days 2-7 were also correctly identified as being infected (median = 42.9; range = 17.6 - 204.0). However, results from a commercial kit (Fungitell®, cutoff = 80pg/mL) failed to ID 2 out of 5 mice for day 1 and 1 out of 5 for day 2. Samples from later time points (days 3 - 7) remained positive in infected mice as judged by results from both assays (microfluidics and Fungitell®).**Conclusion:** In this study, our assay detects serum BDG as early as day 1 in the course of IA in infected mice. This assay has the potential of being a useful tool for detecting IA in at risk patient population, with several advantages over existing assays, including a shorter assay time (~ 2 min), low reagent consumption, robotic liquid and sample handling, and automated runs with a single or multiple samples. Future studies will include the assessment of the performance of this assay using a larger pool of human specimens.

Author Disclosure Block:

R. Kapoor: None. **N. Wiederhold:** A. Board Member; Self; Astellas, Merck, Toyama, and Viamet. I. Research Relationship; Self; Received research support from Astellas, bioMerieux,

Dow, F2G, Merck, Merz, Revolution Medicines, and Viamet. **L.K. Najvar:** None. **T.F. Patterson:** C. Consultant; Self; Astellas, Merck, Toyama, Viamet, and Scynexis. I. Research Relationship; Self; Received research grants from UT Health Science Center San Antonio from Astellas, Merck, and Revolution Medicine. **W.P. Chang:** None.

Poster Board Number:

MONDAY-229

Publishing Title:

Evaluation of Beta-D-Glucan Assay at Different Cut-off Values for the Diagnosis of Invasive Fungal Infections in Pediatric Cancer Patients from India

Author Block:

P. Gupta, A. Ahmad, A. Kumar, M. Singh; King George's Med. Univ., Lucknow, India

Abstract Body:

Background: Limited specific data and investigations are available for invasive fungal infection (IFI) in pediatric cancer patients. Beta D glucan assay (BDG) has been approved by EORTC-MSG for the diagnosis of IFI in cancer patients. But its role in the diagnosis of IFI in pediatric cancer patient is controversial. Typical cut-off values for BDG range from 80 to 150 pg/ml. A cut-off of 80 pg/ml is currently approved by FDA. Different cut-off values needs to be evaluated for the diagnosis of IFI in pediatric cancer population.**Methods:** 64 pediatric cancer patients suspected of IFI were enrolled. 70 blood samples were collected. Blood samples were tested for serum BDG assay (Fungitell, Cape Cod, USA). BDG was evaluated at four different cut off values: 80, 100, 120 and 150.**Results:** According to EORTC-MSG guidelines 64 patients were classified as follows: 8 (12.5%) proven, 17 (26.5%) probable and 39 (76.5%) no IFIs. Receiver operating curve (ROC) analysis showed that the most reasonable cut off value in our study population is also 80 pg/ml. The sensitivity, specificity, PPV and NPV of BDG at cut-off 80 pg/ml was 71%, 29%, 51% and 50% respectively.**Conclusions:** The most reliable cut-off value for BDG assay for the diagnosis of IFI in pediatric cancer patient in our settings is 80 pg/ml. BDG assay is not a very promising test for the diagnosis of IFI in pediatric cancer patients.

Author Disclosure Block:

P. Gupta: None. **A. Ahmad:** None. **A. Kumar:** None. **M. Singh:** None.

Poster Board Number:

MONDAY-230

Publishing Title:

Role of Broad Range Pan Fungal Real Time Pcr in the Diagnosis of Ifi in Pediatric Cancer Patieints from India

Author Block:

M. Singh, P. Gupta, A. Ahmad, A. Kumar; King George's Med. Univ., Lucknow, India

Abstract Body:

Background: Invasive fungal infection (IFI) in pediatric cancer patients is a serious threat to their life. We evaluated a broad range pan fungal real time PCR with two reactions covering almost 80 genera of fungi, which included yeast as well as filamentous fungi. This assay has been developed by Landlinger C et al in year 2010. An excellent correlation was found between PCR positivity and the presence of proven, probable or possible fungal infections. We evaluated this assay at our settings on single blood samples. **Methods:** 64 pediatric cancer patients suspected of IFI were enrolled. Patients were classified as proven, probable, possible and no IFI according to the definitions of European Organization for Research and Treatment of Cancer criteria. 64 blood samples were collected from each patient. Real time PCR was done according to the protocol of Landlinger C et al.**Results:** According to EORTC-MSG guidelines 64 patients were classified as follows: 8 (12.5%) proven, 17 (26.5%) probable and 39 (76.5%) no IFIs. Sensitivity of the assay was found to be 78%. The negative predictive value of this assay was 74%, while the specificity and the positive predictive value were 46% and 51% respectively. Diagnostic accuracy was found to be 60%. LR positive and negative values were 1.4 and 0.4 respectively.**Conclusions:** The results indicate that broad range pan fungal real time PCR during febrile neutropenic episodes could be of significant value in diagnosing IFI in pediatric cancer patients, even in single samples. This assay also has significance in ruling out the disease.

Author Disclosure Block:

M. Singh: None. **P. Gupta:** None. **A. Ahmad:** None. **A. Kumar:** None.

Poster Board Number:

MONDAY-231

Publishing Title:

Development of a Molecular Assay for the Identification of *Histoplasma capsulatum* from Tissue

Author Block:

J. L. Cox, **E. N. Tessier**, A. G. Freifeld, P. C. Iwen; Univ. of Nebraska Med. Ctr., Omaha, NE

Abstract Body:

Background: Many current testing protocols to identify and diagnose histoplasmosis have low sensitivity and specificity, which hinders clinicians from delivering appropriate treatment to their patients[1]. While histologic examination is relatively sensitive, morphologic mimics of *Histoplasma* are common, limiting the specificity. Conversely, fungal culture, as the gold standard in diagnosis, is not always performed and when done leads to a delayed diagnosis due to the extended time required for culture diagnosis[2]. To address these shortcomings, polymerase chain reaction (PCR) may provide a sensitive and specific rapid diagnosis of *H. capsulatum* infection. **Methods:** Surgical biopsies and autopsy specimens that had corresponding tissue culture diagnosis of *H.capsulatum* and/or had budding-yeast organisms on histologic examination were identified. Also included was a negative specimen as a negative control. Tissue blocks selected had been stored in similar conditions. DNA was extracted from the formalin fixed, paraffin embedded tissue, and was used in downstream analysis. Real-time PCRs were performed using primers and a fluorescent probe specific for a 99-bp portion of the H. capsulatum-specific 100-kDa protein gene.**Results:** Three specimens with a corresponding positive tissue culture diagnosis tested positive by our PCR assay. We also examined autopsy tissues from a patient with pre-mortem culture positive Histoplasmosis. Interestingly, 6 of 7 (86%) tissues collected from various sites at autopsy demonstrated positivity on our assay, corroborating pre-mortem tissue culture, and suggest that different input tissues do not affect the outcome of our assay. Moreover, cycle threshold values recorded corresponded with organism burden seen histologically. A negative PCR result was found in 100% of 6 specimens diagnosed on histopathologic examination as having budding-yeast organisms, supporting this technique as an appropriate tool to rule-out *H.capsulatum* infection. Overall sensitivity was 90% (n=10) and specificity was 100% (n=7).**Conclusions:** Real-time PCR has the ability to provide a sensitive and specific rapid diagnosis, and may provide a new tool for rapid assessment of budding-yeast forms seen histologically. Additional studies are being performed to evaluate more samples as a means to validate this assay for approved clinical diagnostics.

Author Disclosure Block:

J.L. Cox: None. **E.N. Tessier:** None. **A.G. Freifeld:** None. **P.C. Iwen:** None.

Poster Board Number:

MONDAY-232

Publishing Title:

A Point-Of-Care Lateral Flow Assay For The Diagnosis Of Histoplasmosis In Hiv/Aids

Author Block:

S. Noor-Mohammadi, B. Crider, B. Neary, S. Bauman; IMMY, Norman, OK

Abstract Body:

Progressive disseminated histoplasmosis is a common and life-threatening fungal infection among patients with HIV/AIDS in the United States and Latin America. Incidence rates in HIV/AIDS can be >20%, with mortality rates >30% in resource-limited countries where the fungus, *Histoplasma capsulatum*, is endemic. Early diagnosis and treatment are essential to reducing this high mortality rate. Diagnosis currently depends on culture or histopathology, which have low sensitivity, are time consuming, expensive, and require trained personnel. IMMY has developed a point-of-care (POC) diagnostic test in the lateral flow (LFA) format for the rapid identification of disseminated histoplasmosis in the urine samples of patients. The LFA detects the polysaccharide antigen of *H. capsulatum* (HGM) that is shed into urine during infection using high-affinity monoclonal antibodies and follows the World Health Organization's ASSURED criteria by providing rapid results in under 30 min. Analysis of HGM-spiked urine samples demonstrates that the HGM LFA has a limit of antigen detection (LOD) of 0.5 ng/ml. Furthermore, comparison of archived infected patient urine specimens in the HGM LFA and IMMY's HGM EIA kit, had a positive and negative agreement of 100% [95% CI: 0.69-1.00] and 95% [95% CI: 0.59-1.00] respectively [n=21]. The HGM LFA offers a rapid, accurate, and sensitive method for qualitatively detecting *Histoplasma* antigen in immune compromised patients.

Author Disclosure Block:

S. Noor-Mohammadi: D. Employee; Self; IMMY. **B. Crider:** D. Employee; Self; IMMY. **B. Neary:** D. Employee; Self; IMMY. **S. Bauman:** D. Employee; Self; IMMY.

Poster Board Number:

MONDAY-233

Publishing Title:

Performance Characteristics of the Mvista® Histoplasma Antigen Eia Developed as an Ivd

Author Block:

P. A. Connolly, M. C. Minderman, L. J. Wheat; MiraVista Diagnostics, Indianapolis, IN

Abstract Body:

Background: The MVista® *Histoplasma* Antigen EIA has been offered as a lab developed test (LDT) for 30 years. As an LDT, it is only available as a service at MiraVista Diagnostics (MVD) and cannot be offered at other laboratories. An *in vitro* diagnostic (IVD) test kit has been developed and is at clinical trial with planned submission to FDA. Advantages of the IVD compared to the LDT include production under good manufacturing practices (GMP), and opportunity for other laboratories to perform the MVD EIA resulting in improved clinician and patient access. This report describes the analytical performance of the IVD and compares it to the LDT.**Methods:** The IVD kit uses microplate wells coated with anti-*Histoplasma* rabbit polyclonal IgG. Urine samples or controls are incubated in the wells, after which the wells are washed and a biotinylated IgG with streptavidin HRP solution is added to the wells and incubated. The wells are again washed and TMB chromogen is added followed by an acid stop solution. The plate is read at 450 nm with 620-630 nm reference. The EIA uses a cutoff calibrator to calculate the assay cutoff which is defined as 1.0 EIA Units (EU); the reportable range is $\geq$1.0 EU with a reference value of <1.0 EU.**Results:** Evaluating multiple IVD kit lots, the LoB was 0.50 EU with a LoD of 0.59 EU. In 24 assays with samples run in triplicate by two operators, each graded positive sample had repeatability and within-lab precision results of <10% CV. The negative and high negative samples had <15% CV for both repeatability and within-lab precision. A group of clinical residual samples from the MVD LDT were banked frozen and re-tested in the LDT and tested in the IVD. Of 45 urines that were originally negative, 43 (95.6%) were negative in the LDT and 44 (97.8%) were negative in the IVD. Of 39 histoplasmosis patient urines, 38 of which were initially positive in the LDT, 38 (97.4%) were positive in the LDT retest and 35 (89.7%) were positive in the IVD. McNemar's Test for paired proportions shows no significant difference between the LDT and IVD results, P=0.1250.**Conclusions:** The MVista® *Histoplasma* Antigen IVD assay is capable of detecting low level antigen demonstrating the cutoff is appropriately set with consistent interpretation. Precision and repeatability meet design goals at all levels of detection. The IVD exhibits similar clinical performance to the LDT.

Author Disclosure Block:

P.A. Connolly: D. Employee; Self; MiraVista Diagnostics. **M.C. Minderman:** D. Employee; Self; MiraVista Diagnostics. **L.J. Wheat:** K. Shareholder (excluding diversitied mutual funds); Self; MiraVista Diagnostics.

Poster Board Number:

MONDAY-234

Publishing Title:

Retrospective Analysis of *Candida parapsilosis* Species Complex Comparing Molecular and Maldi-Tof-Ms Identification Methods

Author Block:

L. M. Leach, X. Li, S. Chaturvedi; Wadsworth Ctr., Albany, NY

Abstract Body:

Background: The *Candida parapsilosis* complex was the second most prevalent species isolated from patients with invasive candidiasis or superficial candidiasis in several studies. The *Candida parapsilosis* complex contains three species: *C. parapsilosis*, *C. metapsilosis*, and *C. orthopsilosis* that exhibit differences in epidemiology, virulence, biofilm formation and antifungal susceptibility. The Matrix Assisted Laser Desorption/Ionization-Time Of Flight-Mass Spectrometry (MALDI-TOF-MS) is an emerging technology for rapid and accurate identification of microorganisms. In this study, we compared the MALDI-TOF-MS method to that of conventional PCR targeting exon-primed intron of the manganese superoxide dismutase (*MnSOD*) gene for the identification of *C. parapsilosis* species complex.**Method:** Sixty clinical isolates that were previously identified as *C. parapsilosis* using conventional or biochemical methods were obtained from the NYS Mycology Culture Collection Repository. The DNA was isolated and subjected to a previously established conventional PCR targeting an exon-primed intron of the manganese superoxide dismutase (*MnSOD*) gene followed by restriction enzyme digestion. Proteins from the isolates were extracted by first treatment with 75% ethanol followed by formic acid/acetonitrile mixture. The extracted proteins were analyzed by MALDI-TOF-MS (Bruker Daltonics).**Results:** The majority of the isolates, 56 (93.3%), were identified as *C. parapsilosis*. Interestingly, one isolate each was identified as *C. metapsilosis* (1.7%) and *C. orthopsilosis* (1.7%) and two isolates were identified as *Lodderomyces elongisporus* (3.3%). The species level identifications obtained with MALDI-TOF-MS was 100% concordance with PCR method.**Conclusions:** The MALDI-TOF based method was proved to be rapid, reproducible and accurate, with low consumable costs and minimal preparation time. The MALDI-TOF-MS has the potential to positively impact patient care and laboratory efficiency.

Author Disclosure Block:

L.M. Leach: None. **X. Li:** None. **S. Chaturvedi:** None.

Poster Board Number:

MONDAY-235

Publishing Title:

identification of Filamentous Fungi by Maldi-Tof Mass Spectrometry

Author Block:

A. Bateman, L. Bourassa, L. Curtis, S. Butler-Wu; Univ. of Washington, Seattle, WA

Abstract Body:

Background: Traditional identification of filamentous fungi can be slow and requires extensive experience. MALDI-TOF mass spectrometry has revolutionized bacterial and yeast identification in the clinical laboratory, and could improve the turn-around-time for mycology laboratories. Building on previous work in our laboratory, we evaluated the ability of the Bruker microflex LT mass spectrometer to identify fungi, using two databases: the Bruker Filamentous Fungal Library and an augmented library developed at the University of Washington.**Methods:** The augmented library consists of the Bruker library (Bruker Daltonics, Billerica, MA) plus 95 strains (50 species) of fungi identified by 28S and ITS sequencing. Fungi grown on Inhibitory Mold Agar (Remel, Lennexa, KS) were extracted with 70% ethanol, 70% formic acid, and acetonitrile, and run on the MALDI-TOF instrument. To evaluate the libraries, we tested 124 strains that were identified by morphology. To evaluate the usefulness of MALDI-TOF in a real-world setting, a prospective study is underway to compare the time to an accurate identification by MALDI-TOF versus morphology.**Results:** Using the Bruker library, 37/124 (30%) isolates were identified to the genus level (score value ≥1.7), with 13/37 (10% of the total) identified to the species level (score value ≥2.0). The augmented library showed improved performance: 71/124 (57%) were identified to the genus level, with 56/71 (45% of the total) identified to the species level. *Aspergillus fumigatus*, *Trichophyton rubrum*, and *Pseudallascheria boydii* complex are three of the most common fungi identified in our laboratory. The augmented library identified 36/36 (100%) isolates of these fungi to the genus level and 92% to the species level, but identification of other fungi varied by species. Data acquisition and analysis are ongoing for the prospective study.**Conclusions:** The augmented library outperformed the Bruker Library with the simplified extraction protocol. The augmented library identified common fungi well, and we plan to add additional isolates to the database to improve its performance for identifying less commonly isolated fungi.

Author Disclosure Block:

A. Bateman: None. **L. Bourassa:** None. **L. Curtis:** None. **S. Butler-Wu:** None.

Poster Board Number:

MONDAY-236

Publishing Title:

Impact of Maldi-Tof Ms on the Cost and Time to Identification of Filamentous Fungi

Author Block:

E. S. Slechta[1], **M. V. Powers-Fletcher**[2], T. Lunt[1], A. P. Barker[2], K. E. Hanson[2]; [1]ARUP Lab., Salt Lake City, UT, [2]Univ. of Utah, Salt Lake City, UT

Abstract Body:

Background: MALDI-TOF MS is used routinely to identify bacteria and yeast isolated from clinical specimens. The purpose of this study was to determine the impact of using MALDI-TOF MS, with an in-house-developed spectral database, on the accuracy, time and cost of mould identification (ID) in a national mycology reference laboratory.**Methods**: Moulds isolated from clinical specimens as a part of routine clinical practice were identified using conventional phenotypic and/or genotypic methods based on standard operating procedures. Isolates were then sub-cultured and reanalyzed by MALDI-TOF MS. Comparisons of IDs made by different methods, the time required for results and the cost of labor and reagents was determined. rDNA sequencing was considered the diagnostic gold standard for ID.**Results**: A total of 261 isolates were identified during this study; 253 (96.9%) isolates had high quality spectra using MALDI-TOF MS and were included in this analysis. The most commonly encountered fungi were hyaline hyphomycetes (≈ 50%) followed by dematiaceous moulds (≈ 18%), with approximately 80% of isolates representing established human pathogens. **Table 1** shows the frequency of IDs using phenotypic, genotypic, or MALDI-TOF MS. There were 9 isolates for which the conventional method and MALDI-TOF MS did not agree. Resolution testing is underway. The median time to ID was 2 d (interquartile range [IQR] = 1 d), 4 d (IQR = 3 d) and 6 d (IQR = 2 d) using MALDI-TOF MS, phenotype or genotype, respectively. The cost of MALDI-TOF MS was $12.41/isolate as compared to phenotypic testing ($22.09/isolate) and sequence analysis ($67.58/isolate).**Table 1**

	Phenotypic ID (%)	Genotypic ID (%)	No ID (%)	**Total (%)**
MALDI-TOF MS Genus-Level Only (%)	7 (2.8)	2 (0.8)	0 (0)	9 (3.6)
MALDI-TOF MS Species-Level (%)	123 (48.6)	44 (17.4)	5 (2.0)	173 (68.4)
MALDI-TOF MS No ID (%)	40 (15.8)	20 (7.9)	12 (4.7)	71 (28.1)

Total (%)	170 (67.2)	66 (26.1)	17 (6.7)	**253**

Conclusion: Compared to conventional methods, MALDI-TOF MS provides rapid results with similar accuracy, even in a laboratory with a high-level of mycology expertise. Implementation of MALDI-TOF MS for mould ID has the potential to reduce costs and improve the turn-around-time (TAT) to critical results. However, a small proportion of isolates may require repeat extraction to obtain acceptable spectra for analysis.

Author Disclosure Block:

E.S. Slechta: None. **M.V. Powers-Fletcher:** None. **T. Lunt:** None. **A.P. Barker:** None. **K.E. Hanson:** None.

Poster Board Number:

MONDAY-237

Publishing Title:

Rapid One-step Extraction Method for the Identification of Molds Using Maldi-Tof Ms

Author Block:

P. Luethy, A. Zelazny; NIH, Bethesda, MD

Abstract Body:

Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) has revolutionized the identification of clinically important molds. Previously, our group developed an extraction procedure to generate a comprehensive clinically relevant mold database (Lau et al, 2013, JCM). Although easy to perform, this extraction method is time consuming, requiring bead-beating and several chemical processing steps. In this study, we optimized and validated a rapid one-step method for protein extraction from filamentous fungi. Excised mold (~5 mm) was placed into tubes containing zirconia-silica beads and 100 µL of extraction solution without prior washing or ethanol soaking. Different ratios of acetonitrile, formic acid, and water were compared. Inoculated tubes were processed by a PowerLyzer high power bead based homogenizer and supernatants spotted for MALDI-TOF MS. Viability studies were performed before (extraction solution alone) and after bead beating by culturing in liquid and solid media for 7 days at 27 °C. The rapid method was evaluated prospectively and in parallel with our current mold extraction method. Preliminary analysis of 50 clinical mold isolates (28 hyaline molds, 12 dematiaceous molds, 3 mucorales, 6 basidiomycetes, and 1 dermatophyte) resulted in a decrease of extraction time by 30 minutes (current = 35 mins, new rapid = 5 mins). Some molds were not represented in MALDI-TOF databases. All organisms were found to be non-viable after 5 mins in the extraction solutions (bead beating was not required). Acceptable identification score (≥ 2.0) was achieved for up to 64% of isolates by the rapid method compared with 54% of isolates by the current method (Table 1). One dermatophyte and three hyaline mold isolates identified by the current method but with lower scores (< 2.0) by the rapid method were still identified correctly by either method. The rapid method was more sensitive for mucorales, basidiomycetes, and dematiaceous molds. This rapid method significantly reduces processing time while maintaining excellent scores for the identification of clinical mold isolates.

Table 1

	Current	Rapid
Hyaline	19/28 (67.9%)	16/28 (57.1%)
Dematiaceous	7/12 (58.3%)	8/12 (66.7%)
Dermatophyte	1/1 (100%)	0/1 (0%)
Mucorales	0/3 (0%)	2/3 (66.7%)

Basidiomycetes	0/6 (0%)	6/6 (100%)

Author Disclosure Block:

P. Luethy: None. **A. Zelazny:** None.

Poster Board Number:

MONDAY-238

Publishing Title:

Evaluation of Maldi-Tof Mass Spectrometry for Identification of 342 Clinical Isolates of *Aspergillus* Species from 11 Korean Hospitals

Author Block:

S. Kim[1], J. Park[1], J. Shin[1], J. Kim[1], M. Choi[1], M. Shin[1], S. Suh[1], S. Jang[2]; [1]Chonnam Natl. Univ. Med. Sch., Gwangju, Korea, Republic of, [2]Chosun Univ. Med. Sch., Gwangju, Korea, Republic of

Abstract Body:

Background: Matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) has become increasingly recognized as an alternative to morphological and molecular-based identification (ID) of *Aspergillus* species. The aim of our study was to evaluate the commercial mold database, entitled Filamentous Fungi Library 1.0 (Bruker Daltonics) of the Bruker MALDI Biotyper to identify 342 clinical isolates of *Aspergillus* species. **Methods:** A total of 342 clinical isolates (collected in 2012-2013) from 11 hospitals in Korea were assessed. Bruker MALDI Biotyper ID featured liquid cultivation followed by ethanol-formic acid extraction, according to the manufacturer's instructions. The results yielded by the Filamentous Fungi Library version 1.0 were compared to those obtained upon sequencing of internal transcribed spacer (which affords ID to the species complex level) and the β-tubulin region (which affords ID to the species level within the complex). **Results:** For the 342 isolates, the frequencies of correct ID to the complex level by the MALDI Biotyper were 94.2% and 98.5% at cutoff values of 2.0 and 1.7, respectively. Sixteen (4.6%) isolates (6 *A. fumigatus,* 2 *A. lentulus,* 5 *A. flavus,* 2 *A. tamarii,* and 1 *A. terreus* isolates) were not identified at a cutoff of 2.0, but only one *A. fumigatus* isolate was not identified at a cutoff value of 1.7. Only four isolates (1.2%) were misidentified at either cutoff value: these were of the *A. versicolor* complex and were misidentified as members of the *A. fumigatus* complex. The rates of correct ID at the species level were 74% and 78.1% at cutoff values of 2.0 and 1.7, respectively. Of isolates of cryptic species (*A lentulus, A. awamori, A. tubingensis, A. tamarii,* and *A. calidoustus*), only two *A. tamarii* isolates were identified correctly for the species cutoff 1.7, and none at a cutoff value of 2.0. **Conclusions:** The Bruker MALDI Biotyper using the Filamentous Fungi Library version 1.0 seems very reliable when used to identify clinical isolates of *Aspergillus* species to the complex level. However, the database must expanded to identify isolates correctly at the species levels, and to identify cryptic species.

Author Disclosure Block:

S. Kim: None. **J. Park:** None. **J. Shin:** None. **J. Kim:** None. **M. Choi:** None. **M. Shin:** None. **S. Suh:** None. **S. Jang:** None.

Poster Board Number:

MONDAY-239

Publishing Title:

Prospective Evaluation of a New *Aspergillus* IgG EIA Kit for the Diagnosis of Chronic and Allergic Pulmonary Aspergillosis

Author Block:

C. Dumollard[1], S. Bailly[1], S. Perriot[1], M-P. Brenier-Pinchart[1], C. Saint-Raymond[1], B. Camara[1], J-P. Gangneux[2], F. Persat[3], S. Valot[4], F. Grenouillet[5], H. Pelloux[1], C. Pinel[1], **M. Cornet**[1]; [1]Grenoble Alpes Univ. Hosp., Grenoble Cedex 9, France, [2]Rennes Univ. Hosp., Rennes, France, [3]Hospices civils de Lyon, Lyon, France, [4]Dijon Univ. Hosp., Dijon, France, [5]Besançon Univ. Hosp., Besançon, France

Abstract Body:

Background: Anti-*Aspergillus* IgG antibodies are important biomarkers for the diagnosis of chronic pulmonary aspergillosis (CPA) and allergic bronchopulmonary aspergillosis (ABPA).**Methods:** In a prospective multicentre study, we compared the performance of a new commercial EIA (Bordier Affinity Products) with those of the Bio-Rad and Virion\Serion EIAs. This assay is novel in the association of two recombinant antigens with somatic and metabolic antigens of *A. fumigatus*. **Results:** A total of 436 serum samples from 147 patients diagnosed with CPA (136 sera/104 patients) or ABPA (94 sera/43 patients) and from 205 controls (206 sera) were tested. We obtained sensitivities of 97%, 91.7%, and 86.1%, and specificities of 90.3%, 91.3%, and 81.5% for the Bordier, Bio-Rad and Virion\Serion test, respectively. The Bordier kit was more sensitive than the Bio-Rad kit ($p<0.01$), which was itself more sensitive than the Virion\Serion kit ($p=0.04$). The Bordier and Bio-Rad kits had similar specificities ($p=0.8$), both higher than that of the Virion\Serion kit ($p=0.02$). The areas under the ROC curves confirmed the superiority of the Bordier kit over the Bio-Rad and Virion\Serion kits (0.977, 0.951 and 0.897, respectively; $p<0.01$ for each comparison). In a subset analysis of 279 serum samples tested with the Bordier and Bio-Rad kits and an in-house immunoprecipitin assay (IPD), the Bordier kit had the highest sensitivity (97.7%), but the IPD had the highest specificity (96.6%). **Conclusions:** The use of recombinant, somatic and metabolic antigens in a single EIA improved the compromise between sensitivity and specificity, resulting in an assay highly suitable for use in the diagnosis of chronic and allergic aspergillosis

Author Disclosure Block:

C. Dumollard: None. **S. Bailly:** None. **S. Perriot:** None. **M. Brenier-Pinchart:** None. **C. Saint-Raymond:** None. **B. Camara:** None. **J. Gangneux:** None. **F. Persat:** None. **S. Valot:** None. **F. Grenouillet:** None. **H. Pelloux:** None. **C. Pinel:** None. **M. Cornet:** None.

Poster Board Number:

MONDAY-240

Publishing Title:

Immunohistochemical (Ihc) and In-Situ Hybridization (Ish) Detection of *Aspergillus spp.* in Human Tissue Samples

Author Block:

K. C. Dee, C. J. Clancy, S. Dacic, K. Cieply, S. Yousem, M. H. Nguyen; Univ. of Pittsburgh Med. Ctr., Pittsburgh, PA

Abstract Body:

Background: Invasive aspergillosis (IA) is a disease which carries significant morbidity and mortality to a broad range of patients. The diagnosis of IA is limited by poor sensitivity and specificity of microbiologic cultures, and the poor ability of conventional histopathologic tests to distinguish IA from other invasive fungal infections (IFI). *Aspergillus*-specific IHC and ISH could facilitate direct diagnosis of IA within tissue. **AIM:** The aim of our study was to assess the sensitivity and specificity, via retrospective chart review with tissue culture as gold standard, of *Aspergillus* IHC and ISH. From 5/2010 through 10/2015, 44 tissue specimens from distinct patients with histopathologic evidence of mold were cultured for fungus and processed for IHC and/or ISH. Positive cultures (21 samples; 48%) grew *A. fumigatus* (11), *A. terreus* (2), *A. versicolor* (2), *A. niger* (1), *Scedosporium* (3), *Fusarium* (1) and *Alternaria* (1), while 23 cultures (52%) were with no growth. Tissue source was lung (72%), followed by sinus (17%), bone (6%), larynx and skin (4%). 28 of the patients were immunosuppressed including solid organ transplant recipients (N=13), hematologic malignancy (5), and solid tumor patients receiving chemotherapy (1). **Results:** Overall sensitivity for all *Aspergillus sp.* was 69% for IHC and 67% for ISH. Sensitivity for *A. fumigatus* alone was 80% and 71% for IHC and ISH respectively. If both tests were performed and either IHC or ISH was positive, sensitivity increased to 70% for all *Aspergillus sp*. and to 82% for *A. fumigatus*. The specificity for culture negative specimens was 65% for IHC and 76% for ISH. For the 5 specimens that grew other mold, specificity was 0% for IHC and 20% for IHC. There were 3 false positive samples (IHC and ISH) that grew *Scedosporium apiospermum* (note: 2 of these patients had also grown *A. fumigatus* on recently obtained cultures) and a single false positive each from *Fusarium sp*. and *Alternaria*. **Conclusions:** The sensitivity of IHC and ISH make these tests potentially valuable adjuncts to culture for diagnosing IA. Specificity may be understated in this study, as culture negative samples that were positive by IHC/ISH may represent IA cases that were missed by cultures. Assays require optimization in order to differentiate *Aspergillus* spp. from other acute-angled branching molds.

Author Disclosure Block:

K.C. Dee: None. **C.J. Clancy:** None. **S. Dacic:** None. **K. Cieply:** None. **S. Yousem:** None. **M.H. Nguyen:** None.

Poster Board Number:

MONDAY-241

Publishing Title:

Comparative Performances Of 2 Pcr Assays For The Detection Of *aspergillus* In Bronchoalveolar Lavage Fluid During The Diagnosis Of Invasive Aspergillosis

Author Block:

P. COMACLE, S. BELAZ, F. ROBERT-GANGNEUX, S. CHEVRIER, **J-P. GANGNEUX**; CHU Pontchaillou, Rennes, France

Abstract Body:

Background: *Aspergillus* detection in the BAL fluid is one of the criteria for the diagnosis of proven and probable invasive aspergillosis (IA). Beside mycology (direct examination and/or culture) and galactomannan (GM) detection that are identified as EORTC/MSG criteria for probable IA (de Pauw 2008), PCR on pulmonary samples has become an essential argument for the laboratory diagnosis. Various amplification methods have been published but without consensus.**Methods:** The aim of this work was to compare the performance of two amplification targets: *A. fumigatus* mitochondrial gene (AFmito) and 28S rRNA. From 01/2012 to 10/2015, 324 BALF were prospectively collected and tested for mycology examination, GM detection (EIA Platelia assay Biorad, cutoff 1.0 if isolated, and 0.8 if associated to serum GM > 0.5) and AFmito PCR assay. Beside, 28S PCR assay was retrospectively performed on the same DNA extracts (312/324 available DNA). **Results:** According to the 2008 modified criteria of EORTC/MSG for classification of IA, patients were classified as: 1 proven, 47 probable and 11 possible IA. Other patients were classified as 11 with colonization and 256 non-infected. Sensitivity of BALF GM detection, 28S PCR assay and AFmito PCR assay was 58%, 61% and 50%, respectively. Mycology testing (ED +/- culture) had a sensitivity of 33%. Specificity of BALF GM detection, 28S PCR assay, AFmito PCR assay and culture was 94%, 97%, 97% and 98%, respectively. Negative predictive value was 93%, 95%, 92% and 89%, respectively. Concordance between the 2 PCR assays was 97% (kappa coefficient 0.84), whereas it was 88% (kappa 0.40) between 28S PCR and BALF GM and 88% (kappa 0.43) between Afmito PCR and BALF GM. **Conclusions:** 28S PCR assay had the highest performance for the molecular detection of *Aspergillus* in BALF compared to AFmito. The combination of 28S PCR assay and GM detection in BALF allowed to reach a sensitivity of 80% with a conserved high specificity (91%).

Author Disclosure Block:

P. Comacle: None. **S. Belaz:** None. **F. Robert-gangneux:** None. **S. Chevrier:** None. **J. Gangneux:** None.

Poster Board Number:

MONDAY-242

Publishing Title:

Assessment of the Critical Components of a Pan-fungal Pcr Assay

Author Block:

P. D. Khot, **E. S. Slechta**, E. Kish-Trier, K. E. Hanson; ARUP Lab., Salt Lake City, UT

Abstract Body:

Background: Broad-range detection of fungal nucleic acid directly in human tissue has potential utility for the diagnosis of invasive fungal infections. The purpose of this study was to assess impact of primer design, master mix composition and DNA extraction method on interference from environmental contamination.**Methods:** Real Time PCR was performed on the Quant Studio (Life Technologies), with amplicons analyzed by post-PCR melt-curve analysis and Sanger sequencing. Two master mixes were evaluated including a standard mix and a fungal DNA-free custom mix, both from Promega. Primers targeted the 18S, 28S and ITS2 regions. Automated (Promega and Chemagen) and manual (Zymo and MoBio) extraction methods using fresh tissue with were also compared.**Results:** Initial studies were performed with 62 different fungal organisms and the standard master mix. All organism identifications were correct, but the number of specimens detected at <30 qPCR cycles varied by primer set (18S [54/62], 28S [60/62] and ITS2 [61/62]).Background fungal DNA was detected in 0 of 30, 3/30 (40 to 44 cycles) and 29/30 (37 to 43 cycles) of the no template custom mix controls using the 18S, 28S and ITS2 primers, respectively. Replicate testing of the no template standard mix control showed that 0 of 11, 12/12 (33 to 39 cycles) and 11/11 (33 to 43 cycles) contained fungal DNA using the 18S, 28S and ITS2 primers, respectively.Fungal culture-negative tissue and tissue spiked with a suspension of *Aspergillus fumigatus, Candida albicans, Cryptococcus neoformans, Exophiala dermatitidis* or *Absidia corymbifera* were also extracted and analyzed using the ITS2 primers. Results are presented in the **Table**.**Conclusions:** PCR primers targeting the fungal 28S and ITS2 regions, use of a fungal-free master mix and DNA extraction with either the Promega or MoBio kits were associated with optimal test performance.**Table**

Specimen	Extraction Kit Ct (range)			
	Chemagen	Promega	MoBio	Zymo
A.fumigatus	Negative	20.4-21.7	20.5-21.0	18.6-20.8
C.albicans	Negative	19.1-19.7	19.2-19.9	17.3-19.0
C. neoformans	Negative	22.1-23.5	20.0-20.2	18.8-19.4
A. corymbifera	Negative	25.6-25.8	21.0-21.4	20.2-20.9
E. dermatitidis	Negative	25.1-25.6	24.3-37.4	26.0-27.5

Tissue only	Negative	36.7-38.1	37.9-42.2	34.5-35.0
Water	Negative	38.6-42.8	37.6 (5 Negative)	36.8-40.3

Author Disclosure Block:

P.D. Khot: None. **E.S. Slechta:** None. **E. Kish-Trier:** None. **K.E. Hanson:** None.

Poster Board Number:

MONDAY-243

Publishing Title:

Comparison Of Sequencing The D2 Large-Ribosomal-Subunit (Lsu) Gene (Microseq^{tm}) Versus The Internal Transcribed Spacer (Its) Regions For Identification Of Common And Uncommon Clinically Relevant Fungal Species

Author Block:

S. Arbefeville, A. Harris, P. Ferrieri; Univ. of Minnesota Med. Sch., Minneapolis, MN

Abstract Body:

Fungal infections are still a cause of considerable morbidity and mortality in immunocompromised patients, and their precise identification (ID) can aid in directing antifungal therapy. With the advent of molecular methods, clinical laboratories have alternative approaches for fungal IDs to supplement traditional phenotypic methods, which can be challenging, and may not be accurate. The commercially available MicroSEQ™ D2 LSU rDNA Fungal Identification assay can streamline acquisition of readable D2 LSU rDNA; however, much of the evaluation of D2 LSU has focused on commonly encountered fungi, leaving clinical laboratories uncertain of the ability to differentiate a broader range of fungi. Thus, a comparison of D2 LSU sequence versus the more commonly used ITS regions, as taxonomic identifiers, was performed. Isolates of hyaline, dematiaceous, dermatophytes, mucorales, and other fungi from clinical specimens were investigated. D2 LSU identified 92.5% or 96.2% of the 53 isolates to the genus level and ITS-1+ITS-2 (f-ITS): 94.3% or 98.1%, using GenBank or MycoBank, respectively, when compared to assigned ID. When comparing species-level designations to assigned ID, D2 LSU aligned with 43.4% (23/53) or 49.1% (26/53) of these isolates in GenBank or MycoBank, respectively. By comparison, f-ITS possessed greater specificity, followed by ITS-1, then ITS-2 (species-level ID 73.6% (39/53), 66.0% (35/53), and 60.4% (32/53), respectively) using GenBank; for MycoBank, these values were 71.7% (38/53), 69.8% (37/53), and 71.7% (38/53). Comparing D2 LSU to ITS regions in GenBank or MycoBank at the species level against the assigned ID, f-ITS (23/53 vs. 39/53; $p = 0.0016$ or 26/53 vs. 38/53; $p = 0.017$, respectively) and ITS-1 ($p = 0.019$ or 0.03), exceeded D2 LSU's performance, but ITS-2 ($p = 0.08$ or 0.017) was comparable to D2 LSU using GenBank. Using either GenBank or MycoBank, D2 LSU outperformed phenotypic based ID at the genus-level (92.5% (49/53) vs. 69.8% (37/53); $p = 0.003$ or 96.2% (51/53) vs. 69.8% (37/53); $p = 0.0003$, respectively). Our results indicated that D2 LSU was equivalent to ITS and its components at genus level ID and identified several complex fungal species.

Author Disclosure Block:

S. Arbefeville: None. **A. Harris:** None. **P. Ferrieri:** None.

Poster Board Number:

MONDAY-244

Publishing Title:

Clinical Benefit of DNA Sequencing for Difficult to Identify Fungi Recovered from Paitents

Author Block:

T. S. Murray[1], R. Feinn[1], V. Ryan[1], D. R. Peaper[2]; [1]Frank H Netter MD Sch. of Med. Quinnipiac Univ., Hamden, CT, [2]Yale Univ. Sch. of Med., New Haven, CT

Abstract Body:

Background: The identification of fungi relies mostly on morphology. Environmental fungi recovered from clinical specimens that fail to form fruiting bodies are often considered contaminants and identified as mold. There is little data regarding the value of DNA sequencing in identifying pathogens when small amounts of these fungi are present. The purpose of this study is to determine the clinical value of DNA sequencing in identifying fungal pathogens for organisms not identified by traditional morphology. We hypothesized DNA sequencing applied to difficult to identify fungi is more likely to identify environmental contaminants when 1 colony forming unit (CFU) is present and more likely to identify pathogens when fungi are present in greater numbers.**Methods:** Over 2 years, sequential clinical fungal isolates from Yale New Haven Hospital sent for DNA sequencing were reviewed to determine whether pathogens were identified. Partial sequencing of the 28sRNA ribosomal subunit identified organisms using either Microseek or GenBank databases. PubMed searches determined whether the organism was a pathogen, probable pathogen (1-3 case reports) or not reported to cause infection. Fischer's exact test compared outcomes between groups using the dichotomous variable of pathogen or not.**Results:** 75 sequences of unique isolates were recovered from 74 patients; 48 respiratory specimens, 17 skin/ hair, 8 tissue , and 2 recovered from urine. 47 likely pathogens, 4 possible pathogens and 24 unlikely pathogens were sequenced. 25 isolates were identified at the species level, 43 at the genus level, and 2 at the level of family. Six were not definitively identified despite quality sequence data. Fungi recovered from tissue (7/8, 87%, p=.06) and skin/hair (14/17,82%, p=.03) were more likely to be pathogenic compared with respiratory fungi (24/48, 50%). There was no correlation between the amount of fungi recovered and the probability of identifying a pathogen (1 CFU, 14/27 pathogens and >4 CFU, 8/16, p=1.0).**Conclusions:** 50% of fungi recovered as a single colony from a clinical specimen and not identified by traditional morphology were likely pathogens. The original hypothesis is incorrect as there is clinical value in applying molecular techniques such as DNA sequencing in the mycology lab to fungi recovered in small numbers.

Author Disclosure Block:

T.S. Murray: None. **R. Feinn:** None. **V. Ryan:** None. **D.R. Peaper:** None.

Poster Board Number:

MONDAY-245

Publishing Title:

Sequencer-Based Capillary Gel Electrophoresis (SCGE) Targeting the rDNA Internal Transcribed Spacer (ITS) Regions for Accurate Identification of *Candida, Cryptococcus, Trichosporon* and Other Yeast Species

Author Block:

X. Hou[1], M. Xiao[1], S. C. Chen[2], H. Wang[1], L. Zhang[1], X. Fan[1], Z-P. Xu[1], F. Kong[2], Y-C. Xu[1]; [1]Peking Union Med. Coll. Hosp., Beijing, China, [2]Westmead Hosp., Univ. of Sydney, Sydney, Australia

Abstract Body:

Accurate species identification of *Candida*, *Cryptococcus*, *Trichosporon* and other yeast species is important for clinical management. In the present study, we evaluated a yeast species identification scheme by determining the rDNA internal transcribed spacer (ITS) region length types (LTs) using a sequencer-based capillary gel electrophoresis (SCGE) approach. A total of 156 yeast isolates encompassing 32 species (151 clinical isolates and five reference strains) were first used to establish a reference SCGE ITS LT database. Clinical evaluation of the SCGE ITS LT database was then performed using a further 95 isolates (seven *Candida* and one *Cryptococcus* species). In the initial database, 41 ITS LTs were identified, which distinguished 29 of 32 (90.6%) studied yeast species, with the exception of *Trichosporon asahii*, *Trichosporon japonicum* and *Trichosporon asteroides*. Isolates of eight species revealed different electropherograms and were subtyped into 2-3 different ITS LTs each. Of 95 test isolates representing eight species, 94 (98.9%) were correctly identified to species level, and the remaining one isolate had a novel ITS LT. The SCGE ITS LT assay was able to distinguish between the species of *Candida parapsilosis* species complex, *Candida glabrata* species complex, *Candida haemulonii* species complex and *Cryptococcus neoformans* species complex, but cannot differentiate the three *Trichosporon* species of *T. asahii*, *T. japonicum* and *T. asteroids*, which need further IGS1 region sequencing. In conclusion, yeast identification by SCGE ITS LT is a reproducible, fast and inexpensive alternative for the identification of clinical important yeast species.

Author Disclosure Block:

X. Hou: None. **M. Xiao:** None. **S.C. Chen:** None. **H. Wang:** None. **L. Zhang:** None. **X. Fan:** None. **Z. Xu:** None. **F. Kong:** None. **Y. Xu:** None.

Poster Board Number:

MONDAY-247

Publishing Title:

Prevalence of Acute Hepatitis A Virus and Hepatitis E Virus in Urban Cities of Sindh, Pakistan

Author Block:

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Abstract Body:

Background: Hepatitis A virus <HAV> and Hepatitis E virus <HEV> are both transmitted by route, resulting in acute viral hepatitis <AVH> in developing countries. They pose major health problems in our country. This study was done to determine prevalence of HAV and HEV in patients presenting with AVH.**Methods:** A cross-sectional study of 1-year duration was conducted in urban cities of Sindh, Pakistan. Samples were collected from general population of different areas of cities. Serum samples were analyzed for IgM anti-HAV and IgM anti-HEV for the detection of HAV and HEV, respectively using commercially available ELISA kits and then confirmed by PCR method.**Results:** : The seroprevalence of HAV and HEV positive patients were 60% and 20% respectively. There were 240 male samples and 260 female samples between the age group of 2 months to 30 years. It was found that young children from the age of 2 months to 10 years were the most affected population.**Conclusions:** Based on the above data, it is identified that young population of urban cities of Sindh is at the high risk of acquiring hepatitis A and hepatitis E virus due to poor sanitary conditions. This data will be helpful in planning for future vaccination and in improvement of hygiene.

Author Disclosure Block:

A. Muneer: None. **T. Siddiqui:** None. **M. Nadeem:** None. **S.U. kazmi:** None.

Poster Board Number:

MONDAY-248

Publishing Title:

Relationship between Socio-Economic and Nutritional Features and Advanced HIV Disease (AHD) in Newly Diagnosed HIV Patients in a Rural Hospital in Mozambique

Author Block:

X. Kortajarena[1], **M. Á. Goenaga**[1], M. Ibarguren[1], H. Azkune[1], M. J. Bustinduy[1], A. Fuertes[2], R. Galvez[2], L. Garcia-Pereña[3], E. Nacarapa[2]; [1]Hosp. Univ.rio Donostia, Donostia, Spain, [2]Hosp. El Carmelo, Chokwe, Mozambique, [3]Hosp. El Carmelo, Chokwe, Spain

Abstract Body:

Background: WHO estimated that there are 35 million people living with HIV in the world (69% in Sub-Saharan Africa). Some reports have shown the relationship between socio-demographic and nutritional features and AHD. The aim of this report is to know the prevalence and that relationship in our cohort. **Methods:** 1170 HIV new diagnosed 15 years old or older patients were enrolled during 2014 in Carmelo Hospital (Chokwe), a rural town located in Gaza province (Mozambique), with more than 25% of HIV prevalence in adults between 15-49 years old. We collected different socio-demographic and basic nutritional data to know the relationship with AHD, defined as WHO stage III or IV or CD4 lymphocyte count <200/mm3. **Results:** Mean age:37 years old. 41.3% were male. WHO stage:I 53,9%, II 23,2%, III 19% and IV 3,9%. 35.8% do not have access to electricity and 34.2% to water. Mean of hospital arrival time was 49 minutes: 42.5% on foot, 55.4% by public transport and 2.1% by their own vehicle. 82.8% do not have any degree, 12.7% primary degree, 4.1% secondary degree and 0.4% university degree. 51.5% were unemployed. Mean height 162 cms and mean weight 58.6%. Body mass index (BMI): 14% desnutrition (BMI<18.5); 66.2% normal (BMI 18.5-25); 15.6% overweight (BMI 25-30) and 4.1% obese (BMI>30). 65.5% had anaemia (Hb<12): 27.8% mild (Hb 11-12); 55.8% moderate (Hb8-11) and 16.4% severe (Hb<8). CD4 lymphocyte levels: 61.2% <350/mm3 and 36.1% <200/mm3. Gender (male 61.1% vs female 31.4%;p=0.00), employment status (employed 31.8% vs unemployed 54.5%;p=0.00), age(39 vs 36 years old;p=0,00), arrival time to the hospital (52 vs 48 minutes;p=0.019), BMI(20.9 vs 23.4;p=0.00) and hemoglobin (10.1 vs 11.7;p=0.00) were the features independently associated with AHD.**Conclusions:** The percentage of people with AHD were 43.5%. The features associated with AHD were male gender, being older, being unemployed, longer arrival time to the hospital, lower BMI and lower hemoglobin level.

Author Disclosure Block:

X. Kortajarena: None. **M.Á. Goenaga:** None. **M. Ibarguren:** None. **H. Azkune:** None. **M.J. Bustinduy:** None. **A. Fuertes:** None. **R. Galvez:** None. **L. Garcia-Pereña:** None. **E. Nacarapa:** None.

Poster Board Number:

MONDAY-249

Publishing Title:

Effects of Pitavastatin on Markers of Arterial Inflammation and Immune Activation in HIV Patients

Author Block:

K. Fitch[1], M. Zanni[1], T. Burdo[2], L. Sanchez[1], C. Sponseller[3], K. Williams[2], J. Aberg[4], S. Grinspoon[1]; [1]Harvard Med. Sch., Boston, MA, [2]Boston Coll., Chestnut Hill, MA, [3]KOWA Pharmaceuticals America, Montgomery, AL, [4]Mt Sinai Sch. of Med., New York, NY

Abstract Body:

Background: HIV patients on chronic ART often exhibit increased arterial inflammation and immune activation. Statin therapy may improve such indices, but few data from randomized, long-term studies are available, particularly with newer statins, such as pitavastatin, which do not have known significant interactions with ART. We previously reported greater effects of pitavastatin than pravastatin to reduce LDL-C in this study (-31% vs. -21% pitavastatin vs. pravastatin, CROI 2014). We now compare changes in markers of arterial inflammation and immune activation. **Methods:** In a randomized, double-blind study, 252 HIV patients on ART > 6 mos (CD4 > 200 cells/μL and HIV RNA <200 copies), and dyslipidemia [(LDL-C ≥130 and ≤220 mg/dL) and TG ≤400 mg/dL)] were randomly assigned 1:1 to receive pitavastatin 4 mg or pravastatin 40 mg PO QD. Inflammatory biomarkers were assessed at 12 and 52 weeks.**Results:** Neither demographics such as age [50 (45, 56) yrs], sex (87% male), BMI [27 (24, 30) kg/m^2], log HIV RNA [1 (1, 1)], Framingham risk score [5 (3, 9) %] nor baseline inflammatory markers were different between groups. Subjects randomized to pitavastatin demonstrated significantly greater reductions in sCD14 (P=0.01), OxLDL (P=0.02) and Lp-PLA2 (P=0.01) compared to subjects randomized to pravastatin, whereas other markers were not different at 52 weeks (Table, P value for Change Between Groups, *P<0.05 for Change Within Groups).**Conclusions:** Pitavastatin significantly lowered sCD14, OxLDL and Lp-PLA-2 over 52 weeks in dyslipidemic HIV patients. These effects were more prominent than seen with pravastatin, a commonly prescribed statin in HIV. Combined with more potent efficacy on LDL-C, these results suggest that pitavastatin may be an effective statin to improve lipids and inflammatory markers in HIV patients. Further studies are needed to determine the effects of pitavastatin on CVD prevention in HIV.

Change in Biomarkers Over 52 Weeks

	Baseline Pravastatin	Baseline Pitavastatin	Change Pravastatin	Change Pitavastatin	P Value(Change Between Groups)
sCD163, ng/mL	948.7 (736.8, 1139.4)	1030.2 (731.1, 1369.3)	-26.8 (-133.4, 128.7)	13.2 (-137.7, 129.9)	0.84

IL-6, pg/mL	1.1 (0.7, 1.7)	0.9 (0.8, 1.4)	0.1 (-0.3, 0.4)	0.0 (-0.3, 0.3)	0.39
MCP-1, pg/mL	142.6 (107.4, 180.7)	142.2 (110.9, 189.2)	-2.5 (-22.3, 22.0)	-2.2 (-30.3, 27.7)	0.89
sCD14, ng/mL	1752.1 (1381.9, 2057.4)	1801.3 (1348.0, 2275.8)	10.22 (-281.2, 307.9)	-144.9 (-482.7, 114.5)*	0.01
oxLDL, U/L	77.9 (63.9, 88.6)	76.8 (60.6, 93.3)	-13.3 (-23.7, -4.0)*	-19.3 (-30.8, -6.9)*	0.02
Lp-PLA2, ng/mL	182.6 (136.8, 240.0)	193.8 (157.1, 227.3)	-26.3 (-51.4, -4.5)*	-44.3 (-74.5, -9.2)*	0.01

Author Disclosure Block:

K. Fitch: None. **M. Zanni:** None. **T. Burdo:** E. Grant Investigator; Self; KOWA Pharmaceuticals America. **L. Sanchez:** None. **C. Sponseller:** D. Employee; Self; KOWA Pharmaceuticals America. **K. Williams:** E. Grant Investigator; Self; KOWA Pharmaceuticals America. **J. Aberg:** None. **S. Grinspoon:** E. Grant Investigator; Self; KOWA Pharmaceuticals America.

Poster Board Number:

MONDAY-250

Publishing Title:

Alarming Prevalence of HDV-HBV Co-Infection in Patients at Hepatitis Clinic, Civil Hospital, Karachi, Pakistan

Author Block:

G. Fatima[1], S. Quraishy[2], **S. U. Kazmi**[1]; [1]Immunology and Infectious Disease Res. Lab. - Dadabhoy Inst. of Higher Ed., Karachi, Pakistan, [2]Civil Hosp., Karachi, Pakistan

Abstract Body:

Pakistan is currently facing an epidemic of viral hepatitis caused by B, C and D viruses which share parallel routes of transmission. Hepatitis D virus (HDV) is a small, defective RNA virus that can only replicate in individuals who are already infected with hepatitis B virus. Patients having HBV-HDV co-infection may have more severe outcomes and higher risk of fulminant hepatitis in acute cases and cirrhosis and hepatocellular carcinoma in chronic infections within 5-10 years compared to individuals suffering from HBV mono infection. Worldwide, more than 15 million people are co-infected with this virus. Pakistan and other developing countries still fall among the areas of high endemicity for HDV infection. Since hepatitis B infection is a major public health problem in Pakistan, we investigated the prevalence of hepatitis D virus infection among the HBV positive patients population and associated risk factors. Blood samples were collected aseptically in red top vaccutainer from 12679 patients who reported at hepatitis Clinic of Civil Hospital, , during a period of two years (2013-2014). Demographic data including age, sex and risk behaviors were also recorded. All serum samples were screened for the presence of HBsAg by one step ELISA technique and confirmed by PCR for HBV DNA. Samples from HBV positive patients were processed to determine HDV specific antibodies by Sandwich ELISA technique. A total of 2472 (19.5%) patients with symptoms of hepatitis tested positive for presence HBsAg, HBV DNA .Out of these HBV positive patients, 1954 tested for anti HDV, 693 (35.5%) were found to be positive for HDV specific antibodies . HDV infection was most prevalent in patients on both extremes of age i-e under 10 years and 71-80 years, slightly higher in females than males and majority had a history of drug injections (therapeutic) and previous surgery. **Conclusion:** Our results indicated a very high prevalence of HDV infection among the HBV infected patients which would suggest that all such patients should be tested for HDV infection to avoid serious complications of the disease. Mass screening, HBV vaccination campaigns and awareness sessions about mode of spread can be effective in reducing the burden of HBV and HDV.

Author Disclosure Block:

G. Fatima: None. **S. Quraishy:** None. **S.U. Kazmi:** None.

Poster Board Number:

MONDAY-251

Publishing Title:

Genotype Distribution of Hepatitis C Virus among Men Who Have Sex with Men in Mongolia

Author Block:

H. Uemura[1], K. Tsuchiya[1], M. Takano[1], E. Emch[2], D. Jagdagsuren[2], H. Gatanaga[1], M. Sugiyama[3], M. Mizokami[3], S. Oka[1]; [1]Natl. Ctr. for Global Hlth.and Med., Toyama, Shinjuku, Tokyo, Japan, [2]Natl. Ctr. for Communicable Diseases, Ulaanbataar, Mongolia, [3]Natl. Ctr. for Global Hlth.and Med., Kounodai, Ichikawa, Chiba, Japan

Abstract Body:

Background: Epidemiological data of hepatitis C virus (HCV) infection in Mongolia is limited. Acute HCV hepatitis is issue among men who have sex with men (MSM) with various genotype of HCV in East Asia.**Methods:** We evaluated HCV antibody of Mongolian MSM who visited us to take a screening test for human immunodeficiency virus in Mongolia from December 2013 to September 2015. Among HCV antibody positive subjects, we evaluated HCV genotype with direct gene sequencing method of NS5B region. **Results:** Among 501 subjects, 51 (10.2%) were HCV antibody positive and HCV-RNA was detected in 32 (6.4%). Among HCV-RNA detected subjects, 30 (93.8%) were genotype 1b and 2 (6.3%) were genotype 2a. **Conclusions:** HCV genotype distribution of MSM was almost similar to the previous data among healthy-individuals in Mongolia.

Author Disclosure Block:

H. Uemura: None. **K. Tsuchiya:** None. **M. Takano:** None. **E. Emch:** None. **D. Jagdagsuren:** None. **H. Gatanaga:** None. **M. Sugiyama:** None. **M. Mizokami:** None. **S. Oka:** None.

Poster Board Number:

MONDAY-252

Publishing Title:

How Important Are The HBV DNA Levels Of 2,000 IU/ml In Hbeag-Negative And 20,000 IU/ml In HbeAg-Positive Chronic Hepatitis B Patients with Persistently High Alt Levels to Predict The Liver Damage

Author Block:

E. Yenilmez[1], R. A. Cetinkaya[1], A. Ulcay[1], M. Afyon[1], B. Bektore[1], T. K. Atik[1], M. B. Selek[1], H. Diktas[2], B. Simsek[3], A. Haholu[1], I. Yilmaz[1], V. Turhan[1]; [1]GATA Haydarpasa Training Hosp., Istanbul, Turkey, [2]Tatvan Military Hosp., Bitlis, Turkey, [3]Kasimpasa Military Hosp., Istanbul, Turkey

Abstract Body:

Background: There are few studies of the chronic hepatitis B (CHB) cases with low HBV DNA but persistently high ALT (PHALT) levels..**Methods:** A total of 450 naive patients with CHB (HBeAg-negative:249, HBeAg-positive:201) between 2008 and 2015 who are 19 to 40 years-old and male, were retrospectively enrolled in the study. Liver biopsy was performed after having ALT over 40 U/l persistently at least for six months regardless of the HBV DNA levels. We separated the cases according to the HBV DNA level of 2,000IU/ml for HBeAg-negative and 20,000IU/ml for HBeAg-positive, respectively. Also we formed groups according to HAI scores of 0-3/4-7/8-11/12-18 and fibrosis scores of 0-1/2/3-4/5-6 defined as no-minimal/moderate/significant/ severe, respectively. Than, we tested for the association between these groups using Chi-square test.**Results:** For HBeAg-negative cases, the mean age, ALT, DNA, Ishak's histological activity index (HAI) and fibrosis score was 23.9±0.3, 88.2±5U/l, $1.4x10^7$ IU/ml±$6.4x10^6$IU/ml, 4.08 and 1.44, respectively. For HBeAg-positive cases, the mean age, ALT, DNA, Ishak's HAI and fibrosis score was 22.5±0.2, 107.8±5.8U/l, $1.9x10^9$ IU/ml±$2.6x10^8$IU/ml, 4.72 and 1.61, respectively. For HBeAg-negative cases, there were statistically significant difference between HBV DNA levels <2,000 IU/ml and >2,000 IU/ml groups in HAI and fibrosis level groups defined (p:0.001<0.01 for HAI and p:0.000<0.01 for fibrosis),but the relationship between the groups were concordant but very low in strenght (tau c:0.21 for HAI and tau c:0.24 for fibrosis). For HBeAg-positive cases, there were statistically significant difference between HBV DNA levels <20,000 IU/ml and >20,000 IU/ml groups in HAI and fibrosis levels groups defined (p:0.001<0.01 for HAI and p:0.002<0.01 for fibrosis, but the relationship between these groups were concordant but very low in strenght (tau c:0.15 for HAI and tau c:0.15 for fibrosis).**Conclusions:** Further studies and new guideline recommendations about CHB cases with low HBV DNA are needed to guide clinicians how to monitor these cases.

Author Disclosure Block:

E. Yenilmez: None. **R.A. Cetinkaya:** None. **A. Ulcay:** None. **M. Afyon:** None. **B. Bektore:** None. **T.K. Atik:** None. **M.B. Selek:** None. **H. Diktas:** None. **B. Simsek:** None. **A. Haholu:** None. **I. Yilmaz:** None. **V. Turhan:** None.

Poster Board Number:

MONDAY-253

Publishing Title:

Long-Term Mean Plasma HIV RNA And CD4 Nadir Influence on Liver Stiffness Measurement in HIV-HCV Coinfected Patients and HIV Monoinfected Patients

Author Block:

S. G. Parisi[1], M. Basso[1], R. Scaggiante[1], M. Franzetti[1], S. Andreis[1], A. Cattelan[1], C. Mengoli[1], M. Cruciani[2], M. Andreoni[3], S. Piovesan[1], G. Palù[1], A. Alberti[1]; [1]Padova Univ, Padova, Italy, [2]ULSS 20, Verona, Italy, [3]Roma Univ, Roma, Italy

Abstract Body:

Background: To determine the influence of long-term (36 months before liver stiffness measurement, LSM) mean plasma HIV viremia (mpHIV) and of nadir CD4+ on LSM assessed with transient elastography in HIV monoinfected patients (MOpts) and HIV-HCV coinfected (Copts**).Methods:** The patients had to be HBsAg negative, HCV RNA positive in case of HCV positivity and with a valid LSM performed; pHIV defined undetectable HIV viremia (UV) if no value >50 copies (cps)/ml (no more than 1 blip/year) and as detectable viremia (DV) otherwise. Cut-off for DV mpHIV: less than 1000 cps/ml, (low, L); 1001-10000 cps/ml (intermediate, I), ≥10001 cps/ml (high, H). The cut-off for "normal" LSM was 4 kPa and 7.1 kPa for significant fibrosis. The Chi-squared test ,Fisher test and the Mann-Whitney test were applied as appropriate.**Results:** COpts had higher LSM values than MOpts both in case of UV and of DV ($p<0.0001$). The frequency of pts with normal LSM in higher in MOpts with UV, approaching the significance (34.9% vs 21.3%, $p=0.07$). The difference between Mopts and COpts was significant for each class of pHIV (L $p=0.001$, I $p=0.02$; H $p=0.0003$). Nadir was lower in MOpts with UV respect to MOpts with D ($p=0.01$): in MOpts with normal LSM the difference between UD and DV was not significant (296 vs 310, $p=0.5$) but in MOPS with LSM>4 nadir is lower in pts with UD (270 vs 357, $p=0.01$).**Conclusions:** COpts had higher LSM than MOpts, regardless mpHIV: in MOpts the influence of long-term mpHIV load on LSM seems related to the HIV RNA detectability and not to the HIV RNA level. The possible role of nadir is probably independent from mpHIV.

Figure 1. Main characteristics of the 207 HIV patients, stratified in 4 groups according to HCV serology, HCV RNA and mean plasma HIV viremia of the 36 months before liver stiffness measurement.

	Neg HCV ab Neg HIV RNA 83 pts (40.1%)	Neg HCV ab Pos HIV RNA 61 pts (29.5%)	Pos HCV RNA Neg HIV RNA 33 pts (15.9 %)	Pos HCV RNA Pos HIV RNA 30 pts (14.5%)
Male, n (%)	69 (83.1)	57 (93.4)	25 (75.6)	25 (83.3)
Age, years (median and 95% CI)	50 (46-54)	44 (40-47)	49 (48-51)	48 (46-49)
Body Mass Index (median and 95% CI)	23.7 (22.7-24.3)	24.2 (22.8-25.3)	22.1 (21.3-23.8)	24.7 (23.1-25.7)
CD4+ cell count at nadir (cells/mm^3), median and 95% CI)	271 (199-330)	330 (300-408)	250 (180-340)	240 (171-306)
Pts with LSM ≤ 4 kPa, n (%)	29 (34.9)	13 (21.3)	0	2 (6.7)
Pts with LSM >7.1 kPa, n (%)	9 (10.8)	5 (8.2)	18 (54.5)	20 (66.7)
LSM (kPa) (median and 95% CI)	4.3 (4.1-4.6)	4.5 (4.4-4.8)	7.4 (6.1-11)	8.2 (6.8-10.1)
Mean plasma HIV RNA (copies/ml) (median and 95% CI)	n.a	14922 (8120-32869)	n.a	3015 (950-17556)
LSM (kPa) in pts with mean plasma HIV RNA <1000 copies/ml (median and 95% CI)	n.a	4.2 (3.5 – 4.8)	n.a	11 (5.8 – 24.1)
LSM in pts with mean plasma HIV RNA 1001-10000 copies/ml (median and 95% CI	n.a	4.8 (4.4 – 6.2)	na	7.8 (5.1-15.5)
LSM in pts with mean plasma HIV RNA > 10000 copies/ml (median and 95% CI)	n.a	4.6 (4.4 – 4.8)	na	7.8 (5.8-9.5)

LSM: liver stiffness measurement
na: not applicable

Author Disclosure Block:

S.G. Parisi: None. **M. Basso:** None. **R. Scaggiante:** None. **M. Franzetti:** None. **S. Andreis:** None. **A. Cattelan:** None. **C. Mengoli:** None. **M. Cruciani:** None. **M. Andreoni:** None. **S. Piovesan:** None. **G. Palù:** None. **A. Alberti:** None.

Poster Board Number:

MONDAY-254

Publishing Title:

Role of VACS Index in the Survival of Patients with Substance Use Disorders and HiIV/HCV Infections

Author Block:

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Abstract Body:

Background: The Veterans Aging Cohort Study (VACS) is an index that includes age, CD4+ cell count, HIV-1 RNA and general indicators of organ system injury. It is designed to predict mortality in a wide range of patient population including HIV-negative patients. We aimed to analyze the VACS index in patients seeking treatment of substance use disorder (SUD).**Methods:** cohort study in patients admitted to treatment between 1997 and 2014 in Badalona, Spain. Characteristics of patients and blood samples were obtained at admission. VACS was computed according to http://vacs.med.yale.edu and stratified by the median; for HIV-negative patients, CD4+ cells and HIV-RNA counted 0 points. Patients were followed-up until December 2014 and cross-checks with death registry were obtained.**Results:** 768 patients included (82% M); median age was 35 years (IQR: 29-43 years); SUD was attributable to opiate, cocaine, and alcohol in 372 (48.5%), 108 (14.1%), and 287 patients (37.4%), respectively. Almost 53% had history of injection drug use. Prevalence of HIV and HCV infection was 23.4% (n=180) and 51.9% (n=399) respectively; 22.6% (n=174) patients were HIV/HCV co-infected. Median CD4+ cell count was 402 cells and median RNA-HIV was 5.200 UI/mL. Prevalence of HAART use was 38%. Median VACS score at admission was 12 points (IQR:5-24 points) and the maximum score observed was 95 points. After median follow-up of 10.6 years (IQR 5.8-14.8 yrs) (7.748 person-years), 18% of patients had died (n=140). Table shows mortality rates and Relative Risk (RR) of death according to viral infections and VACS score:

	Patients* (N=768)	**Deaths (N=140)**	**Follow up (p-y)**	**Mortality rate (x 100 p-y) (95% CI)**	**Relative Risk (95% CI)**
HIV(-) HCV(-)- and VACS ≤12	260	20	2398	0.8 (0.5-1.3)	1
HIV(-) HCV(-) and VACS >12	103	22	659	3.3 (2.1-4.9)	4.0 (2.1-7.4)
HIV(-) HCV(+) and VACS ≤12	122	20	1539	1.3 (0.8-2.0)	1

HIV(-) HCV(+) and VACS >12	103	20	1151	1.7 (1.1-2.6)	1.3 (0.7-2.5)
HIV(+) HCV(+) and VACS ≤12	28	5	325	1.5 (0.6-3.4)	1
HIV(+) HCV(+) and VACS >12	146	53	1610	3.3 (2.5-4.2)	2.1 (0.9-6.0)

*Six HIV+/HCV- patients are not shown in TableAdjusted Cox regression models showed that VACS>12 points, Alcohol Use Disorder and HIV/HCV co-infection were independent risk factors of death (HR: 1.9, 95%CI=1.3-2.9; HR=2.1, 95%CI:1.4-3.3 and HR=2.2, 95%CI:1.3-3.7, respectively).**Conclusion:** VACS predicts mortality in SUD patients with and without HIV infection. Alcohol and drug use disorder should be included in comorbidity indexes to better estimate mortality.

Author Disclosure Block:

A. Sanvisens: None. **P. Zuluaga:** None. **D. Fuster:** None. **J. Tor:** None. **R. Muga:** None.

Poster Board Number:

MONDAY-255

Publishing Title:

Hepatitis C Virus Genotypes in Patients Attending a Tertiary Care Hospital, Karachi, Pakistan

Author Block:

G. Fatima[1], S. Kumar[2], S. Quraishy[1], **S. U. Kazmi**[1]; [1]Immunology and Infectious Disease Res. Lab., Karachi, Pakistan, [2]Civil Hosp., Karachi, Pakistan

Abstract Body:

Background: Hepatitis C is a blood borne chronic liver disease, caused by the hepatitis C virus (HCV),which may progress to cirrhosis, hepatocellular carcinoma and even liver failure. It is one of the leading cause of morbidity and mortality and a serious public health problem, worldwide as well as in Pakistan. An estimated 130-170 million people worldwide are infected with hepatitis C. No vaccine against hepatitis C is currently available. There are seven major genotypes of the hepatitis C virus, which are indicated numerically from one to seven. HCV genotype-3 is known to be relatively more responsive to available therapy than other types. **Objective:** In this study investigated the frequency of various HCV genotypes present in patients with liver disorders attending Hepatitis Clinics at Civil Hospital - the largest public sector tertiary care hospital of Pakistan . **Methods:** All patients attending Civil Hospital Karachi, Pakistan, during a period of five years (2010-2014) who were positive for Hepatitis C virus RNA by real time PCR were included in the study. Blood samples were collected from the patients in yellow top vaccutainers, and allowed to clot, centrifuged and serum was separated and saved at -40^0C till further testing. HCV genotyping was done by real time PCR according to manufacturer's instructions. Demographic data of all patients was recorded and results were analysed to determine risk factors. **Results:** In order to know the prevalence of hepatitis C virus genotype in our community we determined HCV Genotype for 951 patients who were positive for HCV RNA by real time PCR. It was observed that the most prevalent HCV genotype was "3" for which 713 (75%) patients tested positive , followed by 1a in 63(6.6%) cases. Genotype 3 was distributed in all age groups but females were slightly more affected by genotype 3 than males. **Conclusions:** High prevalence of HCV Genotype 3 strain among inject able drug users (IDUs) due to use of recycled syringes, unsafe blood transfusion is a cause of concern for public health professionals in Pakistan, however timely diagnosis may reduce the chances of serious complications due to comparatively effective therapeutic response to available antiviral treatment. Our observations call for developing effective control of factors contributing to high incidence of disease.

Author Disclosure Block:

G. Fatima: None. **S. Kumar:** None. **S. Quraishy:** None. **S.U. Kazmi:** None.

Poster Board Number:

MONDAY-256

Publishing Title:

Increasing Incidents of Hbv & Hcv Dual Infection in Sindh Pakistan

Author Block:

B. A. Khan, s. Khan; Dow Univ. of Hlth.Sci., Karachi, Pakistan

Abstract Body:

Introduction: Viral hepatitis, Chronic liver diseases and hepatocellular carcinoma (HCC) are majorly caused by Hepatitis B (HBV) and Hepatitis C (HCV) viruses which shares the same route of transmission thus increasing the risk of dual-infection. The major concern with HBV/HCV dual-infection is that it can cause more severe liver disease and increase risk for the development of HCC. The frequency of HBV & HCV dual infection varies according to the geographical region. Pakistan is highly endemic for HBV & HCV infection. Due to lack of awareness & exposure to high risk activities HBV & HCV infection is significantly boosting. Since both of these viruses shares the same route of transmission, so there is a high risk of HBV & HCV dual-infection which needs to be evaluated **Objective:** To determine the frequency of Hepatitis B virus and Hepatitis C virus dual-infection in Sindh Pakistan using Real Time-PCR. **Methods:** Total 1864 blood samples were collected from suspected male & female patients, ranging from 07 to 70 years. HBV DNA was extracted using Qiagen dsp virus extraction kit and HCV RNA was extracted using kit from Roche followed by real time PCR using Qiagen & roche amplification kit respectively. **Results:** Out of total 1824 patients 1208 (66.2%) were males and 616 (33.7%) were females. HBV DNA was detected in 472 (25.8%) individuals. HCV RNA was found in 464(25.4%) patients. HBV/HCV co-infection was found in 322 (17.6%) samples while 888 (48.6%) were found negative for HBV and HCV. Females were infected more with HCV than HBV while HBV was found more in males. Patients of 21-40 years of age group were most affected for these viral infections as compare to other two age groups. **Conclusions:** This study shows that the frequency of HBV/HCV dual-infection is very high in Sindh Pakistan, the highest prevalence of dual-infection was seen in between 21-40 years of age. The main reason might be that this age group has more exposure to the high risk factors. Therefore extra attention is required to prevent the spread of infection particularly in this age group. Dual-infection of HBV/HCV is significantly boosting in our population. Which causes more severe liver disease therefore special attention is required towards the management and treatment of patients infected with Hepatitis B and C viruses.

Author Disclosure Block:

B.A. Khan: None. **S. Khan:** None.

Poster Board Number:

MONDAY-257

Publishing Title:

Seroprevalence and Phylogeny of Hepatitis B Virus Among Apparently Healthy Blood Donors in Ekiti State, Nigeria

Author Block:

A. O. Oluyege; EKITI STATE Univ. ADO EKITI, Ado-Ekiti, Nigeria

Abstract Body:

Background: Voluntary and non-remunerated blood donors are frequently employed for blood donation and constitute a potential source of transmission of Hepatitis B virus particularly in rural communities in Nigeria. Blood are accepted if the result of the screening for the Hepatitis B surface Antigen and other transfusion transmissible infections is negative. Establishing the phylogeny of HBV is critical to developing infection control strategies. This study was carried out to establish the phylogeny of the HBV among blood donors in Ekiti State. **Methods:** Commercially prepared immunochromatographic test strips used for screening blood donors was employed to detect the seroprevalence of HBsAg in the sera of blood donors. A nested PCR assay targeting a 408 bp stretch within the S ORF was used to detect HBV DNA. The targeted amplicons were purified and sequenced using BigDye chemistry sequencing. HBsAg reference sequences were retrieved from the HBV database and aligned using the CLUSTAL W program in MEGA 5 software. The phylogeny and pair wise distance of the HBsAg were estimated using MEGA 5 software with Kimura-2 parameter model. **Results:** A total of 27 (6.8%) of the 400 sera were positive for HBsAg. The 408 bp amplicon within the S ORF was successfully amplified in only 6 (22.2%) of the HBsAg positive samples. The phylogenetic analysis revealed that all the HBV from blood donors in Ekiti State belong to the genotype E. **Conclusions:** This study confirms the endemicity of HBV, the risk constituted by blood donors and the circulation of genotype E among blood donors in Ekiti State.

Author Disclosure Block:

A.O. Oluyege: None.

Poster Board Number:

MONDAY-258

Publishing Title:

Factors Associated to Co-Infection with Hepatitis B Virus or Hepatitis C Virus in Hiv Patients Under Treatment in Mbujimayi in Drc

Author Block:

E. K. Tshibangu, N. Kayiba, T. M. Tshiswaka, **T. G. Disashi**; Univ. of Mbuji-Mayi, Mbuji-Mayi, Congo, Democratic Republic of the

Abstract Body:

Background: Mbujimayi in DRC is an area of high prevalence of infection by the human immunodeficiency virus (HIV) and hepatitis B virus the virus (HBV) and C (HCV) virus which share the common routes of transmission. This study aimed to determine the prevalence of HIV co-infection with HBV and HCV and to identify the its associated factors among patients on antiretroviral therapy in Mbujimayi. **Methods:** Sectional study involves a random sample of 100 patients under treatment. These patients were interviewed and examined. Detremination of HBe and HBs antigens of HBV and anti-HCV antibodies against HCV, used the immunochromatographic test (SD Bioline®). CD4 count was performed by flow cytometry (Alere Pima®). A logistic regression analysis model was used to assess the determinants of HIV co-infection with HBV and / or HCV. The Epi-Info 3.5.1 (CDC®) was used for statistical analyses. **Results:** The frequency of co-infection of HIV with HBV, HIV with HCV, HIV with HBV and HCV was respectively 21% [13.5 to 30.3], 8.8% [3.6 to 17.2] and 3.0 [1.0 to 9.8]. The HBe Ag frequency was 8.7% [1.1 - 28.0] among HIV patient with HBV. None of these patients has been checked for HBV or HCV or received a vaccination or a treatment against HBV, HIV patients co-infected with HBV or HCV have a more severe immunosuppression than other patients (CD4 cells / mm3: 356.3 ± 254.5 vs 499.9 ± 243.1 cells / mm3; p = 0.019). The most determinants of HIV co-infection with HCV or HBV were the past history of jaundice (OR = 7.7 [1.0 to 57.9]; p = 0.049) and the notion of sex without a condom in the last six months (OR = 4.8 [1.1 to 21.4]; p = 0.039). **Conclusions:** HIV and HBVor HCV coinfection are frequent and associated with a poor immune status of patients Mbujimayi. Unfortunately, these co-infections do not seem to be the target of specific diagnostic and therapeutic measures.

Author Disclosure Block:

E.K. Tshibangu: None. **N. Kayiba:** None. **T.M. Tshiswaka:** None. **T.G. Disashi:** None.

Poster Board Number:

MONDAY-259

Publishing Title:

Emergence of CMY-2-Producing *Proteus mirabilis* in Companion Animals with UTI: 16 Years Study

Author Block:

C. Marques, A. Belas, L. Telo da Gama, C. Pomba; CIISA, Faculty of Vet. Med. - UL, Lisbon, Portugal

Abstract Body:

Proteus mirabilis is frequently associated with complicated and nosocomial urinary tract infections (UTI) in humans and companion animals. ESBL- and pAmpC-producing bacteria are frequently multidrug-resistant (MDR) and their increase is a worldwide concern. The goal of this study was to characterise the antimicrobial resistance of *P. mirabilis* isolated from companion animals with UTI over a period of 16 years.A total of 155 *P. mirabilis* isolated from companion animals with UTI (1999-2014) were included. Disk diffusion antimicrobial susceptibility testing was performed and CLSI breakpoints were applied. Third-generation cephalosporin (3GC) resistant isolates were screened by PCR and sequencing for the presence of ESBL (CTX-M) and pAmpC (CMY, DHA, MOX, ACT, FOX, MIR) genes. Fluoroquinolone resistance genes *qnr* (A, B, C, D and S) were also tested. Fisher exact test was used for statistical analysis.First detected in 2004, *P. mirabilis* 3GC resistance increased significantly ($p<0.001$) from 2.06% (n=2/97) to 20.37% (n=11/54) when comparing 1999-2006 and 2007-2014 time periods. A significant increase in resistance against amoxicillin/clavulanate ($p<0.01$), fluoroquinolones ($p=0.03$) and gentamicin ($p<0.01$) was also detected. In the 2007 to 2014-time period, resistance frequencies were as follow: 21.82% (n=12/55) amoxicillin/clavulanate, 32.14% (n=18/56) fluoroquinolones and 16.07% (n=9/56) gentamicin. Ninety percent (n=9/10) 3GC-resistant *P. mirabilis* harbored CMY-2 enzyme. All but one *P. mirabilis* CMY-2-producer were isolated from dogs. One *P. mirabilis* DHA-1-producer was also found in a dog. All 3GC-resistant isolates were resistant to three or more antimicrobials and thus were considered MDR. A significant increase in MDR was also detected ($p<0.001$). Only five *P. mirabilis qnr*D-producers were detected among 37 fluoroquinone-resistant isolates.This study shows a significant increase in *P. mirabilis* antimicrobial resistance in companion animals with UTI. The emergence of MDR CMY-2-producing *P. mirabilis* in companion animals raises great public-health concerns. Future epidemiological studies on CMY-2-producing *P. mirabilis* fecal colonization should be conduced in dogs to assess their role as reservoirs.

Author Disclosure Block:

C. Marques: None. **A. Belas:** None. **L. Telo da Gama:** None. **C. Pomba:** None.

Poster Board Number:

MONDAY-260

Publishing Title:

Comparison of Beta-Lactam Versus Fluoroquinolone Empiric Treatment in *Pseudomonas aeruginosa* Urinary Tract Infections

Author Block:

N. R. Schwarber[1], S. Schmittling[1], S. Waqar[2], S. J. Bergman[3]; [1]HSHS St. John's Hosp., Springfield, IL, [2]Southern Illinois Univ. Sch. of Med., Springfield, IL, [3]Southern Illinois Univ. Edwardsville Sch. of Pharmacy, Edwardsville, IL

Abstract Body:

Background: Data have emerged that improving empiric therapy in urinary tract infections (UTI) can decrease length of stay (LOS). The aim of this study was to evaluate how empiric therapy impacts LOS in UTI caused by *Pseudomonas aeruginosa* (PA). **Methods:** An IRB approved retrospective cohort study was conducted on inpatients admitted from January 1, 2010 through September 30, 2015. Patients with PA urine cultures were identified from the microbiology laboratory and included if they received empiric treatment with either a beta-lactam (BL) or fluoroquinolone (FQ). Exclusion criteria were patients less than 18 years old or non-urinary bacterial infections. The primary outcome was LOS compared between those who received empiric treatment with a BL versus those who received a FQ. Secondary outcomes included differences in mortality, adverse events, rates of *Clostridium difficile* infection, and appropriateness of empiric therapy based on susceptibility results. Analysis was performed using STATA. Non-normally distributed means were logarithmically transformed. **Results:** A total of 44 patients were included in this study with 24 in the BL group and 20 in the FQ group. Baseline characteristics were similar in both groups with regard to age, gender, and presence of urinary catheter. Four patients (20%) were immunosuppressed in the FQ group compared to only one patient (4%) in the BL group. The mean LOS in the FQ group was 7.6 days compared to 5.3 days in the BL group ($p > 0.05$). After regression analysis including age, gender, immunosuppression, urinary catheter, sepsis, and appropriateness of empiric therapy, the FQ group was associated with a LOS 1.04 days longer than the BL group ($p > 0.05$). FQ was associated with 60% appropriate empiric therapy compared to 42% in the BL group. For antipseudomonal BL, however, this number was 100%. No differences in other secondary outcomes were detected. **Conclusion:** In this pilot study, we found a 30% decrease in LOS with BL empiric therapy compared to FQ use. Although not statistically significant, future studies should include a larger sample size to determine if these results can be replicated and assess reasons for the difference.

Author Disclosure Block:

N.R. Schwarber: None. **S. Schmittling:** None. **S. Waqar:** None. **S.J. Bergman:** None.

Poster Board Number:

MONDAY-261

Publishing Title:

The Effect of Cranberry Juice on the Human Gut Microbiome: A Placebo-Controlled Trial of Women with Recurrent Urinary Tract Infections

Author Block:

W-C. Chou[1], H. Schreiber[2], K. Kaspar[3], M. Hullar[4], O. Kahsai[4], E. Traylor[4], S. Hultgren[2], C. Khoo[3], A. Earl[1]; [1]Broad Inst., Cambridge, MA, [2]Washington Univ. in St. Louis, St. Louis, MO, [3]Ocean Spray, Middleborough, MA, [4]Fred Hutchinson Cancer Res. Ctr., Seattle, WA

Abstract Body:

Urinary tract infections (UTIs) affect 15 million women each year in the United States alone, with 20-30% of women experiencing frequent recurrent UTIs (rUTIs) that do not resolve even with repeated antibiotic treatment. Studies have shown that women who experience rUTIs and consumed cranberry products were 47% less likely to have another UTI. This study aims to understand how cranberry consumption may reduce rUTI incidence by assessing its impact on the composition of the gut microbial community, the reservoir for UTI-associated pathogens. Seventy-one women with a recent history of rUTI were enrolled and received a cranberry beverage or a placebo daily for 24 weeks. Fecal samples were collected prior to study visits at weeks 0 and 24 and prior to any UTI visit. Using 16S rRNA gene-based metagenomic sequencing, we determined the community structure of the gut microbial community and identified genera that were correlated with the effects of daily cranberry beverage consumption. We found that microbial diversity within samples was not significantly different between cranberry juice and placebo groups, and same subject samples tended to be more similar to each other than to other subject samples regardless of cranberry consumption. However, we identified two genera, *Anaerovorax* ($p=0.0003$) and *Flavonifractor* ($p=0.001$), that were significantly associated with cranberry consumption. Ongoing work using whole genome shotgun metagenomic approaches will help us to further elucidate the functional changes within the gut microbiota associated with cranberry consumption.

Author Disclosure Block:

W. Chou: None. **H. Schreiber:** None. **K. Kaspar:** None. **M. Hullar:** None. **O. Kahsai:** None. **E. Traylor:** None. **S. Hultgren:** None. **C. Khoo:** None. **A. Earl:** None.

Poster Board Number:

MONDAY-262

Publishing Title:

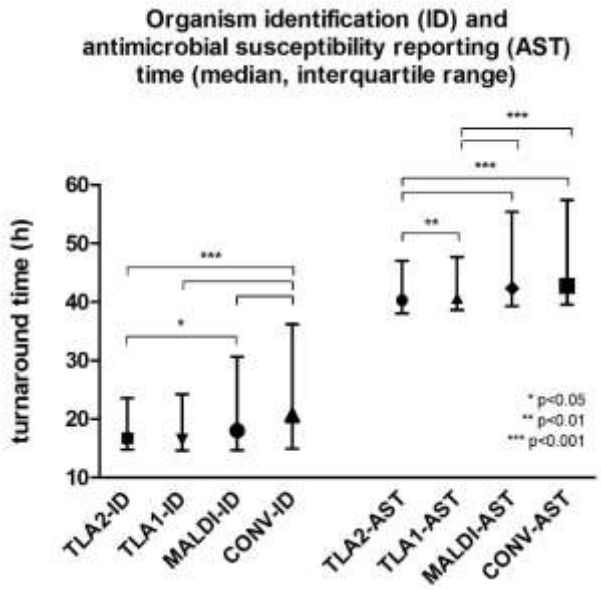
Total Laboratory Automation and Maldi-Tof Improves Turnaround Times for Urine Cultures

Author Block:

T. Theparee, S. Das, R. B. Thomson, Jr; NorthShore Univ. Hlth.System, Evanston, IL

Abstract Body:

Rapid microbiological information is essential for patient care. A Bruker matrix-assisted laser desorption ionization time of flight (MALDI-TOF) system was implemented in our laboratory for organism identification followed later by BD Kiestra total laboratory automation (TLA). We compared turnaround times (TAT) for urine cultures before and after implementation to assess these developments.Urine culture data was extracted from our laboratory information system in one month periods representing manual plating and biochemical identification (CONV), manual plating and MALDI (MALDI), early phase implementation of TLA and MALDI (TLA1), and late phase implementation of TLA and MALDI (TLA2). TAT to organism identification (ID) and antimicrobial susceptibility (AST) report were calculated for positive cultures. Results were compared between time periods and organism groups using the Kruskal-Wallis test and Dunn's post test.A total of 1532, 1330, 1214, and 1326 positive urine cultures were extracted for the CONV, MALDI, TLA1, and TLA2 groups respectively. TLA1 and 2 show significantly improved time to ID and AST and reduced TAT variability (interquartile range) compared to MALDI and CONV ($p<.001$). In addition, TLA2 showed significantly improved time to AST compared to TLA1 ($p<.01$). Time to ID significantly improved with MALDI compared to CONV for organisms not identifiable by spot testing ($p<.001$). Time to ID was similar for organisms identifiable by spot testing for all time periods.**Conclusions:** MALDI-TOF and TLA significantly improved TAT to ID and AST with positive urine cultures. Specifically, MALDI-TOF improves time to ID compared to CONV, and TLA plus MALDI-TOF improves time to ID and AST compared to CONV and MALDI. Our analysis also revealed areas for further improvements with TLA.



Author Disclosure Block:

T. Theparee: None. **S. Das:** None. **R.B. Thomson:** None.

Poster Board Number:

MONDAY-263

Publishing Title:

Risk Factors for Severe Sepsis and Septic Shock in Complicated Pyelonephritis

Author Block:

I. Marquez, V. Buonaiuto, L. Valiente, J. Mora, I. De Toro, A. Plata, B. Sobrino, J. Reguera, J. Colmenero; Hosp. REGIONAL DE MÁLAGA, MÁLAGA, Spain

Abstract Body:

Background: Severe sepsis and/or septic shock are the main factors influencing the prognosis of acute pyelonephritis. However, information about risk factors for severe sepsis in complicated pyelonephritis (CPN) is very scarce. Our aim was to analyze factors associated with the development of severe sepsis or septic shock in a large sample of patients with CPN. **Methods:** We conducted a retrospective cross-sectional observational study including 1507 consecutive patients older than 14 years diagnosed with CPN and admitted to a tertiary hospital between 1997 and 2015. Covariates associated with severe sepsis or septic shock in univariate analysis, were included in a multivariate logistic regression model.**Results:** Of the 1507 patients, 423 (28.1%) fulfilled the criteria for severe sepsis or septic shock at the time of admission. Crude and attributable mortality at 30 days were 17.7% and 11.7% in patients with severe sepsis or septic shock versus 1.7% and 0.6% in patients without severe sepsis or septic shock, $P < .0001$ and $P < .0005$ respectively. An age >65 years, urinary instrumentation in the previous two weeks, the lack of mictional syndrome or costovertebral tenderness, thrombocytopoenia, an ectasia ≥ grade II and bacteraemia were independent risk factors associated with severe sepsis or septic shock.**Conclusions:** The prevalence of severe sepsis and septic shock in patients with CPN is high. Some risk factors for severe sepsis are easy to identify in any Emergency Department. Using these data should simplify the empirical treatment of CPN and avoid unnecessary admissions.

Author Disclosure Block:

I. Marquez: None. **V. Buonaiuto:** None. **L. Valiente:** None. **J. Mora:** None. **I. De Toro:** None. **A. Plata:** None. **B. Sobrino:** None. **J. Reguera:** None. **J. Colmenero:** None.

Poster Board Number:

MONDAY-264

Publishing Title:

Intravenous Eravacycline With Transition To Oral Therapy For Treatment Of Complicated Urinary Tract Infections (Cuti) Including Pyelonephritis: Results From A Randomized, Double-Blind, Multicenter, Phase 3 Trial (Ignite2)

Author Block:

L. Tsai[1], M. Zervos[2], L. Miller[3], P. Tenke[4], A. Marsh[1], J. Mohr[1], K. Luepke[1], P. Horn[1]; [1]Tetraphase Pharmaceuticals, Inc, Watertown, MA, [2]Henry Ford Hosp., Detroit, MI, [3]Harbor-UCLA Med. Ctr., Torrance, CA, [4]Jahn Ferenc South-Pest Teaching Hosp., Budapest, Hungary

Abstract Body:

Background: Eravacycline (ERV) is a novel, fully synthetic fluorocycline antibiotic of the tetracycline class with broad-spectrum activity being developed for the treatment of serious infections, including those caused by multidrug-resistant pathogens.**Methods:** IGNITE2 evaluated the efficacy and safety of ERV vs levofloxacin (LEV) in hospitalized patients with cUTI. Patients were randomized to intravenous (IV) ERV 1.5 mg/kg once daily (QD) or IV LEV 750 mg QD. Patients could transition to ERV 200 mg orally (PO) twice daily or LEV 750 mg PO QD after 3 days if clinically indicated, for a total of 7 days. The primary endpoint was the composite response rate (clinical cure and microbiological success) in the microbiological intent-to-treat (micro-ITT) population at the post-treatment visit (PT, 6-8 days after end of therapy [EOT]) with a non-inferiority margin of 10%.**Results:** 908 patients were randomized, 600 were included in micro-ITT analysis. Although composite response rates were higher for ERV than LEV at EOT (5.5%, 95% CI=-0.5 to 11.4), ERV did not achieve non-inferiority to LEV at PT. In a pre-specified subgroup analysis, the response rate among LEV-resistant pathogens was higher for ERV compared to LEV. Response rates were also higher for ERV among patients who received 7 days of IV study drug. The most common adverse events were nausea and vomiting, 82/455 (18%) and 33/455 (7.3%) for ERV vs 14/450 (3.1%) and 6/450 (1.3%) for LEV, respectively.**Conclusion:** ERV did not achieve statistical non-inferiority compared to LEV but showed higher response rates among patients with LEV-resistant pathogens and those who received 7 days of IV study drug. Further investigation of the change in response between EOT and PT and the efficacy of IV-only therapy is warranted.

Table 1. Composite Response at the PT Visit

Group	ERV	LEV	Difference (95% CI)
All micro-ITT	180/298 (60.4%)	202/302 (66.9%)	-6.5% (-14.1, 1.2)
LEV-R pathogens	43/80 (53.8%)	31/85 (36.5%)	17.3% (2.1, 31.8)
3 days IV	74/131 (56.5%)	91/129 (70.5%)	-14.1% (-25.4, -2.3)

7 days IV	30/51 (58.8%)	26/54 (48.2%)	10.7% (8.5, 29.0)

Author Disclosure Block:

L. Tsai: D. Employee; Self; Tetraphase Pharmaceuticals, Inc. **M. Zervos:** J. Scientific Advisor (Review Panel or Advisory Committee); Self; Tetraphase Pharmaceuticals, Inc. **L. Miller:** J. Scientific Advisor (Review Panel or Advisory Committee); Self; Tetraphase Pharmaceuticals, Inc. **P. Tenke:** J. Scientific Advisor (Review Panel or Advisory Committee); Self; Tetraphase Pharmaceuticals, Inc. **A. Marsh:** D. Employee; Self; Tetraphase Pharmaceuticals, Inc. **J. Mohr:** D. Employee; Self; Tetraphase Pharmaceuticals, Inc. **K. Luepke:** D. Employee; Self; Tetraphase Pharmaceuticals, Inc. **P. Horn:** D. Employee; Self; Tetraphase Pharmaceuticals, Inc..

Poster Board Number:

MONDAY-265

Publishing Title:

Intravenous Eravacycline Compared to Intravenous Levofloxacin for the Treatment of Complicated Urinary Tract Infections (cUTI): Subgroup Analysis from a Randomized, Double-Blind, Phase 3 Trial (IGNITE2)

Author Block:

L. Tsai[1], M. Zervos[2], L. Miller[3], P. Tenke[4], A. Marsh[1], J. Mohr[1], K. Luepke[1], P. Horn[1]; [1]Tetraphase Pharmaceuticals, Inc., Watertown, MA, [2]Henry Ford Hosp., Detroit, MI, [3]Harbor-UCLA Med. Ctr., Torrance, CA, [4]Jahn Ferenc South-Pest Teaching Hosp., Budapest, Hungary

Abstract Body:

Background: In IGNITE2, Eravacycline (ERV), when administered as an intravenous (IV) to oral transition therapy, did not achieve the primary endpoint of statistical non-inferiority compared to levofloxacin (LEV) for the treatment of cUTI. Additionally, fewer days of IV therapy was associated with loss of efficacy between the end of therapy (EOT) and post treatment (PT, 6-8 days after EOT) evaluations, particularly among ERV patients.**Methods:** A subgroup analysis of patients enrolled in IGNITE2 who received only IV ERV 1.5 mg/kg once daily or IV LEV 750 mg once daily was performed. All patients who received IV-only therapy were included, regardless of the duration of treatment.**Results:** There were 178 patients who received IV-only therapy; 121 were included in the microbiological intent-to-treat (micro-ITT) population. Baseline demographics were balanced between the 2 groups and included 67% female, mean age 58.3±16.9 years, and 38% pyelonephritis. *Escherichia coli* was the most common pathogen identified (76.5%). The LEV resistance rate was 34.7%. Composite responder rates (clinical cure and microbiological success) at PT are presented in the table. Responder rates among patients with LEV-resistant and LEV-susceptible pathogens are also presented. The most common adverse events were nausea and vomiting, reported in 19/91 (20.9%) and 8/91 (8.8%) subjects in the ERV arm compared to 6/87 (6.9%) and 3/87 (3.4%) subjects in the LEV arm.**Conclusions:** ERV demonstrated comparable efficacy to LEV in a subgroup of patients enrolled in IGNITE2 who received IV-only therapy for the treatment of cUTI and greater efficacy in patients with LEV-resistant pathogens.

Table 1. Composite Response at the PT Visit - IV-Only Therapy

Group	ERV	LEV	Difference (95% CI)
IV-only-micro-ITT	31/57 (54.4%)	27/64 (42.2%)	12.2% (-5.7, 29.3)
LEV-R pathogens	8/19 (42.1%)	3/23 (13.0%)	29.1% (2.1, 53.8)
LEV-S pathogens	23/28 (60.5%)	24/41 (58.5%)	2.0% (-19.5, 23.2)

Author Disclosure Block:

L. Tsai: D. Employee; Self; Tetraphase Pharmaceuticals, Inc. **M. Zervos:** J. Scientific Advisor (Review Panel or Advisory Committee); Self; Tetraphase Pharmaceuticals, Inc. **L. Miller:** J. Scientific Advisor (Review Panel or Advisory Committee); Self; Tetraphase Pharmaceuticals, Inc. **P. Tenke:** J. Scientific Advisor (Review Panel or Advisory Committee); Self; Tetraphase Pharmaceuticals, Inc. **A. Marsh:** D. Employee; Self; Tetraphase Pharmaceuticals, Inc. **J. Mohr:** D. Employee; Self; Tetraphase Pharmaceuticals, Inc. **K. Luepke:** D. Employee; Self; Tetraphase Pharmaceuticals, Inc. **P. Horn:** D. Employee; Self; Tetraphase Pharmaceuticals, Inc..

Poster Board Number:

MONDAY-266

Publishing Title:

Risk Factors for Change in Microbiological Outcomes between the End of Therapy (Eot) and Post-Treatment (Pt) Evaluations in Patients with Complicated Uti (Cuti) Treated with Eravacycline (Erv): Analysis from a Randomized, Double-Blind, Phase 3 Trial (Igni

Author Block:

L. Tsai[1], A. Das[2], J. Mohr[1], P. Scoble[1], P. Horn[1], A. Shorr[3]; [1]Tetraphase Pharmaceuticals, Inc, Watertown, MA, [2]InClin, Inc, San Mateo, CA, [3]Medstar Washington Hosp. Ctr., Washington, DC

Abstract Body:

Background: IGNITE2 compared ERV to levofloxacin (LEV) for cUTI. Although ERV achieved a higher response rate (clinical cure and microbiological success) at the end of therapy (EOT), ERV did not demonstrate non-inferiority to LEV at the post-treatment evaluation (PT, 6-8 days after EOT). Since response was driven by microbiological outcome, we investigated factors associated with microbiological failure at PT despite initial response at EOT.**Methods:** Outcomes in patients classified as microbiological success at EOT were examined. We compared those who remained successes at PT to patients who converted to failure. Risk factors including demographics, type of infection, study drug, and duration of IV therapy while on study drug were assessed. We employed logistic regression to identify variables independently associated with microbiological failure at PT despite success at EOT.**Results:** In the microbiological-ITT population, at EOT, 90.6% (270/298) and 84.8% (256/302) subjects were microbiological successes in the ERV and LEV groups, respectively. Crude failure rates between EOT and PT were 25.5% and 14.3 %, for ERV and LEV, respectively. Independent variables associated with conversion to failure at PT are shown in the table below. The adjusted Odds Ratios (OR) for loss of efficacy between EOT and PT among patients who received ≤ 3 days IV study drug were 1.61 (95% CI=0.90, 2.88, p=0.1102) and 1.34 (95% CI=0.67, 2.67, p=0.4135) for ERV and LEV, respectively, suggesting an interaction between duration of IV therapy and study drug.

Table. Factors associated with failure at post-treatment visit in multivariate analysis

Factor	**Adjusted OR**	**95% CI**	**p-value**
Treatment	0.68	0.44 - 1.06	0.0900
Diagnosis (AP vs other cUTI)	0.57	0.36 - 0.92	0.0200
Gender	1.55	0.96 - 2.50	0.0716
IV study drug ≤ 3 days	1.45	0.93 - 2.26	0.0977

Conclusions: Treatment with ≤ 3 days of IV ERV was identified as a potentially modifiable, independent risk factor for a change in microbiologic outcome between EOT and PT in patients enrolled in this study. Further investigation of IV-only ERV for the treatment of cUTI is warranted.

Author Disclosure Block:

L. Tsai: D. Employee; Self; Tetraphase Pharmaceuticals, Inc. **A. Das:** J. Scientific Advisor (Review Panel or Advisory Committee); Self; Tetraphase Pharmaceuticals, Inc. **J. Mohr:** D. Employee; Self; Tetraphase Pharmaceuticals, Inc. **P. Scoble:** D. Employee; Self; Tetraphase Pharmaceuticals, Inc. **P. Horn:** D. Employee; Self; Tetraphase Pharmaceuticals, Inc. **A. Shorr:** J. Scientific Advisor (Review Panel or Advisory Committee); Self; Tetraphase Pharmaceuticals, Inc..

Poster Board Number:

MONDAY-267

Publishing Title:

Outcomes and Development of Resistance of Fosfomycin Use in Extended-spectrum *Beta-Lactamases (Esbl) Enterobacteriaceae* Urinary Tract Infection (Uti) in Geriatric Patients

Author Block:

J. Quek[1], T. Ng[1], C. Teng[2]; [1]Tan Tock Seng Hosp., Singapore, Singapore, [2]Natl. Univ. of Singapore (NUS), Singapore, Singapore

Abstract Body:

Background: There is limited clinical data on oral fosfomycin in the treatment of urinary tract infections caused by *ESBL Enterobacteriaceae*. These *ESBL* organisms are often resistance to ciprofloxacin and co-trimoxazole, limiting the availability of oral treatment. In the geriatric population, renal impairment often limits the use of nitrofurantoin. We evaluated the treatment outcomes and development of resistance of fosfomycin use, for *ESBL Enterobacteriaceae* UTI, in geriatric inpatients in a Singapore hospital.**Methods:** A retrospective study was conducted on all patients who received oral fosfomycin from 1st March 2013 to 30th June 2014 in Tan Tock Seng Hospital. Only patients with *ESBL K. pneumoniae* and *E.coli* UTI were included. Data on patient characteristics, microbiology and outcomes were collected.**Results:** One hundred and fifteen patients received oral fosfomycin. The median age was 79 (IQR, inter-quartile range; 70 - 86) years old and 43 (37.4%) were males. The median Charlson score was 6 (IQR; 4 - 8). 39 (42.6%) had diabetes and median creatinine clearance was 40.4 (IQR; 27.6 - 56.5) mls/min. 77 (67.0%) were cystitis and 38(33.0%) complicated UTI. 71 (67.1%) patients had *E.coli* UTIs while 44 (38.3%) had *K.Pneumoniae* UTIs. 30 (26.1%) received active empiric antibiotics while 37 (32.2%) received an active definitive antibiotic prior to fosfomycin. Majority of patients received a single dose of fosfomycin [88 (76.5)%] with the remaining patients receiving up to 3 doses. Mortality rate was 5.2%. 81 (70.4%) patients had microbiological cure and 33 (28.7%) had recurrence within 3 months; of which, fosfomycin resistant *enterobactericeae* UTI developed in 11 (9.6%) patients. No patient developed adverse drug reactions to fosfomycin.**Conclusions:** Fosfomycin is safe and effective in treating *ESBL Enterobacteriaceae* UTI in the geriatric population. It achieved comparable microbiological cure rates as reported in other studies[1]. However resistance development may be a cause of concern.

Author Disclosure Block:

J. Quek: None. **T. Ng:** None. **C. Teng:** None.

Poster Board Number:

MONDAY-268

Publishing Title:

Activity of Ceftazidime-avibactam (CAZ-AVI) Against ESBL Positive *Enterobacteriaceae* (Entb) and CAZ-resistant *Pseudomonas aeruginosa* from Urinary Tract Infections (UTI) in Asia/South Pacific, Europe, Middle East/Africa and Latin America in the 2013 INFORM Surveillance Program

Author Block:

G. STONE[1], E. Reiszner[1], R. Badal[2], M. Hackel[2]; [1]AstraZaeneca Pharmaceuticals, Waltham, MA, [2]IHMA, Schaumburg, IL

Abstract Body:

Background: Resistance to β-lactams is increasing worldwide due in part to extended-spectrum β-lactamases (ESBL) among Gram-negative pathogens that cause UTIs, thus reducing treatment options for these infections. Avibactam (AVI) is a novel, non-β-lactam, β-lactamase inhibitor that inhibits Ambler class A, C, and some class D β- lactamases. Combining AVI with ceftazidime (CAZ) may have utility in the treatment of such UTI pathogens.**Methods:** Isolates were collected as a part of the International Network for Optimal Resistance Monitoring (INFORM) global surveillance program. UTI isolates were collected during 2013 as follows: Asia/South Pacific (AP): 766 Entb and 62 *P. aeruginosa* from 8 countries; Europe (E): 1,715 Entb and 184 *P. aeruginosa* from 19 countries; Middle East/Africa (MEA): 323 Entb and 29 *P. aeruginosa* from 5 countries; Latin America (LA): 581 Entb and 55 *P. aeruginosa* from 6 countries. MICs were determined by CLSI broth microdilution method. CAZ-AVI MICs were determined using a fixed concentration of 4 µg/ml of AVI. Entb ESBL+ phenotype was confirmed according to CLSI guidelines. CAZ-AVI FDA susceptible breakpoints: ≤8 µg/mL for Entb and *P. aeruginosa*.**Results:** MIC_{90} values (µg/ml) of CAZ-AVI against selected Gram-negative species (including phenotypically characterized ESBL+ and CAZ-NS *P. aeruginosa*) are shown below.

	MIC_{90} (µg/mL)			
Organism	AP (n)	E (n)	MEA (n)	LA (n)
Klebsiella pneumoniae ESBL -	0.5 (208)	1 (530)	1 (89)	2 (133)
K. pneumoniae ESBL+	1 (68)	1 (233)	1 (50)	2 (81)
Escherichia coli ESBL -	0.25 (334)	0.25 (679)	0.25 (137)	0.25 (240)
E. coli ESBL+	0.5 (121)	0.5 (161)	0.25 (31)	0.5 (75)
P. aeruginosa CAZ-S	4 (54)	8 (149)	32[b] (22)	8 (41)
P. aeruginosa CAZ-NS	NA[a] (8)	64 (35)	NA[a] (7)	16 (14)

[a]NA - Not Applicable[b]CAZ-AVI MICs were ≤8 μg/ml (susceptible FDA breakpoint) for 82% of *P. aeruginosa* isolates in MEA**Conclusions:** CAZ-AVI showed excellent *in vitro* activity against ESBL+ Entb, CAZ-NS and *P. aeruginosa* isolates from urinary tract infections worldwide.

Author Disclosure Block:

G. Stone: D. Employee; Self; AstraZeneca. **E. Reiszner:** D. Employee; Self; AstraZeneca. **R. Badal:** D. Employee; Self; IHMA. **M. Hackel:** D. Employee; Self; IHMA.

Poster Board Number:

MONDAY-269

Publishing Title:

Exploring Antibody Responses to Uropathogenic *Escherichia coli* in Patients with Uncomplicated Utis

Author Block:

C. A. Sarkissian, V. DeOrnellas, A. Sintsova, H. H. L. Mobley; Univ. of Michigan, Ann Arbor, MI

Abstract Body:

Urinary tract infection (UTI) is the second most common infection in humans after those involving the respiratory tract. Uropathogenic *Eschericia coli* (UPEC) is the most common causative agent of UTIs in healthy individuals. Although antibiotics have generally been an effective treatment for UPEC infections, the continuous rise in antibiotic resistant strains adds significant urgency to vaccine development programs. Efforts to license an effective vaccine have so far been unsuccessful, and are in part hampered by gaps in our understanding of the immune response to UPEC infection. While some studies, as well as the high incidence of recurrent disease, suggest that antibody responses to bladder infections are weak and ineffective, these responses have not been investigated in sufficient detail. To explore UPEC-induced antibody responses, we obtained sera and bacterial specimens from a patient cohort with uncomplicated UTIs that included both individuals with no prior history of UTIs, as well as those with recurrent infections. When patients' sera were tested against a lysate of UPEC strain CFT073, we found that most patients produced antibodies that are able to recognize multiple CFT073 antigens. The specificity of the responses varied dramatically between patients with recurrent infection and those with no history of UTI. Additionally, using whole cell ELISAs, we found that each patient produced significant levels of antibodies that were specific for that patient's clinical UPEC isolate, and that antibody responses showed varied levels of cross reactivity against clinical strains isolated from other patients. In conclusion, we demonstrate that patients with uncomplicated UTIs show high levels of specific antibodies in their sera, and our future studies will focus on the ability of this antibody response to mediate bacterial clearance.

Author Disclosure Block:

C.A. Sarkissian: None. **V. DeOrnellas:** None. **A. Sintsova:** None. **H.H.L. Mobley:** None.

Poster Board Number:

MONDAY-270

Publishing Title:

***Pseudomonas aeruginosa* Urinary Tract Infections in Hospitalized Patients: Mortality and Prognostic Factors**

Author Block:

J. Lamas, L. González, J. Álvarez, A. Arca, J. Bermúdez, I. Rodríguez, M. Fernández, J. De la Fuente; Hosp. Povisa, Vigo (Pontevedra), Spain

Abstract Body:

Background: The aim of this study was to analyze the mortality and predictors of 30-day mortality among hospitalized patients with *Pseudomonas aeruginosa* urinary tract infection (PAUTI), as well as the impact of antibiotic treatment on survival.**Methods:** Patients admitted to our hospital with PAUTI or those diagnosed of PAUTI during hospitalization for other disease between September 2012 and September 2014 were included. Repeated episodes from the same patient and episodes of asymptomatic bacteriuria were excluded. Database with demographic, clinical and laboratory ítems was created. Empirical and definitive antibiotic therapy, antimicrobial resistance and all-cause mortality at 30 and 90 days were included. Kaplan-Meier curves and log-rank test were used to analyze the association of variables with 30 days survival. Multivariate analysis was made using Cox regression.**Results:** 62 hospitalized patients with PAUTI were included, with a mean age of 75 years. 51% were male. PAUTI were comunity adquired in 27% of cases. 8% meet criteria for severe sepsis or septic shock at the time of diagnosis. *Pseudomonas aeruginosa* isolates were multiresistant in 27% of cases. Definitive antibiotic treatment was inadequated in 24,2% of patients. Mortality was 17.7% at 30 days and 33.9% at 90 days. Factors associated with reduced survival at 30 days were chronic liver disease with portal hypertension (P <0.01), diabetes mellitus (P = 0.04) chronic renal failure (P = 0.02), severe sepsis or septic shock (P <0.01), Charlson index > 3 (P = 0.02) and inadequated definitive antibiotic treatment (P <0.01). Independent risk factors for mortality in multivariate analysis were advanced chronic liver disease (HR 77,4; P<0,01), diabetes mellitus (HR 3,6; P=0,04), chronic renal failure (HR 4,1; P=0,03) and inadequated definitive antimicrobial treatment (HR 6,8; P=0,01).**Conclusions:** PAUTI are associated with high mortality in hospitalized patients, which increases significantly in those with severe comorbidity such as chronic renal failure, advanced liver disease or diabetes mellitus. Inadequated antibiotic treatment is associated with poor outcome, which remarks the importance of adjusting empirical antibiotic treatment based on the microbiological susceptibility results.

Author Disclosure Block:

J. Lamas: None. **L. González:** None. **J. Álvarez:** None. **A. Arca:** None. **J. Bermúdez:** None. **I. Rodríguez:** None. **M. Fernández:** None. **J. De la Fuente:** None.

Poster Board Number:

MONDAY-271

Publishing Title:

Persistence and Quiescence in Uropathogenic *Escherichia coli*

Author Block:

E. Pelton, William Law, Paul S. Cohen and Jodi Camberg; Univ. of Rhode Island, Kingston, RI

Abstract Body:

Uropathogenic *Escherichia coli* (UPEC) are the leading cause of urinary tract infections, and 27% of patients experience recurrent infection even after treatment with antibiotics. The bacteria associated with recurrent infections are thought to enter dormancy and survive exposure to antibiotics. Persister cells, which are a subpopulation of cells that are resistant to the cytotoxic effects of antibiotics, have been identified in several pathogenic organisms including UPEC strains. Several mechanisms for stimulating persister cell formation in *E. coli* include toxin-antitoxin systems, starvation, gene regulation by ppGpp, and stochasticity. Here we utilize an assay to promote the development of persistence in the prototypic UPEC strain CFT073 and monitor persistence by measuring survival after treatment with ampicillin. We performed minitransposon mutagenesis using a miniTn5 to generate mutants of CFT073 that are defective for persistence. We screened 300 mutants and identified three that are defective for persistence. In addition to forming persister cells, CFT073 also undergoes metabolite-induced quiescence. Quiescence is a state of dormancy where bacteria exit from the growth and division cycle but remain viable. To understand if the two states of dormancy, persistence and quiescence, are controlled by overlapping or redundant mechanisms, we tested if the persistence mutants are also defective for quiescence. We found that all three mutants isolated undergo quiescence on minimal media when plated at low density, similar to CFT073, but are defective for persistence. These results suggest that persistence and quiescence represent two distinct states of dormancy and may further uncover the mechanisms by which recurrent UPEC infections survive antibiotic treatment in patients with urinary tract infections.

Author Disclosure Block:

E. Pelton: None.

Poster Board Number:

MONDAY-272

Publishing Title:

Evaluation of a Biomarker Based Algorithm for Diagnosis of Urinary Tract Infection in Hospitalized Patients

Author Block:

S. Das, E. Usacheva, R. Thomson, L. Peterson; NorthShore Univ. Hlth.System, Evanston, IL

Abstract Body:

Background: Diagnosis of urinary tract infection (UTI) is dependent on the accurate interpretation of a positive urine culture and careful evaluation of symptoms and can be difficult in elderly and patients with comorbidities. Despite best efforts, overtreatment of asymptomatic bacteriuria has reached epidemic proportions jeopardizing antibiotic stewardship efforts. We hypothesized that true infection is associated with host response and evaluated the utility of a urinary biomarker (microRNA 223-3p) of host response as a predictor of UTI among hospitalized patients. **Methods:** In a previous study, we derived our UTI diagnostic algorithm on 113 patients with a spectrum of findings with and without UTI. For this study, we validated the concept on 101 urine specimens from patients admitted for acute care. Clinical and laboratory data was collected from all patients. Total RNA was extracted from 1ml of whole urine and expression of microRNA-223-3p was quantified. Briefly, 10ng of RNA was reverse transcribed using specific primers followed by Taqman® assay. Signals were normalized to U6 housekeeping gene and a comparative threshold cycle (Ct) method ($2^{-\Delta Ct}$) was used to calculate relative miR expression.**Results:** Using clinical and laboratory data, patients were grouped as: a) having UTI per NHSN guidelines or b) with no evidence of UTI. Clinical and/or radiologic evidence of UTI or pyelonephritis was present in 34 patients and 7 patients had evidence of sepsis in addition to UTI. Urine culture was positive in all patients with clinical UTI, but was also positive in 73% of patients without UTI. Similarly, abnormal urinalysis had no correlation with clinical diagnosis of UTI ($p>0.5$). The median expression of miR-223-3p was 5.99 in the UTI group compared to 0.44 in the non-UTI group ($p<0.0001$). When the cut-off value for a positive relative miR 223-3p expression was set at a >2.0, the test was 91% sensitive and specific for detection of UTI. The negative predictive value of the assay was found to be 0.95 (0.86-0.98, 95% CI). The area under the receiver operating characteristic curve (ROC) was 0.905.**Conclusions:** A diagnostic algorithm of miR-223-3p levels in urine could be a valuable tool in screening patients with suspected UTI, with culture being done only on patients exceeding a defined threshold.

Author Disclosure Block:

S. Das: None. **E. Usacheva:** None. **R. Thomson:** None. **L. Peterson:** None.

Poster Board Number:

MONDAY-273

Publishing Title:

Urinary Tract Infection in Renal Transplant Recipients in India

Author Block:

S. Sharma, R. K. Nair, D. Mukherjee; Army Hosp. Res. & Referral, Delhi, India

Abstract Body:

Background: Urinary tract infection (UTI) is the most common bacterial infection faced by renal transplant recipients(RTR). Our objective was to determine the incidence, predisposing factors and microbiological profile of UTI in renal transplant recipients in our centre.**Methods:** This was a cross-sectional observational study in which 210 renal transplant recipients were studied over one year. All patients were analysed in detail and cultures were taken when there was significant WBC count in urine microscopic examination.**Results:** Out of 210 transplant recipients, 69(32.86%) had UTI. Majority(59/69) had undergone live renal transplantation and ten cases had received cadaveric grafts. Forty nine patients had a primary infection while twenty patients had recurrences. The mean age of patients with UTI was 38.63 ± 10 years. The incidence of UTI was higher in females(42.25%) as compared to males(28.06%). Most common causative agent of UTI in RTR was Escherichia coli(72.46%), followed by Klebsiella pneumoniae(13.04%). Staphylococcus aureus(03/69), Pseudomonas aeruginosa(02/69), Proteus mirabilis(02/69), Salmonella enterica(01/69), Acinetobacter spp(01/69) and Enterococcus fecalis(01/69) were also isolated. Gram negative bacilli accounted for 94.20%(65/69) while Gram positive cocci for 5.8%(4/69) of positive cultures. Multidrug resistance was highest in Klebsiella pneumonia(100%). Fifteen transplant recipients with UTI were detected to have underlying urinary tract abnormalities, most common being urethral stricture(60%) and ureteral stricture(13.33%). One patient was detected to have broken double J stent in renal pelvis. Forty eight patients(69.57%) developed acute graft dysfunction secondary to UTI. Female sex($p=0.038$), urinary tract abnormality($p<0.01$), prolonged Foley's catheterization($p<0.01$), prolonged post-transplant hospitalization($p<0.01$), New onset diabetes after transplantation(NODAT)($p<0.01$) and coexisting HCV infection($p=0.012$) were statistically significant predisposing factors for UTI in RTR.**Conclusions:** The incidence of UTI is related strongly with female sex, urinary tract abnormality, prolonged hospitalization/indwelling catheterization and immunomodulatory conditions like NODAT & HCV infection. Patients with these predisposing factors should undergo regular surveillance for UTI.

Author Disclosure Block:

S. Sharma: None. **R.K. Nair:** None. **D. Mukherjee:** None.

Poster Board Number:

MONDAY-274

Publishing Title:

Antibiotic and Bacteriophage Susceptibility of Uropathogenic "*Klebsiella pneumoniae*"

Author Block:

M. Fursov[1], A. Lev[2], E. Astashkin[2], V. Myakinina[2], N. Kartsev[2], N. Volozhantsev[2], N. Fursova[2]; [1]RCB RAS, Moscow, Russian Federation, [2]SRCAMB, Obolensk, Russian Federation

Abstract Body:

Background: "*Klebsiella pneumoniae"* is one of the causative agents of urinary tract infections (UTI). The progressive increasing antimicrobial multi drug resistance (MDR) among uropathogens during last decades become a healthcare problem worldwide. Phage therapy considered as alternative and complementary method for antibiotic therapy as part of personalized medicine.**Methods:**Three "*K. pneumoniae*" isolates were collected from 23-year man with chronic inflammation of the bladder. Bacterial identification was done using VITEK-2 (bioMerieux, France), MALDI-TOF Biotyper (Bruker, Germany). The antibiotic susceptibility was determined by VITEK-2 (bioMerieux, France). The susceptibility to 30 Klebsiella phages was determined by the spot test. Resistance genes (bla_{TEM}, bla_{SHV}, bla_{CTX-M}, int1) and virulence genes (*rmpA, aer, uge2, wabG, kfu, fimH, allS*) were detected by PCR. Strain genotyping was performed by Random Amplified Polymorphic DNA (RAPD-PCR) method.**Results:** Three "*K. pneumoniae*" isolates X1, X2 and X3 had MDR phenotype and were resistant to 7 functional classes of antibacterials: beta-lactams, aminoglycosides, quinolones, tetracycline, nitrofurantoin, chloramphenicol, and sulfonamides; sensitive to cefoxitin, tigecycline and carbapenems. The mechanism of MDR determined by beta-lactamase genes bla_{CTX-M}, bla_{TEM} and bla_{SHV}, and class 1 integrons. There are three virulence genes in the genomes of all three isolates: *allS, fimH,* and *wabG*. Virulence gene *uge* was detected additionally in X1 and X3 isolates. Intraspecies differences of isolates were identified by genotyping and phagotyping. Isolates X1 and X3 belong to subtype A, and X2 isolate - to subtype B. Subtype A was sensitive to 17 of 30 used phages, and subtype B - to only one phage. Latter bacteriophage had the ability to lyse all three "*K. pneumoniae*" isolates. It was recommended for phage therapy.**Conclusions:** Three studied uropathogenic "*K. pneumoniae*" isolates demonstrate combining of MDR and virulence potentials. Genetic heterogeneity of UTI pathogen was detected in one patient. Definition of resistance spectrum and phage sensitivity allows to combine antibiotic and phage therapy for realization of personalized approach to the treatment of UTI.

Author Disclosure Block:

M. Fursov: None. **A. Lev:** None. **E. Astashkin:** None. **V. Myakinina:** None. **N. Kartsev:** None. **N. Volozhantsev:** None. **N. Fursova:** None.

Poster Board Number:

MONDAY-275

Publishing Title:

European Multicenter Study on Antimicrobial Resistance in Companion Animal Urinary Tract Infection

Author Block:

C. Marques, UTIR-VNET, C. Pomba; CIISA (UIC/CVT/00276/2013), Faculty of Vet. Med.-UL, Lisbon, Portugal

Abstract Body:

Despite the growing concerns about antimicrobial resistance in companion animals (CA), there is a lack of studies comparing resistance between countries. This multicenter study aimed to investigate the geographical and temporal trends of antimicrobial-resistant bacteria in CA with urinary tract infection (UTI) in Europe.Susceptibility data on 22256 bacterial isolates obtained, between 2008-2013, from CA with UTI from 14 European countries were analyzed. The antimicrobial resistance frequency for the most common uropathogens was determined for each country individually for 2012-2013 using clinical breakpoints used in each laboratory, following the example of EARS-Net surveillance program reports. Fully-resistant isolates to three or more antimicrobial categories were considered multidrug resistant (MDR). Temporal trends of resistance were established by logistic regression.Overall, Southern countries (SC) showed higher percentage of antimicrobial resistance in *Escherichia coli*, *Proteus* spp. and *Staphylococcus* spp. than Northern countries (NC). Resistance to third generation cephalosporins (3GC) was high for *E. coli* in SC: Portugal (31.2%), Italy (24.6%) and Spain (21.2%). *E. coli* fluoroquinolone (FLU) resistance was high in Spain (29.7%), Italy (29.0%) and Portugal (24.0%). The lower percentages of 3GC-resistant *E. coli* were found in the Netherlands (3.8%), Denmark (4.3%) and Austria (5.6%). Sweden, Denmark, Belgium and the Netherlands had less than 10% FLU resistant *E. coli*. Less than 16% *E. coli* were MDR in all countries, except Portugal (24.0%), Spain (29.7%) and Italy (29.0%). The Netherlands and Switzerland had a significant increase in *E. coli* amoxicillin/clavulanate (AMC) and gentamicin (CN) resistance, respectively. *Proteus* spp. from Belgium also showed a rising trend in FLU resistance. Belgium had a significant decrease in *E. coli* resistance to all antimicrobials. Denmark (AMC, FLU, trimethoprim/sulfamethoxazole [SXT]), France (3GC, FLU), the Netherlands (3GC, FLU, SXT, MDR) and Sweden (CN, MDR) also had significant decreases in *E. coli* resistance over time.This work brings new insights into the current European antimicrobial resistance scenario of CA UTI-pathogens and reinforces the need for strategies aiming to reduce resistance, especially in SC, where the highest resistance levels occur.

Author Disclosure Block:

C. Marques: None. **C. Pomba:** None.

Poster Board Number:

MONDAY-276

Publishing Title:

Rapid and Joint Detection of Three Childhood Pneumoniapathogens Using AllGlo Quadruplex Quantitative PCR

Author Block:

d. Yu, Sr.; Hangzhou First People's Hosp., Hangzhou, China

Abstract Body:

Background: Pneumonia caused by an acute respiratory infection (ARI) is a common disease and continues to be the leading killer of children worldwide. Co-infection of *Mycoplasma pneumoniae* (MP), Epstein-Barr virus (EBV) and human cytomegalovirus (HCMV) is a recently developed ARI cause that poses a serious threat to children's health. Therefore, an accurate and rapid test that can simultaneously detect and differentiate among MP, EBV and HCMV should be established to aid in diagnosis. **Methods:** In this study, we established a quadruplex real-time quantitative PCR (qPCR) method to rapidly identify and simultaneously detect a single infection or co-infection of these three widespread children pneumonia pathogens and an internal control in a single tube using AllGlo fluorescent probes. The quadruplex qPCR system was optimized and evaluated in this assay. **Results:** The analysis demonstrated a high sensitivity of 10^1 copies/test, a wide linear range of detection from 10^1 to 10^8 copies/test, and a low coefficient of variation of less than 5%. The amplification efficiencies for MP, EBV, HCMV and the internal control were 1.04, 1.06, 1.03 and 0.96, respectively, and the correlation coefficients (r^2) of the standard curves were all greater than 0.99. In a comparative reagent test, we found no significant difference between TaKaRa™ and Vazyme™ ($p > 0.05$). Moreover, the results of tests on clinical samples using AllGlo quadruplex qPCR and TaqMan uniplex qPCR were in near perfect agreement (kappa value = 0.97). **Conclusions:** The diagnosis of a single infection or co-infection with three children pneumonia pathogens (MP, EBV, HCMV) in a single tube by multiplex quantitative PCR is possible. This assay will improve clinical differential diagnosis capability, and allow better disease surveillance and controlled outcomes. Application of this promising technique for evaluating children with pneumonia as a screening or confirmation tool might enhance clinical decision making during the early stage of pathogen infection, thereby reducing the overuse of antibiotics and improving patient prognosis.

Author Disclosure Block:

D. Yu: None.

Poster Board Number:

MONDAY-277

Publishing Title:

Performance and Cost-effectiveness of Verigene Rp Flex

Author Block:

A. Silva, R. LaCount, L. Ballard, B. Milham; St. Marys Hosp. and Regional Med. Ctr., Grand Junction, CO

Abstract Body:

Background: Until recently, none of the multiplex respiratory pathogen tests alone could provide the flexibility to satisfy the clinician's desire for both targeted and broad respiratory pathogen testing in a cost-effective manner for the lab and patient. Verigene RP *Flex* allows the user to choose any combination of the 16 viral and bacterial targets for an individual sample at the time of test ordering based on the clinician and patient's needs and pay just for the targets reported. In this study, we determined the analytical performance of RP *Flex* using NP washes and NP eswabs and analyzed the cost-effectiveness of flexible testing with RP *Flex* relative to St. Mary's previous respiratory algorithm and use of broad respiratory viral panel (RVP) only. **Methods:** A combination of clinical (n=22), contrived (n=28) and characterized control (n=6) specimens were tested. The clinical NP eswabs and NP wash specimens were prospectively collected between December 2014 and May 2015 and were frozen at -80°C. Contrived specimens were made by spiking UTM, eswab and NP washes with known positive specimens provided by Zeptometrix. The characterized controls were provided by Zeptometrix. The financial analysis was performed using testing volumes and pricing from 2013 and list pricing for RP *Flex* and a comparable RVP. **Results:** Results of the method comparison are presented in Table 1. RP Flex positive and negative agreement was 100% across all sample types. In 2013, a total of 1,234 flu EIA, 76 flu serotypings, 232 RSV EIA, 2 flu/RSV PCR, 1 Adv PCR, 37 EV PCR, 135 pertussis PCR, and 77 respiratory viral panel tests were sent to a reference lab for testing in 2013, totaling $191,523 in charges. Use of a broad RVP in-house at $130 per test for this testing would have cost $226,690. Use of RP *Flex* with flexible pricing would have cost only $94,860.

Target	**TP**	**TN**	**FP**	**FN**	**PPA**	**NPA**
Adv	17	39	0	0	100%	100%
hMPV	17	39	0	0	100%	100%
Flu A	17	39	0	0	100%	100%
Flu A/H1	13	43	0	0	100%	100%
Flu A/H3	17	39	0	0	100%	100%

Flu B	23	33	0	0	100%	100%
Para 1	17	39	0	0	100%	100%
Para 2	17	39	0	0	100%	100%
Para 3	13	43	0	0	100%	100%
Para 4	8	48	0	0	100%	100%
hRhv	16	40	0	0	100%	100%
RSV A	20	36	0	0	100%	100%
RSV B	30	16	0	0	100%	100%
B. pertussis	13	43	0	0	100%	100%
B. parapertussis/ brochiseptica	6	50	0	0	100%	100%
B. holmesii	14	42	0	0	100%	100%

Conclusion: With RP *Flex*, labs of all sizes can offer a respiratory pathogen testing algorithm that fully addresses clinician and patient needs in a format that minimizes the financial and resource burden on the laboratory.

Author Disclosure Block:

A. Silva: None. **R. LaCount:** None. **L. Ballard:** None. **B. Milham:** None.

Poster Board Number:

MONDAY-278

Publishing Title:

Comparison of the Simplexa™ and Solana™ Group A Strep Assays

Author Block:

M. England, P. Erdman, J. Merlo, C. Lowe, D. Myers, D. Craft; Penn State Hershey Med. Ctr., Hershey, PA

Abstract Body:

Introduction: Group A *Streptococcus* (GAS) is the most common cause of bacterial pharyngitis and may lead to non-suppurative sequelae associated with high morbidity and mortality. POC rapid antigen tests are highly specific but have poor sensitivity. Backup conventional cultures require 24-48 hours to final report. Molecular assays provide a means for faster and more sensitive reporting. We evaluated the Simplexa™ (Focus Diagnostics) Group A Strep Direct assay and Solana™ (Quidel Corp) GAS assay for their ability to replace culture for rapid antigen negative specimens.**Methods:** We received 252 throat swab specimens for throat culture. Swabs were rinsed in 250 µl of Amies buffer (sample) and planted to BAP and SSA plates for routine workup. The Simplexa™ and Solana™ were then set up with 50 µl of sample each. A second SSA plate was planted with 50 µl of sample to control for proper rinsing. An additional 50 µl was kept for discrepant analysis by the Lyra® Direct Strep Assay (Quidel Corp).**Results:** Overall, the positivity rate was 11-12%. The average patient age was 21.9y (18m-73y), and the highest positivity rate came from patients aged 5-17y (16/98). There were 3 culture positive/molecular assay negative specimens. MALDI-TOF identified 1 as *S. constellatus* and 2 as *S. pyogenes*. The sensitivity and specificity of the Solana™ were 84.4% and 97.7% respectively, whereas the Simplexa™ had a sensitivity of 93.8% and specificity of 98.2%.

	Solana™	Simplexa™	Culture
True Pos	27	30	30
False Pos	5	4	1
True Neg	215	216	219
False Neg	5	2	2
Total	252	252	252

Conclusions: The overall positivity rate (11-12%) of culture and molecular assays suggest that the number of POC false-negative rapid antigen tests is consistent with reported sensitivities (70-85%). Backup culture proved to be a sensitive method for testing GAS and fared better than or equal to the molecular assays. Both molecular assays had high specificity, but were not as

sensitive as expected in this study. Although we analyzed both molecular assays according to an agreed upon procedure, the overall protocol differed slightly from the manufacturers' package insert. We cannot rule out that the changes had an effect on the sensitivity of either assay.

Author Disclosure Block:

M. England: None. **P. Erdman:** None. **J. Merlo:** None. **C. Lowe:** None. **D. Myers:** None. **D. Craft:** None.

Poster Board Number:

MONDAY-279

Publishing Title:

Comparison Of Methods For Dispersion Of *mycoplasma Pneumoniae* Aggregates

Author Block:

A. H. Totten, L. Xiao, D. M. Crabb, T. P. Atkinson; Univ. of Alabama at Birmingham, Birmingham, AL

Abstract Body:

Background: *Mycoplasma pneumoniae* (Mpn), one of the smallest self-replicating prokaryotes, is known to readily adhere to host cells and to form aggregates in suspension. The fact that the organism has only a single membrane, like eukaryotic cells, presents questions as to optimal aggregate disruption method while minimizing cell death *in vitro*. We compared conventional vortex mixing with other methods for disruption of bacterial aggregates and for its effect on cell viability.**Methods:** UAB PO1, a clinical Mpn isolate, was dispersed via vortex mixer, a probe-type ultrasonicator, repeated 27 gauge needle passage and nonionic detergent (0.1% Tween-20). The resulting suspensions were then plated to measure recoverable CFU / mL *in vitro*. Flow cytometry was carried out to examine membrane integrity with the transmembrane potential dye $DiBAC_4$. Wet Scanning Transmission Electron Microscopy (Wet-STEM) was also performed for high resolution imaging of the resultant cell suspensions.**Results:** Comparison between dispersion methods with Mpn showed a 10-fold enrichment of recoverable CFU / mL *in vitro* with sonication compared to other methods. Time course analysis showed significantly lower bacterial CFU with vortexing compared to sonication at all time points. Flow cytometric analysis showed less cellular membrane damage via $DiBAC_4$ staining in sonicated suspensions. Wet-STEM imaging showed markedly improved dispersion with sonication compared to conventional vortex treatment.**Conclusions:** Sonication is superior to vortexing, repeated 27 gauge needle passage, or nonionic detergent for dispersion of Mpn aggregates while preserving cell viability. Preparation of Mpn suspensions for experiments should be done using brief sonication due to the dramatic decrease in CFU induced by extended vortexing.

Author Disclosure Block:

A.H. Totten: None. **L. Xiao:** None. **D.M. Crabb:** None. **T.P. Atkinson:** None.

Poster Board Number:

MONDAY-280

Publishing Title:

The Evaluation of Two Single-Analyte Assays and One Multi-Analyte Assay Utilizing Specimens Submitted for *B. pertussis* PCR

Author Block:

L. M. Kirkpatrick, A. M. Budgin, J. J. Manaloor, T. E. Davis, R. R. Relich; Indiana Univ. Sch. of Med., Indianapolis, IN

Abstract Body:

Background: In recent years, the laboratory diagnosis of pertussis has dramatically changed. Once considered the gold standard, culture has largely been replaced by molecular methods. To diagnose *B. pertussis* infection, our institution performs a laboratory-developed assay, which is performed once per day. Although it provides a rapid turnaround time and better sensitivity compared to culture, delays in patient care are possible. Recently, other molecular assays that offer faster turnaround times have become available. To evaluate the clinical impact and cost savings of these tests, we compared our assay to the *illumigene*® Pertussis Assay, the AmpliVue® Bordetella Assay, and the FilmArray® Respiratory Panel (RP). **Methods:** A total of 120 de-identified nasopharyngeal swab specimens submitted for *B. pertussis* PCR and known to be positive or negative were tested using the *illumigene*® and AmpliVue® assays. A smaller subset of these specimens (n = 60) were tested using the RP assay. Flocked nylon swabs submitted in either M6 or BD universal transport media (UTM) were first tested by the standard of care method and then stored at -80°C until testing. Analytical parameters, including sensitivity, specificity, and negative and positive predictive values, were calculated for each assay and compared. In addition, a cost-benefit analysis was conducted.**Results:** The sensitivity and specificity for the *illumigene* and AmpliVue assays were 69% and 85%, and 99% and 98%, respectively. The sensitivity and specificity of the RP for *B. pertussis* were 70% and 100%. Of the positive samples tested by the RP, 40% were also positive for other respiratory pathogens. Of the negative samples, 67% of specimens were positive for respiratory pathogens, including human rhinovirus/enterovirus, *M. pneumoniae*, and human parainfluenza viruses.**Conclusions:** All assays were easy to perform and provided rapid turnaround times. Of the two single-analyte assays, the AmpliVue assay performed best when utilized with UTM, and was the most cost effective alternative to in-house testing. The RP assay provided the most diagnostic value, but was the most costly. Overall, rapid single-plex and multi-plex nucleic acid tests provide clinically-meaningful information in a short turnaround time and offer a cost effective means to diagnose pertussis.

Author Disclosure Block:

L.M. Kirkpatrick: None. **A.M. Budgin:** None. **J.J. Manaloor:** None. **T.E. Davis:** None. **R.R. Relich:** None.

Poster Board Number:

MONDAY-281

Publishing Title:

Comparison of Molecular Methods and Rapid Antigen Tests with Culture in Detection of Group A Streptococcus from Throat Swab Samples

Author Block:

N. Putkuri, L. Ivaska, J. Niemelä, V. Peltola, **K. Rantakokko-Jalava**; Turku Univ. Hosp., Turku, Finland

Abstract Body:

Background: The diagnosis of streptococcal pharyngitis is most commonly confirmed with bacterial culture or a rapid antigen test. Neither of the approaches is perfect since either the time span for the final result (24-48h in culture) or the sensitivity of the methods (rapid antigen tests) is insufficient. In this study, rapid and accurate molecular methods were tested for detection of group A streptococcus (GAS) from throat swab samples and compared to five rapid antigen tests and the gold standard culture method. **Methods:** Altogether 85 throat swab samples were collected from children (1 to 16 yrs) presenting at emergency department with acute pharyngitis. Throat swab samples were collected in ESwab™ (Copan), and the leftover was stored in -70°C prior testing. Molecular methods tested were Simplexa™ GAS assay using 3M Integrated Cycler (Focus Diagnostics) and Illumigene® GAS assay (Meridian Bioscience). Rapid antigen tests included QuickVue Strep A Dipstick (Quidel), OSOM Strep A (Sekisui Diagnostics), Alere TestPack +Plus Strep A (Alere Medical Co., Ltd.), 75-75STREPA (Salofa Oy) and Ultimed Strep A (Ultimed) tests. Tests were performed as manufacturer's instructions except that the swab samples were replaced with 50µl of the Eswab transport media. **Results:** Out of 85 samples, 14 GAS positive samples were detected by culture. Both molecular assays proved to be sensitive and specific to detect GAS when compared to culture (Simplexa sensitivity 100% and specificity 94 %, Illumigene 94 % and 96 %). Specificity values were affected by the better sensitivity of the assays compared to culture; both assays established more positive results than had been initially indicated by culture. In practice, Simplexa assay required less hands on time and was easier to set up for testing. All rapid antigen tests were specific but sensitivity varied between 43-91%. QuickVue Strep A Dipstick, 75-75STREPA and Ultimed Strep A tests were the most sensitive tests (sensitivity values 86 %, 91 % and 91 %, respectively). **Conclusion:** Molecular methods are an applicable choice when considering fast and sensitive assay to replace conventional bacterial culture for the detection of GAS.

Author Disclosure Block:

N. Putkuri: None. **L. Ivaska:** None. **J. Niemelä:** None. **V. Peltola:** None. **K. Rantakokko-Jalava:** None.

Poster Board Number:

MONDAY-282

Publishing Title:

Improved Lower Respiratory Pathogen Testing from Clinical Sputum Specimens with No Pre-Processing on a Fully Automated Multiplex Molecular Testing System

Author Block:

J. Green[1], B. Graham[1], U. Spaulding[1], M. Rogatcheva[1], M. Jones[1], A. Clark[1], E. Huynh[1], D. Goldgar[1], S. Thatcher[1], B. Buchan[2], M. Faron[2]; [1]BioFire Diagnostics, Salt Lake City, UT, [2]Med. Coll. of Wisconsin, Milwaukee, WI

Abstract Body:

Background: Quantitative Culture (QC) is a reliable method used to identify bacteria or fungi in sputum specimens, but requires extensive procedures (pre-treatments, colony isolation & identification) and time. Semi-quantitative culture (SQC), while less accurate, is commonly used because it is easier and faster. Both methods are limited in their ability to distinguish multiple species, either from background oropharyngeal flora or co-infections (both common in sputum) without extra labor. BioFire is developing a multiplex molecular test to detect lower respiratory pathogenic bacteria, viruses, fungi, and several antibiotic resistance markers in an hour without sample pre-treatments.**Methods:** A prospective study was conducted using 48 residual fresh sputum and endotracheal aspirate specimens submitted for standard of care testing at the Medical College of Wisconsin, USA. Detections and quantity were characterized by 4 independent methods: SQC, QC (not normally performed), and prototype molecular tests with sputum and digested sputum.**Results:** 67% of specimens were positive by prototype FilmArray compared to 44%/52% (SQC/QC) by culture. FilmArray detections were 100%/96% (SQC/QC) concordant with culture while also providing viral (9%) and antibiotic resistance marker (8%) detections not available by culture. The total number of pathogens detected at a meaningful level (≥10^5 cfu/ml) was higher for sputum (treated & untreated) tested on FilmArray than traditional culture; co-detection rates of 2.4 (FA), 0.9 (SQC), and 1.7 (QC).**Conclusions:** Sputum specimens tested without pre-processing on the FilmArray system provide more comprehensive results compared to traditional SQC. QC provides expanded results as compared to SQC, but at the cost of increased processing and time to result. FilmArray identifies more meaningful LRTI pathogens than culture methods, and requires only 2 minutes to load sample into a fully automated proprietary sputum specimen preparation; all of which improves pathogen identification and patent care.*The FilmArray LRTI Panel has not been evaluated by the FDA or other regulatory agencies for* in vitro *diagnostic use*.

Author Disclosure Block:

J. Green: D. Employee; Self; BioFire Diagnostics, LLC. **B. Graham:** D. Employee; Self; BioFire Diagnostics, LLC. **U. Spaulding:** D. Employee; Self; BioFire Diagnostics, LLC. **M.**

Rogatcheva: D. Employee; Self; BioFire Diagnostics, LLC. **M. Jones:** D. Employee; Self; BioFire Diagnostics, LLC. **A. Clark:** D. Employee; Self; BioFire Diagnostics, LLC. **E. Huynh:** D. Employee; Self; BioFire Diagnostics, LLC. **D. Goldgar:** D. Employee; Self; BioFire Diagnostics, LLC. **S. Thatcher:** D. Employee; Self; BioFire Diagnostics, LLC. **B. Buchan:** F. Investigator; Self; BioFire Diagnostics, LLC. **M. Faron:** F. Investigator; Self; BioFire Diagnostics, LLC.

Poster Board Number:

MONDAY-283

Publishing Title:

A Novel Lamp Platform Enabling Rapid Poc Diagnosis Using a Multi-Well Chip

Author Block:

M. Sato, A. Shoji, S. Semba, N. Hosaka, T. Yonekawa, Y. Segawa, H. Watanabe, T. Notomi; Eiken Chemical Co.,Ltd., Tochigi, Japan

Abstract Body:

Background: In infectious diseases, rapid identification of pathogens is very important for prompt and appropriate treatment. Recently many technologies for Point-of-Care (POC) diagnosis by nucleic acid testing have been emerging. However, there are still high expectations for further improvement of accuracy, rapidity and coverage of pathogens in medical practice. Therefore, we have been developing a novel system of Loop-mediated isothermal amplification (LAMP) platform which enables a 15 minutes detection of multiple pathogens genomes. The system is characterized by a microfluidic chip having 25 wells with dry form reagent for LAMP and an amplification-to-detection device optimized for the chip.**Methods:** The analytical performance of this system was evaluated by using newly designed LAMP primers and probes for the respiratory infectious diseases panel; *Bordetella pertussis* (BP), *Mycoplasma pneumoniae* (MP), *Chlamydophila pneumoniae* (CP) and *Legionella* (LG). Purified DNA was injected into the chip automatically with pumpless injection technology and the reaction completed after 15 minutes under isothermal condition (64°C) in the device. The amplification signal of each well was measured in real time by quenching method. **Results:** Limit of Detection (LoD) test was performed with plasmid DNA having target sequence of BP, CP, MP and LG and LoD for each target was 1.56, 1.88, <0.63 and <0.63 copies per test respectively (5 wells were used for 1 test and 1 well equals to 5µl). The test of specificity and cross-reactivity was performed with purified bacteria genomes from ATCC strains and clinical isolates. As a result of specificity test for 10 BP strains, 5 MP strains, 6 CP strains and 23 LG strains, only the target genome was detected and there was no cross-reaction. There was also no cross-reaction with the other 46 pathogenic bacteria species genome. **Conclusions:** These findings indicate the capability of the system to detect multiple pathogens simultaneously, rapidly and easily with high sensitivity and specificity. Although we reported 4-pathogens panel in this study, the chip is applicable to test up to 25 pathogens including controls. Furthermore our goal is set to develop the fully automated system enabling rapid POC diagnosis (<30min), which integrates this system and nucleic acid extraction system under development.

Author Disclosure Block:

M. Sato: None. **A. Shoji:** None. **S. Semba:** None. **N. Hosaka:** None. **T. Yonekawa:** None. **Y. Segawa:** None. **H. Watanabe:** None. **T. Notomi:** None.

Poster Board Number:

MONDAY-284

Publishing Title:

Semi-quantitative Analysis of Bacterial Pathogens in Bronchial Alveolar Lavage (Bal) Specimens Using the Pilot Filmarray Lower Respiratory Tract Infection (Lrti) Multiplex Molecular Test

Author Block:

M. L. Faron[1], N. A. Ledeboer[1], **H. Seliger**[1], J. Connolly[1], M. Rogatcheva[2], J. J. Gilbreath[2], B. W. Buchan[1]; [1]Med. Coll. of Wisconsin, MIlwaukee, WI, [2]BioFire Diagnostics, LLC, Salt Lake City, UT

Abstract Body:

Background: Pneumonia is a serious medical condition associated with bacterial, viral and fungal agents. Some bacterial agents are normal inhabitants of the upper airway and have fastidious growth requirements. These factors complicate interpretation of bacterial culture results. Quantitative bacterial thresholds have been proposed to aid in assigning clinical significance. We evaluate a pilot, Research Use Only (RUO) version of the FilmArray LRTI Panel (LRTI) and correlate results with quantitative culture.**Methods:** BAL specimens (n=73) were tested using the LRTI and quantitative bacterial culture. LRTI reports semi-quantitative results for 15 bacterial targets, in addition to qualitative results for 14 other bacterial, viral, or fungal agents. LRTI results are reported as "Not Detected" or values of "10^4", "10^5", "10^6", or ">10^7". Quantitative cultures were reported with quantitation of potential pathogens present at 10^3 to 10^5 and ">10^5" CFU/mL. Potential pathogens were not specifically identified if normal flora was present at >10^5 CFU/mL.**Results:** Culture identified 13 potential pathogens, 10 of which were reported 10^4 CFU/mL or greater (i.e. clinically significant). LRTI identified all 13 culture positive specimens; however, semi-quantitative results were at least 10-fold higher for 11/13 targets. This included 3 targets that were reported at a concentration of 10^3 by culture (one each, *S. aureus, S. agalactiae, P. aeruginosa*). An additional 8 targets were reported as positive by LRTI at 10^4-10^7 that were not detected by culture. Five of these (*H. influenza, M. catarhalis, P. aeruginosa, S. marcescens, S. pneumoniae*) were in cultures containing normal oral flora at >10^5 CFU/mL. The remaining 3 cultures had a second pathogen that was predominant, potentially resulting in failure to identify the pathogen of lesser quantity.**Conclusions:** The LRTI Panel was in categorical agreement (i.e. 10^4 or greater) with culture results for 10/13 bacteria reported by culture method. LRTI also identified 8 bacterial targets not reported by culture. The significance of these additional positive results require correlation with clinical outcomes.

Author Disclosure Block:

M.L. Faron: None. **N.A. Ledeboer:** None. **H. Seliger:** None. **J. Connolly:** None. **M. Rogatcheva:** D. Employee; Self; BioFire Diagnostics. **J.J. Gilbreath:** D. Employee; Self; BioFire Diagnostics. **B.W. Buchan:** H. Research Contractor; Self; BioFire.

Poster Board Number:

MONDAY-285

Publishing Title:

Surveillance for Low Pathogenic Avian Influenza Virus Antibodies in Domestic and Peri-Domestic Birds in Two States of Nigeria

Author Block:

A. Adebiyi, D. Oluwayelu, O. Gamra, a. Jagun-Jubril; Univ. of Ibadan, Ibadan, Nigeria

Abstract Body:

Flock surveillance for avian influenza (AI) plays a crucial role in establishing the epidemiological characteristics of the disease especially in countries where vaccination is not practiced. The recent cases of AI in Nigeria are of concern not only to the poultry industry but also to public health. However, current AI surveillance is tilted towards the highly pathogenic disease which may bias understanding of the true extent of involvement of less pathogenic strains such as low pathogenic AI virus (LPAIV) H5, H7 and H9 that have been reported to cause outbreaks of highly pathogenic AI. Therefore, as part of on-going AI surveillance in southwest Nigeria, sera from domestic and peri-domestic birds including Japanese quails, village weavers and laughing dove were screened for antibodies to LPAI viruses (LPAIV).A competitive ELISA was used for detecting AIV-specific antibodies in the sera of 101 unvaccinated Japanese quails from ten flocks in Osun state, as well as 30 village weavers from a live-bird market and one laughing dove caught for human consumption in Oyo state, Nigeria. Haemagglutination inhibiting antibodies against LPAIV were then detected in the ELISA-positive sera using H3N8, H5N2 and H9N7 subtype-specific antigens. In quails, the AI seroprevalence obtained by ELISA was 18.8% (19/101). Of the 19 ELISA-positive samples, 5 were positive for anti-LPAIV H9N7 antibodies only while 11 were positive for anti-LPAIV H3N8 and H9N7 antibodies, and 3 were positive for antibodies against the three LPAIV subtypes, indicating a mixed infection. The laughing dove serum was positive for only anti-LPAIV H9N7 antibodies while all sera from village weavers had no detectable LPAIV antibodies.This study provides serologic evidence of infection with LPAIV H3N8, H5N2 and H9N7 in Japanese quails and laughing dove in southwest Nigeria. The findings highlight the potential role of Japanese quails in the epidemiology of AI and stress the need for continuous monitoring of different avian species in order to provide an early warning system for implementation of AI control strategies. We recommend that quails, which are rapidly gaining popularity as a cheap source of animal protein in Nigeria, should not be reared together with commercial or backyard chickens.

Author Disclosure Block:

A. Adebiyi: None. **D. Oluwayelu:** None. **O. Gamra:** None. **A. Jagun-Jubril:** None.

Poster Board Number:

MONDAY-286

Publishing Title:

Verification and Impact of a New Influenza A/B Platform at a Major Military Medical Center

Author Block:

L. E. NIELSEN, G. Brusstar, B. Pagaoa, J. Anderson, C. Lanteri, E. Ager; San Antonio Military Med. Ctr., San Antonio, TX

Abstract Body:

Background: Respiratory illnesses caused by Influenza (Flu) A and B are common between Nov-April. Symptoms of Flu are similar to other respiratory viruses, therefore, detection becomes necessary for antiviral therapy, epidemiology, and vaccine development purposes. Several diagnostics platforms are available; many being an enzyme-linked immunoassay (EIA) or real-time polymerase chain reaction (RT-PCR) based systems. During the 2014-15 season, our laboratory tested >5,500 specimens from across the hospital and its six associated outlying clinics using the Quidel Sofia EIA and the GeneXpert Infinity (Cepheid) RT-PCR system with a combined turn-around-time (TAT) of 2 hrs. This year, all suspected Influenza specimens are tested solely on the point-of-care, CLIA-waived Cobas (Roche) lab-in-a-tube (LIAT) system that has combined the TAT (20 min) of an EIA with the sensitivity of RT-PCR. The purpose of this study was to evaluate methods to determine which system(s) are most advantageous for routine Flu testing and highlight the transformation of workload for multi-center patient testing laboratories. **Methods:** Nasopharyngeal swab specimens were tested for the presence of Influenza A and B virus using the Quidel Sofia EIA, the Cepheid RT-PCR, and the Roche LIAT diagnostic platforms following manufacturer's recommendations. Extracted RNA was used to determine limits of detection (LOD) for each platform. Test results were compared for accuracy, sensitivity, TAT, workflow, LOD and cost. **Results:** Concordance between specimens tested using RT-PCR vs. EIA assays were 80% for negative samples, 50% for Influenza A, and 80% for Influenza B supporting the lower sensitivity of EIA platforms and validating the need for RT-PCR testing. TAT was decreased by 83% and cost from $60 using both the Sofia and Cepheid systems to $30 per sample. The lower LODs for Sofia EIA was 1000-times higher than the LIAT system. **Conclusions:** The LIAT RT-PCR assay offers a similar cost and complexity to EIA platforms but increases accuracy and sensitivity of Influenza A/B detection making reliable and accurate point-of-care, CLIA-waived testing possible. This new technology has allowed us to increase the time to patient result and provide testing at all outlying clinics, allowing the provider to prescribed anti-viral therapy while the patient is still in the clinic.

Author Disclosure Block:

L.E. Nielsen: None. **G. Brusstar:** None. **B. Pagaoa:** None. **J. Anderson:** None. **C. Lanteri:** None. **E. Ager:** None.

Poster Board Number:

MONDAY-287

Publishing Title:

Evaluation of Novel Respiratory Assays on a Fully Automated System for Competitive Inhibition with Co-infection, Cross Reactivity, and Vtm Compatibility

Author Block:

P. Douglass, M. Jost, A. Narwold, D. Li, J. Zowalki, P. Quinones, **C. R. Hentzen**, C. Nugent, D. P. Kolk; Hologic, San Diego, CA

Abstract Body:

Background: Respiratory viral infections remain a leading cause of infectious diseases worldwide. Most respiratory viruses present with similar symptoms and many patients have more than one viral agent. Clinicians rely on accurate and rapid molecular diagnostics to identify virus(es) to ensure appropriate patient management. For multiplexed panels such as the Panther Fusion Flu A/B/RSV and Paraflu Assays (in development), demonstrating reliability in detecting the appropriate virus is required. In this study, the effects of clinically relevant co-infections and commonly found micro-organisms and viruses were assessed to evaluate inhibition and performance in the Flu A/B/RSV and Paraflu Assays. A variety of commercial viral transport media (VTM) were also evaluated to demonstrate assay compatibility. **Methods:** To evaluate competitive interference in clinically relevant co-infections, viral isolates for different target analytes were spiked into simulated clinical matrix (SCM). High (3 log > LoD) and low concentration (0.5 log > LoD) of one or more target were evaluated (12 conditions; n=6/condition). 51 (Flu A/B/RSV) and 56 (Paraflu 1/2/3/4) microorganisms and viruses were tested to assess cross-reactivity. VTM equivalency was evaluated in five commercial and one in-house VTM (n=12 or 24). **Results:** For Flu A/B/RSV and Paraflu Assays, the co-infection/interference study had 100% agreement to expected result for all conditions. No cross reactivity was observed with any non-targeted organism while all control panels were positive for intended target in SCM. The positivity rate for each intended target at a level near LoD was 100% in all six VTMs. **Conclusions:** The Panther Fusion Flu A/B/RSV and Paraflu Assays demonstrated excellent reliability in detecting intended targets at clinically-relevant concentrations in simulated co-infection samples where no competitive inhibition was observed. The assays did not exhibit any cross reactivity or inhibition in the presence of other micro-organisms and viruses. In addition, the assays are compatible with the most commonly used VTMs. These data suggest the Panther Fusion Respiratory Assays are suitable for a modular approach to syndromic testing.

Author Disclosure Block:

P. Douglass: None. **M. Jost:** None. **A. Narwold:** None. **D. Li:** None. **J. Zowalki:** None. **P. Quinones:** None. **C.R. Hentzen:** None. **C. Nugent:** None. **D.P. Kolk:** None.

Poster Board Number:

MONDAY-288

Publishing Title:

Quantification of Bacterial Pathogens in Lower Respiratory Specimens with a Sample to Answer Automated Multiplexed Molecular Detection System

Author Block:

U. K. Spaulding, D. Nicholes, I. Kavetska, O. Cham, B. Graham, J. Cloud, C. Baker, J. Peterson, A. Demogines, N. Fish, M. Rogatcheva; BioFire Diagnostics, LLC, Salt Lake City, UT

Abstract Body:

Background & Objective: Typical bacteria associated with lower respiratory tract infections (LRTI) are also frequently commensals of the oropharyngeal passage. Semi-quantitative or quantitative culture methods routinely used in labs to distinguish pathogenic loads from commensal carriage have poor sensitivity, and are slow and subjective. Subject to FDA clearance, the FilmArray® LRTI Panel (FA-LRTI; BioFire Diagnostics) is expected to be a rapid (~ 1 hr), sensitive, and objective alternative for identification of bacteria, viruses, & fungi and select antibiotic resistance genes. Additionally, FA-LRTI will provide semi-quantitative results for 15 typical bacteria. This study compared titers estimated by a development version of FA-LRTI to titers obtained from culture and qPCR methods in respiratory samples (bronchoalveolar lavage (BAL) and sputum). **Method**: Performance of FA-LRTI was compared to other methods in a variety of matrices. Initially, titers of 5 bacteria (*H. influenzae, K. pneumoniae, P. aeruginosa, S. agalactiae, S. aureus*) spiked into normal saline (NS) were determined by FA-LRTI and 5 comparator methods including culture and turbidity, as well as 3 qPCR assays/organism. Next, contrived respiratory matrices spiked with a mix of same bacteria at different titers, and 38 residual specimens characterized by clinical labs were tested with FA-LRTI and qPCR. **Results**: FA-LRTI titers of organisms spiked into NS were reported to be within 0.5 to 1 log units of titers from microbiological and qPCR methods. Similar accuracy of quantification was achieved in contrived matrices. In 38 clinical samples (with 37 reported detections), FA-LRTI had 49 reportable detections ($\geq 10^4$ copies/mL) compared to 54 by qPCR assays with 95% agreement of results (within 1 log window for reportable titers) between the two methods for 14 out of 15 targeted bacteria. All but 2 of 37 bacteria reported by clinical lab were detected by FA-LRTI and qPCR assays. **Conclusion**: This study highlights the ability of FA-LRTI to provide meaningful results to aid diagnoses of bacterial LRTI.*The FilmArray LRTI Panel has not been evaluated by the FDA or other regulatory agencies for* In Vitro *Diagnostic use*.

Author Disclosure Block:

U.K. Spaulding: None. **D. Nicholes:** None. **I. Kavetska:** None. **O. Cham:** None. **B. Graham:** None. **J. Cloud:** None. **C. Baker:** None. **J. Peterson:** None. **A. Demogines:** None. **N. Fish:** None. **M. Rogatcheva:** None.

Poster Board Number:

MONDAY-289

Publishing Title:

Comparison Between the Alere Binaxnow Influenza A+b Card and the Quidel Sophia Influenza A+b Fia for the Detection of Seasonal Influenza in Rural Hospitals

Author Block:

M. Dodd[1], J. Schmetterer[2], I. Mallawaarachchi[1], K. Culbreath[3]; [1]Univ. of New Mexico, Albuquerque, NM, [2]Presbyterian Hlth.care Services, Albuquerque, NM, [3]TriCore Reference Lab., Albuquerque, NM

Abstract Body:

Background: The diagnostic accuracy of rapid influenza tests very widely with sensitivities greater than 60% for detection of seasonal influenza infections. Utilization of rapid influenza diagnostic tests (RIDTs) may be useful in timely identification of influenza infection enabling appropriate prescribing of antivirals. The purpose of this study is to evaluate the performance of RIDTs used in rural hospitals and determine if there is clinical value to implementing a more accurate test.**Method:** This retrospective study compared the performance of the Alere BinaxNOW Influenza A+B Card (Binax) from Oct 2013-Mar 2014 to the Quidel Sophia Influenza A+B FIA (Sophia) from Oct 2014-Mar 2015 for two rural New Mexico hospitals. Comparing the RIDTs results to RT-PCR, the gold standard, the percent positivity captured and sensitivity was calculated. Descriptive statistics were used to compare demographics between respiratory seasons. Two sample proportion test was used to compare percentages of positive influenza detected by the Binax and Sophia.**Results:** A total of 973 patients were tested during the 2013-2014 respiratory season and 1365 during the 2014-2015 respiratory season with no significant difference between mean age, 45.3 ± 19.8 vs. 46.0 ± 21.4 respectively ($p = 0.432$). Of the 233 influenza A+B positives, the Binax detected 74 (32.5%) positive influenza A and 2 (40%) positive influenza B while the RT-PCR captured an additional 154 (67.5%) influenza A and 3 (60%) influenza B. The Sophia detected 156 (60.5%) positive influenza A and 30 (53.6%) positive influenza B while the RT-PCR captured an additional 101 (39.5%) influenza A and 26 (46.4%) influenza B from the total 313 influenza A+B positives. The overall sensitivity of the Binax was 32.6% and the Sophia was 59.4% ($p = <0.001$).**Conclusion:** Overall, the Sofia was more sensitive than the Binax for rapid detection of seasonal influenza. However, the Sofia assay was significantly less sensitive than the molecular-based assay. These findings suggest that the Sophia may provide clinicians in rural hospitals with a more clinically reliable rapid antigen-based result for their decision to treat when molecular based testing is not as easily accessible.

Author Disclosure Block:

M. Dodd: None. **J. Schmetterer:** None. **I. Mallawaarachchi:** None. **K. Culbreath:** None.

Poster Board Number:

MONDAY-290

Publishing Title:

Evaluation of *Illumigene* mycoplasma Direct Dna Amplification Assay

Author Block:

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Abstract Body:

Background: *Mycoplasma pneumoniae* (MP) is a common cause of community acquired pneumonia in humans. The investigational use only (IUO) ***illumi****gene* Mycoplasma Direct (iMD) DNA amplification assay (Not FDA-Cleared) is a qualitative *in vitro* diagnostic test utilizing loop-mediated isothermal amplification (LAMP) technology for the direct detection of MP DNA in human respiratory specimens.**Objective:** The aim of this multicenter study was to evaluate the performance characteristics of the iMD assay as compared with the FDA-cleared ***illumi****gene* Mycoplasma (iM) assay. Both assays utilize the same LAMP technology and targets. The major improvement of iMD over iM assay is that the Qiagen DNA extraction step is not required for iMD assay. **Methods:** Three sites across United States participated in this prospective study. Subjects with symptoms of upper respiratory illness suggestive of MP were enrolled. Two throat swabs were obtained from each subject, one in M4 transport medium and one in liquid Amies. The liquid Amies swab was tested at each enrollment site by the iMD assay within 72 hours of sample collection. Reference testing with iM was performed by the manufacturer from the non-nutritive liquid Amies media within 14 days of sample collection.**Results:** Among the 385 specimens tested, the iM reference method detected MP in 24 (6.2%) specimens while iMD assay detected 23 (6.0%) samples as MP positive. There were nine false positive and one false negative detections with the iMD assay prior to discrepant evaluation. The overall positive percent agreement and the negative percent agreement was 95.8% (79.8-99.3%) and 97.5% (95.3-98.7%), respectively. The overall percent agreement was determined to be 97.4% (95.3-98.6%).**Conclusions:** The IUO ***illumi****gene* Mycoplasma Direct test results were comparable with the iM assay. The removal of the Qiagen DNA extraction step in the iMD assay simplifies testing, saves time and reduces the expense of detecting MP from throat swabs when compared to iM assay.

Author Disclosure Block:

N. Kanwar: None. **M.A. Pence:** None. **D. Mayne:** None. **R. Selvarangan:** None.

Poster Board Number:

MONDAY-291

Publishing Title:

Laboratory Evaluation of a Point of Care Molecular Test for the Detection of Influenza A and B

Author Block:

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Abstract Body:

Introduction: Rapid laboratory identification of respiratory viral agents is becoming increasingly important because of the development and use of antiviral agents and the continued emergence of new respiratory virus strains with potentially far reaching global impact. The Carolinas Medical Center has used PCR for diagnostics on in-patient and immunocompromised patients. In addition culture and Direct Fluorescent antibody (DFA) methods for the detection of respiratory viral agents were used for outpatients not sick enough to be admitted to the hospital. Testing at the point of care (physician office laboratories [POC]) was discouraged due to the poor performance of rapid cartridge methods. Recently, PCR methods have become available that are touted as more accurate than previous POC methods and easy to use.**Objective:** In this study we compared conventional PCR (real time polymerase chain reaction assays- bead based DNA amplification tests (xTAG RVP FAST)] and a rapid PCR method designed to be used in the POC (Roche LIAT).**Method:** All samples were banked previously confirmed strains or fresh collected using nasal wash, flocked swab NP, bronchial washes, or tracheal aspirates samples. The banked strains were confirmed as to their identity using PCR technology or obtained from the NC state public health laboratory. A total of 121 samples were tested. Including 92 strains of Flu A (seasonal flu strains and H1N1 strains), Flu B and all negative Flu samples were positive for other viral agents such as Parainfluenzae, RSV and adenovirus, and metapneumovirus agents were included as samples negative for influenza.**Results:** Overall, the LIAT was tested in 94 positive and 25 Flu negative samples demonstrating a sensitivity/ specificity of 100% (95% CI 95-100%)/ 93% (95% CI= 74-98.7%) respectively as compared to the xTAG RVP FAST assay.**Conclusion:** The LIAT assay is an accurate and reliable method for detecting both FLU A and B infections and offers the advantage of detecting FLU at the point of care in a timely fashion.

Author Disclosure Block:

D. Meadows: F. Investigator; Self; Roche. **J. Longshore:** F. Investigator; Self; Roche. J. Scientific Advisor (Review Panel or Advisory Committee); Self; Roche. **R.L. Sautter:** E. Grant Investigator; Self; Roche. L. Speaker's Bureau; Self; Roche.

Poster Board Number:

MONDAY-292

Publishing Title:

Laboratory Evaluation of the Biogx Flu A, Flu B, RSV Assay on the Bd Max™ System

Author Block:

C. Kubasek, S. Silbert, A. Gostnell, **R. Widen**; Tampa Gen. Hosp., Tampa, FL

Abstract Body:

Due to low sensitivity of respiratory viral rapid antigen tests and the long turn-around time of viral culture, many laboratories are using PCR-based methods for detection of respiratory viruses. The objective of this study was to evaluate the detection of Flu A, Flu B and RSV on the BD MAX™ System, using the BioGX Flu A, Flu B, RSV Sample-Ready™ kit (BioGX, Birmingham, AL) and to compare the results obtained to the Simplexa™ Flu A/B & RSV Direct assay (Focus Diagnostics, Cypress, CA), our standard of care procedure. **Methods:** A total of 200 frozen specimens (NP swabs) submitted for routine testing during the 2015 influenza/respiratory season were tested (50 Flu A, 50 Flu B and 50 RSV positive, as well as 50 negative NP samples). All frozen samples were thawed and re-tested by the Simplexa Flu A/B & RSV Direct assay following manufacture's recommendation. In parallel, a 400µL aliquot from each sample was inoculated into a BD MAX Sample Buffer Tube. Extraction and multiplex PCR were performed on the BD MAX System, using the BD MAX™ ExK™ TNA-2 extraction kit (BD, Quebec, Canada) and the BioGX assay. PCR cycling conditions were: 48°C for 900s and 99°C for 120s followed by 15 cycles of 99°C for 10s, 55°C for 26s, 72°C for 12.6s, and another 30 cycles of 99°C for 5s, 55°C for 15s and 72°C for 8.6s. The total run time (extraction and PCR) took approximately 2.5 hours. In the end, results from the standard of care Simplexa Flu A/B & RSV test were compared to the BioGX assay. **Results:** Out of 200 samples tested, 49 were Flu A, 48 were Flu B and 47 were RSV positives, and 56 were negative for Flu A, Flu B and RSV by both PCR based methods tested. Discrepant results between Simplexa and BioGX were observed in just 2 RSV samples, one of them was RSV positive just by the Simplexa assay and the other one, just by the BioGX assay. Samples previously identified as positive for Flu A (n=1), Flu B (n=2) and RSV (n=3), and negative by the BioGX and Simplexa re-testing may have undergone DNA degradation after the freezing-thawing process. **Conclusion:** The BioGX assay performed on the BD MAX System and the Simplexa Flu A/B & RSV Direct assay displayed an excellent overall agreement for the detection of Flu A, Flu B and RSV. Ultimately, the BioGX assay performed on the BD MAX System is an alternative method for respiratory virus detection in clinical samples.

Author Disclosure Block:

C. Kubasek: None. **S. Silbert:** None. **A. Gostnell:** None. **R. Widen:** None.

Poster Board Number:

MONDAY-293

Publishing Title:

Test Performance Of The Cobas Influenza A/B (Liat), Xpert Flu And The Simplexa Flu A/B Assay For The Direct Detection Of Influenza Virus In Respiratory Specimens

Author Block:

C. Y. Ying, K. Molina, A. Tavares, T. Koyamatsu, W. Kim, M. J. Bankowski; DLS (The Queen's Med. Ctr.), Aiea, HI

Abstract Body:

Background: Influenza is an acute respiratory illness that requires the most sensitive and specific test detection method from the clinical laboratory in the shortest turn-around-time. Molecular amplification testing has offered the best approach over cell culture and antigen testing. Rapid molecular assays can identify patients with Influenza and aid in more effective infection control, prevention and the decision to use anti-viral treatment. This study challenges the test performances of three molecular assays: cobas ® Influenza A/B assay, the Xpert® Flu and Simplexa™ Flu A/B Assay. **Methods:** Nasopharyngeal swabs (n=44 in M4) were collected from patients presenting with upper respiratory tract infections. Specimens were de-linked after initial testing using the Simplexa™ Flu A/B Assay (ASR) in routine use along with extraction using the MagNA Pure LC System. The cobas® Influenza A/B (Liat) Nucleic acid test assay and the Xpert® Flu A/B test performance was challenged with clinical specimens and limit of detection (LOD). Both are direct, rapid *in vitro* diagnostic tests (Liat is also CLIA-waived), which integrate nucleic acid extraction, amplification, and detection in a closed system.**Results:** A total of 44 specimens were tested using the cobas ® Influenza A/B assay and the Xpert® Flu and compared to the Simplexa™ Flu A/B Assay (ASR). The cobas ® Influenza A/B (Liat) assay showed 96.6% sensitivity and 100% specificity compared to the Xpert® Flu with 62.1% sensitivity and 100% specificity. The mean LOD for the Xpert® Flu was approximately 100-fold less sensitive than the Simplexa™ Flu A/B Assay. The cobas® Influenza A/B (Liat) assay showed 97.7% (43/44) agreement with Simplexa™ Flu A/B Assay.**Conclusions:** The cobas® Influenza A/B (Liat) was comparable in test performance to the Simplexa™ Flu A/B Assay compared to the Xpert Flu, which was less sensitive (100-fold). The cobas® Influenza A/B (Liat) assay also offers the best turn-around-time (20 minutes) compared to either the Xpert® Flu (75 minutes) or Simplexa™ Flu A/B Assay (ASR) (150 minutes).

Author Disclosure Block:

C.Y. Ying: None. **K. Molina:** None. **A. Tavares:** None. **T. Koyamatsu:** None. **W. Kim:** None. **M.J. Bankowski:** A. Board Member; Self; Roche Molecular Diagnostics.

Poster Board Number:

MONDAY-294

Publishing Title:

Multicenter Evaluation of Alere™ I Influenza A & B Assay Using Respiratory Specimens Collected in Viral Transport Media

Author Block:

F. Hassan[1], L. Hays[1], A. B. Bonner[2], J. Connolly[3], N. A. Ledeboer[3], R. Selvarangan[1]; [1]Children's Mercy Hosp., Kansas City, MO, [2]Veritas, P.A., Belton, TX, [3]Med. Coll. of Wisconsin, Milwaukee, WI

Abstract Body:

Background: Rapid and accurate detection of influenza virus is critical for proper patient management, especially in out-patient settings during influenza season. Alere™ i Influenza A & B assay is an isothermal nucleic acid amplification test capable of detecting influenza A and B viruses directly from respiratory specimens.**Objectives:** To evaluate the performance characteristics of Alere™ i Influenza A & B assay using nasal and nasopharyngeal swab specimens eluted and transported in viral transport media (M4, M6 and UTM).**Study design**: A prospective, multicenter, clinical trial was conducted at three different sites across USA to evaluate the performance of the Alere™ i Influenza A & B assay using fresh (within 24 hours of sample collection), left over, de-identified clinical specimens obtained from patients (Children =1084 and Adults= 159). The performance of Alere™ i Influenza A & B assay was compared with ProFlu(+) real-time RT-PCR assay considered as the "gold standard". All specimens with discrepant test results were further tested by FDA cleared PCR assay (CDC InfAB PCR) at Alere Scarborough, Inc.**Results:** A total of 1273 samples were enrolled in this study; 30 were excluded from final analysis. Influenza test results by Alere™ i Influenza A & B assay (250 Flu A positive, 105 Flu B positive, 6 Flu A and B positive, and 882 Flu A/B negative) were compared with ProFlu(+) real time RT-PCR results to determine test performance (see table). Following discrepant analysis by CDC developed Inf AB real-time PCR, the sensitivity and specificity of the Alere™ i Influenza A & B assay increased to 98.1% and 99.6% for influenza A, and 93.1% and 98.6% for influenza B, respectively.**Conclusions:** The Alere™ i Influenza A & B assay is an ideal assay for influenza virus detection in patients of all age groups due to its high sensitivity and specificity and ability to produce results within 15-20 minutes from time of sample collection with minimal hands on time.

Target	**True positive**	**True negative**	**False positive**	**False negative**	**%Sensitivity (95% CI)**	**%Specificity (95% CI)**
Flu A	221	982	35	5	97.8 (94.6-99.2)	96.6 (95.2-97.5)

Flu B	92	1125	19	7	92.9 (85.5-96.9)	98.3 (97.4-98.0)

Author Disclosure Block:

F. Hassan: None. **L. Hays:** None. **A.B. Bonner:** None. **J. Connolly:** None. **N.A. Ledeboer:** None. **R. Selvarangan:** None.

Poster Board Number:

MONDAY-295

Publishing Title:

Development and Evaluation of a Molecular Assay for Improved Detection and Quantification of *Human rhinovirus* Viral Load

Author Block:

K-T. Ng, J-B. Chook, X-Y. Oong, Y-F. Chan, K-G. Chan, N-S. Hanafi, Y-K. Pang, A. Kamarulzaman, K-K. Tee; Univ. of Malaya, Kuala Lumpur, Malaysia

Abstract Body:

Background: *Human rhinovirus* (HRV), a member of the *Picornaviridae* family, continues to dominate as the major cause of acute respiratory tract infections worldwide. Several studies attempted to investigate the correlation between HRV viral load and severity of respiratory illnesses. However, limitations that may affect accurate HRV quantification exist.**Methods:** We developed a one-step real-time reverse transcription-PCR (RT-PCR) assay using oligonucleotides designed based on a comprehensive list of global HRV sequences. The performance of the newly developed assay was first assessed using a 2013 rhinovirus External Quality Assessment (EQA) panel. The assay was then tested on 315 recently collected human enterovirus-positive clinical nasopharyngeal specimens, in parallel with a commercial HRV quantification kit.**Results:** The newly designed oligonucleotides highly specific to the 5′-UTR region showed high PCR efficiency (E=99.6%) and Pearson correlation coefficient (R^2=0.996), detecting and quantifying HRV RNA as low as -0.29 and 0.05$\log_{10}$ copies/µl, respectively. Performance evaluation using the EQA panel yielded a detection rate of 90%. When tested on 315 human enterovirus-positive nasopharyngeal swabs, comprising at least 84 genetically distinct HRV types/serotypes (as identified by the *VP4/VP2* gene phylogenetic analysis), the newly developed assay yielded a detection rate of 100%, detecting all HRV species and types, as well as other non-polio enteroviruses. In contrast, the commercial quantification kit, which failed to detect any of the EQA specimens, only produced a detection rate of 13.3% (42/315) among the clinical specimens. Using the improved assay, it was shown that HRV persists in the upper respiratory tract and sheds for more than a week following acute infection. In addition, HRV-C had a significantly higher viral load than HRV-A and HRV-B at 2-7 days after the onset of symptoms (p=0.001).**Conclusions:** A rapid real-time RT-PCR assay for improved HRV detection and quantification was developed. The availability of such assay for HRV detection and quantification is crucial to facilitate disease management, antiviral development, and infection control.

Author Disclosure Block:

K. Ng: None. **J. Chook:** None. **X. Oong:** None. **Y. Chan:** None. **K. Chan:** None. **N. Hanafi:** None. **Y. Pang:** None. **A. Kamarulzaman:** None. **K. Tee:** None.

Poster Board Number:

MONDAY-296

Publishing Title:

***Mycoplasma pneumoniae*: Only Who Seeks, Will Find**

Author Block:

S. Zimmermann, P. Schnitzler, A. H. Dalpke; Heidelberg Univ. Hosp., Heidelberg, Germany

Abstract Body:

Background: Underdiagnosing of specific infections has been put forward as an argument for use of broad-spectrum multiplex panels. Moreover, pathogens that have been known to occur in typical patient populations night nowadays spread as a consequence of the achievements of modern medicine. In an independent trial 2352 LRT and sputum samples, which were requested for respiratory viruses, were analysed by a multiplex PCR that also included detection of *Mycoplasma pneumoniae* (Mp). This assay revealed an incidence of up to 1.5% which was in contrast to the previously known incidence of 0.5% in our hospital. To clarify this discrepancy we performed a retrospective study of the participating wards to find out, why *Mycoplasma pneumoniae* was apparently underdiagnosed over the last years. **Methods:** From November 2014 to June 2015 all 2352 respiratory samples that were submitted to the virology department were investigated using the new FTD21 Respiratory PCR panel (Fast Track Diagnostics). This assay included detection of *Mycoplasma pneumoniae*, which normally was diagnosed in the bacteriology department upon specific request. Mycoplasma positive samples of the FTD21 assay were confirmed by the routine in-house Mp-PCR. Demographic data and clinical requests were analysed to find out, why *Mycoplasma pneumoniae* was obviously underdiagnosed in our hospital. **Results:** 23 of 2352 respiratory samples were PCR positive for *Mycoplasma pneumoniae* (1.0%). The incidence peaked in the second quarter 2015 with 1.5%. 21 of these samples were confirmed by our routine in-house PCR (91%); 2 samples were not available for retesting. 16 patients were male (76%) and 11 came from the pediatric hospital (52%). Only in 3 cases the Mycoplasma investigation had been requested specifically by the clinician (14%). In all the other cases the positive finding was only due to the use of a multiplex PCR panel. **Conclusion:** *Mycoplasma pneumoniae* is underdiagnosed in our hospital, especially in the pediatric clinic. This is not due to an insufficient sensitivity of the PCR assay, but the main reason is an absence of clinical suspicion. Amendments in the preanalytics are obligatory to improve the situation.

Author Disclosure Block:

S. Zimmermann: J. Scientific Advisor (Review Panel or Advisory Committee); Self; Roche Molecular Systems, Sanofi Pasteur MSD. L. Speaker's Bureau; Self; Becton Dickinson. **P. Schnitzler:** None. **A.H. Dalpke:** L. Speaker's Bureau; Self; Becton Dickinson.

Poster Board Number:

MONDAY-297

Publishing Title:

Irida'S Genomic Epidemiology Application Ontology: Genomic, Clinical and Epidemiological Data Standardization and Integration

Author Block:

E. Griffiths[1], M. Courtot[2], D. Dooley[3], J. Adam[4], F. Bristow[4], J. A. Carrico[5], A. Keddy[6], T. Matthews[4], A. Petkau[4], R. G. Beiko[6], L. M. Schriml[7], E. Taboada[8], G. Van Domselaar[4], M. Graham[4], F. Brinkman[1], W. Hsiao[3]; [1]Simon Fraser Univ., Burnaby, BC, Canada, [2]EMBL-EBI, Hinxton, United Kingdom, [3]BC PHMRL, Vancouver, BC, Canada, [4]PHAC, Winnipeg, MB, Canada, [5]Univ. of Lisbon, Lisbon, Portugal, [6]Dalhousie Univ., Halifax, NS, Canada, [7]Univ. of Maryland Sch. of Med., Baltimore, MD, [8]PHAC, Lethbridge, AB, Canada

Abstract Body:

Background: Successful use of genomics for microbial typing, surveillance and outbreak investigation requires integration with contextual information. Contextual data such as sample information and epidemiology exposure data are often institution-specific and non-standardized, hampering their analysis on a larger scale. Canada's Integrated Rapid Infectious Disease Analysis (IRIDA) project is developing open-source, user-friendly tools such as SNVPhyl, IslandViewer, GenGIS and CARD for incorporating genomic data into epidemiological analyses to support real-time infectious disease surveillance and investigation. Our research efforts include the development of a Genomic Epidemiology Ontology (GenEpiO), a standardized vocabulary interconnected by logical relationships, which is crucial for providing the framework for public health and genomics data integration.**Methods:** To determine the scope and priorities of GenEpiO development, we interviewed public-health stakeholders and domain experts and surveyed reporting forms and databases. Existing, related, ontologies were reused when possible to promote interoperability.**Results:** An OWL file containing harmonized metadata fields and terms describing source attribution, clinical data, sequencing processes, patient histories and exposures was created adhering to the best practices of the Open Biomedical and Biological Ontology Consortium (OBO). GenEpiO was then made more robust through testing using real public health data.**Conclusions:** Implementation of GenEpiO will facilitate data standardization and integration, interoperability, improve querying and facilitate automation of many analyses. Since harmonization of the genomic epidemiology ontology can only be achieved by consensus and wide adoption, IRIDA is currently forming an international consortium to build partnerships and solicit domain expertise.

Author Disclosure Block:

E. Griffiths: None. **M. Courtot:** None. **D. Dooley:** None. **J. Adam:** None. **F. Bristow:** None. **J.A. Carrico:** None. **A. Keddy:** None. **T. Matthews:** None. **A. Petkau:** None. **R.G.**

Beiko: None. **L.M. Schriml:** None. **E. Taboada:** None. **G. Van Domselaar:** None. **M. Graham:** None. **F. Brinkman:** None. **W. Hsiao:** None.

Poster Board Number:

MONDAY-298

Publishing Title:

Nist Canidate Microbial Genomic Reference Materials

Author Block:

N. D. Olson, J. M. Zook, S. A. Jackson, D. Catoe, M. L. Salit, L. Vang; Natl. Inst. of Standards & Technology, Gaithersburg, MD

Abstract Body:

Advances in DNA sequencing has paved the way for the use of whole genome sequencing (WGS) in clinical and public health settings. Genomic reference materials (RMs) are needed to evaluate and benchmark sequencing methods to improve confidence in WGS results. To address this need, the National Institute of Standards and Technology (NIST) is developing candidate genomic DNA microbial RMs for four bacterial strains: Salmonella enterica Typhimurium LT2, Staphylococcus aureus,Pseudomonas aeruginosa, and Clostridium sporogenes PA3679. The four strains were selected with input from the scientific community based on clinical and public health relevance and to represent a range of GC content. A commercial vendor produced 1000 vials from a single lot of genomic DNA for each of the four strains. The genomic material was characterized for homogeneity, purity, and stability. To evaluate the material homogeneity and purity, eight replicate vials were sequenced using two short-read sequencing technologies. Long-read sequencing and two optical mapping technologies were used to generate a de novo genome assembly for each strain. In the absence of an existing pipeline, "Pipelines for Evaluating Prokaryotic References" (PEPR) was developed, ensuring reproducibility and transparency in the characterization process. Whole genome base-level purity was assessed based on the proportion of sequencing data supporting the reference assembly using data from replicate vials. Material homogeneity was assessed based on the similarity in the base-level purity data between replicate vials. Genomic purity, the presence of genomic DNA from organisms other than the RM genus, was assessed using the short-read sequencing data. Overall over 99.9999% of the bases had purity values > 95%, and > 99.5% of the DNA was from the expected organism. Additionally, the material stability was evaluated using pulse-field gel electrophoresis. No DNA degradation was detected after 8 weeks at 4℃. The NIST candidate microbial genomic DNA reference materials and the data generated during the characterization process can be used to evaluate sequencing methods and bioinformatic pipelines, increasing confidence when used in applied settings such as foodborne outbreak investigations.

Author Disclosure Block:

N.D. Olson: None. **J.M. Zook:** None. **S.A. Jackson:** None. **D. Catoe:** None. **M.L. Salit:** None. **L. Vang:** None.

Poster Board Number:

MONDAY-299

Publishing Title:

1000 Genomes and Counting: Preliminary Results from Nctc3000, a Type Culture Reference Genome Project

Author Block:

S. Alexander[1], M. Fazal[1], E. Burnett[2], A. Deheer-Graham[3], K. Oliver[4], N. Holroyd[4], S. Picton[5], J. Parkhill[4], J. Russell[1]; [1]Publ. Hlth.England, London, United Kingdom, [2]Publ. Hlth.England, Salisbury, United Kingdom, [3]Publ. Hlth.England, Potters Bar, United Kingdom, [4]Wellcome Trust Sanger Inst., Cambridge, United Kingdom, [5]Pacific BioSci.s, Menlo Park, CA

Abstract Body:

Background: The NCTC3000 project aims to generate 3000 high quality reference genomes from strains within the National Collection of Type Cultures (NCTC). Annotated and assembled genomes are being made publically available via an E-resource which links sequencing data to all other available strain metadata (provenance, phenotypic characteristics and authentication data). **Methods:** High molecular weight DNA from 1075 bacterial strains was extracted using either the Masterpure or Qiagen midi kit and analysed using the Agilent TapeStation to ensure sufficient quality (>60kb) and quantity (>3 µg). Whole Genome Sequencing (WGS) was performed using the PacBio SMRT® DNA Sequencing technology followed by genome assembly via an automated pipeline. **Results**: To date WGS has been completed on 1075 bacterial strains, from 21 families: Alcaligenaceae (2), Bacillaceae (3), Bartonellaceae (1), Brachyspiraceae (2), Brucellaceae (2), Campylobacteraceae (8), Corynebacteriaceae (1), Enterobacteriaceae (567), Flavobacteriaceae (12), Fusobacteriaceae (6), Helicobacteraceae (3), Legionellaceae (11), Moraxellaceae (8), Mycobacteriaceae (1), Neisseriaceae (11), Nocardiaceae (1), Pasteurellaceae (24), Pseudomonadaceae (23), Rhizobiaceae (1), Sphingobacteriaceae (3), Sphingomonadaceae (1), Staphylococcaceae (167), Streptococcaceae (201) and Vibrionaceae (7) within the NCTC collection. Of those strains sequenced thus far, 161 (15%) are type strains. All WGS data is made publically available (http://www.sanger.ac.uk/resources/downloads/bacteria/nctc/) and 499 genomes have been assembled and annotated. Where assembly has been possible 55.8% of genomes have been closed into a single contig. 55.1% of strains had evidence of one or more plasmids. **Conclusion**: The NCTC3000 project has generated over 1000 reference genomes from strains of medical importance. All sequences generated and associated metadata are being made publically available and it is hoped that these sequences will be used by scientists to support and enhance genomic studies globally.

Author Disclosure Block:

S. Alexander: None. **M. Fazal:** None. **E. Burnett:** None. **A. Deheer-Graham:** None. **K. Oliver:** None. **N. Holroyd:** None. **S. Picton:** D. Employee; Self; PACIFIC BIOSCIENCES. K. Shareholder (excluding diversitied mutual funds); Self; PACIFIC BIOSCIENCES. **J. Parkhill:** None. **J. Russell:** None.

Poster Board Number:

MONDAY-300

Publishing Title:

Towares a Reference Material for Mixed Pathogen Detection via High-Throughput Sequencing

Author Block:

J. G. Kralj, N. D. Olson, S. Jackson; Natl. Inst. of Standards and Technology, Gaithersburg, MD

Abstract Body:

Background: Sequencing-based culture-independent detection of pathogens continues to garner attention in clinical, environmental, and biomanufacturing. Regulatory agencies, such as FDA and CAP/CLIA, seek best practices for establishing competency and effectiveness in sequencing-based pathogen detection methods. We have proposed to establish a standard pool of microorganisms using input from a consortium. The provenance and identity of the microbes would be well-documented, enabling regulatory bodies to "stress-test" device claims. Further, this standard pool will benefit those designing new devices by enabling integration of reference materials during pre-submission development.**Methods**: Our proof-of-concept samples were mixtures of DNA from four sources (NIST candidate microbial genomic reference materials for *S. enterica* subspecies enterica serovar Typhimurium strain LT2, *S. aureus* clinical isolate, *P. aeruginosa* clinical isolate, and *C. sporogenes* PA3679) in a background of human DNA from the "Genome in a Bottle" project. Two mixtures were generated (BioProject PRJNA297045), including an equigenomic (contstant number of genomes) and a log-10 genome copy dilution series. The resulting data was then subjected to 5 taxonomic identification tools to identify and quantify the composition. The number of species detected varied by classification algorithm.**Results**: The equigenomic sample analyses typically identified and quantified all constituents at the genus level. For the log-10 genome dilution series, identifying down to 0.1x (~several hundred reads, or 0.01% of the sample) was possible on all platforms for species-level ID. The number of species detected varied by classification algorithm. Perhaps notable by its absence, E. coli did not appear to contaminate the samples.**Conclusions**: The differences in results generated from highly-characterized DNA were important in so much that it highlights how reference materials can enlighten where sequencing-based identification tools generate uncertainty. Future work will focus on additional organisms that can be used in sequencing-based diagnositics for identifying and examining multiple variables such as near-taxonomic "neighbor" species, G+C content, genome size, and extremophiles. Despite the fact that others have observed similar trends, this ground-up approach will enable

Author Disclosure Block:

J.G. Kralj: None. **N.D. Olson:** None. **S. Jackson:** None.

Poster Board Number:

MONDAY-301

Publishing Title:

Genetic Diversity of Georgian and Turkey *Brucella* Isolates

Author Block:

K. Sidamonidze[1], W. Su[2], E. Zhgenti[1], N. Trapaidze[3], F. Buyuk[4], M. Sahin[4], E. Celik[4], O. Celebi[4], S. Otlu[4], L. Baillie[5], P. Imnadze[1], A. Kotorashvili[1], M. Nikolich[2]; [1]Natl. Ctr. for Disease Control and Publ. Hlth., Tbilisi, Georgia, [2]Walter Reed Army Inst. of Res., Silver Spring, MD, [3]U.S. Army Med. Res., Tbilisi, Georgia, [4]Univ. of Kafkas, Kars, Turkey, [5]Cardiff Univ., Cardiff, United Kingdom

Abstract Body:

The genus *Brucella* consists of a number of species that infect different mammalian hosts including humans. *Brucella* infection can lead to abortion in many domestic animals, causing economic harm. Human brucellosis is one of the most globally widespread and debilitating zoonotic diseases, and it remains endemic in both Georgia and Turkey. Due to the high infectivity in mammals *Brucella* is classified as a Category B biological threat agent by the United States Department of Homeland Security. Precise genetic characterization of *Brucella* isolates by molecular typing methods can provide useful analysis for the description of strain diversity, detection of clonal groups, trace-back to sources of infection and discrimination of naturally occurring outbreaks from a bioterrorist event. Recent studies have demonstrated that multiple-locus variable-number tandem-repeat analysis (MLVA) is a high resolution genetic subtyping tool that can provide valuable information for brucellosis epidemiological investigations.In this study we assessed the genetic variability of 29 cattle and sheep isolates of *Brucella* isolated in Turkey and 72 animal and human *Brucella* isolates from Georgia, all identified as *B. melitensis* or *B. abortus* based on microbiological and Bruce-Ladder PCRresults. Genotypes were revealed using a MLVA approach with 15 mini-satellite markers based on Huynh et al. 2008, possessing discriminatory capability. Data obtained were analyzed to construct a phylogenic tree using BioNumerics Software (Applied Maths) and relationships between Turkish and Georgian isolates described.Our data showed that these strains are grouped by origin of country and species, but there are two *B. melitensis* of Turkey strains are mixed in Georgian group. This might suggest these two *B.melitensis* strains in Turkey come from Georgia. Our future research will further elucidate the geospatial localities and transmission patterns of *Brucella* strains we have reported here.

Author Disclosure Block:

K. Sidamonidze: None. **W. Su:** None. **E. Zhgenti:** None. **N. Trapaidze:** None. **F. Buyuk:** None. **M. Sahin:** None. **E. Celik:** None. **O. Celebi:** None. **S. Otlu:** None. **L. Baillie:** None. **P. Imnadze:** None. **A. Kotorashvili:** None. **M. Nikolich:** None.

Poster Board Number:

MONDAY-302

Publishing Title:

Pan-Genome Wide Association Study to Identify Pneumococcal Genomic Variation Associated with Meningitis

Author Block:

Y. Li, B. J. Metcalf, S. Chochua, R. Gierke, T. Pilishvili, L. Mcgee, B. W. Beall, Active Bacterial Core Surveillance Team; CDC, Atlanta, GA

Abstract Body:

Background: *Streptococcus pneumoniae* causes several types of invasive pneumococcal disease (IPD).Specific bacterial factors that predispose the pathogen to causing meningitis over non-meningitis IPD in humans remain unclear.**Methods:** Invasive pneumococcal isolates from 183 meningitis and 2442 non-meningitis patients were sequenced on an Illumina platform. The short reads were mapped onto 3225 non-redundant coding DNA sequences extracted from 20 complete pneumococcal genomes. Three types of variation--single nucleotide polymorphisms (SNPs), insertion/deletions (INDELs), and gene presences/absence (GPA)--were called from mapped sequences and confirmed by *de novo* genome assembly. Association with meningitis was assessed by Mantel-Haenszel test conditioned on patient age. The effect size of candidate variant was examined by logistic regression and stratified analysis.**Results:** A total of 10258 non-synonymous SNPs, 44 INDELs, and 289 GPAs passed quality control. The genomic inflation factor for all 10591 tests was 1.02. After Bonferroni correction, only snp1783187 in the *pbp1b* gene (causing N214T variation in the transglycosylase domain) was significantly associated with meningitis. Other top hits included SNPs in *folE*, *clpC*, *GrpE*, and GPA of *zmpB*. The snp1783187C allele was enriched in meningitis isolates after adjusting for patient age, serotype group (PCV13, PCV13 minus PCV7, and non-PCV13), and penicillin (PEN) susceptibility (adjusted OR=3.10, 95% CI 1.98-4.80). In the serotype-stratified analysis, snp1783187C allele was also enriched in meningitis isolates (common OR = 2.46, 95% CI=1.14-5.30), and no evidence of OR difference among serotypes was found (Breslow-Day test p=0.83).**Conclusion:** Pneumococcal genome variation appears to contribute modestly yet detectably to the development of meningitis over non-meningitis IPD in humans. A *pbp1b* variant showed association with meningitis isolates, which was apparently independent from PEN resistance and incompletely explained by linkage to serotype. The effect of the variant remains to be confirmed by replication studies.*The findings and conclusions in this report are those of the author(s) and do not necessarily represent the official position of the Centers for Disease Control and Prevention.*

Author Disclosure Block:

Y. Li: None. **B.J. Metcalf:** None. **S. Chochua:** None. **R. Gierke:** None. **T. Pilishvili:** None. **L. Mcgee:** None. **B.W. Beall:** None.

Poster Board Number:

MONDAY-303

Publishing Title:

Comparative Genomics and Antimicrobial Resistance Determinants of Community-associated *Staphylococcus aureus* Strains in South Texas

Author Block:

G. C. Lee[1], S. D. Dallas[2], Y. Wang[3], R. J. Olsen[4], K. A. Lawson[5], J. P. Wilson[5], C. R. Frei[1]; [1]UT Hlth.Sci. Ctr. and UT Austin, San Antonio, TX, [2]UT Hlth.Sci. Ctr. San Antonio, San Antonio, TX, [3]Univ. of Texas San Antonio, San Antonio, TX, [4]Houston Methodist Res. Inst., Houston, TX, [5]UT Austin, Austin, TX

Abstract Body:

Background: The emergence of CA-MRSA has resulted in an epidemic of skin and soft tissue infections (SSTI) in the U.S. This investigation sought to describe the molecular epidemiology and antimicrobial resistance patterns of *S. aureus* strains in the South Texas community. **Methods:** Whole genome sequencing (WGS) was performed on *S. aureus* SSTI and nasal colonization isolates from patients within 14 primary care clinics in the South Texas from 2007 to 2015. The bacterial genomes were compared to a reference genome FPR3757 (USA300 *S. aureus* strain) to identify single nucleotide polymorphisms (SNPs). SNP-based phylogenetic analyses were conducted using concatenated SNP nucleotides in the core genomes in reference to FPR3757. Multi-locus sequence type (MLST) was determined from WGS data using the *S. aureus* MLST database. The resistome was assembled by identifying antimicrobial resistance determinants related to the phenotypically derived antibiogram. **Results:** Overall, 144 *S. aureus* isolates (112 from SSTIs and 32 from nasal colonization) were sequenced. Seventy-three strains were MRSA and 71 were MSSA. When compared to the reference FPR3757, the strains differed by an average of 3,548 SNPs (range 15 to 47,574). *S. aureus* SSTI strains were predominantly ST-8 (83%) which clustered tightly within a similar clade containing the reference FPR3757, differing by an average of 160 SNPs (range 15-1,215). The remaining isolates comprised mostly of nasal colonization strains and were more divergent from the reference with an average of 12,422 SNPs (2,808-47,574). The majority of *S. aureus* strains harbored genes encoding for resistance to beta lactams (80%) and erythromycin (71%). High-level mupirocin (*ileS)* was only present in 3 isolates (2%). Notably, isolates harboring mutations conferring fluoroquinolone resistance (*gyrA*) clustered tightly together only in the ST-8 clade. **Conclusions:** This study highlights the genomic diversity of *S. aureus* strains in the South Texas community and demonstrates the utility of next generation sequencing to define the diversity and distribution of resistance mechanisms within *S. aureus*.

Author Disclosure Block:

G.C. Lee: None. **S.D. Dallas:** None. **Y. Wang:** None. **R.J. Olsen:** None. **K.A. Lawson:** None. **J.P. Wilson:** None. **C.R. Frei:** None.

Poster Board Number:

MONDAY-304

Publishing Title:

Resolution Of *legionella Pneumophila* Outbreak-Associated St1 Isolates Using Genome Sequence Analysis

Author Block:

B. H. Raphael, S. Morrison, J. Mercante, N. Kozak-Muiznieks, J. Winchell; CDC, Atlanta, GA

Abstract Body:

Background: Legionella *pneumophila* (Lp) is the causative agent of Legionnaires' Disease (LD), a severe pneumonia that occurs in susceptible populations exposed to environmental sources of Lp. During outbreak investigations, rapid and accurate subtyping of isolates from clinical specimens and potential environmental samples can confirm the source and direct measures to stop the outbreak. Sequence-based typing (SBT) for Lp is based on sequence variations among 7 alleles, and >2,000 sequence types (ST) have been identified. ST1 is the most common ST identified from isolates submitted to CDC from sporadic LD cases and environmental sources. **Methods:** To evaluate the ability of next generation sequencing technologies to improve the resolution of ST1 isolates, genome sequencing using the Illumina MiSeq instrument was performed with ST1 isolates (N=50). These isolates were selected from various clinical and environmental sources including both sporadic LD cases and outbreaks where multiple clinical and/or source isolates collected during an investigation were examined. **Results:** Single nucleotide polymorphism (SNP) analysis of draft genome sequences demonstrated that ST1 isolates form a distinct clade and that isolates associated with clinical and/or environmental sources within individual outbreaks generally form outbreak-specific subclades. Moreover, a whole genome multilocus sequence typing (wgMLST) approach based on sequence variation of 4,472 alleles revealed similar outbreak-specific subclades. Analysis of isolates from 3 LD outbreaks occurring in New York (2-4 isolates per outbreak), revealed that core SNPs within 2 of the outbreaks differed by 0-1 nucleotides while the core SNPs shared between isolates from the third outbreak (determined to be associated with multiple sources) differed by 6 to >400 SNPs. Correspondingly, isolates associated with these outbreaks shared ~93% identical alleles using the wgMLST scheme while isolates within the outbreaks sharing the fewest SNP differences shared >99% identical alleles. **Conclusions:** This work demonstrates that genome sequencing provides improved resolution of ST1-associated outbreak isolates. Finally, use of a wgMLST approach with a centralized allele database provides a robust and reproducible method for genomic analysis of Lp.

Author Disclosure Block:

B.H. Raphael: None. **S. Morrison:** None. **J. Mercante:** None. **N. Kozak-Muiznieks:** None. **J. Winchell:** None.

Poster Board Number:

MONDAY-305

Publishing Title:

Genomic Dissection of Australian *Bordetella pertussis* Isolates from the 2008-2012 Epidemic

Author Block:

A. Safarchi[1], S. Octavia[1], S. Wu[1], S. Kaur[1], V. Sintchenko[2], L. Gilbert[2], N. Wood[2], P. McIntyre[2], H. Marshall[3], A. Keil[4], **R. Lan**[1]; [1]Univ. of New South Wales, Sydney, Australia, [2]Univ. of Sydney, Sydney, Australia, [3]Univ. of Adelaide, Adelaide, Australia, [4]Princess Margaret Hosp. for Children, Perth, Australia

Abstract Body:

Despite high pertussis vaccination coverage, Australia experienced a prolonged epidemic in 2008-2012. The predominant *Bordetella pertussis* genotype harboured pertussis toxin promoter allele, *ptxP3*, and pertactin gene allele, *prn2*. The emergence and expansion of non-expressing *prn* isolates (Prn negative), was also observed. In this study, whole genome sequencing was used to investigate the microevolution and genomic diversity of 22 epidemic *B. pertussis* isolates, including 10 Prn-negative strains with three different modes of silencing of pertactin gene (*prn*::IS*481*F, *prn*::IS*481*R and *prn*::IS*1002*). Five pre-epidemic strains were also sequenced for comparison. Five single nucleotide polymorphisms (SNPs) were common in the epidemic isolates and differentiated them from pre-epidemic isolates. The epidemic SP13 isolates can be divided into five lineages (EL1-EL5) with EL1 containing only Prn negative isolates. Comparison with global isolates showed that three lineages remained geographically and temporally distinct whereas two lineages mixed with isolates from 2012 UK outbreak. Our results suggest significant diversification and the microevolution of *B. pertussis* within the 2008-2012 Australian epidemic.

Author Disclosure Block:

A. Safarchi: None. **S. Octavia:** None. **S. Wu:** None. **S. Kaur:** None. **V. Sintchenko:** None. **L. Gilbert:** None. **N. Wood:** None. **P. McIntyre:** None. **H. Marshall:** None. **A. Keil:** None. **R. Lan:** None.

Poster Board Number:

MONDAY-306

Publishing Title:

Influenza Throughout the 2014-2015 Season, with Focus on Influenza A(H3n2)

Author Block:

W. E. Gruner[1], S. H. Sarria[1], L. E. Sinclair[2], S. L. Jones[1], M. L. Powell[2], J. F. Hanson[2], J. P. Smith[1], R. A. Yohannes[2], K. F. Fumia[2], M. A. Edwards[2], H. Vireak[3], J. Dejli[3], D. W. Ellison[4], E. A. Macias[2]; [1]Henry M. Jackson Fndn., Wright-Patterson AFB (WPAFB), OH, [2]United States Air Force Sch. of Aerospace Med. (USAFSAM), Epidemiology Lab. Service, WPAFB, OH, [3]US Navy Med. Res. Unit No. Two (NAMRU-2), Phnom Penh, Cambodia, [4]Armed Forces Res. Inst. of Med. Sci. (AFRIMS), Bangkok, Thailand

Abstract Body:

Background: The DoD Global, Laboratory-based, Influenza Surveillance Program conducts testing on respiratory specimens collected from a world-wide network of sentinel and participating sites using viral culture, molecular diagnostics, and sequencing to detect and characterize influenza viruses.**Methods:** Around 5,700 respiratory specimens were analyzed at USAFSAM from September 2014 to June 2015 for the presence of influenza using the CDC RT-PCR based assays. About 50% of influenza positive specimens were selected based on epidemiological factors for Sanger-based hemagglutinin (HA) sequence analysis. Additional HA sequences were analyzed from partner DoD laboratories at AFRIMS and NAMRU-2. DNASTAR and BioEdit software were used to assemble the raw data, build phylogenetic trees, and perform cluster analysis of influenza viruses. **Results:** 1,009 out of 1,177 isolates were identified as influenza A (86%); 168 were influenza B (14%). Among influenza A, 94% were A(H3N2) and 6% were A(H1N1)pdm09. Based on the observed mutations, 80% of A(H3N2) viruses analyzed were subclade 3C.2a and 20% were clade 3C.3. Within 3C.3, 30% were subclade 3C.3a. Throughout the season, the number of isolates classified as subclade 3C.2a increased from 22% in October 2014 to 100% in August 2015. All of theA(H1N1)pdm09 isolates classified as clade 6B. Seventy percent of influenza B specimens were Yamagata lineage clade 3 and 30% Victoria lineage clade 1A.**Conclusions:** For the 2014-2015 season, influenza A(H3N2) was the predominant circulating subtype at 80% of the specimens sequenced. Influenza A(H1N1)pdm09 and influenza B constituted the remaining 20%. The A(H3N2) subclade 3C.2a, which contains strains that underwent mutational drift from the 2014-2015 A(H3N2) vaccine component, increased throughout the season and was the dominant subclade going into the 2015-2016 season. This DoD surveillance program provided data to the CDC and to the Vaccines and Related Biological Products Advisory Committee.

Author Disclosure Block:

W.E. Gruner: None. **S.H. Sarria:** None. **L.E. Sinclair:** None. **S.L. Jones:** None. **M.L. Powell:** None. **J.F. Hanson:** None. **J.P. Smith:** None. **R.A. Yohannes:** None. **K.F. Fumia:** None. **M.A. Edwards:** None. **H. Vireak:** None. **J. Dejli:** None. **D.W. Ellison:** None. **E.A. Macias:** None.

Poster Board Number:

MONDAY-307

Publishing Title:

Whole Genome Sequencing as a Tool for Epidemic Investigation of Tattoo-Associated Nontuberculous Mycobacterial Skin Infections

Author Block:

K. Chou[1], D. M. Williams-Hill[1], J. Pettengill[2], E. Strain[2], S. Torres[1], W. B. Martin[1]; [1]US FDA, Irvine, CA, [2]US FDA, College Park, MD

Abstract Body:

Background: Thirty-eight cases of nontuberculous *Mycobacterium* infections were reported associated with tattooing at a tattoo parlor in Florida between December 2014 and April 2015. In an investigation, all tattoo artists involved in the incidents reportedly used distilled water for diluting grey wash inks. Additionally, none of the artists at other parlors who used the same brand of tattoo inks reported any tattoo related illnesses. To determine the source of contamination, we embarked on a trace-back investigation centered on the use of whole genome sequencing and subsequent analysis.**Methods:** Selective recovery of nontuberculous *Mycobacterium* was performed using the following samples: tap water, environmental swabs, and an opened container of tattoo ink directly related to the skin infections collected from the tattoo parlor, as well as 5 unopened containers of tattoo inks of the same brand and lot number collected from other tattoo parlors. Suspect colonies isolated were screened and identified using methodology developed previously. Selected isolates from the above procedures as well as clinical isolates from the skin infections were subjected to whole genome sequencing.**Results:** *Mycobacterium chelonae* was isolated from all 5 of the unopened container of tattoo inks. *M. abscessus* and *M. fortuitum* were isolated from the opened container of ink. *M. phocacium*, *M. mucogenicum*, and *M. abscessus*, were isolated from the tap water. Clinical isolates consist of 3 strains of *M. abscessus* and 1 strain of *M. fortuitum.* One of the most interesting findings from the whole genome sequencing analysis was that there were 2 perfectly matched groups of *M. abscessus* strains each consisted of isolates from the tap water, tattoo ink and skin biopsy.**Conclusions:** In the absence of complete and accurate epidemiological information, our trace-back investigation centered on the use of whole genome sequencing was able to provide data suggesting that the incidents of skin infections were highly likely and at least partially due to use of tap water to dilute tattoo inks for subsequent application. The outcome of the current study confirmed that whole genome sequencing could serve as a useful tool for epidemic investigation of illnesses caused by bacterial pathogens.

Author Disclosure Block:

K. Chou: None. **D.M. Williams-Hill:** None. **J. Pettengill:** None. **E. Strain:** None. **S. Torres:** None. **W.B. Martin:** None.

Poster Board Number:

MONDAY-308

Publishing Title:

Comparison of *Propionibacterium spp.* Detection in Skin Swabs by Variable Regions of the 16s Rrna Gene

Author Block:

M. D. Gonzalez[1], M. A. Wallace[2], A. E. Schriefer[2], D. K. Warren[2], S. A. Fritz[2], C-A. Burnham[2]; [1]Children's Hlth.care of Atlanta, Atlanta, GA, [2]Washington Univ., St. Louis, MO

Abstract Body:

Propionibacterium spp. (*Propi*) are an important component of the skin microbiota. Our objective was to compare the detection of *Propi* from skin using genomic- and culture-based methods. We used Eswabs to sample 5 skin sites: the anterior nares, axilla, inguinal fold, forearm, and lower leg. Of the 15 subjects (10 female) sampled, 8 were community-dwelling and 7 were in the intensive care unit. Specimens were plated to a variety of culture media and incubation environments; specifically for *Propi* anaerobic incubation on Brucella blood agar, and broth enrichment were used. All cultured isolates were identified by MALDI-TOF MS. For all Eswab specimens, DNA sequencing data was obtained for the V2 (n=216), V3 (n=213), V4 (n=200) and V6 (n=219) variable regions of the 16S rRNA gene. Taxonomic classification of the rarified sequencing reads was performed using QIIME against the GreenGenes database, and all subsequent analysis was done on genus level classification data. We found that *Propi* was detected more frequently, and with higher abundance, from the V2, V3, and V6 regions, but was nearly absent in the V4 region (Table 1). A similar bias in V regions was not observed with *Staphylococcus* spp. and *Corynebacterium* spp., which are also members of the skin microbiota (Table 1). A subanalysis of the V3 sequencing data with relative *Propi* spp. read counts at ≥1% (n=114) indicated that 69% (n=79) of these specimens were culture positive for *Propi* spp, confirming the V3 data. None of the culture positive specimens for *Propi* (n=118), had ≥1% of read counts for V4. Our results indicate that the choice of V region for sequence-based skin microbiota studies greatly influences the relative abundance of *Propi* spp. that is detected.

Table 1. Average relative abundance assigned to *Propi* spp.

V region (n)	*Propionibacterium* spp.[a]	*Staphylococcus* spp.[a]	*Corynebacterium* spp.[a]
V2 (216)	0.0443	0.251	0.1226
V3 (213)	0.0578	0.238	0.1490
V4 (200)	0.0002	0.292	0.1525
V6 (219)	0.0856	0.212	0.1479

[a] Average relative abundance is the sum of relative abundance assigned to The specific taxonomic group divided by the total number of specimens with sequencing data for that V region

Author Disclosure Block:

M.D. Gonzalez: None. **M.A. Wallace:** None. **A.E. Schriefer:** None. **D.K. Warren:** None. **S.A. Fritz:** None. **C. Burnham:** None.

Poster Board Number:

MONDAY-309

Publishing Title:

Analysis of the Manila Family of *Mycobacterium tuberculosis* Linage 1

Author Block:

X. Wan, K. Koster, L. Qian, S. Hou, **J. T. DOUGLAS, PhD**; Univ. of Hawaii, Honolulu, HI

Abstract Body:

Tuberculosis (TB) was recognized by WHO in 2014, as world public health emergency, even though significant efforts have been put into TB control programs. With airborne transmission and prolonged latency period, *Mycobacterium tuberculosis* continues to spread worldwide as one of the most successful bacterial pathogens and kills an estimated 1.5 million people every year. In the Pacific region two linages are dominant: lineage 1 characterized by the Manila family found in Filipinos and linage 2 by the Beijing family found in Northern Asians. In this presentation, we present the complete genome sequence and analysis of *Mycobacterium tuberculosis* Manila family in Lineage 1 and a deep comparative analysis of this family with the complete genomes in Lineage 2-4 and animal strains. Manila strain genomes were assembled using gsMapper with Manila 96121 complete genome. Mauve and MUMmer 3 were used for whole genome alignments. BLAST and Markov cluster algorithm were used to cluster CRISPR spacers. Pan-genome was analyzed using both orthoMCL and Roary pipeline. SNP analysis and synonymous/nonsynonymous substitution rates were calculated using kSNP and ParaAT respectively. Principal components analysis were carried out using R. This reveals the unique evolution of this ancestrally derived family and sheds light on virulence an attenuation of TB. *Mycobacterium tuberculosis* lineage 1 is inferred to originate ancestrally on account of presence of 52-bp TbD1 sequence and analysis of single nucleotide polymorphisms. Manila family genomes are closely clustered with animal outgroups based on whole genome alignment, providing further evidence to support the ancestral lineage hypothesis. Principal component analysis of presence-absence of CRISPR spacers suggest that Manila family is in distinct distance from Lineage 2-4. We further identify the truncated *wbi5* gene, an additional operon consisting of paralogous STPK PknH coupled ABC transporter, and 6 uniquely conserved SNPs, which are fixed in Manila strains. These unique features can further serve as biomarkers to identify Manila strains and may explain attenuated virulence and limited transmission in this ancestral lineage.

Author Disclosure Block:

X. Wan: None. **K. Koster:** None. **L. Qian:** None. **S. Hou:** None. **J.T. Douglas:** None.

Poster Board Number:

MONDAY-310

Publishing Title:

Whole-Genome Sequencing Reveals the Origin and Rapid Evolution of an Emerging Outbreak Strain *Streptococcus pneumoniae* 12F

Author Block:

W. Deng[1], G. Peirano[2], E. Schillberg[3], T. Mazzulli[1], S. D. Gray-Owen[1], J. L. Wylie[4], D. Robinson[5], S. M. Mahmud[6], D. R. Pillai[2]; [1]Univ. of Toronto, Toronto, ON, Canada, [2]Univ. of Calgary, Calgary, AB, Canada, [3]Winnipeg Hlth.Authority, Winnipeg, MB, Canada, [4]Cadham Provincial Lab., Winnipeg, MB, Canada, [5]Univ. of Mississippi, Jackson, MS, [6]Univ. of Manitoba, Winnipeg, MB, Canada

Abstract Body:

Background: *Streptococcus pneumoniae* is a major cause of community-acquired pneumonia and septicemia in adults. The global drug-susceptible capsular serotype 12F, clonal complex 218 caused several outbreaks in the USA between 1989-2008, as well as a recent large outbreak in Manitoba, Canada that resulted in 36 cases of septicemia and 3 deaths. The evolutionary origin of the Canadian outbreak strain and its relationship to the historical US outbreak strains are not known. **Methods:** Whole-genome deep sequencing was performed on isolates from the Canadian outbreak (n=36), the US outbreaks (n=9), and non-outbreak surveys (n=21). Phylogenomic analysis and comparative genomics were used to assess evolutionary relationships and to detect gene content differences between the isolates.**Results:** The Canadian outbreak was closely related to sporadic cases that occurred pre-outbreak in cross-border geographic regions in North Dakota, Iowa and Manitoba. The emerging Canadian strain differed from US ones by acquisition of cell-surface protein and macrolide resistance determinants via incorporation of a 5.3kb mega cassette harboring *msrD* and *mefE*. Furthermore, during 11 months of transmission, this clone evolved rapidly and acquired fluoroquinolone resistance through precise step-wise mutations in both *parC* and *gyrA* , and putative compensatory mutations in *uraA* or *IMPDH* under drug selection. Alarmingly, this drug-resistant clone appears to have spread quickly to other regions of Canada and the USA, and transforms bacterial population structure.**Conclusion:** Whole-genome sequencing revealed an independent emergence and secondary adaptation of a new virulent and drug resistant pneumococcal epidemic clone. Ongoing molecular surveillance is required and measures to prevent its spread should be developed.

Author Disclosure Block:

W. Deng: None. **G. Peirano:** None. **E. Schillberg:** None. **T. Mazzulli:** None. **S.D. Gray-Owen:** None. **J.L. Wylie:** None. **D. Robinson:** None. **S.M. Mahmud:** None. **D.R. Pillai:** None.

Poster Board Number:

MONDAY-311

Publishing Title:

Epidemiology of the Pandemic Multidrug-Resistant *E. coli* ST131-H30 and H30Rx Subclones among US Children

Author Block:

A. Miles-Jay[1], S. J. Weissman[2], A. L. Adler[2], X. Qin[2], V. Tchesnokova[1], E. V. Sokurenko[1], D. M. Zerr[2]; [1]Univ. of Washington, Seattle, WA, [2]Seattle Children's Hosp., Seattle, WA

Abstract Body:

E. coli ST131-*H30* and its sublineage, *H30Rx*, have abruptly emerged as the dominant cause of multidrug-resistant extraintestinal *E. coli* infections. The epidemiologic significance of these subclones in children is undefined. We quantified the burden and identified host correlates of infection with ST131-*H30* and *H30Rx* among US children. We conducted a prospective surveillance study at 4 US children's hospitals from 2009-2013. All third-generation cephalosporin-resistant (3GCR) *E. coli* isolated from urine or normally sterile sites of patients ≤ 21 years old were collected. For each 3GCR isolate, 3 subsequent third-generation cephalosporin-susceptible (3GCS) *E. coli* isolates were collected. Demographic and clinical data were abstracted from medical records. ST131-*H30* isolates were identified using *fumC/fimH* genotyping; *H30Rx* isolates were identified using a single nucleotide polymorphism-based PCR. Associations between host factors and infection with ST131-*H30* or *H30Rx* were analyzed using univariable and multivariable log-binomial regression stratified by 3GCR status. In total, 21,799 *E. coli* isolates were identified; 334 3GCR and 994 3GCS isolates were further analyzed. Of the 3GCR isolates, 119 were ST131-*H30*, and of those, 82 were *H30Rx*. Of the 3GCS isolates, 47 were ST131-*H30*, and of those, 5 were *H30Rx*. The estimated prevalence of ST131-*H30* and *H30Rx* among all extraintestinal *E. coli* infections was 5.2% and 0.88%, respectively. Among children with 3GCR *E. coli* infections, age 0-5 years was the only factor associated with ST131-*H30* or *H30Rx* (relative risk [RR]: 1.99, 95% confidence interval [CI]: 1.31-3.03; RR: 2.28 95% CI: 1.37-3.82). Among children with 3GCS infections, age and several factors related to underlying illness were associated with ST131-*H30* in the univariable analyses; in the multivariable analysis, only a negative association with age 0-5 years persisted (RR: 0.49, 95% CI: 0.26-0.94). 3GCS *H30Rx* were not separately analyzed due to small numbers. These are the first estimates of the burden of *E. coli* ST131-*H30* and *H30Rx* among US children. We observed that in children with 3GCR *E. coli* infections, ST131-*H30* is more dominant among children age 0-5 years, while in children with 3GCS infections, ST131-*H30* is more dominant among older children.

Author Disclosure Block:

A. Miles-Jay: None. **S.J. Weissman:** F. Investigator; Self; The Joint Commission. N. Other; Self; Party to patent application pertaining to tests for specific E. coli strains. **A.L. Adler:** None. **X. Qin:** None. **V. Tchesnokova:** N. Other; Self; Party to patent applications pertaining to tests for specific E. coli strains. **E.V. Sokurenko:** N. Other; Self; Party to patent applications pertaining to tests for specific E. coli strains. **D.M. Zerr:** None.

Poster Board Number:

MONDAY-312

Publishing Title:

Diversity of Plasmids Harboring *bla*$_{kpc-2}$ in *Klebsiella pneumoniae*

Author Block:

D. Cejas, A. Elena, M. Nastro, C. H. Rodriguez, A. Famiglietti, **G. Gutkind**, M. A. Radice; Univ. de Buenos Aires, Buenos Aires, Argentina

Abstract Body:

Background: Sporadic emergence of KPC-2 producing *Klebsiella pneumoniae* (KPCKp) displaying susceptibility profiles different to the pandemic ST258, as susceptibility to aminoglycosides and/or fluoroquinolones and/or trimetoprim-sulfametoxazol, has been recently reported.The aim of this study was to characterize *bla*$_{KPC}$ coding plasmids from KPCKp clones different from ST258 in our region.**Methods:** Plasmids obtained from 9 KPCKp isolates recovered during 2010-2014 at a single teaching hospital were included. The isolates showed different pulsotypes in X*ba*I-PFGE and belonged to different sequence types (2/9 were ST392, while the rest were ST502, ST1458, ST17 and ST11). Purified plasmids were characterized by replicon typing according to Carattoli *et al*., and plasmid addiction systems were detected as suggested by Mnif *et al.* MOB-P and MOB-F relaxases coding genes identification was carried out by PCR according to Alvarado *et al.* The genetic context of *bla*$_{KPC-2}$ was investigated by PCR mapping in order to identify the reported transposon Tn*4401*.**Results:** Inc L/M group was detected in 4/9 *bla*$_{KPC-2}$ harboring plasmids, being also identified the Inc F_{IIS} group in one of them. The other plasmids were non typable by the methodology proposed by Carattoli *et al.*, unlike Inc F related replicons were characterized in KPCKp ST258 plasmids. The addiction system ParD-ParE was identified in all studied plasmids. In 2/9 plasmids *p*51 of MOB-P family could be identified, however no MOB-P or MOB-F relaxases could be detected in the others. The genetic context of *bla*$_{KPC-2}$ corresponded to Tn*4401* in 2/9 plasmids. However, the genetic environment of *bla*$_{KPC-2}$ seems to be different in the others, given that the upstream region of *bla*$_{KPC-2}$ could not be amplified.**Conclusions:** This study contributes to the knowledge of *bla*$_{KPC-2}$ plasmids epidemiology in our region. Diversity of plasmid backbones could be responsible for the presence of *bla*$_{KPC-2}$ in different clones of *K. pneumoniae*. In addition to previous reports, this work highlights the continuing evolution of the genetic environment of the *bla*$_{KPC-2}$ gene.

Author Disclosure Block:

D. Cejas: None. **A. Elena:** None. **M. Nastro:** None. **C.H. Rodriguez:** None. **A. Famiglietti:** None. **G. Gutkind:** None. **M.A. Radice:** None.

Poster Board Number:

MONDAY-313

Publishing Title:

Molecular Epidemiology of *Providencia* spp. Harbouring Ndm Carbapenemase in Argentina

Author Block:

D. F. Faccone, F. Pasteran, E. Albornoz, M. Rapoport, P. Ceriana, C. Lucero, S. Gomez, NDM-Argentina Group, A. Corso; Antimicrobianos, INEI-ANLIS Dr. Malbran, Natl. Reference Lab. (NRL), Buenos Aires, Argentina

Abstract Body:

Background: NDM-producing organisms emerged in Argentina in 2013. Since then NDM spread was mainly due to *Providencia* spp (Prov) isolates. Between 2013-2015 the NRL confirmed 39 Prov: 23 *P. stuartii* (Pst) and 16 *P. rettgeri* (Pre) harboring NDM. **Methods:** Isolates non-susceptible to carbapenems (CBP) and with a positive synergy test between CBP and EDTA discs, were suspicious of MBL production. Strains were identified using MALDI-TOF. NDM-1 was confirmed by PCR and DNA sequencing, genetic relatedness was assessed by NotI-PFGE. Susceptibility was evaluated by disc diffusion and dilution methods (CLSI). Statistical analyses were performed with Statistics Pro. **Results.** Pre was mainly from urine (14/16, 88%) unlike Pst (8/23, 35%) ($p<0.01$). CBP non-susceptibility: 100% imipenem and meropenem and 77% ertapenem (100% Pre vs. 61% Pst [$p<0.01$]). 4/14 (29%) hospitals (hosp) reported Pre or Pst during more than one calendar year. 10/39 (26%) isolates co-produced ESBLs (7 CTXM, 3 PER). Susceptibility only to amikacin was mostly linked to Pst (31% vs 6% [$p <0.01$]). By NotI-PFGE, 4 Pre and 5 Pst clonal types were defined. Each dominant clone included 8/16 (3 hosp) and 11/23 (5 hosp) isolates of Pre and Pst, respectively. 56% of Pre (clones A and B) were found at the same hosp. Pst clone A (5 hosp/4 cities) was associated with increased likelihood of severe infection (bloodstream [$p<0.01$]), to co-produce CTXM [$p <0.01$], or to display an extreme-resistant phenotype. **Conclusions:** Two Pre clones were widely found in a single hosp, while a dominant Pst clone was found in several hosp. The spread of NDM-producing Prov is of high concern due to the limited available therapeutic options.

	Year			Total (%)
	2013	2014	2015	2013-2015
P. stuartii (Pst)				
No. of isolates		6	17	23
No. of hospitals / cities		4 / 4	7 / 6	9 / 6
No. and types (%) of ESBL coproduction		0	7 (CTXM)	7 (30%)
No. (%) of isolates susceptible to:				
>=3 drugs		6	6	12 (52%)
2 drugs		0	4	4 (17%)
1 drug		0	7	7 (31%)
NotI-PFGE clonal types (No. of isolates)		B (5); C (1)	A (11); B (2); C (2); D (1); E (1)	A (11)(48%); B (7)(30%); C (3)(13%); D (1)(4%); E (1)(4%)
P. rettgeri (Pre)				
No. of isolates	3	8	5	16
No. of hospitals	2 / 1	1 / 1	5 / 1	6 / 1
No. and types (%) of ESBL coproduction	2(PER)	1 (PER)	0	3 (18%)
No. (%) of isolates susceptible to:				
>=3 drugs	2	6	3	11 (69%)
2 drugs	1	1	2	4 (25%)
1 drug	0	1	0	1 (6%)
NotI-PFGE clonal types (No. of isolates)	A (3)	A (3); B (5)	B (3); C (1); D (1)	A(6)(38%); B(8)(50%); C(1)(6%); D(1)(6%)

Author Disclosure Block:

D.F. Faccone: None. **F. Pasteran:** None. **E. Albornoz:** None. **M. Rapoport:** None. **P. Ceriana:** None. **C. Lucero:** None. **S. Gomez:** None. **A. Corso:** None.

Poster Board Number:

MONDAY-314

Publishing Title:

Prevalence of *qnr* and *aac(6´)-Ib-cr* Genes in Extended-Spectrum β-Lactamase-Producing *Enterobacteriaceae* Clinical Isolates from a Spanish Hospital

Author Block:

M. M. Tavío, F. Artiles, I. Anacarso, C. Poveda, J. B. Poveda; Las Palmas G. C. Univ., Las Palmas G. C., Spain

Abstract Body:

Genes *qnr* and *aac(6´)-Ib-cr* are widespread among *Enterobacteriaceae* clinical isolates producing extended-spectrum beta-lactamases (ESBLs) and may facilitate the selection of quinolone resistance mutations.[1] We evaluated their prevalence in ESBL-positive *Enterobacteriaceae* clinical isolates and ciprofloxacin resistant strains.ESBLs were detected by the double-disk method. The 56 studied ESBL-producing strains included 24 *Escherichia coli*, 19 *Klebsiella pneumoniae*, 5 *Enterobacter cloacae*, 4 *Morganella morganii* and 4 *Proteus mirabilis*. Antibiotic MICs and the effect of the proton motive force inhibitor carbonyl cyanide *m*-chlorophenyl hydrazone (CCCP) on ciprofloxacin MIC were assesed by microdilution method. The presence of *qnr* (A, B, C, D, S) and *aac(6´)-Ib-cr* genes and possible mutations in the Quinolone Resistance Determining Regions (QRDRs) of *gyrA* and *parC* genes were studied.The gene *qnrB1* (7 strains) was predominant, followed by *qnrB6* (3 strains), *qnrS1* (3 strains), and *qnrA1* (1 strain). The *qnr* and *aac(6´)-Ib-cr* genes were more prevalent in plasmids from *K. pneumoniae* strains (53% and 84%) than in those from *E. coli*, *E. cloacae* and *M. morganii* strains (9% and 36%, respectively). No other *qnr* and *aac(6´)-Ib-cr* positive isolate was detected. The *qnr*-positive plasmids were identified nearly 2.3-fold more in ciprofloxacin resistant isolates than in susceptible strains, whereas *aac(6´)-Ib-cr* was spread equally among resistant and susceptible strains. Ciprofloxacin MICs 8- 512 μg/ml were found in 84% of the strains that were associated with single and double mutations in QRDRs at positions 83 and 87 in GyrA and 80 and 84 in ParC. In addition, four mutations at codons Met-52, Ser-83, Asp-87 and Asp-115 in GyrA were found in one *K. pneumoniae* isolate. CCCP induced 2- to 8-fold decreases in ciprofloxacin MICs in 33/56 strains.Our results confirmed that multipe mutations in QRDRs of GyrA and ParC played the most relevant role in ciprofloxacin resistance. The *qnr* genes were more prevalent than *aac(6´)-Ib-cr* gene in ciprofloxacin resistant strains. *K. pneumoniae* clinical isolates were the main reservoir of *qnr* genes. The highest number of mutations in GyrA was found in one *K. pneumoniae* isolate carrying *qnrB1*, *qnrS1* and *aac(6´)-Ib-cr* genes.

Author Disclosure Block:

M.M. Tavío: None. **F. Artiles:** None. **I. Anacarso:** None. **C. Poveda:** None. **J.B. Poveda:** None.

Poster Board Number:

MONDAY-315

Publishing Title:

Detection of Extended Spectrum Beta Lactamases Resistance Genes Among Bacteria Isolated from Selected Drinking Water Distribution Channels in Southwestern Nigeria

Author Block:

A. T. Adesoji[1], A. A. Ogunjobi[2]; [1]Federal Univ. Dutsin-Ma, Dutsin-Ma, Dutsin-Ma, Nigeria, [2]Univ. of Ibadan, Ibadan, Nigeria

Abstract Body:

Antibiotic-resistant bacteria present an important public health challenge worldwide. Aquatic ecosystems are recognized reservoir for antibiotic resistant bacteria. Important antibiotic resistance traits include those encoding the Extended Spectrum Beta-Lactamases (ESBL) that provide high level resistance to beta-lactam antibiotics. Information about the molecular basis of ESBL resistant bacteria is scarce for Drinking Water Distribution systems (DWDS) in southwestern Nigeria. Previously described multidrug resistant bacteria from raw, treated and municipal taps of DWDS from selected dams in southwestern Nigeria were assessed for the presence of ESBL resistance genes which include bla_{TEM}, bla_{SHV} and bla_{CTX} by PCR amplification. A total of 164 bacteria spread across treated (33), raw (66) and municipal taps (68), belonging to α-proteobacteria, β-proteobacteria, ɣ-proteobacteria, flavobacteria, bacilli and actinobacteria group were isolated from this study. Among these bacteria, the most commonly observed resistance was for ampicillin and amoxillin/clavulanic acid (61 isolates). Sixty-one isolates carried at least one of the targeted ESBL genes with bla_{TEM} being most abundant (50/61) and bla_{CTX} being detected least (3/61). *Klebsiella* was the most frequently identified genus (18.03%) to harbor ESBL gene followed by *Proteus* (14.75%). Moreover, combinations of two ESBL genes, bla_{SHV}+bla_{TEM} or bla_{CTX}+bla_{TEM}, were observed in 11 and 1 isolates, respectively. The classic bla_{TEM} ESBL gene was present in multiple bacterial strains that were isolated from DWDS sources in Nigeria. These environments may serve as foci exchange of genetic traits between a diversity of Gram-negative bacteria.

Author Disclosure Block:

A.T. Adesoji: None. **A.A. Ogunjobi:** None.

Poster Board Number:

MONDAY-316

Publishing Title:

Limited Clonal Expansion of Extended Spectrum ß-Lactamase-Producing *Escherichia coli* in a Community

Author Block:

M. Jinnai[1], R. Kawahara[1], D. T. Khong[2], T. N. Nguyen[2], H. T. Tran[2], H. V. Le[2], H. T. Nguyen[2], K. N. Pham[2], S. Ueda[3], Y. Kumeda[1], T. Hamabata[1], T. Nakayama[4], Y. Sumimura[4], Y. Yamamoto[4]; [1]Osaka Prefectural Inst. of Publ. Hlth., Osaka, Japan, [2]ThaiBinh Univ. of Med. and Pharmacy, ThaiBinh, Viet Nam, [3]Univ. of the Ryukyus, Nishihara, Japan, [4]Osaka Univ., Suita, Japan

Abstract Body:

Background: Wide dissemination of the extended spectrum β-lactamase-producing *Escherichia coli* (ESBL-*E. coli*) in communities, particularly in developing countries, is a threat to public health. Recent studies have indicated that the transmission of ESBL genes may be a mechanism underlying the wide dissemination of ESBL-*E. coli*. However, clonal expansion of ESBL-*E.coli* is not studied well. Therefore, we investigated the relationship between ESBL-*E. coli* isolates from different sources such as food, community residents, and clinical samples obtained from patients with urinary-tract infection (UTI) in the same area, Thai Binh, Vietnam.**Method:** In total, 253 samples of retail food (pork, poultry, fish, and shrimp), feces of 194 residents, and urine of 205 clinically diagnosed UTI patients were collected. ESBL production, ESBL-genotype, and phylogeny of all *E. coli* isolates from the samples were examined. Further, we analyzed the diversity of 112 ESBL-*E. coli* isolates by pulsed-field gel electrophoresis (PFGE).**Results and Conclusions:** In total, 69.7%, 89.7%, and 5.6% of food samples, residents, and UTI patients, respectively, were ESBL-*E. coli* positive. Additionally, 95.9% of 642 isolates harbored genes belonged to CTX-M-1 and/or CTX-M-9 group. PFGE analysis revealed the diversity of the ESBL-*E. coli* isolates. However, some isolates from different sources were closely related PFGE patterns. In particular, one cluster contained identical isolates from a food and human. These isolates belonged to the phylogenetic group B2 and harbored the $bla_{\text{CTX-M-27}}$ gene. These results indicate that clonal expansion may have occurred in the community, at least on a limited scale. This is the first report of the identification of the same ESBL-*E. coli* clone in a food and human. Furthermore, close relatedness of ESBL-*E. coli* isolates from different sources indicated the transmission of ESBL- *E. coli* between foods and humans.

Author Disclosure Block:

M. Jinnai: None. **R. Kawahara:** None. **D.T. Khong:** None. **T.N. Nguyen:** None. **H.T. Tran:** None. **H.V. Le:** None. **H.T. Nguyen:** None. **K.N. Pham:** None. **S. Ueda:** None. **Y. Kumeda:** None. **T. Hamabata:** None. **T. Nakayama:** None. **Y. Sumimura:** None. **Y. Yamamoto:** None.

Poster Board Number:

MONDAY-317

Publishing Title:

The Virulence of Ctx-M-15-Producing *Escherichia coli* Isolates of Different Clonal Lineages at a Tertiary Hospital in Nairobi, Kenya

Author Block:

B. Ghebremedhin[1], D. Mania[2], A. Kimang'a[2], G. Revathi[3], W. König[4]; [1]Witten/Herdecke Univ., Clinic Wuppertal, Witten, Germany, [2]Aga Khan Univ. Hosp., Nairobi, Kenya, [3]Aga Khan Univ. Hosp. Nairobi, Nairobi, Kenya, [4]Univ. Clinic Magdeburg, Magdeburg, Germany

Abstract Body:

Background: Multidrug-resistant *Escherichia coli* other gram-negative isolates have been increasing in rate in sub-Saharan Africa. We investigated the correlation between the prevalent extended-spectrum beta-lactamase (ESBL), the virulence factor genes (VFGs) and the genetic lineages of E. coli at the Aga Khan University Hospital in Nairobi within 1-year period.**Methods:** The *E. coli* isolates (n=130) were gained from clinical specimens, including urine (n=104), blood culture and pus (n= 15), skin and wound swabs. Identification and susceptibility testing of were performed by VITEK 2 and E-test. ESBL and quinolone resistance genes (*aac-(6')-lb-cr*) were characterized by PCR and sequencing. Different virulence factor genes (*cdt, cnf, felA, fimA, hlyA, hlyF, papC, nfaE*) were analyzed by PCR. Clonal analysis was performed by multilocus sequence typing (MLST).**Results:** Among the 130 CTX-M-15-producing *E. coli* isolates 43 were recovered from inpatients and 83 from outpatients. Resistance to ciprofloxacin and gentamycin co-existed in these ESBL isolates. All isolates carried the insertion sequence IS*EcpI*. The predominant ST131 ESBL isolates (n=49; 37.7%) harbored the *aac-(6')-lb-cr* gene and were positive for the virulence factor genes *fimA* (81.6%), *papC* (38.8%), *cnf* (8.1%) and *hlyF* (6.1%). Nearly all ST131 *E. coli* (89.8%) were isolated from urine samples, followed by pus and respiratory specimens. Other clones were determined as ST405 (9.2%) and ST648 (8.5%) which were positive for the virulence factor genes *fimA* (16% and 36%), and *papC* (16% and 9%). These two STs were also mostly isolated from urine samples. Less frequent STs were ST10 and ST23 complex, ST156, ST393, ST940, ST1284 and ST1642 which were mostly negative for all VFGs.**Conclusion:** This is the first comprehensive report on CTX-M-15-producing *E. coli* ST131, ST405 and ST648 strains in Nairobi, found predominantly among outpatients. The high diversity of STs is remarkable among the CTX-M-15 *E. coli*. Noteworthy is the higher virulence of the most prevalent ST131 *E. coli* as compared to other STs. This study highlights the need to intensively investigate the diverse ESBL dissemination in Kenya.

Author Disclosure Block:

B. Ghebremedhin: None. **D. Mania:** None. **A. Kimang'a:** None. **G. Revathi:** None. **W. König:** None.

Poster Board Number:

MONDAY-318

Publishing Title:

Ctx-M-27 Producing *Salmonella enterica* Serotypes Typhimurium and Indiana Are Prevalent among Livestock in China

Author Block:

W-H. Zhang, Z-L. Zeng, **H-X. Jiang**; South China Agricultural Univ., Guangzhou, China

Abstract Body:

Background: *Salmonella spp*. are one of the most important food-borne pathogens causing digestive tract and invasive infections in both humans and animals. Extended-spectrum β-lactamases (ESBLs) especially the CTX-M-type ESBLs are increasingly being reported worldwide and in China. These studies seldom focused on *Salmonella* isolates from food-producing animals. The aim of this study was to characterize the antimicrobial resistance profiles, serotypes and ESBLs and in particular, CTX-M producing Salmonella isolates from chickens and pigs in China.**Methods:** *Salmonella* isolates were identified by API20E system and polymerase chain reaction (PCR) assay; serotypes were determined using slide agglutination with hyperimmune sera; antimicrobial susceptibility was tested using the ager dilution method; the prevalence of ESBLs genes were screened by PCR; CTX-M-producing isolates were further characterized by conjugation along with genetic relatedness and plasmid replicon type.**Results:** In total, 159 *Salmonella* strains were identified, among which 95 strains were *S. enterica* serovar Typhimurium, 63 strains were *S. enterica* serovar Indiana and 1 strain was *S. enterica* serovar Enteritidis. All of these isolates presented multi-drug resistant phenotypes. Forty-five isolates carried CTX-M genes, the most common CTX-M-27(34), followed by CTX-M-65(7) and CTX-M-14(4).Most $bla_{\text{CTX-M}}$ genes were transmitted by non-typeable or IncN/IncFIB/IncP/IncA/C/IncHI2 plasmids with sizes ranging from 80 to 280 kb. In particular, all the 14 non-typeable plasmids were carrying $bla_{\text{CTX-M-27}}$ gene and had a similar size. PFGE profiles indicated that CTX-positive isolates were clonally related among the same serotype, whilst the isolates of different serotypes were genetically divergent. This suggested that both clonal spread of resistant strains and horizontal transmission of the resistance plasmids contributed to the dissemination of $bla_{\text{CTX-M-9G}}$-positive *Salmonella* isolates.**Conclusions:**The presence and spread of CTX-M, especially the CTX-M-27-positive serovars Typhimurium and Indiana in food-producing animals poses a great threat for public health. Control strategies to limit the dissemination of these strains through the food chain are necessary.

Author Disclosure Block:

W. Zhang: None. **Z. Zeng:** None. **H. Jiang:** None.

Poster Board Number:

MONDAY-319

Publishing Title:

Whole Genome Sequencing Of Shiga Toxin-Producing *e. Coli* In The New York State Public Health Laboratory To Identify Serogroup, Virulence Factors, And Genomic Clusters

Author Block:

S. E. Wirth, T. Quinlan, D. J. Baker, D. Bopp, T. Halse, P. Lapierre, W. J. Wolfgang, K. A. Musser; Wadsworth Ctr., NYSDOH, Albany, NY

Abstract Body:

In the United States, it is estimated that shiga toxin producing *E.coli* (STEC) is responsible for at least 265,000 infections each year. STEC in non-O157 serogroups cause approximately 75% of these infections. At the Wadsworth Center, certain virulence factors and the more common serogroups are currently characterized for each isolate by using up to seven real-time PCR assays. Concurrently, pulsed-field gel electrophoresis (PFGE) is performed to identify the PFGE subtype to aid in epidemiological investigations. The use of multiple molecular tests for identification and typing is time-consuming and expensive.Whole Genome Sequencing (WGS) can yield information regarding virulence factors, serogroup, and genomic subtyping from a single dataset, potentially leading to reduced costs and improved turn around time. Furthermore, genomic subtyping can enhance cluster resolution compared to PFGE information alone. To evaluate WGS as a "one-stop-shop" for STEC identification and typing, we have sequenced and analyzed 200 sporadic and outbreak-associated STEC isolates within serogroups O26, O45, O103, O111, and O145. WGS was performed on an Illumina Miseq^{TM} using 2 x 250 paired end chemistry. After passing quality control metrics, sequence reads were analyzed using an in-house developed bioinformatic pipeline. Serogroup and virulence profiles were assigned from raw reads and assembled genomes. In addition, the pipeline mapped raw reads to a single O26 serogroup reference genome and a SNP-based phylogenetic tree was produced. Isolates belonging to the same serogroup were diverse with 0 to >300 SNPs difference while isolates belonging to different serogroups could be thousands of SNPs apart. However, isolates from five different outbreaks clustered tightly with only 0 to 4 SNPs difference. Furthermore, there is a high degree of concordance between SNP-based phylogenetic clusters, PFGE-defined clusters, and epidemiologically defined outbreaks. Our next steps are to refine the pipeline, investigate the impact of using reference genomes from different serogroups to construct our SNP-based tree, and validate for New York State clinical laboratory approval to report WGS-derived serogroup and virulence data.

Author Disclosure Block:

S.E. Wirth: None. **T. Quinlan:** None. **D.J. Baker:** None. **D. Bopp:** None. **T. Halse:** None. **P. Lapierre:** None. **W.J. Wolfgang:** None. **K.A. Musser:** None.

Poster Board Number:

MONDAY-320

Publishing Title:

PCR-RFLP Based Screening Method for *Salmonella enterica* Serovar Typhi to Detect the H58 Lineage and Related Mutations in *gyrA, gyrB, parC* and *parE* Genes

Author Block:

A. M. Tanmoy[1], S. Baker[2], S. P. Luby[3], S. K. Saha[1]; [1]Child Hlth.Res. Fndn., Dhaka, Bangladesh, [2]Oxford Univ. Clinical Res. Unit, Ho Chi Minh City, Viet Nam, [3]Stanford Univ., Stanford, CA

Abstract Body:

Typhoid fever, caused by *Salmonella* Typhi, is a major cause of severe illness particularly among children and adolescents in South Asia. Since the early 1990s, a dominant multidrug-resistant (MDR) lineage (H58) of this organism has been emerged and spread globally. In addition to the MDR phenotype, a high percentage of H58 isolates also exhibit reduced susceptibility against fluoroquinolones via mutations in DNA Gyrase (*gyrA* & *gyrB*) and Topoisomerase IV (*parC* & *parE*) genes. Currently, detection of lineage H58 depends on genome sequencing, which is prohibitively expensive and time consuming. To address this limitation, we have developed a PCR-RFLP based method to identify the H58 lineage among *S.* Typhi. In addition, we have developed a method to screen the mutations in *gyrA*, *parC*, *gyrB* and *parC*. PCR primers were designed from the NCBI-Genbank *S.* Typhi CT18 sequence. Using NEB-cutter tool, appropriate restriction enzymes were selected based on their cutting site and specificity for the mutations in the PCR generated amplicons. *Spe*I was selected to detect H58, targeting the glpA-C1047T mutation. In case of *gyrA* gene, a previously described method using *Hinf*I enzyme to detect the *gyrA*-83 & 87 mutations was adapted with the addition of *parE*-420. Using the same principle, *Bsa*JI was selected to detect *gyrB*-464 and *Hae*II for *parC*-57 & 80 mutations. These methods were validated using a library of 86 *S.* Typhi isolates, with and without MDR and differing degrees of susceptibility to ciprofloxacin. Our method detected 67 isolates (78%) with H58 lineage of *S.* Typhi. Among them 78% were MDR and 97% had reduced susceptibility or resistance against ciprofloxacin. The H58 isolates had mutation at *gyrA*-83 (97%), *gyrA*-87 (1.5%), *gyrA*-83, 87 (1.5%), *gyrB*-464, (0%), *parC*-57 (0%) and *parC*-80 (2%). Among the non-H58 isolates, the mutation rate was 68%, 26%, 0%, 11%, 5% and 0% to the corresponding genes and codons. Overall, this low-cost and simple method to detect H58 *S.* Typhi and mutations in four genes responsible for reduced susceptibility against fluoroquinolones will be of significance for surveillance and monitoring of drug resistance of *S.* Typhi, specifically in resource poor setting.

Author Disclosure Block:

A.M. Tanmoy: None. **S. Baker:** None. **S.P. Luby:** None. **S.K. Saha:** None.

Poster Board Number:

MONDAY-321

Publishing Title:

Epidemiological Investigation on Uropathogenic *Escherichia coli* to Identify Potential Vaccine Candidate for Urinary Tract Infection

Author Block:

M. Mally[1], C. DebRoy[2], V. Gambillara Fonck[1]; [1]LimmaTech Biologics AG, Schlieren, Switzerland, [2]ECRC (E. coli Reference Ctr.), Pennsylvania, PA

Abstract Body:

Urinary tract infections (UTI) are one of the most prevalent infectious diseases worldwide that have significant impact on quality of life and health care costs. Originating from the gut flora, *Escherichia coli* is the most common cause of community and nosocomially acquired infections leading to acute cystitis, pyelonephritis or urosepsis. While the current standard of care is antimicrobial therapy, uropathogens are getting increasingly resistant to certain types of antimicrobials. Hence, alternative treatment strategies are gaining significant importance. GlycoVaxyn developed a proprietary *in vivo* conjugation technology that enzymatically transfers a complex polysaccharide to a consensus protein sequence that allows the efficient production of a multivalent glycoconjugate vaccine against the relevant O-antigen of *E. coli*. In order to evaluate the prevalence of *E. coli* associated with UTI-and the predominant O-serogroups of the strains in community and hospital settings, GlycoVaxyn (now LimmaTech) performed an epidemiological study in Switzerland characterizing the pathogen causing more than 3,000 cases of- UTI and collected over 1800 *E. coli* strains isolated from urines of humans in order to identify the main O-serogroups associated with urinary tract infection. Our findings confirmed that *E. coli* is the predominant pathogen causing UTI infection and showed that a limited subset of O-serogroups accounted for more than 63% of the *E. coli* isolates. Therefore, the O-antigens associated with the prevalent serogroups may be considered as potential vaccine candidates for UTI. Many of these strains were highly resistant to antimicrobials. While the incidence of fluoroquinolone resistance was 24% among the isolates, 4% of all isolates produced extended-spectrum beta-lactamase. In addition, we observed that the antibiotic-resistance is specifically widespread in *E. coli* O25. These findings support the profile and development of O-antigen specific vaccine for the prevention of E.coli infections. Moreover, the glycoengineering technology has the suitable applicability for the development of such a multivalent vaccine candidate.

Author Disclosure Block:

M. Mally: None. **C. DebRoy:** None. **V. Gambillara Fonck:** None.

Poster Board Number:

MONDAY-322

Publishing Title:

Usefulness of a Pcr Assay for the Identification of the *Klebsiella pneumoniae* st258 Epidemic Clone in Latin American Clinical Isolates

Author Block:

S. A. Gomez, M. Rapoport, N. Piergrossi, D. Faccone, F. Pasteran, ReLAVRA-Group, A. Petroni, A. Corso; Serv. Antimicrobianos, Laboratorio Natl. de Referencia (LNR), INEI-ANLIS "Dr. Carlos G. Malbrán"., Buenos Aires, Argentina

Abstract Body:

Background: The worldwide dissemination of ST258 of *Klebsiella pneumoniae* (KP)-KPC producers encouraged investigators to develop a rapid typing method based on the identification of ST258 unique gene *pilv-l,* and phage related protein (*prp)* which is neither specific nor ubiquitous of ST258 *(*Adle*r et. al. DMID 2014, 78:12-15)*. Prompt identification of ST is invaluable for some Latin American (LA) countries where this clone has become endemic. **Objective:** to evaluate a PCR developed for ST258 in KP-KPC clinical isolates from LA. **Methods:** KPC, pilv-l and prp genes were detected by PCR and sequenced following standard procedures. Specific primers were used to detect the 3´end of PilV-like gene which is highly specific of ST258 (product size 320 bp) and *prp* gene (product size 544 bp). ST258 and non-ST258 were defined by Xba-I-PFGE and MLST. A total of 107 KP clinical isolates from 9 LA countries recovered from 2006 to 2015 where tested, of which 44 were ST258 and 63 non-ST258. **Results:** The *pilv-l* gene was present only in ST258 isolates regardless of the presence of KPC (positive predictive value 100%). All *pilvl* negative isolates were non-ST258 (negative predictive value 100%). *prp* results were variable. Remarkably, four ST258 isolates were positive for *pilv-l*, negative for KPC and carried CTX-M (n=3) or NDM (n=1). **Conclusions:** The evaluation of a specific PCR for ST258 unique gene that could replace time and resource consuming methods (MLST and PFGE) was successful for the LA isolates tested. In our setting, *pilv-l* detection is enough to discriminate ST258 from other STs.

K. pneumoniae	KPC	n	*pilvl+/prp+*	*pilvl+/prp-*	*pilvl-/prp+*	*pilvl-/prp-*
ST258	+	40	16	24	0	0
n=44	-	4	3	1	0	0
non-ST258	+	40	0	0	6	34
n=63	-	23	0	0	1	22

Author Disclosure Block:

S.A. Gomez: None. **M. Rapoport:** None. **N. Piergrossi:** None. **D. Faccone:** None. **F. Pasteran:** None. **A. Petroni:** None. **A. Corso:** None.

Poster Board Number:

MONDAY-323

Publishing Title:

Fda-*Escherichia coli* Identification (Fda-Ecid) Microarray: A Pan-genome Molecular Toolbox for Serotyping, Virulence Profiling, Molecular Epidemiology, and Phylogeny

Author Block:

I. R. Patel[1], J. Gangiredla[1], D. W. Lacher[1], M. K. Mammel[1], S. A. Jackson[2], K. A. Lampel[1], C. A. Elkins[1]; [1]FDA, Laurel, MD, [2]NIST, Gaithersburg, MD

Abstract Body:

Most *Escherichia coli* are non-pathogenic and some have a beneficial effect on humans. However, it is important to identify pathogenic strains for clinical diagnosis as well as food safety analysis. Commonly used methods for the identification of pathogenic *E. coli* are either time-consuming and/or provide limited and sometimes incomplete information to make a definitive determination of the potential risk to human health. A high-density custom DNA microarray was designed with informative genetic features extracted from 368 whole genome sequences (WGS) for rapid and high-throughput pathogen identification. The FDA-ECID microarray contains three sets of molecularly informative features that function together to stratify strain identification and relatedness. First, 53 known flagellin alleles, 103 alleles of *wzx* and *wzy*, and 5 alleles of *wzm*, provide extensive utility to perform molecular serotyping. Second, 41,932 probe sets representing the pan-genome of *E. coli* provide strain-level gene content information. Third, approximately 125,000 single nucleotide polymorphisms (SNP) of available WGS were distilled to the most discriminatory 9984 SNPs capable of recapitulating the phylogeny of *E. coli* with greater detail than previously reported for available typing methods. Using this platform, we analyzed 103 diverse *E. coli* isolates with available WGS data, including those associated with past foodborne illnesses, to determine robustness and accuracy. The array was able to accurately identify the molecular O and H serotypes of all isolates tested. In addition, molecular risk assessment was possible with virulence maker identifications, as exemplified with the targeted *stx* and *eae* alleles. Epidemiologically, each strain had a unique comparative genomic fingerprint that was extended to an additional 507 strains with strain-level resolution demonstrated for food and clinical examples. Finally, a 99% phylogenetic concordance was established between microarray analysis and WGS using SNP-level data for advance genome typing. The current study confirms the FDA-ECID microarray as a powerful tool for epidemiology and molecular risk assessment with the capacity to profile the global landscape and diversity of *E. coli*.

Author Disclosure Block:

I.R. Patel: None. **J. Gangiredla:** None. **D.W. Lacher:** None. **M.K. Mammel:** None. **S.A. Jackson:** None. **K.A. Lampel:** None. **C.A. Elkins:** None.

Poster Board Number:

MONDAY-324

Publishing Title:

Molecular Characterization of Enteropathogenic *Escherichia coli* from Children under Than 5 Years with and without Diarrhea in a Rural Area of Southern Mozambique

Author Block:

D. C. Vubil, A. Amos, M. Garrine, T. Nhampossa, I. Mandomando; Manhica Hlth.Res. Ctr., Manhica, Mozambique

Abstract Body:

Introduction: Enteropathogenic *Escherichia coli* are the major cause of infant diarrhea in developing countries. The pathogenicity of EPEC strains has been associated with expression of diverse virulence genes therefore it is possible that different EPEC isolates hold different pathogenic potentialities.**Objectives:** To determine molecular variability of EPEC virulence genes (*eae*, *bfpA* and *perA*) recovered from children under than 5 years with diarrhea in Manhiça District, southern Mozambique.**Material and Methods:** We genetically characterized a collection of EPEC isolates recovered from children under than 5 years admitted to Manhiça District Hospital from 2007-2010 as part of multi-centric case/ control study of etiology of diarrhea in developing countries. EPEC identification and typing of virulence genes was done by conventional PCR.**Results:** total number of 151 EPEC isolates (48 cases of diarrhea, 103 controls) have been characterized. Typical EPEC (*bfpA* with or without eae) was identified in 97(64%) while atypical EPEC (*eae* only) was found in 54(36%). The operon regulator gene *perA* was found in 73(48%). Fifteen different eae types have been identified. The most found were *eae*-λ with 15(31%) for cases and 23(22%) for controls, followed by *eae*-β1 with 12(25%) for cases and 21(20%) for controls, *eae*-ξR/β2B‡§ with 9(18%) for cases and 12(11%) for controls and *eae*-μB# with 9(18%) for cases and 9(8%) for controls, although were not statistical different.**Conclusions:** This is the first report on molecular characterization of EPEC strains in Mozambique, showing that some virulence genes are likely to be more common among cases of diarrhea than in controls although, its implication in severity remain unclear.

Author Disclosure Block:

D.C. Vubil: None. **A. Amos:** None. **M. Garrine:** None. **T. Nhampossa:** None. **I. Mandomando:** None.

Poster Board Number:

MONDAY-325

Publishing Title:

Variation in the Distribution of Shiga Toxin-Producing *Escherichia coli* O157 Genotypes from Humans and Cattle

Author Block:

P. Singh[1], R. Mosci[1], M. Moore[1], K. Jernigan[1], J. T. Rudrik[2], G. H. Loneragan[3], S. D. Manning[1]; [1]Michigan State Univ., East Lansing, MI, [2]Michigan Dept. of Hlth.and Human Services, Lansing, MI, [3]Texas Tech Univ., Lubbock, TX

Abstract Body:

Escherichia coli O157:H7 is an important serotype of Shiga toxin (stx)-producing *E. coli* (STEC), a foodborne pathogen responsible for bloody diarrhea and hemolytic uremic syndrome. According to the CDC, there has been a decline in STEC O157 cases in 2014 as compared to 2006-2008, however it is still predominantly associated with outbreaks relative to other foodborne pathogens. Here, we characterized clinical (N=212) and cattle (N=101) isolates from Michigan (MI) and Texas (TX) in 2007-2012 using single nucleotide polymorphism (SNP) genotyping. SNP genotypes were classified into previously defined clades using a modified Illumina-based GoldenGate assay targeting 80 loci and subsequent phylogenetic analyses. Among all isolates examined, the cattle and clinical isolates clustered into six clusters, which correlated with specific virulence genes. Isolates in clusters 1 and 2 were significantly ($p<0.0001$) more likely to harbor *stx1,2* and *eae*, whereas isolates in clusters 4-6 only had *stx*2 and *eae*. Although previously defined clades (1&2 and 7&9) clustered together, new SNP genotypes within the existing clades were identified. As formerly reported among clinical isolates from MI, clade 8 (39%) and clades1&2 (37%) were still dominant from 2007-2012 followed by clade 3 (21%). Relative to the isolates recovered from 1998-2006, the frequency of clade 8 (22%) isolates has continued to increase in MI, while clade 2 (53%) has decreased. Clade 2 (40%) in cattle predominated followed by clades 8 (27%) and 7 (21%), though the distribution varied between MI and TX. Cattle isolates from MI most commonly belonged to clade 2 (80%) versus 28% from TX. None of the cattle-derived isolates from MI were classified as clade 8 compared to 35% from TX. Further, during the same time period, the high clade 2 frequency among bovine isolates may partly explain the increase in clade 2 frequency observed in clinical isolates. This study demonstrates variation in the circulation of *E. coli* O157 genotypes in MI patient and cattle populations in both MI and TX, which could impact disease frequencies in each location.

Author Disclosure Block:

P. Singh: None. **R. Mosci:** None. **M. Moore:** None. **K. Jernigan:** None. **J.T. Rudrik:** None. **G.H. Loneragan:** None. **S.D. Manning:** None.

Poster Board Number:

MONDAY-326

Publishing Title:

Isolation of Six *Enterobacteriaceae* Producing New Delhi Metallo-Beta-Lactamase (Ndm-1) in a Pediatric Patient from Argentina

Author Block:

F. Martino[1], N. Tijet[2], R. Melano[2], F. Pasteran[1], M. Rapoport[1], D. Faccone[1], E. Biondi[3], M. Vazquez[3], A. Corso[1], **S. A. Gomez**[1]; [1]Serv. Antimicrobianos, Laboratorio Natl. de Referencia (LNR), INEI-ANLIS "Dr. Carlos G. Malbrán", Ciudad Autonoma de Buenos Aires, Argentina, [2]Publ. Hlth.Ontario Lab., Toronto, Ontario, Canada, Toronto, ON, Canada, [3]Hosp. de Niños Dr. Ricardo Gutiérrez, Ciudad Autonoma de Buenos Aires, Argentina

Abstract Body:

BACKGROUND: In Argentina, KPC is endemic and represents a severe public health problem, but since 2013, NDM metallo-β-lactamase has emerged in our country, and since then, its detection has been increasing **OBJECTIVES:** To characterize *bla*$_{NDM}$ carrying plasmids recovered from six *Enterobacteriaceae* (ETB) isolated from a single patient **METHODS:** Specie identification was done by MALDI-TOF (Bruker Co). MBL-production were evaluated by the Blue-Carba test (BCT) and synergism between carbapenems and EDTA/SMA discs, respectively. MICs were determined by agar dilution (CLSI 2015). PCR, sequencing and conjugation were performed by standard methods. *bla*$_{NDM-1}$ carrying plasmids were extracted from the transconjugants (TC) with the Qiagen Large-Construct kit and sequenced with the Illumina-MiSeq. Assembly of the contigs was done with the CLC Genomics Workbench software (CLC bio, Qiagen). Open reading frames were predicted and annotated by the RAST server (rast.nmpdr.org), followed by manual curation and searches in the NCBI (www.ncbi.nlm.nih.gov/BLAST) **RESULTS:** A 4 y.o. boy cursing erythrodermic psoriasis with previous hospitalizations and multiple antimicrobial treatments was hospitalized for 137 days until his death due to septic shock. Between day 39 and 132, *K. pneumoniae, E. coli (n=2), C. freundii, E. cloacae and S. marcescens* were isolated from blood, retroculture and rectal swabs. All isolates and TC were resistant to carbapenems. Fosfomycin and minocycline were the most active drugs. All harboured the same ca139 Kb IncF plasmid with 176 ORFs carrying *bla*$_{NDM-1}$, *bla*$_{CMY-6}$, *rmtc, aacA4, aac(6´)Ib-cr, sul1* resistance genes. *bla*$_{NDM-1}$ environment gene order was: *rmtc*-IS*Kpn14*-delta IS*Aba125*-*bla*$_{NDM-1}$-*ble*$_{MBL}$-*trp*F-*tat*-*dct*-GroES-GroEL-*ins*A. These plasmids had 99% identity with 98% of coverage with pNDM-US (CP006661) **CONCLUSIONS:** As far as we know, this is the first report of *bla*$_{NDM}$ in the same IncF plasmid in 6 ETB from a patient, alerting of the potential of the dissemination of this plasmid

Author Disclosure Block:

F. Martino: None. **N. Tijet:** None. **R. Melano:** None. **F. Pasteran:** None. **M. Rapoport:** None. **D. Faccone:** None. **E. Biondi:** None. **M. Vazquez:** None. **A. Corso:** None. **S.A. Gomez:** None.

Poster Board Number:

MONDAY-327

Publishing Title:

Global Molecular Epidemiology of Imp and Vim-producing *Enterobacteriaceae*

Author Block:

Y. Matsumura[1], G. Peirano[2], M. Hackel[3], D. J. Hoban[3], M. R. Motyl[4], R. DeVinney[1], J. Pitout[2]; [1]Univ. of Calgary, Calgary, AB, Canada, [2]Calgary Lab. Service, Calgary, AB, Canada, [3]IHMA, Schaumburg, IL, [4]Merck Sharp & Dohme Corp., Kenilworth, NJ

Abstract Body:

Background: Carbapenemase-producing Enterobacteriaceae (En) is an emerging global threat. IMP and VIM carbapenemases are endemic in certain regions, although international data on molecular epidemiology are lacking. We investigated clinical IMP and VIM-producing En collected from the SMART worldwide surveillance program during 2008 and 2013. **Methods:** Genomic sequencing of 34 IMP and 81 VIM-producing En was performed on Illumina next generation sequencing systems. Carbapenemase genes and their flanking regions were analyzed using ARG-ANNOT database and BLASTN. In silico multilocus sequence typing was used to identify international lineages. **Results:** Most IMP-producing En were obtained from South Pacific (n=18) and Asia (n=14), whereas most VIM-producing En were obtained from Europe (n=71) followed by Africa (n=4). *Klebsiella pneumoniae* and *Enterobacter cloacae* complex were the predominant species, and IMP-26 and VIM-1 were the predominant carbapenemases. *K. pneumoniae* clonal complex (CC) 14/15 was the most prevalent lineage associated with bla_{IMP} (IMP-26, n=9 from Philippines; IMP-6, n=2 from Japan). Among 59 VIM-1-producing En isolates, *K. pneumoniae* CC147 was detected in Greece and Italy and CC17 was detected in Greece and South Africa. *E. cloacae* complex CC78 was present in Greece, Italy, and Spain and CC114 in Greece, Taiwan, and USA. All of the bla_{IMP} and bla_{VIM} genes were situated within class 1 integrons; $bla_{\text{VIM-1}}$-*aacA4* were the most common cassettes being present in 6 species. *K. pneumoniae* with $bla_{\text{IMP-26}}$ contained a novel $bla_{\text{IMP-26}}$-*qacG-aacA4* structure. **Conclusions:** IMP-producing En were distributed among South Pacific and Asia. VIM-1-producing En had global distribution and specific clones (CC 147 and 17) contributed to its spread. This study highlights the importance of surveillance programs using molecular techniques in providing insight into characteristics and global distribution of STs with carbapenemases.

Author Disclosure Block:

Y. Matsumura: None. **G. Peirano:** None. **M. Hackel:** None. **D.J. Hoban:** None. **M.R. Motyl:** D. Employee; Self; Merck Sharp & Dohme Corp.. **R. DeVinney:** None. **J. Pitout:** None.

Poster Board Number:

MONDAY-328

Publishing Title:

Intestinal Colonization with Extended-Spectrum Cephalosporin-Resistant *Enterobacteriaceae* in HIV-Positive Individuals in Switzerland

Author Block:

J. Pires[1], S. Kasraian[1], R. Tinguely[1], C. Hauser[2], A. Rauch[1], H. Furrer[1], **A. Endimiani**[1]; [1]Univ. of Bern, Bern, Switzerland, [2]Bern Univ. Hosp., Bern, Switzerland

Abstract Body:

Background: The intestinal microbiota of HIV-positive individuals has been shown to be in a state of dysbiosis. This might influence the susceptibility of this population to develop specific gastrointestinal diseases. However, nothing is known about the influence of HIV infection on the intestinal colonization with extended-spectrum cephalosporin resistant *Enterobacteriaceae* (ESC-R-*Ent*). In this study, we aimed to address this research question. **Methods:** So far, 67 HIV-positive volunteers followed by Bern University Hospital have been enrolled (from March 2015 to January 2016). Stools of individuals and their pets (if any) were enriched overnight in LB broth with cefuroxime and plated on BLSE, ChromID ESBL, and Supercarba selective plates. At least 5 ESC-R-*Ent* colonies per sample were recovered. Species ID was assessed using the MALDI-TOF MS. Microarray CT103XL and PCR/DNA sequencing were used to characterize the *bla* genes. Clonality was assessed by MLST. An epidemiological questionnaire was obtained from the volunteers.**Results:** The prevalence of intestinal colonization was 3% (n=2). All ESC-R-*Ent* recovered were *E. coli.* Interestingly, one of the volunteers was colonized with two different *E. coli* clones: a CTX-M-15-producing ST405 and a CTX-M-1-producing ST127. The other one was colonized with a CTX-M-1-producing ST410. All these STs are usually defined as hyperepidemic clones (HiRC). Remarkably, the individual colonized with two clones presents as the only case where the pets living within the same household were also colonized with ESC-R-*Ent*. In particular, one of the cats was colonized with the same CTX-M-1-producing ST127 clone identified in the human, while the other cat with unrelated clone (ST73) and ESBL (CTX-M-15).**Conclusions:** The prevalence of intestinal colonization with ESC-R-*Ent* in the Swiss HIV-population is low. In spite of this, ESBLs and clones identified resemble those frequently associated with HiRC causing serious infections. Moreover, the identification of the same clone within the same household also highlights the potential silent spread of these pathogens between pets and humans in close contact.

Author Disclosure Block:

J. Pires: None. **S. Kasraian:** None. **R. Tinguely:** None. **C. Hauser:** None. **A. Rauch:** None. **H. Furrer:** None. **A. Endimiani:** None.

Poster Board Number:

MONDAY-329

Publishing Title:

Very High Prevalence of Multidrug-Resistant (MDR) *Enterobacteria* in Hospitalized Bacteriemic Patients from Mali

Author Block:

S. A. Sangare[1], A. I. Maiga[1], I. Guindo[2], A. Maiga[3], N. Camara[1], O. Dicko[3], S. Diallo[2], F. Bougoudogo[2], E. Rondinaud[4], A. Andremont[4], I. I. Maiga[3], **L. Armand-Lefevre**[4]; [1]Gabriel Toure Hosp., Bamako, Mali, [2]Univ. of Sci., Techniques, and Technologies of Bamako, Bamako, Mali, [3]Point G Hosp., Bamako, Mali, [4]Bichat Hosp. and UMR 1137 INSERM IAME, Univ. Paris Diderot, Paris, France

Abstract Body:

The worldwide diffusion of MDR enterobacteria, *i.e.* extended spectrum beta-lactamase (ESBL-PE) and carbapenemase producing enterobacteria (CPE), is alarming. Limited data concerning the prevalence of such strains in patients from Sub-Saharan Africa are available. We determined, here, the prevalence of MDR among enterobacteria in bacteriemic patients in two teaching hospitals from Bamako (Mali). **Methods:** During one year, all enterobacteria isolated from blood cultures of patients hospitalized in the Point G Hosp. and in the pediatric units of G. Toure Hosp. (550 and 110 beds) were collected. Identifications and antibiotic susceptibility testing were performed using API 20E ID system and VITEK 2 (Biomérieux) in Mali, and confirmed respectively using mass spectrometry (Bruker) and disk diffusion method in France (Bichat hospital). Results were interpreted according to the EUCAST recommendations. PCR and sequencing were performed on ESBL-PE and CPE to determine the type of enzyme. **Results:** 82 enterobacteria were isolated from 77 patients, [mean age 41.5 year (15-81y) for Point G Hosp. and 3.5 y (3m-13y) for G. Toure Hosp.; sex ratio 1.3], 31 (37.8%) *E. coli*, 26 (31.7%) *K. pneumoniae*, 15 (18.3%) *E. cloacae*, 5 *S.* Enteritidis and 5 others. In all, 58.5% (48/82) of the strains produced an ESBL [20/31 (64.5%) *E. coli*, 20/26 (76.9%) *K. pneumoniae* and 8/15 (53.3%) *E. cloacae*] without any difference between the two hospitals. One *E. coli* produced an OXA-181 carbapenemase. Among the ESBL enzyme, CTX-M groupe 1 was highly prevalent (43/48, 89.6%), the remaining being SHV-7 (n=3) and TEM (n=2). Except for amikacin (2.4%), resistance rates to other antibiotic classes were high, 52.4 % to gentamicin, 75.6% to co-trimoxazol and 53.7% to fluoroquinolones. **Conclusion:** A very high prevalence of ESBL-PE and one CPE were observed in enterobacteria isolated in bacteriemic patients hospitalized in Bamako. Among antibiotics, only carbapenem and amikacin remained frequently susceptible. Theses data need to be taken into account to establish guidelines for bacteremia treatment.

Author Disclosure Block:

S.A. Sangare: None. **A.I. Maiga:** None. **I. Guindo:** None. **A. Maiga:** None. **N. Camara:** None. **O. Dicko:** None. **S. Diallo:** None. **F. Bougoudogo:** None. **E. Rondinaud:** None. **A. Andremont:** None. **I.I. Maiga:** None. **L. Armand-Lefevre:** None.

Poster Board Number:

MONDAY-330

Publishing Title:

***bla*$_{\text{CTX-M-15}}$ Carried by IncF-Type Plasmids Is the Dominant ESBL Gene in *Escherichia coli* and *Klebsiella pneumoniae* at a Hospital in Ghana**

Author Block:

A. Agyekum[1], A. Fajardo-Lubián[1], D. Ansong[2], S. Partridge[1], T. Agbenyega[2], J. Iredell[1]; [1]Ctr. for Infectious Diseases and Microbiol., The Westmead Inst. for Med. Res., The Univ. of Sydney, Westmead Hosp., New South Wales, Westmead, Australia, [2]Malaria Res. Ctr., Agogo Presbyterian Hosp., Komfo Anokye Teaching Hosp., Sch. of Med. Sci., Kwame Nkrumah Univ. of Sci. and Technology, Kumasi, Ghana, Kumasi, Ghana

Abstract Body:

Escherichia coli and *Klebsiella pneumoniae* producing extended-spectrum β-lactamases (ESBLs) are among the most multidrug-resistant pathogens in hospitals and are spreading worldwide. ESBLs are capable of hydrolysing penicillin, broad-spectrum cephalosporins and monobactams and are mostly of the TEM, SHV or CTX-M-type with the later becoming more predominant over the last decade. ESBLs are often located on plasmids and horizontal transfer of these plasmids, as well as spread of high risk clones, are involved in their dissemination. Investigation of the resistance phenotypes of 101 consecutive clinical *E. coli* (n=58) and *K. pneumoniae* (n=43) isolated at the Komfo Anokye Teaching Hospital in Ghana over three months revealed 63 (62%) with an ESBL phenotype. All 63 isolates had a *bla*$_{\text{CTX-M}}$ gene and sequence analysis showed that 62 of these were *bla*$_{\text{CTX-M-15}}$. *bla*$_{\text{CTX-M-15}}$ was linked to IS*Ecp1* and orf477Δ in all isolates and most isolates also carried *bla*$_{\text{TEM}}$, *aac(3)-II*, *aacA4*cr and/or *bla*$_{\text{OXA-30}}$ genes on IncF plasmids. XbaI/pulsed-field electrophoresis showed heterogeneity among isolates of both species, suggesting that *bla*$_{\text{CTX-M-15}}$ dissemination is caused by horizontal gene transfer rather than clonal spread of these species. The study revealed the dominance and polyclonal spread of *bla*$_{\text{CTX-M-15}}$ among *Enterobacteriaceae* with an ESBL phenotype in a teaching hospital in Ghana.

Author Disclosure Block:

A. Agyekum: None. **A. Fajardo-Lubián:** None. **D. Ansong:** None. **S. Partridge:** None. **T. Agbenyega:** None. **J. Iredell:** None.

Poster Board Number:

MONDAY-331

Publishing Title:

Molecular Characteristics of CTX-M β-Lactamase-Producing Clinical Isolates of *Escherichia coli* in Japan

Author Block:

R. Nakano[1], K. Hikosaka[2], A. Nakano[1], S. Endo[3], K. Kasahara[1], Y. Ono[4], H. Yano[1]; [1]Nara Med. Univ., Kashihara-shi, Nara, Japan, [2]Graduate Sch. of Med., Chiba Univ., Chiba, Japan, [3]Tohoku Univ. Graduate Sch. of Med., Miyagi, Japan, [4]Teikyo Univ. Sch. of Med., Tokyo, Japan

Abstract Body:

Background: Plasmid-mediated β-lactamase CTX-M-type has become the most prevalent extended-spectrum β-lactamase (ESBL) identified in both nosocomial and community settings. Among CTX-M-type ESBL-producing *Escherichia coli*, CTX-M-15 (which belongs to the CTX-M-1 group)-producing *E. coli* have spread worldwide. In this study, we investigated molecular characteristics of clinical isolates of CTX-M-producing *E. coli* in Japan. **Methods:** Fifty non-duplicate CTX-M-producing *E. coli* isolates were obtained from various hospitals throughout Japan between 2013 and 2015. These specimens were isolated from hospital patients and outpatients. The antimicrobial susceptibility profiles of these strains were determined by CLSI guidelines. β-lactamase genes and MLST were identified by PCR analysis and DNA sequencing. The transferability of $bla_{\text{CTX-M}}$-harboring plasmids was confirmed by transconjugation, and plasmid Inc groups were determined by PCR-based replicon typing. **Results:** Of the 50 isolates, 22 (44%) and 28 (56%) were positive for CTX-M-1 and CTX-M-9 groups, respectively. The other CTX-M group β-lactamases were not detected. Of the CTX-M-1 group, CTX-M-15 was the most prevalent (14/22), followed by CTX-M-55 (5/22) and CTX-M-1 (3/22). Of the CTX-M-9 group, CTX-M-14 was the most prevalent (17/28), followed by CTX-M-27 (9/28) and CTX-M-24 (2/28). ST131 was dominant in the CTX-M-1 group (17/22) and the CTX-M-9 group (20/28). Forty-five isolates were transferable and mostly belonged to the IncF family (30/45), which included some highly conjugative plasmids (conjugation frequency, 10^{-1} per recipient). Distinct molecular characteristics were not observed between isolates from hospital patients and outpatients. **Conclusions:** Our findings indicate that CTX-M-14 and CTX-M-27 of the CTX-M-9 group were predominant in CTX-M-producing *E. coli* in Japan. Subsequently, CTX-M-15 (CTX-M-1 group)-producing *E. coli* was identified as a worldwide pandemic clone. ST131 and IncF-type plasmids were predominant among CTX-M-producing *E. coli* isolates. These findings may lend insight into the spread of these resistance genes among *E. coli*.

Author Disclosure Block:

R. Nakano: None. **K. Hikosaka:** None. **A. Nakano:** None. **S. Endo:** None. **K. Kasahara:** None. **Y. Ono:** None. **H. Yano:** None.

Poster Board Number:

MONDAY-332

Publishing Title:

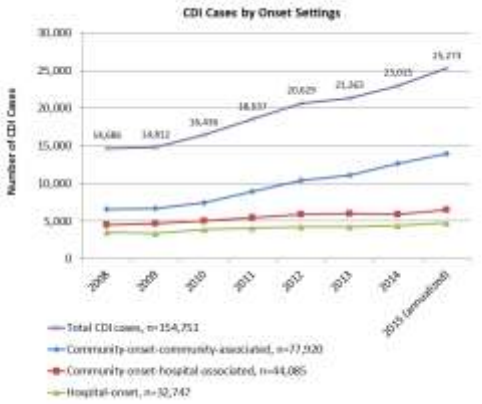
Trends Of Extended-Spectrum β-Lactamases In U.S. Hospitals: 2008-2015

Author Block:

C. A. DeRyke[1], V. Gupta[2], X. Sun[2], D. D. DePestel[1], S. Merchant[1], Y. P. Tabak[2]; [1]Merck & Co. Inc., Rahway, NJ, [2]Becton, Dickinson & Company, Franklin Lakes, NJ

Abstract Body:

Background: We sought to determine the trends in susceptibility of non-duplicate extended spectrum cephalosporin-resistant *E coli, K pneumoniae* (KP), and *P mirabilis* (PM) isolates in the ambulatory, admission, and hospital-onset settings.**Methods:** We analyzed automated electronic data in a research database of Becton Dickinson and Company from the same 154 USA hospitals (2008-2015). All non-duplicate *E coli*, KP, and PM isolates (first isolate of a species per 30 day period) from all sources were categorized as Extended-Spectrum β-Lactamases (ESBLs) if confirmed as ESBL-positive per commercial panels or intermediate/resistant to any of the 4 extended spectrum cephalosporins (ceftriaxone, cefotaxime, ceftazidime or cefepime). Non-duplicate positive isolates were categorized into three settings by the specimen collection time: a) Admission: within 3 days of an inpatient admission and no previous admission within 14 days; b) Hospital-onset: 3 days post-admission or within 14 days of discharge; c) Ambulatory (neither a or b).**Results:** Of the 116,415 ESBLs isolates, 70.7 % were *E coli*, 22.4% KP, and 6.9% PM. For settings, 53.6%, 20.6%, and 25.8% were ambulatory, admission, and hospital-onset. The overall ESBL rate was 4.8%; pathogen specific ESBL rates were 4.6% (*E coli*), 6.5% (KP), and 3.5% (PM). From 2008 to 2015, the ESBL rates increased from 2.3% to 6.9% (*E coli*, $p < 0.0001$), 6.2% to 7.1% (KP, $p=0.09$), and 1.8% to 4.2% (PM, $p<0.0001$). The overall ESBLs rates increased in all three settings (all $p<0.0001$, see figure). **Conclusions:** ESBL resistance rates increased steadily from 2008 through 2015 in ambulatory, admission, and hospital settings in the U.S. *E coli* accounted for the majority of all the ESBLs.



Author Disclosure Block:

C.A. DeRyke: D. Employee; Self; Merck & Co. Inc. **V. Gupta:** D. Employee; Self; BD. **X. Sun:** D. Employee; Self; BD. **D.D. DePestel:** D. Employee; Self; Merck & Co. Inc. **S. Merchant:** D. Employee; Self; Merck & Co. Inc. **Y.P. Tabak:** D. Employee; Self; BD.

Poster Board Number:

MONDAY-333

Publishing Title:

Identification of β-Lactamase Genes in Clinical Isolates of *Acinetobacter spp.* Collected from Saudi Arabia

Author Block:

S. Schroeder[1], B. Abdalhamid[2], H. Hassan[2], N. D. Hanson[1]; [1]Creighton Univ., Omaha, NE, [2]King Fahad Specialist Hosp, Dammam, Saudi Arabia

Abstract Body:

Background: Gram-negative organisms resistant to β-lactam antibiotics are a growing problem worldwide. Carbapenems are the most potent β-lactam antibiotics available for therapeutic use, but the spread of carbapenemases are threating the utility of these drugs. Enterobacteriaceae, *Pseudomonas aeruginosa*, and *Acinetobacter spp.* (*AS*) are the most prevalent organisms displaying carbapenem resistance. There are several different types of carbapenemases however, data on the occurrence of OXA carbapenemases in isolates of *Acinetobacter spp.* from the Middle East are lacking. The purpose of this study is to identify the types of β-lactamases in clinical isolates of *AS* collected from Saudi Arabia. **Methods:** 25 *AS* isolates where collected from a hospital in Saudi Arabia between January and September of 2013 and identification was completed using API 20E strips (biomerieux). Meropenem, imipenem, and doripenem susceptibilities were evaluated using agar dilution and interpreted using CLSI criteria. PCR amplification was performed using the Philisa rapid amplification system (Streck) and an in house multiplex PCR to identify $bla_{OXA\text{-}like}$ genes. The ARM-D for β-lactamase ID kit (Streck) was used to detect other β-lactamase genes. **Results:** 20/25 *AS* isolates were resistant to all 3 carbapenems tested while 5 isolates were susceptible. $bla_{OXA\text{-}24\text{-}like}$, $bla_{OXA\text{-}48\text{-}like}$, $bla_{OXA\text{-}58\text{-}like}$, and $bla_{OXA\text{-}143\text{-}like}$ genes where not detected in any isolate. 19 isolates were $bla_{\text{OXA-23-like}}(+)/bla_{\text{OXA-51-like}}(+)$, 5 isolates were $bla_{OXA\text{-}23\text{-}like}(-)/bla_{OXA\text{-}51\text{-}like}(+)$, and 1 isolate was $bla_{OXA\text{-}23\text{-}like}(+)/bla_{OXA\text{-}51\text{-}like}(-)$.In addition to $bla_{OXA\text{-}23\text{-}like}$ and $bla_{OXA\text{-}51\text{-}like}$, bla_{KPC} was detected in 1 isolate. **Conclusions:** This is the first report of a KPC β-lactamase in an *AS* isolate outside of Puerto Rico. 80% of these isolates were resistant to all of the carbapenems tested. These data indicate that many *AS* isolates collected in Saudi Arabia are carbapenem resistant and carry the $bla_{OXA\text{-}23\text{-}like}$ gene . In order to understand the mechanisms associated with carbapenem resistance in *AS*, adequate surveillance is key. Molecular detection of genes responsible for β-lactamase production can help identify the presence and spread of OXA carbapenemase genes in isolates of *Acinetobacter*.

Author Disclosure Block:

S. Schroeder: F. Investigator; Self; STRECK. **B. Abdalhamid:** None. **H. Hassan:** None. **N.D. Hanson:** E. Grant Investigator; Self; STRECK.

Poster Board Number:

MONDAY-334

Publishing Title:

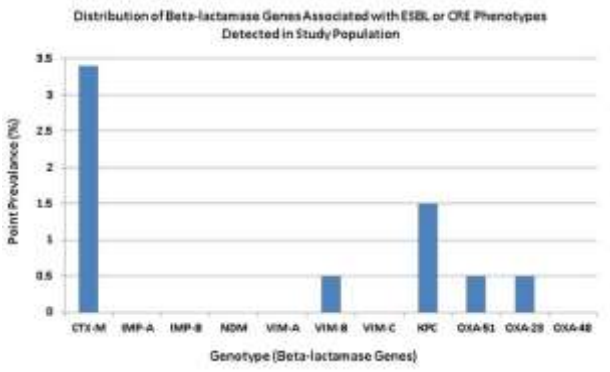
Prevalence And Genotype Characterization Of Multi-Drug Resistant Gram Negative Organism Colonization In Patients Presenting To An Oncology Unit

Author Block:

H. Michael, B. Yen-Lieberman, M. Sekeres, T. Fraser; Cleveland Clinic, cleveland, OH

Abstract Body:

Background: Multidrug-resistant organisms (MDRO), such as extended beta-lactamase (ESBL) and carbapenem-resistant Enterobacteriaceae (CRE) are an increasing burden. Emerging rapid molecular tests can implicate resistance genes, and may be more sensitive than culture. We describe our experience using a novel PCR-based test to characterize the point prevalence and genotype distribution of MDRO colonization in oncology patients. **Methods:** Surveillance peri-rectal swabs were collected within 24 hours of admission from 205 patients presenting to an oncology unit. Sample size was defined by swab manufacturer. Real-time PCR detected beta-lactamase genes associated with ESBL (CTX-M) and CRE (IMP, NDM, VIM, KPC, OXA-51, OXA-23, OXA-48). Swabs also underwent selective CRE culture. Positive CRE screens underwent ID/AST testing on Vitek2.**Results:** Figure 1 shows the point prevalence of gram negative MDRO genes. CTX-M was most common at 3.4% (N=7). CRE-associated genes had a point prevalence of 2.9% (N=5). Selective CRE culture was positive on 4 swabs. Specifically, 3/3 swabs with KPC gene and 1/7 swabs with CTX-M yielded growth of carbapenem-resistant *Klebsiella pneumonia.* Selective culture was negative in 6/7 CTX-M swabs, 1/1 VIM-B swab, and a swab with CTX-M, OXA-23, OXA-51.**Conclusions:**A PCR-based screening test identified a low prevalence of gram negative MDRO genotypes in a select oncology population and identified relevant resistance genes. While the KPC gene has traditionally been associated with the CRE phenotype, we observed heterogeneity in CRE determinants.Screening based on selective culture may be insensitive for non-KPC organisms. Multiplex PCR may be a promising strategy.



Author Disclosure Block:

H. Michael: None. **B. Yen-Lieberman:** None. **M. Sekeres:** None. **T. Fraser:** None.

Poster Board Number:

MONDAY-335

Publishing Title:

Enterprise-Wide Clinical Implementation Of Next Generation Sequencing For Quality Improvement & Infection Control: Managing Expectations

Author Block:

E. Lesho[1], R. Clifford[1], F. Onmus-Leone[1], L. Appalla[1], E. Snesrud[1], Y. Kwak[1], A. Ong[1], R. Maybank[1], P. Waterman[2], P. Rohrbeck[2], M. Julius[1], A. Roth[1], J. Martinez[1], L. Nielsen[3], E. Steele[3], P. McGann[1], M. Hinkle[1]; [1]Walter Reed Army Inst. of Res., Silver Spring, MD, [2]Armed Forces Hlth.Surveillance Ctr., Silver Spring, MD, [3]San Antonio Military Med. Ctr., San Antonio, TX

Abstract Body:

Objective: Next generation sequencing (NGS) is increasingly acclaimed to revolutionize clinical practice, but most reports involved a single facility or were supported by heavily resourced genomics institutes. Based on our recent experience, large, geographically dispersed healthcare networks and government-funded public health laboratories are likely to encounter constraints and challenges implementing NGS across their health systems. To increase awareness of these and of the public availability of the sequences, isolates, and specialized database architecture of our health system, we highlight the genomic epidemiology of an extensive collection of carbapenemase-producing bacteria (CPB).**Design:** Prospective surveillance**Setting:** 288-hospital healthcare network of the Department of Defense**Intervention:** Enterprise -wide implementation of NGS for quality improvement**Methods:** All CPB underwent NGS. Laboratory capacity, throughput, and response time were assessed.**Results:** From 2009 to present, 25,000 multidrug-resistant Gram-negative isolates were submitted. 225 contained carbapenemase-encoding genes (most commonly bla_{KPC} bla_{NDM}, and bla_{OXA23}) and were found in 15 species, 146 inpatients (53% male; average age 36.1 years) from 19 facilities. Genetically related CPB were found in more than one hospital. Other clusters or outbreaks were not clonal, and involved genetically related plasmids, while others involved several unrelated plasmids. Relatedness depended on clustering algorithm used. Transmission patterns of plasmids and other mobile genetic elements could not be determined without additional, expensive, and rarely available, single-molecule real-time sequencing.**Conclusion:** If not addressed, lack of standardized definitions and quality control metrics, limitations of short-read sequencing, insufficient bandwidth, and limited availability of ultra-long read platforms will constrain enterprise-wide NGS implementation efforts.

Author Disclosure Block:

E. Lesho: None. **R. Clifford:** None. **F. Onmus-Leone:** None. **L. Appalla:** None. **E. Snesrud:** None. **Y. Kwak:** None. **A. Ong:** None. **R. Maybank:** None. **P. Waterman:** None. **P.**

Rohrbeck: None. **M. Julius:** None. **A. Roth:** None. **J. Martinez:** None. **L. Nielsen:** None. **E. Steele:** None. **P. McGann:** None. **M. Hinkle:** None.

Poster Board Number:

MONDAY-336

Publishing Title:

Clinical Characteristics and Outcome in Patients with Bacteremia Caused by Extended-Spectrum β-Lactamase (ESBL)- and Non ESBL-Producing *Escherichia coli*

Author Block:

K. Nagaoka, S. Nakakubo, K. Kamada, S. Konno, N. Ishiguro, M. Nishimura; Hokkaido Univ. Sch. of Med., Sapporo, Japan

Abstract Body:

Background: Extended-spectrum beta-lactamase-producing *Escherichia coli* (ESBL-EC) are recently-emerging pathogens worldwide as a cause of community-acquired infection as well as nosocomial infection. Some previous studies suggested high mortality in those patients with bacteremia caused by ESBL- EC bacteremia. However, there have been few studies which compared the clinical features and/or the outcomes between the patients with bacteremia caused by ESBL-EC and those with bacteremia caused by non ESBL-EC. In this study, we attempted to make a direct comparison between the two groups. **Methods:** We retrospectively analyzed 169 consecutive patients with positive blood culture of EC, who had been diagnosed in Hokkaido University between January 2011 and December 2014. We classified the subjects into two groups; ESBL group (N=39) and non ESBL group (N=130), and compared clinical characteristics of the subjects, laboratory data, and regimens of antibiotics at the diagnosis of bacteremia and finally the clinical outcome between the two groups. **Results:** Compared with the non ESBL-EC group, the ESBL-EC group had received any antibiotics more frequently within 3 months before onset of bacteremia (61.5% vs. 33.1% of all the subjects for each; P<0.01), and was being administered inadequate empirical therapy more frequently (58.0% vs. 4.7%; P<0.01). The prevalence of abscess formation in any site of body was significantly higher in the ESBL-EC compared with the non ESBL-EC group (23.1% vs 4.8%; P<0.01). However, the 30 days' mortality was not significantly different between the two groups (13.5% vs. 14.7%; P=1.00). **Conclusions:** Patients with ESBL-producing EC bacteremia tended to be received inadequate empirical therapy at the diagnosis as compared with those with non ESBL EC bacteremia. Higher prevalence of abscess formation might be influenced by the initial treatment failure in ESBL group. However, the 30 days' mortality rate was not significantly different between ESBL-EC bacteremia and that of non ESBL-EC.

Author Disclosure Block:

K. Nagaoka: None. **S. Nakakubo:** None. **K. Kamada:** None. **S. Konno:** None. **N. Ishiguro:** None. **M. Nishimura:** None.

Poster Board Number:

MONDAY-337

Publishing Title:

The Epidemiology of *bla*$_{CMY-2}$ in *Escherichia coli*: A Change Over Time?

Author Block:

K. Tagg, A. Ginn, S. Partridge, J. Iredell; The Westmead Inst. for Med. Res., Westmead, Australia

Abstract Body:

Escherichia coli is a leading cause of urinary tract infections, diarrheal diseases and septic shock worldwide and resistance rates, particularly to the β-lactam antibiotics, are rising globally. Resistance to β-lactams in *E. coli* is predominantly mediated by plasmid-borne β-lactamase genes and among the most common is *bla*$_{CMY-2}$ (and minor variants), conferring resistance to both cephalosporins and β-lactamase inhibitors. Understanding the transmission dynamics of genes such as *bla*$_{CMY-2}$ is essential for limiting their further spread, but patterns may vary geographically and local studies are essential. We investigated the epidemiology of *bla*$_{CMY-2}$-like genes in *E. coli* isolates from Sydney, Australia over two time periods (2005-09; 2013-14) to gain insight into the dynamics of their spread. Clinical *E. coli* isolates (n=101) were characterised by multilocus sequence typing (MLST), PCR-based replicon typing, multiplex resistance gene PCR and S1 Southern hybridisation and subsets were analysed using pulsed-field gel electrophoresis (PFGE) or Illumina MiSeq technology. Horizontal spread of IncI1 plasmids appeared to be the predominant mode of transmission in 2005-09, as *bla*$_{CMY-2}$ was carried by closely related plasmids in diverse *E. coli* strains. In contrast, *bla*$_{CMY-2}$ was found in the chromosome in half of the recent *E. coli* isolates (2013-14) and very few of these contained IncI1 plasmids. The majority of these isolates were related by PFGE, likely representing clonal expansion of an ancestor strain. However, at least two corresponded to entirely different sequence types by MLST, suggesting that the movement of this gene to the chromosome has occurred on several occasions. This may be a reflection of persistent antibiotic pressure driving mobile resistance genes to more stable genetic locations or simply the natural tendency of these genes to mobilise to different sites. In either case, the apparent increase in the prevalence of *E. coli* strains with a chromosomal *bla*$_{CMY-2}$-like gene is of major therapeutic concern. These findings suggest that the epidemiology and transmission of *bla*$_{CMY-2}$-like genes in Sydney are complex and may be changing over time, and that multiple surveillance strategies are necessary to effectively control their spread.

Author Disclosure Block:

K. Tagg: None. **A. Ginn:** None. **S. Partridge:** None. **J. Iredell:** None.

Poster Board Number:

MONDAY-338

Publishing Title:

Multidrug-Resistant *pseudomonas Aeruginosa* (Mdr-Psa) By Source: 2008-2015

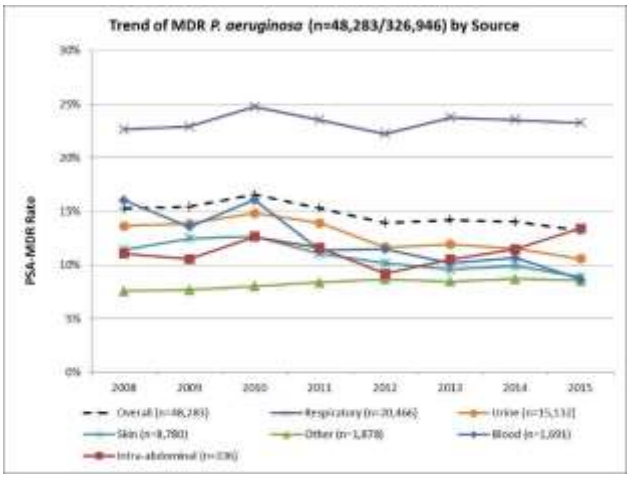
Author Block:

D. D. DePestel[1], C. A. DeRyke[1], X. Sun[2], S. Merchant[1], J. Till[2], V. Gupta[2]; [1]Merck & Co. Inc., Rahway, NJ, [2]Becton, Dickinson & Company, Franklin Lakes, NJ

Abstract Body:

Background: We sought to determine the trends in MDR-PSA isolates by source in the ambulatory, inpatient admission, and hospital-onset settings.**Methods:** We analyzed electronic data in a research database of Becton Dickinson from the same 154 USA hospitals (2008 - 2015). All non-duplicate PSA isolates (first isolate of a species per 30 day period) from all sources were identified as MDR if intermediate or resistant to at least 1 in 3 of the 5 drug classes: ceftazidime or cefepime; ciprofloxacin or levofloxacin; aminoglycosides, carbapenems; and piperacillin or piperacillin/tazobactam. Non-duplicate isolates were categorized into three settings by the specimen collection time: a) Admission: < 3 days of an inpatient admission and no previous admission within 14 days; b) Hospital-onset: 3 days post-admission or within 14 days of discharge; c) Ambulatory (neither a or b)**.Results:** Overall, 14.8% (48,283/326,946) of non-duplicate PSA isolates were MDR-PSA. The MDR-PSA rates by ambulatory, admission, hospital-onset settings were 10.2% (14,950/147,109), 14.9% (10,425/70,067) and 20.9% (22,908/109,770), respectively. The proportion of MDR-PSA isolates by source was 42.4% (respiratory), 31.3% (urine), 18.2% (skin), 3.9% (other), 3.5% (blood), and 0.7% (intraabdominal). While a decrease of MDR-PSA for the overall and for blood, skin, and urine sources was observed (all $p<0.01$), the respiratory source remained high (22.6% to 23.3% ($p=0.78$) and the absolute difference was 10% higher than other sources (all $p<0.01$).**Conclusions:** From 2008 to 2015 the MDR-PSA rates were highest for the hospital-onset settings in the US hospitals. Over 40% of all MDR-PSA were respiratory isolates with an

MDR rate of 23% which was consistent across the study period.



Author Disclosure Block:

D.D. DePestel: D. Employee; Self; Merck & Co. Inc. **C.A. DeRyke:** D. Employee; Self; Merck & Co. Inc. **X. Sun:** D. Employee; Self; Becton, Dickinson & Company. **S. Merchant:** D. Employee; Self; Merck & Co. Inc. **J. Till:** D. Employee; Self; Becton, Dickinson & Company. **V. Gupta:** D. Employee; Self; Becton, Dickinson & Company.

Poster Board Number:

MONDAY-339

Publishing Title:

Differential Incidence of *Acinetobacter baumannii* Bacteremia Based on Bodily Source of Colonization

Author Block:

R. Rosa[1], A. Jimenez[2], J. Castro[1], D. Kett[1], N. Namias[1], L. Munoz-Price[3]; [1]Univ. of Miami Miller Sch. of Med., Miami, FL, [2]Jackson Hlth.System, Miami, FL, [3]Med. Coll. of Wisconsin, Milwaukee, WI

Abstract Body:

Background: We previously described the strong impact of colonization with carbapenem-resistant *Acinetobacter baumannii* (CRAB) on the subsequent development of any clinical infection with this pathogen. Now we aim to determine the impact of the initial bodily source of colonization on the subsequent development of bacteremia. **Methods:** Retrospective cohort performed at a 1500 bed county teaching hospital, from November 30, 2010 to November 30, 2011. We included all patients consecutively admitted to the trauma intensive care unit (TICU) who had at least one surveillance culture performed. Rectal and respiratory cultures (if intubated) were obtained upon admission and weekly thereafter. Relative risks were determined using the entire cohort. The outcome variable was CRAB bacteremia, defined as at least one positive blood culture in a four year follow up period.**Results:** A total of 364 patients were included in this analysis: 315 (86.5%) remained surveillance negative and 49 (13.4%) tested positive for CRAB. There were 18 CRAB bloodstream infections detected. The relative risk (RR) for the development of bacteremia among surveillance positive patients against surveillance negative patients was 12.8 (95% CI: 5.05-32.6; $p<0.0001$). There were 21 patients whom tested initially positive in the respiratory tract: 6 developed CRAB bacteremia and 15 remained bacteremia free (RR: 15; 95% CI: 5.3-42.5; $p<0.0001$). Among the 16 patients with only rectal colonization at onset, 4 developed bacteremia and 12 remained bacteremia free (RR: 13.1; 95% CI: 4.1-41.9; $p<0.0001$). Twelve patients were concomitantly positive in the rectum and respiratory tract, two of which developed CRAB bacteremia and the remaining 10 were bacteremia free (RR: 8.7; 95% CI: 1.9-38.9; $p=0.004$).**Conclusions:** The bodily source of CRAB colonization appears to impact the risk to develop CRAB bacteremia; the source with highest risk was the respiratory tract.

Author Disclosure Block:

R. Rosa: None. **A. Jimenez:** None. **J. Castro:** None. **D. Kett:** None. **N. Namias:** None. **L. Munoz-Price:** C. Consultant; Self; Xenex, Clorox. L. Speaker's Bureau; Self; Ecolab.

Poster Board Number:

MONDAY-340

Publishing Title:

Increasing Trends of Antimicrobial Susceptibilities of *Escherichia coli* and *Klebsiella pneumoniae* from Intra-Abdominal Infections in South Korea from 2005 to 2014

Author Block:

I-G. BAE[1], J. Lee[2], S. Kim[1], M. Lee[3], M. Kim[4]; [1]Gyeonsang Natl. Univ., Jinju, Korea, Republic of, [2]Hallym Univ. Med. Ctr., Seoul, Korea, Republic of, [3]Merck Sharp & Dohm Korea Ltd., Seoul, Korea, Republic of, [4]Korea Univ. Coll. of Med., Seoul, Korea, Republic of

Abstract Body:

Background: The *Enterobacteriaceae*, including *Escherichia coli* and *Klebsiella pneumoniae*, are major pathogens of intra-abdominal infections (IAIs), and their antimicrobial resistances are increasing worldwide. We evaluated the antimicrobial susceptibilities of *E. coli* and *K. pneumoniae* from patients with IAIs in South Korea.**Methods**: A total of 1,739 aerobic Gram-negative IAIs isolates were collected from two or three medical centers in South Korea participating in the Study for Monitoring Antimicrobial Resistance Trends (SMART) program from 2005 to 2014. We determined the antimicrobial susceptibilities to 12 antimicrobials and the extended-spectrum beta-lactamase (ESBL) status using CLSI broth microdilution. **Results**: Of 1,739 IAI isolates, 548 (31.5%) were *E. coli* and 402 (23.1%) were *K. pneumoniae*. The *E. coli* isolates have retained the excellent activities for amikacin and carbapenems with susceptibility rates of > 90%, but fluoroquinolones showed the lowest susceptibility rates of < 60%. With the exception of cefepime and fluoroquinolones, the trends of antimicrobial susceptibilities of *E. coli* isolates were significantly increased over the study period. The *K. pneumoniae* isolates had the modest activities to most antibiotics except for imipenem. Susceptibility rates to most antibiotics were the lowest between 2005 and 2008, and the rates have been increasing since 2012. There was a statistically significant increase in *E. coli* and *K. pneumoniae* susceptibility in isolates from hospital-associated IAIs. There was no significant change in regards to community-associated IAIs. The rates of ESBL-positive *E. coli* and *K. pneumoniae* were not statistically significant changed. **Conclusions**: Our study showed that antimicrobial susceptibility rates of *E. coli* and *K. pneumoniae* isolates from IAIs have been significantly increased from 2005 to 2014, although prevalence of ESBL-positive isolate were not statistically significant changed.

Author Disclosure Block:

I. Bae: None. **J. Lee:** None. **S. Kim:** None. **M. Lee:** None. **M. Kim:** None.

Poster Board Number:

MONDAY-341

Publishing Title:

Exploring the Underlying Mechanism of Action and Ultra-structure Distortion Accomplishes by Newly Synthesized Chalcone Derivatives on *Plasmodium falciparum* Infected Erythrocytes

Author Block:

S. Sinha[1], D. I. Batovska[2], B. Medhi[1], B. D. Radortra[1], A. Bhalla[1], R. Sehgal[1]; [1]Post Graduate Inst. of Med. Ed. and Res., Chandigarh, India, [2]Bulgarian Academy of Sci., Sofia, Bulgaria

Abstract Body:

Background: *Plasmodium falciparum* is the deadliest *Plasmodium* that causes human malaria among other five species and for thousands of year traditional herbs are widely used for the cure of this disease. Chalcones (1, 3, diaryl-2-propen-1-ones), secondary metabolite that's belong to the family of flavonoid has been known for its diverse range of pharmacological activities including anti-*Plasmodial* activity. Though several studies illustrates about their mechanisms by which it shows anti-*Plasmodial* action, but its exact mechanism of action is still in the illusion.**Methods**: The hemozoin (b-hematin) inhibition assay was performed in *Plasmodium falciparum* cultures to determine hemozoin content using drug concentrations at IC_{50} values at the end of 48 h and hemozoin content was estimated by determining the absorbance at 400 nm. Further to observe changes at ultra-structure level, ring-stage parasite-infected erythrocytes of both susceptible and resistant strain were treated with chalcone derivatives at twice their respective IC_{50} values for 24 h and then processed, stained to examined through Transmission electron microscopy.**Results**: The level hemozoin production in case of chloroquine and chalcone treated *Plasmodium* culture was almost same, that is, < 350.00 ng/ml, however the hemozoin content was found to be much less as compared to non-treated *Plasmodium* culture that shows statistically significant data ($p<0.05$). Observation through Transmission Electron Microscopy elucidate about the formation of appendages, vacuole formation and condensation of chromatin as compared to non-treated control. This observation is also compared with chloroquine and artemisnin.**Conclusion**: The present analysis suggests these new chalcone derivatives have same mechanism of action as that of chloroquine, which acts on food vacuole of *Plasmodium*. Also, ultra-structural study of *Plasmodium falciparum* strains in the presence of these chalcone derivatives gives the detail analysis of happening inside the *Plasmodium* that will be beneficial while selecting drug target site.

Author Disclosure Block:

S. Sinha: None. **D.I. Batovska:** None. **B. Medhi:** None. **B.D. Radortra:** None. **A. Bhalla:** None. **R. Sehgal:** None.

Poster Board Number:

MONDAY-342

Publishing Title:

Population-Dependency of Atovaquone Resistance in *Plasmodium chabaudi* Malaria

Author Block:

J. T. Bram, M. Acosta, D. G. Sim, M. J. Jones, A. F. Read; Pennsylvania State Univ., University Park, PA

Abstract Body:

In choosing treatment regimens that best manage drug resistance, it is important to understand the differences between the origin of resistance and the spread of resistance through microbial populations. It is well known that once resistance has arisen, strong drug use places selective pressure on microbes and leads to the proliferation of resistant phenotypes, often over short treatment periods. However, we know little about the factors that contribute to the origin of drug resistance, particularly within hosts. In this study using in an in-vivo rodent malaria model, we test the basic population genetics principle that mutational events occur more frequently in larger populations, and this contributes to increased rates of evolution (i.e. rate of resistance emergence). Mice were inoculated with equal parasite counts and subsequently drug treated at varying time points to simulate different population sizes, allowing us to examine the effects of population size on resistance emergence. Using the rodent malaria model and the antimalarial (Atovaquone), we found that resistance emergence does depend on parasite population size: proportion of mice harboring resistant parasites increased with parasite densities at the onset of treatment. Thus, treatment of larger parasite populations is more likely to lead to resistance emergence. We hypothesize that this may be due to an increased genetic load found within large populations, increasing the probability of the parasite population containing a resistance-conferring allele.

Author Disclosure Block:

J.T. Bram: None. **M. Acosta:** None. **D.G. Sim:** None. **M.J. Jones:** None. **A.F. Read:** None.

Poster Board Number:

MONDAY-344

Publishing Title:

***Plasmodium* Aldolase Detection Using a Smart Phone and a Microfluidic Chip: Electricity-free Elisa for Malaria Monitoring**

Author Block:

N. S. Gopal[1], R. Raychaudhuri[2], L. M. Brzustowicz[3]; [1]Lawrenceville Sch., Lawrenceville, NJ, [2]Med. Coll. of Wisconsin, Milwaukee, WI, [3]Rutgers Univ., Piscataway, NJ

Abstract Body:

Background: Malaria control efforts are limited in rural areas due to lack of monitoring methods for infected patients. A low-cost system to monitor response without the use of expensive enzyme linked immunosorbent assay (ELISA) equipment and electricity is needed. *Plasmodium* aldolase is a key enzyme in the metabolic pathway of the malaria parasite and is a well-characterized biomarker. However aldolase can only be detected with standard ELISA techniques, which requires the use of expensive antibodies, equipment and training. A three part system to enable electricity-free ELISA was developed consisting of a microfluidic chip, hand crank centrifuge and a cell phone based app to read color values. This new system could offer the same sensitivity as ELISA but with a fraction of the costs. **Methods:** A circular microfluidic chip was fabricated using 3 mm optically clear acrylic and a CO_2 laser. Lamination was performed with 3M 501FL adhesive. A series of passive valves released reagents at precise times based upon centrifugal force generated by a hand crank. Color change was measured with a smart-phone camera application programmed in Java. The microchip was compared to a 96 well sandwich ELISA. The ELISA contained: 1) primary: mouse anti-plasmodium aldolase 2) antigen - recombinant aldolase and 3) conjugate: rabbit polyclonal anti-plasmodium aldolase-horse radish peroxidase. **Results:** Results from standard ELISA were compared to microchip at varying concentrations (1-20 ng/mL). Over 15 different microfluidic patterns were tested, and a final prototype of the chip was created. The prototype microchip was compared to standard sandwich ELISA (n=20) using samples of recombinant aldolase. Color readings of standard ELISA and microfluidic microchip showed equivalent results. We found the lower level of the detection limit to be 0.2 ng/mL (200 parasites/uL).**Conclusions:** The cost of the microfluidic system is approximately $10 per sample compared to over $1000 per sample for a standard ELISA. A low cost microfluidic system could improve access to monitoring in rural areas and help identify resistant strains more quickly. Next steps include validating the assay in malaria patients and endemic malaria negative patients.

Author Disclosure Block:

N.S. Gopal: None. **R. Raychaudhuri:** None. **L.M. Brzustowicz:** C. Consultant; Self; Janssen Pharmaceutical Companies of Johnson & Johnson.

Poster Board Number:

MONDAY-345

Publishing Title:

Complete Telomere-to-Telomere *de novo* Assembly of the *Plasmodium falciparum* Genome Using Long-Read Sequencing

Author Block:

S. S. Vembar[1], **M. Seetin**[2], C. Lambert[2], P. Baybayan[2], A. Scherf[1], M. L. Smith[2]; [1]Inst. Pasteur, Paris, France, [2]PacBio, Menlo Park, CA

Abstract Body:

Sequence-based estimation of genetic diversity of *Plasmodium falciparum*, the most lethal malarial parasite, has proved challenging due to a lack of a complete genomic assembly. The skewed AT-richness (~80.6% (A+T)) of its genome and the lack of technology to assemble highly polymorphic sub-telomeric regions that contain clonally variant, multigene virulence families (i.e. *var* and *rifin*) have confounded attempts using short-read NGS technologies. Using single molecule, real-time (SMRT) sequencing, we successfully compiled all 14 nuclear chromosomes of the *P. falciparum* genome from telomere-to-telomere in single contigs. Specifically, amplification-free sequencing generated reads of average length 12 kb, with ≥50% of the reads between 15.5 and 50 kb in length. A hierarchical genome assembly process (HGAP), was used to assemble the *P. falciparum* genome *de novo*. This assembly accurately resolved centromeres (~90-99% (A+T)) and sub-telomeric regions, and identified large insertions and duplications in the genome that added extra genes to the *var* and *rifin* virulence families, along with smaller structural variants such as homopolymer tract expansions. These regions can be used as markers for genetic diversity during comparative genome analyses. Moreover, identifying the polymorphic and repetitive sub-telomeric sequences of parasite populations from endemic areas might inform the link between structural variation and phenotypes such as virulence, drug resistance and disease transmission.

Author Disclosure Block:

S.S. Vembar: None. **M. Seetin:** D. Employee; Self; PacBio. **C. Lambert:** D. Employee; Self; PacBio. **P. Baybayan:** D. Employee; Self; PacBio. **A. Scherf:** None. **M.L. Smith:** D. Employee; Self; PacBio.

Poster Board Number:

MONDAY-346

Publishing Title:

Efficacy of Rapid Diagnostic Tests for Malaria in Children of Rural Ghana

Author Block:

N. Griswold[1], T. Dickerson[1], J. Reynolds[1], E. Roberts[1], D. Ansong[2], C. Nkyi[2], J. M. Boaheng[2], E. Amuzu[2], S. Gren[1], M. V. M. Morhe[2], P. A. Kontor[2], O. A. Owusu[2], M. G. Quansah[2], R. Buxton[1]; [1]Univ. of Utah, Salt Lake City, UT, [2]Kwame Nkrumah Univ. of Sci. and Technology, Kumasi, Ghana

Abstract Body:

In 2015, 88% of new cases and 90% of deaths due to malaria were in the WHO African region. WHO recommends prompt parasite-based diagnosis in all patients suspected of malaria before treatment, but the absence of accurate diagnostic tests can result in the treatment of patients who do not actually have malaria, contributing to overtreatment, wasted resources and drug resistance. Historically, making a definitive diagnosis of malaria required microscopic examination of a carefully prepared blood sample by a skilled laboratorian. Malaria rapid diagnostic tests (RDTs) have become available to the public for diagnosis of malaria in rural Ghana and require no laboratory infrastructure, allowing them to be deployed to remote settings. It is unknown how well these locally available RDTs perform compared to standard microscopy for diagnosis of asymptomatic malaria in children.A community based, cross sectional study was performed to establish the prevalence of asymptomatic malaria in children of rural Ghana and determine efficacy of locally available RDTs in the diagnosis of asymptomatic malaria as compared to microscopy.Caregivers of children aged 6 months to 5 years in 6 communities in the Barekese sub-District were informed of the study and invited to participate. Samples of capillary blood were used for RDTs and preparation of thick and thin blood smears for microscopy. RDT results were read and recorded on-site. Slides were sent to the research lab for examination.293 participants enrolled in the study. 112 tested positive, 180 tested negative, and 1 test was inconclusive. Compared to the blood smear, the sensitivity and specificity of the RDT were 96% and 74% respectively. The positive and negative predictive values were 43% and 99% respectively. Additionally, 49 of 64 individuals with false positive tests reported having taken medication for malaria within 14 days of RDT analysis, suggesting a possibility of the subjects having remaining antigens after successful treatment of malaria.There was agreement among 77% of the diagnostic tests, making RDTs a fair alternative to microscopy for diagnosing asymptomatic malaria in young children of rural Ghana.

Author Disclosure Block:

N. Griswold: None. **T. Dickerson:** None. **J. Reynolds:** None. **E. Roberts:** None. **D. Ansong:** None. **C. Nkyi:** None. **J.M. Boaheng:** None. **E. Amuzu:** None. **S. Gren:** None. **M.V.M.**

Morhe: None. **P.A. Kontor:** None. **O.A. Owusu:** None. **M.G. Quansah:** None. **R. Buxton:** None.

Poster Board Number:

MONDAY-347

Publishing Title:

Efficacy of Artemether-Lumefantrine (ACT) for the Treatment of Uncomplicated *P. falciparum* Malaria in Nepal

Author Block:

P. Ghimire[1], N. Singh[2], G. Thakur[3], K. Rijal[1], B. Karki[4], B. Marasini[5], C. Kafle[1]; [1]Tribhuvan Univ.; WHO Nepal, Kathmandu, Nepal, [2]WHO country office for Nepal, Kathmandu, Nepal, [3]Ministry of Hlth., Kathmandu, Nepal, [4]KIST Med. Coll.; Tribhuvan Univ., Lalitpur, Nepal, [5]Epidemiology & Disease Control Div., MoHP, Nepal, Kathmandu, Nepal

Abstract Body:

Background: A single-arm prospective study to assess the therapeutic efficacy of antimalarial drug Artemether-Lumefantrine in Nepal was conducted during 2013-2014. The efficacy of the ACT was assessed based on clinical and parasitological response to directly observed treatment for uncomplicated *P falciparum* malaria. **Methods:** Giemsa stained thick and thin blood smear, prepared from uncomplicated *P falciparum* malaria patients visiting the selected sentinel sites in Nepal during 2013-2014 and meeting the inclusion criteria including parasitemia of between 1000 to 100,000/µl of blood, were evaluated on day 0,1,2,3,7,14,21 and 28 after ACT treatment, following WHO standardized TES Protocol. Based on the reappearance of fever and or increased parasitemia, the study subjects were classified as resistant or susceptible. Blood specimens collected in specified filter papers during first day of microscopic evaluation were also further analyzed by PCR, looking for K13 propeller gene mutation in parasites, a recently identified molecular marker for ACT resistance, for further confirmation of the resistance.**Results:** A total of 56,013 suspected malaria cases only 106 cases were found to be infected with P falciparum, while 1083 were P vivax cases. Out of 28 P falciparum cases meeting inclusion criteria for enrollment in the study only 24 cases completed the study, and 4 lost to follow up after the enrollment in the study. One out of 24 cases (4%) resulted to be late treatment failure (LTF). However, K13 mutation, a proxy indicator for ACT resistance in parasites was not detected on the day-I blood specimens of the cases, indicating the resistance is not yet reached at molecular level and still at phenotypic resistance.**Conclusions:** Detection of ACT resistant parasite on microscopy is a clear indication of phenotypic resistance in the circulating *P falciparum* parasite. The strengthening the sensitivity of the antimalarial drug resistance surveillance is important to contain the resistance as it appears and to mitigate the effects of emerging antimalarial resistance in the south East Asia region.

Author Disclosure Block:

P. Ghimire: None. **N. Singh:** None. **G. Thakur:** None. **K. Rijal:** None. **B. Karki:** None. **B. Marasini:** None. **C. Kafle:** None.

Poster Board Number:

MONDAY-348

Publishing Title:

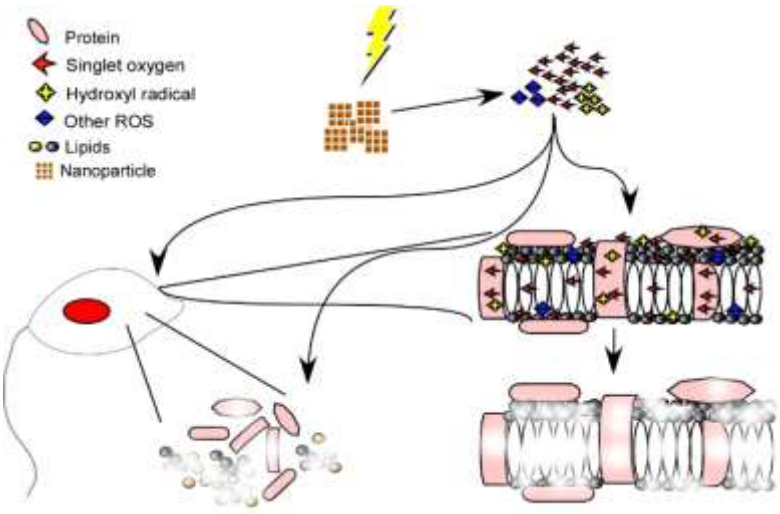
Annihilation of *Leishmania* Promastigotes by Daylight Responsive Pegylated ZnO Nanoparticles: A Temporal Relationship of ROS Induced Lipid and Protein Oxidation

Author Block:

A. NADHMAN[1], M. Khan[2], S. Nazir[3], M. Khan[4], G. Shahnaz[5], A. Rao[2], D. Shams[6], M. Yasinzai[7]; [1]NORI Hosp., Islamabad, Pakistan, [2]NORI, Islamabad, Pakistan, Islamabad, Pakistan, [3]Natl. Ctr. for Physics, Islamabad, Pakistan, Islamabad, Pakistan, [4]Khyber Med. Univ., Peshawar, Pakistan, Peshawar, Pakistan, [5]Quaid-i-Azam Univ., Islamabad, Pakistan, Islamabad, Pakistan, [6]Abdul Wali Khan Univ., Mardan, Pakistan, Mardan, Pakistan, [7]Intl. Islamic Univ., Islamabad, Pakistan, Islamabad, Pakistan

Abstract Body:

Background: Lipid and protein oxidation are well-known manifestations of free radical activity and oxidative stress. The current study investigates extermination of *Leishmania tropica* promastigotes persuaded by lipid and protein oxidation because of reactive oxygen species (ROS) produced by PEGylated metal based nanoparticles.**Methods:** Zinc oxide nanoparticles doped with different concentrations of silver (1, 5 and 9%) and copper (1%) were synthesized by a modified Masayuki method. The synthesized Photodynamic therapy (PDT) based doped and non-doped zinc oxide nanoparticles were activated in daylight that produced ROS in the immediate environment.**Results:** The ROS produced by the PDT based nanoparticles were found as the main cause of lipid and protein oxidation. However, no lipid and protein oxidation production was observed in the dark experiments. The major lipid peroxidation derivatives comprise of conjugated dienes, lipid hydroperoxides and malondialdehyde, whereas water, ethane, methanol and ethanol were found as the end products. Proteins were oxidized to carbonyls, hydroperoxides and thiol degrading products. Interestingly, lipid hydroperoxides were produced more than 2-fold of the protein hydroperoxides ($p<0.01$), indicating higher degradation of lipids compared to proteins.**Conclusions:** The *in-vitro* evidence represents a significant contribution of the involvement of both lipid and protein oxidation in the annihilated anti-promastigotes effect of nanoparticles.



Author Disclosure Block:

A. Nadhman: None. **M. Khan:** None. **S. Nazir:** None. **M. Khan:** None. **G. Shahnaz:** None. **A. Rao:** None. **D. Shams:** None. **M. Yasinzai:** None.

Poster Board Number:

MONDAY-349

Publishing Title:

***Leishmania amazonensis* Rhomboid Proteases**

Author Block:

O. Gonzalez, S. Ganguly, A. Kucknoor; Lamar Univ., Beaumont, TX

Abstract Body:

Leishmaniasis, is a tropical disease caused by species of Leishmania, which include a wide spectrum of disease in humans characterized by lesions in the skin, mucosal surfaces, and visceral organs. Over 10 million people are affected worldwide by this disease that causes significant morbidity and mortality. *Leishmania* parasites have two developmental stages; amastigotes that live intracellularly in macrophages, and promastigotes that multiply inside the insect vector. Although several studies have looked into the actual mechanisms of transition of promastigotes into amastigotes, details are still unknown. Rhomboids are trans-membrane proteins (serine-like proteases), and are able to cleave substrates within their trans-membrane domains. In this study, we have characterized rhomboid like genes in a cutaneous species- *Leishmania amazonensis,* whose genomes have not been sequenced yet. We used the *Leishmania* genome network database to identify Rhomboid-like gene sequences from the available genome sequences. BLAST, ClustalW, and TargetP servers were used to analyze the sequences. Gene specific primers were synthesized based on the sequence comparison and the species of *Leishmania*. Genomic DNA was isolated from a fresh isolate of *L. amazonensis.* PCR amplification was performed and the amplified products were verified for the size. Total RNA was isolated and mRNA was purified. RT-PCR analysis was performed to confirm gene expression. PCR from genomic DNA resulted in amplification of two putative rhomboid-like genes, from *L. amazonensis (LaRom-2 and LaRom-4).* Sequence analyses revealed that except for the catalytic domain, sequences were significantly different from rhomboids in related species. Using the prediction models, LaRom-2 showed two trans-membrane domains with an N-terminal extension towards the cytoplasmic end while LaRom-4 showed four trans-membrane domains with N-terminal extension towards the extracellular end. Interestingly, RT-PCR analyses showed that these genes showed increased expression during stationary phase of growth compared to early logarithmic stages. The stage-regulated expression of these genes suggests a role during the transition of promastigotes to amastigotes, and possibly during the infection process. Functional characterization of these genes is underway.

Author Disclosure Block:

O. Gonzalez: None. **S. Ganguly:** None. **A. Kucknoor:** None.

Poster Board Number:

MONDAY-350

Publishing Title:

Visceral Leishmaniasis in Benishangul-Gumuz Regional State, Western Ethiopia: Re-Emerging or Emerging?

Author Block:

A. A. Hirpa[1], G. Tasew[2], T. Tsegaw[1], A. Kejella[3], A. Mulugeta[4], D. Worku[5], A. Aseffa[1], E. Gadisa[1]; [1]Armauer Hansen Res. Inst., Addis Ababa, Ethiopia, [2]Ethiopian Publ. Hlth.Inst., Addis Ababa, Ethiopia, [3]Benishangul Gumuz regional Hlth. bureau, Assosa, Ethiopia, [4]World Hlth.Organization-Ethiopia, WHO-Ethiopia, Addis Ababa, Ethiopia, [5]KalaCORE-Ethiopia, Addis Ababa, Ethiopia

Abstract Body:

Kala-azar is a growing public health problem in Ethiopia. Benishangul-Gumuz regional state is previously not known to be endemic for the disease. In response to a case report fromthe region, we conducted a rapid assessment survey. A pre-tested questionnaire was used to capture socio-demographic and clinical histories pertinent to Kala-azar. Study participants with complaints of fever and headache for two or more weeks were tested for Kala-azar and malaria. All participants were screened with the leishmanin skin test (LST) and the direct agglutination test (DAT) for exposure to Leishmania, defined as a positive result with either or both tests. Twenty of 275 participants were exposed giving an overall leishmaniasis sero-prevalence rate of 7.3%. Among the 20 positive individuals, 19 were farmers and nine of them reported no travel history outside their district. It appears that Kala-azar is emerging in Dangur and Guba districts of Benishangul-Gumuz regional state, probably in connection with human encroachment into one or several previously out of reach zoonotic foci. We recommend integrated epidemiological surveys for confirmation and early containment of disease transmission in the area.

Author Disclosure Block:

A.A. Hirpa: None. **G. Tasew:** None. **T. Tsegaw:** None. **A. Kejella:** None. **A. Mulugeta:** None. **D. Worku:** None. **A. Aseffa:** None. **E. Gadisa:** None.

Poster Board Number:

MONDAY-351

Publishing Title:

Epidemiological Studies on Opportunistic Intestinal Parasites of HIV Patients on Antiretroviral Therapy in Imo State

Author Block:

T. N. Njoku-Obi, Spouse; Imo State Univ.,Owerri Imo State, Owerri, Nigeria

Abstract Body:

Background: The major health challenges among HIV-positive individuals is opportunistic intestinal parasites infection due to low immunity. The objective of this study was to determine the prevalence of intestinal parasites infection in HIV-infected patient on anti retroviral therapy in Imo state,Nigeria between February 2013 and December 2014.**Methods:** Stools samples were collected from 220 HIV on cART, 200 HIV not on cART and 100 control group. A structured biodata was used to collect socio-demographic data of the patients. Stool samples were **examined by direct saline, iodine wet mount, formol-ether concentration method, modified Ziehl-Neelsen staining techique and modified Trichrome staining method.Results:** Out of 520 stool samples examined 314 were positive for opportunistic intestinal parasites with overall prevalence rate of (60.3%). The highest rate 200(98.5%) of intestinal parasites were observed among HIV positive non cART patients, followed by HIV positive on cART 200(51.3%) and HIV negative individuals 100(4.0%). *Cryptosporidium parvum* 13(2.5%), *Isospora belli* 14(2.6%), *Microsporidium spp* 14 (2.6%), *Giardia lamblia* 91(17.5%) and *Entamoeba histolytica* 182(35%).**Conclusions:** The prevalence of intestinal parasitic infections remains significicant in HIV-infected patients with or without low CD4 counts in the cART era. Cryptosporidium parvum, Isospora belli and Microsporidium spp are the major opportunistic intestinal parasites observed in HIV / AIDS patients. Therefore,early detection and treatment of these intestinal parasites are very important to enhance the life and well -being of HIV/AIDS patients with or without diarrhoea**.**

Author Disclosure Block:

T.N. Njoku-Obi: None.

Poster Board Number:

MONDAY-352

Publishing Title:

Impact of *Entamoeba histolytica* Infection on Acute Appendicitis among HIV-1 Infected Individuals in Japan

Author Block:

T. Kobayashi[1], K. Watanabe[2], Y. Murata[1], H. Gatanaga[1], Y. Kikuchi[1], H. Yano[1], T. Igari[1], S. Oka[1]; [1]Natl. Ctr. for Global Hlth.and Med., Tokyo, Japan, [2]Univ. of Virginia, Charlottesville, VA

Abstract Body:

Background: Amoebic appendicitis is rare, but sometimes life-threatening disease of *Entamoeba histolytica* infection. However, its pathogenesis is still unclear. Here, we assessed the impact of *E. histolytica* infection on the pathogenesis of acute appendicitis among HIV-1 infected individuals in Japan.**Methods:** HIV-1 infected appendicitis cases who received appendectomy from 1996 to 2014 were analyzed. Periodic acid-Schiff (PAS) stain in addition to hematoxylin and eosin (HE) stain were used for histopathological examination. PCR for identifying *Entamoeba histolytica* was performed using extracted DNA from formalin fixed paraffin embedded appendix. Serum cytokines before and at the onset of appendicitis were evaluated by ELISA for whom stock serum samples were available.**Results:** Appendectomy was performed in 57 appendicitis patients in the study period. Anti-amoebic antibody was positive in 32.6%. *E. histolytica* infection was confirmed in 9 cases (15.8%). Sequencing of S^{TGA}-D locus showed 4 different genotypes from 9 positive samples. White blood cell counts were slightly higher in *E. histolytica* positive group (13,760/μL vs. 10,385/μL, p=0.02), although no differences were seen in other clinical, imaging and laboratory findings between *E. histolytica* positives and negatives. One case of *E. histolytica* positives, who didn't receive metronidazole, developed intestinal perforation after surgery. Serum samples before and at the onset of appendicitis were available in 3 cases of *E. histolytica* positives. Interleukin-4 was positive in 2 cases both at the onset and before appendicitis, suggesting chronic subclinical *E. histolytica* infection prior to the appendicitis.**Conclusions:** *E. histolytica* infection was confirmed in 15.8% of the appendicitis cases. After surgery, 1 case developed severe complication by *E. histolytica*. Chronic subclinical infection of *E. histolytica* prior to appendicitis was suspected in some cases, which might influence on the development of acute appendicitis. Further investigations on pathogenesis of amoebic appendicitis are needed to improve the morbidity and mortality.

Author Disclosure Block:

T. Kobayashi: None. **K. Watanabe:** None. **Y. Murata:** None. **H. Gatanaga:** None. **Y. Kikuchi:** None. **H. Yano:** None. **T. Igari:** None. **S. Oka:** None.

Poster Board Number:

MONDAY-353

Publishing Title:

Prevalence and Determinant Factors of Intestinal Parasites among Yekolo Temari Children Attending Traditional Education in the Ethiopian Orthodox Churches in Northern Ethiopia

Author Block:

G. B. Hailu, D. T. Mengiste, A. A. Fissiha, S. B. Dasissa; Mekelle Univ., Mekelle, Ethiopia

Abstract Body:

Background: Yekolo temari are children who are studying traditional education in the Ethiopian Orthodox Churches. These special groups of children are characterized by migration, begging and hardship.**Objective**: To determine the prevalence of intestinal parasites and determinant factors among Yekolo temari children of the Ethiopian Orthodox Churches in Northern Ethiopia. **Method**: A cross sectional study design was employed to assess the prevalence and factors associated with parasitic infection among Yekolo temari children in 2015.Wet mount and kato-katz techniques were used to detect *S.mansoni* and other intestinal parasites. Intensity of infection was estimated from the number of eggs per gram of faeces. SPSS version 20 was used to analyze data. **Result**: 361 children participated in the study with a response rate of 85.6%. Of the study participants, 77.8% were in the age group 16 years and above. One hundred eighty three (50.7%) children were positive for at least one parasite. *E.histolytica* was the predominant parasite followed by *S.mansoni* which were detected in 108(29.9%) and 60(16.6%) of study subjects, respectively. Of the study participants, 139(38.5%) and 37(10.2%) harbored single and dual infections, respectively. The mean intensity of *S.mansoni* infection was found to be 118 eggs per gram (epg) of stool and 38(71.7%) of the study participants had light infection (<epg). Majority (82.5%) used to defecate on open fields and 253(70.1%) did not wash their hands after defecation. Moreover, 308(85.3%) of them reported that they get their food by begging and 58.4% trimmed their fingers. Significant relationships were observed between parasitic infection and environmental/behavioral factors. The likely hood of washing hand after defecation was found to be more protective against parasitic infection by 31.8 % (OR=0.68, 95% CI (1.249, 3.132). Children who used to wear shoes were less likely to be infected by hookworm by 3.7 times (OR=3.649, 95%CI (0.005, 0.147). The presence of dirty materials on finger nails was also found to be a risk factor for infection by 53% (AOR=0.47, 95%CI (1.043, 2.45). **Conclusion**: intestinal parasites are very common among this group of children. Therefore, multiple intervention strategies should be implemented to reduce the burden of these infections.

Author Disclosure Block:

G.B. Hailu: None. **D.T. Mengiste:** None. **A.A. Fissiha:** None. **S.B. Dasissa:** None.

Poster Board Number:

MONDAY-354

Publishing Title:

Evaluation of a New FDA Approved Multiplex Molecular Assay for High Throughput Screening of *giardia Lamblia*, C*ryptosporidium spp*., and *entamoeba histolytica* in the Parasitology Clinical Laboratory

Author Block:

C. A. Lanteri, L. Hamilton, R. Ybarra, C. Valenzuela, A. Maramag, R. Tapia, E. Ager; Brooke Army Med. Ctr., San Antonio, TX

Abstract Body:

Enteric pathogens are responsible for significant morbidity and mortality, causing more than 2 million deaths annually worldwide and >375 million cases of diarrhea leading to 900,000 hospitalizations and 6,000 deaths per year in the United States. Giardia infection can be difficult to diagnose using standard microscope examination and often requires a patient to submit multiple stool samples to achieve optimal sensitivity. More recently, molecular methods, such as polymerase chain reaction (PCR), are becoming increasingly more common in clinical microbiology as they offer superior sensitivity relative to traditional methods. Reliance on labor-intensive techniques, such as acid-fast staining, direct fluorescent antibody, or enzyme immunoassays, is no longer required. We evaluated workflow characteristics and sensitivity of the Becton Dickinson (BD) Max™ Enteric Parasite Panel (EPP), a new FDA approved automated multiplex real-time PCR method to screen stool specimens for *Giardia lamblia*, *Cryptosporidium* (*parvum* and *hominis*), and *Entamoeba histolytica*, relative to standard procedures. Previously, processing and analyzing a typical day's workload (32 stools) required 5 hours of technician time, whereas PCR using the BD Max™ system reduced hands-on time to <30 min for sample preparation (<1 min/sample) and resulted in only 3 hrs total turn-around-time (90 min per batch of 24 samples). In this study, we directly compare sensitivity of the EPP versus traditional methods for detecting parasites in clinical specimens and samples spiked with parasite DNA in unpreserved, Carey-Blair, and 10% formalin fixed stool. Results to date suggest formalin interferes with EPP results, whereas we observed better correlation in detecting positives between traditional methods and the EPP in unpreserved or Carey-Blair specimens. Findings from this study are critical to developing a new testing paradigm incorporating rapid molecular testing to replace and/or augment traditional parasitology diagnostics to improve patient care. Since 95% of stools submitted for examination are negative, use of the EPP to rapidly screen for positives improves workflow, eliminates unnecessary processing, and greatly reduces turn-around-time.

Author Disclosure Block:

C.A. Lanteri: None. **L. Hamilton:** None. **R. Ybarra:** None. **C. Valenzuela:** None. **A. Maramag:** None. **R. Tapia:** None. **E. Ager:** None.

Poster Board Number:

MONDAY-355

Publishing Title:

***Dirofilaria* as the Etiologic Culprit of an Orbital Pseudotumor**

Author Block:

T. B. Walls, D. Gondim, B. H. Schmitt, T. E. Davis, R. F. Relich; Indiana Univ. Sch. of Med., Indianapolis, IN

Abstract Body:

Background: The zoonotic nematode genus *Dirofilaria*, is a frequent infective roundworm of dogs, cats, raccoons and other animals. Humans are uncommon incidental hosts of dirofilariae, and infections generally present as lesions of the breast, genitals, lungs, or conjunctiva. This report describes a case of an orbital pseudotumor caused by *Dirofilaria* infection. **Case:** A 68-year-old man with no significant past medical history presented to his primary care physician with a 3-week intermittent history of redness and swelling in his left eye that began after mowing his lawn. The patient was prescribed an antihistamine, but there was no improvement in his swelling and he subsequently developed a vesicle on the left sclera that increased in size. The patient's eye eventually became swollen shut, prompting a visit to the ED. The patient was given steroids and a CT scan, which demonstrated proptosis with periorbital soft tissue swelling. Differential diagnoses of an orbital pseudotumor or neoplasm were considered. An excision of the lesion was performed and the tissue was sent for histopathologic examination. **Results:** Histopathologic examination of the tissue revealed conjunctival stroma infiltrated by acute inflammation and areas of granulation tissue associated with a parasitic nematode. The cross section of nematode demonstrated the characteristic longitudinal ridges, lateral chords, and multilayered cuticle consistent with *Dirofilaria*. **Discussion:** The removal of the scleral lesion was curative and the patient underwent orbital reconstruction surgery. The microfilariae of *Dirofilaria* spp. are transmitted to hosts through a mosquito vector. Humans are dead-end hosts since the nematodes cannot achieve sexual maturity. Four species of *Dirofilaria* have been identified as pathogens of humans: *D. immitis, D. repens, D. tennius*, and *D. ursi*. While definitive identification is not possible on histologic sectioning, *D. immitis*, commonly known as the dog heartworm, is the most common species found in Europe and North America, and it is the most likely culprit in this case.

Author Disclosure Block:

T.B. Walls: None. **D. Gondim:** None. **B.H. Schmitt:** None. **T.E. Davis:** None. **R.F. Relich:** None.

Poster Board Number:

MONDAY-356

Publishing Title:

***Ixodes persulcatus* is the Principal Vector for U.S. Lineage Parasites of *Babesia microti*-Group in Japan**

Author Block:

A. Zamoto-Niikura[1], H. Hirata[2], C. Ishihara[2], S. Morikawa[1], K. Hanaki[1]; [1]Natl. Inst. of Infectious Diseases, Tokyo, Japan, [2]Rakuno Gakuen Univ., Hokkaido, Japan

Abstract Body:

Protozoan parasites belong to U.S. lineage in *Babesia microti* group are known as main causative agents of human babesiosis in the Northeastern and the upper Midwestern United States. The parasites in the U.S. lineage are distributed as well in various small wild mammals throughout the temperate zone of Eurasia, where at least 5 human cases (1 and 4 from Germany and China, respectively) were caused by the lineage, so far. In Northern Asian countries, including Russia, China and Japan, nucleotide sequences of the U.S. lineage were exclusively reported from *Ixodes persulcatus* tick. Whereas evidence of biologically active parasites in *I. persulcatus* is lacking. To confirm vector competence of this tick species, we attempted to isolate infectious parasites from salivary glands of the field collected female ticks in Japan. Five out of 41 ticks were PCR-positive, and 4 isolates were established by inoculating the specimens into hamsters. Phylogenetical analysis based on the nucleotide sequences of *18S rRNA, β-tubulin* and *CCT7* genes revealed that all isolates were classified in U.S. lineage. The sequences of the 4 isolates and those of the isolates from rodents captured in Japan were identical each other. Antigenically all U.S. isolates from *I. persulcatus* and rodents in japan appeared to be same, demonstrating natural transmission in the endemic region in Japan. To our knowledge, direct evidence that *I. persulcatus* tick carries infectious *B. microti* U.S. lineage was provided for the first time. Together with the previous epidemiological and transmission studies, we concluded *I. persulcatus* is at least one of the principal vectors for U.S. lineage in Japan and presumably in Northeastern Eurasia.

Author Disclosure Block:

A. Zamoto-Niikura: None. **H. Hirata:** None. **C. Ishihara:** None. **S. Morikawa:** None. **K. Hanaki:** None.

Poster Board Number:

MONDAY-357

Publishing Title:

Benchmark Results and Trending Analysis Following Introduction of Nucleic Acid Tests for Diagnosis of Anaplasmosis, Ehrlichiosis and Babesiosis in Central and Northern Wisconsin (2013-2015)

Author Block:

T. R. Fritsche[1], T. N. Marti[1], M. E. Nedd[1], A. M. Schotthoefer[2], B. J. Olson[2], T. S. Uphoff[1]; [1]Marshfield Clinic, Marshfield, WI, [2]Marshfield Clinic Res. Fndn., Marshfield, WI

Abstract Body:

Background: Nucleic acid amplification tests (NATs) for detection of the zoonotic pathogens *Anaplasma phagocytophilum* (AP), *Ehrlichia* spp., and *Babesia microti* (BM) are largely replacing blood smear and serologic analyses as the diagnostics of choice for these tick-borne diseases (TBD). Improved diagnostics are facilitating our understanding of the public health significance and expanding distributions of both pathogens and tick vectors. Here we present results following introduction of NATs in 2013 for the diagnosis of AP, *Ehrlichia* spp. and BM in a regional upper Midwest healthcare system.**Methods:** EDTA blood specimens submitted for testing as part of management of patients with possible TBD included 21,236 and 20,185 requests, respectively, for *Anaplasma/Ehrlichia* spp. and BM during the period 1/1/2013-12/15/2015. Tests included two real-time PCR assays, one targeting the *gro*EL operon with melt-curve analysis for differentiation of AP, *E. chaffeensis* (EC), *E.ewingii*, and a novel pathogen identified tentatively as the *Ehrlichia muris*-like agent (EML; Pritt, N Engl J Med 365:422, 2011; Schotthoefer, J Clin Microbiol 51:2147, 2013), and another targeting a variable region of the BM 18s rRNA gene (Teal, J Clin Microbiol 50:903, 2012).**Results:** Overall, positive tests included 654 (3.08%) AP; 17 (0.08%) EML; 2 (0.01%) EC; and 104 (0.52%) BM. Prevalence rates varied little between 2013, 2014 and 2015 (AP, 3.61%, 2.86% and 2.86%, respectively; EML, 0.06%, 0.11% and 0.06%; EC, 0.03%, 0.00%, 0.00%; and BM, 0.63%, 0.44% and 0.50%). These results confirm the expanding range of the rarer *Ehrlichia* spp. in our state as well as the endemic nature of AP and BM in the upper Midwest.**Conclusions:** Use of NATs for diagnosing certain TBD is a paradigm shift demonstrating superior performance compared to traditional methods. While prevalence rates of these TBD in our largely rural service area remain low (AP, 3.08%; EML, 0.08%; EC, 0.01%; and BM, 0.52%), their presence along with recently described geographic spread of competent tick vectors creates a worrisome public health threat superseded only by Lyme disease, also known to be both endemic and enzootic in our region.

Author Disclosure Block:

T.R. Fritsche: None. **T.N. Marti:** None. **M.E. Nedd:** None. **A.M. Schotthoefer:** None. **B.J. Olson:** None. **T.S. Uphoff:** None.

Poster Board Number:

MONDAY-358

Publishing Title:

Genital *Chlamydia trachomatis* Infection among Women of Reproductive Age Attending the Gynecology Clinic of Hawassa University Referral Hospital, Southern Ethiopia

Author Block:

E. Tadesse, T. Shimelis, M. Teshome; Hwassa Univ., Hawassa, Ethiopia

Abstract Body:

Urogenital infection with *Chlamydia trachomatis* is one of the most common bacterial STIs world-wide, especially in developing nations where routine laboratory diagnosis is unavailable. This study is, therefore, aimed at assessing the prevalence of *C. trachomatis* infection and its associated factors among women of reproductive age group. A cross-sectional study was conducted in a total of 322 consecutive women aged 15-49 years attending the gynecology clinic of Hawassa University Referral Hospital from November 2014 to April 2015. Structured questionnaires were used to collect data on socio-demography and potential risk factors for the genital infection. Moreover, endocervical swab samples were collected from each patient and analyzed for *C. trachomatis* antigen using RDT kit. The overall prevalence of *C. trachomatis* infection in the study population was 18.9 %. High prevalence rates of *C. trachomatis* infection were in the age group 15-24 years (24.2%), followed by the age group 25-34 years (16.8%). Women who had unprotected sex in the last six months were at higher risk of having the infection compared to those with protected sex . Women educational level, marital status, history of pre-marital sex, and use of contraceptive did not influence the infection status significantly ($p > 0.05$). The high prevalence of *C. trachomatis* infection among women of reproductive age group, especially among those participants who were younger and had practiced unprotected sex highlight the need to target this sub-population with interventions measures so that it's clinical and public health impacts could be minimized.

Author Disclosure Block:

E. Tadesse: None. **T. Shimelis:** None. **M. Teshome:** None.

Poster Board Number:

MONDAY-359

Publishing Title:

Accuracy of Automated Rapid Plasma Reagin Test in the Diagnosis of Syphilis in HIV-Infected Patients

Author Block:

T. Nishijima, S. Tezuka, K. Teruya, K. Tsukada, H. Gatanaga, Y. Kikuchi, S. Oka; Natl. Ctr. for Global Hlth.and Med., Tokyo, Japan

Abstract Body:

Background: Whether automated Rapid Plasma Reagin (RPR) test is as accurate as conventional manual RPR test in the diagnosis of syphilis among HIV-infected patients is unknown. **Methods:** We conducted a prospective study to compare automated RPR test [Mediace RPR (Sekisui Medical, Tokyo, Japan)] and conventional manual RPR card test [RPR test SANKO (Sanko Junyaku, Tokyo, Japan)] among HIV-infected patients. HIV-infected patients who were suspected of syphilis based on clinical symptoms/signs or who were routinely screened for syphilis were tested for automated RPR test, manual RPR card test, and conventional *Treponema pallidum* Latex Agglutination (TPHA) test with the same serum. For automated RPR test, samples with the results exceeding the upper detection limit were diluted according to the manufacturer's protocol and re-tested. Manual RPR test result of ≥8 fold increase (FI) and positive TPHA test were defined as diagnosis of syphilis.**Results:** 194 test results for 189 patients were analyzed. 97% were men and 93% were men who have sex with men (MSM), with median age and CD4 of 42 (IQR 37-48) and 512 (366-674), respectively. 88% were taking antiretroviral therapy. Median value of automated RPR test was 1.8 RPR Unit (range 0-4500, IQR 0-16) and that of conventional RPR was 2 (range 0-256, IQR 0-16). Two RPR tests showed excellent correlation (Spearman's coefficient 0.947). 58 (30%) results of automated RPR tests were ≥8, whereas 66 (34%) of conventional tests were ≥8. 65 (34%) patients fulfilled the diagnostic criteria for syphilis. There were 8 false negative cases with a median of automated RPR 6.3 (range 2.4-7.4, IQR 4.3-7.3), whereas there were no false positives, resulting in sensitivity of 88% and specificity of 100%. CD4 counts were not different between those with false negative results and the others (p=0.885), and among false negative cases, only 1 out of 8 patients had CD4 count <200 /μl.**Conclusion:** The results of automated RPR test were well-correlated with that of manual RPR card test among HIV-infected patients. However, automated RPR tests can yield false negative results.

Author Disclosure Block:

T. Nishijima: None. **S. Tezuka:** None. **K. Teruya:** None. **K. Tsukada:** None. **H. Gatanaga:** None. **Y. Kikuchi:** None. **S. Oka:** None.

Poster Board Number:

MONDAY-360

Publishing Title:

Gen-003, a Therapeutic Vaccine for Genital Herpes, Significantly Reduces Viral Shedding and Lesions for at Least 6 Months

Author Block:

K. FIFE[1], N. Van Wagoner[2], P. Leone[3], D. Bernstein[4], T. Warren[5], L. Panther[6], R. Novak[7], R. Beigi[8], J. Kriesel[9], S. Tyring[10], J. Lalezari[11], W. Koltun[12], G. Lucksinger[13], A. Morris[14], B. Zhang[15], S. Tasker[15], S. Hetherington[15], A. Wald[16]; [1]Indiana Univ., Indianapolis, IN, [2]U. Alabama Birmingham, Birmingham, AL, [3]U. North Carolina, Chapel Hill, NC, [4]Cincinnati Children's Hosp., Cincinnati, OH, [5]Westover Heights Clinic, Portland, OR, [6]Beth Israel Deaconess Hosp., Boston, MA, [7]U. Illinois, Chicago, IL, [8]U. Pittsburgh, Pittsburgh, PA, [9]U. Utah, Salt Lake City, UT, [10]Ctr. for Clinical Studies, Houston, TX, [11]Quest Clinical Res., San Francisco, CA, [12]Med Ctr. for Clinical Res., San Diego, CA, [13]Tekton Res., Austin, TX, [14]IND 2 Results, Atlanta, GA, [15]Genocea BioSci.s, Cambridge, MA, [16]U. Washington, Seattle, WA

Abstract Body:

Background: GEN-003 is a candidate therapeutic vaccine containing recombinant HSV antigens gD and ICP4 with Matrix M-2 (MM) adjuvant. This Phase 2 study was designed to confirm GEN-003 antiviral activity and select the best dose combination for future trials. We previously reported an immediate reduction in virus shedding and lesions. We now present durability of effect. **Methods:** Healthy adults with genital herpes were randomized to receive 30 or 60 µg of each protein antigen and 25, 50, or 75 µg MM, or saline placebo, 3 times 3 weeks apart. Participants collected twice daily genital swabs for HSV-2 DNA PCR for 28 days prior, after the third vaccination, and at 6 and 12 months following immunization and recorded stop and start dates of every outbreak. Placebo recipients were unblinded and re-randomized to one of the 6 active arms after the post-vaccination swabbing period. **Results**: 310 participants were evaluable. Six months after immunization, HSV-2 shedding was significantly reduced in all vaccine groups containing 60 µg of HSV-2 antigens compared to baseline, with the highest reduction in the 60/75 µg group (58%, $p<0.0001$). In subjects receiving 30 µg of antigens, only the 30/50 µg group had a significant reduction in shedding (50%, $P<0.0001$). All active groups (except 30/25 µg) had a reduction in lesions, ranging from 43% to 69% ($p<0.0001$ vs. baseline). Common side effects included injection site discomfort, fatigue and myalgia. No Grade 4 reactogenicity or related SAEs were observed. **Conclusion:** GEN-003 had significant antiviral activity persisting at least 6 months post dosing with an acceptable safety profile.

Author Disclosure Block:

K. Fife: H. Research Contractor; Self; Genocea Biosciences, Vical. **N. Van Wagoner:** H. Research Contractor; Self; Genocea Biosciences. **P. Leone:** C. Consultant; Self; Trinity

Biological, Vical. H. Research Contractor; Self; Genocea Biosciences. L. Speaker's Bureau; Self; Abbott Diagnostics. **D. Bernstein:** C. Consultant; Self; Merck. H. Research Contractor; Self; Genocea Biosciences. **T. Warren:** H. Research Contractor; Self; Genocea Biosciences. **L. Panther:** H. Research Contractor; Self; Genocea Biosciences, Gilead Sciences, Merck. **R. Novak:** H. Research Contractor; Self; Genocea Biosciences. **R. Beigi:** H. Research Contractor; Self; Genocea Biosciences. **J. Kriesel:** H. Research Contractor; Self; Genocea Biosciences, Vical. **S. Tyring:** E. Grant Investigator; Self; Genocea Biosciences. **J. Lalezari:** H. Research Contractor; Self; Genocea Biosciences. **W. Koltun:** H. Research Contractor; Self; Genocea Biosciences. **G. Lucksinger:** H. Research Contractor; Self; Genocea Biosciences. **A. Morris:** H. Research Contractor; Self; Genocea Biosciences. **B. Zhang:** D. Employee; Self; Genocea Biosciences. K. Shareholder (excluding diversitied mutual funds); Self; Genocea Biosciences. **S. Tasker:** D. Employee; Self; Genocea Biosciences. K. Shareholder (excluding diversitied mutual funds); Self; Genocea Biosciences. **S. Hetherington:** D. Employee; Self; Genocea Biosciences. K. Shareholder (excluding diversitied mutual funds); Self; Genocea Biosciences. **A. Wald:** C. Consultant; Self; Aicuris, Amgen. H. Research Contractor; Self; Genocea Biosciences, Vical.

Poster Board Number:

MONDAY-361

Publishing Title:

Prognosis of Ocular Syphilis in HIV-Infected Patients in the Antiretroviral Therapy Era

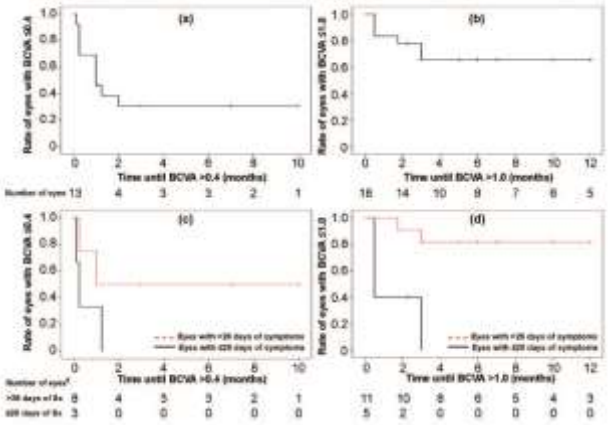
Author Block:

M. Tsuboi, T. Nishijima, S. Yashiro, K. Teruya, Y. Kikuchi, N. Katai, S. Oka, H. Gatanaga; Natl. Ctr. for Global Hlth.and Med., Tokyo, Japan

Abstract Body:

Background: The clinical course < prognosis of visual acuity in HIV-infected patients with ocular syphilis in the antiretroviral therapy (ART) era remain unknown. **Methods:** We conducted a single-center retrospective chart review of ocular syphilis in HIV-1 infected patients diagnosed between August 1997 < July 2015. The prognosis of best-corrected visual acuity (BCVA) was analyzed. **Results:** The study subjects were 30 eyes of 20 Japanese men who had sex with men (median age, 41). Loss of vision < posterior uveitis were the most common ocular clinical features (43%) < location of inflammation at presentation (50%), respectively. The median baseline BCVA was 0.4 (IQR 0.2-1.2), including three eyes with hand motion. BCVA ≤0.4 at diagnosis was significantly associated with posterior uveitis or panuveitis (p=0.044), while scleritis was significantly associated with BCVA >0.4 (p=0.048). Seventy-five percent were treated with intravenous benzylpenicillin < 53% were diagnosed of neurosyphilis. After treatment (median follow-up: 21 months), BCVA improved in 89% of the eyes, including all eyes with hand motion, to a median BCVA of 1.2 (IQR 0.8-1.2). Kaplan-Meier analysis showed that >28 days of ocular symptoms before diagnosis was the only factor associated with poor prognosis of BCVA. Three patients (15%) developed recurrence after treatment.**Conclusions:** Prognosis of BCVA in HIV-infected patients with ocular syphilis in the ART era was favorable after proper treatment. Persistence of ocular symptoms for >28 days was associated with poor prognosis. We should consider ocular syphilis in the differential diagnosis of uveitis in order to diagnose < treat it early.

Figure. Kaplan-Meier curve for prognosis of visual acuity in patients with ocular syphilis



Author Disclosure Block:

M. Tsuboi: None. **T. Nishijima:** None. **S. Yashiro:** None. **K. Teruya:** None. **Y. Kikuchi:** None. **N. Katai:** None. **S. Oka:** None. **H. Gatanaga:** None.

Poster Board Number:

MONDAY-362

Publishing Title:

Genomic Analysis of *Neisseria gonorrhoeae* Transmission Between Male Partners

Author Block:

J. C. Kwong[1], E. P. F. Chow[2], K. Stevens[1], T. Tomita[1], T. P. Stinear[1], T. Seemann[3], C. K. Fairley[2], M. Y. Chen[2], B. P. Howden[1]; [1]Doherty Inst. for Infection & Immunity, Melbourne, Australia, [2]Melbourne Sexual Hlth.Ctr., Carlton, Australia, [3]Victorian Life Sci. Computation Initiative, Carlton, Australia

Abstract Body:

Background: Gonorrhoea (NG) is a major public health problem, with rising antimicrobial resistance rates and limited therapeutic options. Recent population genomics studies of NG have provided insight into the genomic epidemiology of antimicrobial resistant NG. We conducted this prospective cohort study to understand the evolution of resistance and the genomics of NG transmission between male partners. **Methods:** Men who have sex with men (MSM) who attended the Melbourne Sexual Health Centre with their partners were recruited to participate in the study. Urethral, pharyngeal and rectal cultures for NG were obtained from all participants though only couples where both partners cultured NG were included in the analysis. Whole genome sequencing of the cultured isolates was performed on the Illumina MiSeq/NextSeq. **Results:** 33 couples were included in the study. Urethral, pharyngeal and rectal isolates within an individual were clonal by *in silico* NG-MAST[1] and MLST[2], and highly similar using core-genome SNP[3] analyses, with a median pairwise SNP distance = 2 SNPs (range 0-13; IQR 1-6). In 32/33 couples, MSM were infected with the same clone as their partner (same NG-MAST and MLST; median pairwise SNP distance = 2 SNPs; range 0-199; IQR 1-6). Pan-genomic comparisons of gene content showed a similar signal. Recombination events accounted for many genetic differences between clones, and resulted in partner isolates differing by >50 SNPs in two couples. Isolates from 4 couples had mosaic *penA* alleles (NG-MAST types 1407, 4822), with phylogenetic analyses indicating transmission of clones with identical alleles conferring reduced susceptibility to extended-spectrum cephalosporins between partners. **Conclusion:** NG isolates were clonal at different sites within individuals and between MSM partners. Our findings illustrate the potential for resistance to rapidly emerge in NG through homologous recombination and show the propagation of antimicrobial resistance through transmission of resistant clones. The results from this study provide important information for future genomic analyses of NG resistance and transmission.

Author Disclosure Block:

J.C. Kwong: None. **E.P.F. Chow:** None. **K. Stevens:** None. **T. Tomita:** None. **T.P. Stinear:** None. **T. Seemann:** None. **C.K. Fairley:** None. **M.Y. Chen:** None. **B.P. Howden:** None.

Poster Board Number:

MONDAY-363

Publishing Title:

Real-Time Pcr for Rapid Detection of *Neisseria gonorrhoeae* and Resistance Determinants in Clinical Specimens

Author Block:

V. Dona[1], S. Kasraian[1], C. Hauser[2], M. Unemo[3], H. Furrer[2], N. Low[4], **A. Endimiani**[1]; [1]Inst. for Infectious Diseases, Univ. of Bern, Bern, Switzerland, [2]Bern Univ. Hosp., Univ. of Bern, Bern, Switzerland, [3]Örebro Univ. Hosp., Örebro, Sweden, [4]Inst. of Social and Preventive Med., Univ. of Bern, Bern, Switzerland

Abstract Body:

Background: Molecular methods are often used for *N. gonorrhoeae* (NG) detection but antibiotic resistance testing still requires time-consuming phenotypic tests. We developed an assay for use in clinical specimens to both identify NG and detect genetic markers of antibiotic resistance. **Methods:** We designed a mismatch amplification mutation assay (MAMA) real-time PCR targeting sequences for: two NG-specific genes (*opa*, *porA*); *penA* mosaic alleles (D345 deletion, G545S) associated with decreased susceptibility to cephalosporins; single nucleotide polymorphisms conferring resistance to ciprofloxacin (GyrA: S91F), azithromycin (23S rRNA: A2059G and C2611U) and spectinomycin (16S rRNA: C1192U). We applied the real-time PCR to 201 clinical specimens (72 urethral, 56 rectal, and 73 pharyngeal) from 79 patients. We evaluated the performance of our method by comparison with the Aptima CT/NG test and standard MICs obtained with the Etest method. **Results:** The MAMA strategy allowed the detection of resistance mutations by comparing cycle threshold values with reference reactions (*opa* and *porA*). Our assay detected NG-positive samples with the following sensitivity/specificity: urethral, 100/98.2%; rectal, 94.7/100%; and pharyngeal, 90/97.7%. It correctly predicted the ciprofloxacin phenotype (24 with MIC ≤0.032 and 16 with MIC ≥0.064 µg/ml) in all culture isolates from specimens tested by real-time PCR. Two patients tested positive for the presence of a mosaic *penA* XXXIV allele. No samples tested positive for the 16S rRNA gene mutation, in line with the observed spectinomycin MICs. The presence of commensals resulted in false positive results for 23S rRNA gene mutations in 5 pharyngeal specimens. **Conclusion:** Our real-time PCR platform is a promising rapid method to accurately identify NG and predict antimicrobial resistance directly in clinical specimens. The impact of this method on the antibiotic management and outcome of NG infections should be evaluated in the near future.

Author Disclosure Block:

V. Dona: None. **S. Kasraian:** None. **C. Hauser:** None. **M. Unemo:** None. **H. Furrer:** None. **N. Low:** None. **A. Endimiani:** None.

Poster Board Number:

MONDAY-364

Publishing Title:

Resistance Patterns Gram-Negative Uropathogens in St.Paul's Hospital Millennium Medical College

Author Block:

Y. Mamuye, Semaria Solomon; SPHMMC, .Addis Ababa, Ethiopia

Abstract Body:

Background: The resistance of bacteria causing urinary tract infection (UTI) to commonly prescribed antibiotics is increasing both in developing as well as in developed countries. The study was undertaken to report antibiotic resistance pattern among common bacterial uropathogens. **Methods**: A total of 2544 urine samples were processed between September 2012 and 2015. Inoculation was performed onto blood and MacConkey agar symoultaniously. Significant bacteriuia was considered with colony counts greater than 10^5cfu/ml. Antibiotic susceptibility was done by Kirby Bauer disk diffusion method. Data entry and analysis was performed using SPSSv20. **Result**: Of the total 2544 samples, 569(22.4%) showed significant growth. Gram negative organisms totaled 508(20.0%), and 61(2.4%) isolates were gram positive. The most frequently isolated gram negative bacterium was *E. coli* followed by *Protues* and *Klebsiella spp*. 305 (12.5%), 102(4.0%), and 42(1.7%) respectively. Between 2012 and 2013, the resistance rate to Tetracyclin, Ampicilin, Amoxycilin, and Nalidixic Acid was reported as 69%, 64% and 67% respectively. In 2014, the study showed that resistance pattern for all except to tetracycline becomes increased 65%, 78% and 78%. The study also showed an emerging resistance to Ciprofloxacilin and Ceftriaxone especially for common gram-negative bacteria. There was relatively low resistance rate to Nitrofurantoin, Gentamycin, and Trimethoprim-Sulfamethoxazole throughout the years. **Conclusions**: In this study setting resistant rate to Tetracyclin, Ampicilin, Amoxycilin, and Nalidixic Acid were high. Since most isolates were sensitive to Nitrofurantoin, Gentamycin, and Trimethoprim-Sulfamethoxazole, thus clinicians should consider these drugs as appropriate antimicrobials for empirical treatment for UTI.

Author Disclosure Block:

Y. Mamuye: G. Member; Self; ASM member.

Poster Board Number:

MONDAY-365

Publishing Title:

Serogroup and Genotype Distribution of *Chlamydia trachomatis* in Reactive Arthritis and Urogenital Infections in Jamaica

Author Block:

T. R. Butterfield[1], N. Christian[1], J. F. Lindo[1], D. A. Lyn[2], J. Igietseme[3], M. Smikle[1]; [1]Univ. of the West Indies, Kingston 7, Jamaica, [2]Morehouse Sch. of Med., Atlanta, GA, [3]CDC, Atlanta, GA

Abstract Body:

Chlamydia trachomatis is the most frequent triggering agents of reactive arthritis, a sterile joint inflammation. There are no reports on the serogroups or genotypes of *C. trachomatis* in the Jamaican population. The genotypes and serogroups of *C. trachomatis* were determined in joint fluid from patients with suspected reactive arthritis (n=100) and urine samples from a control group of antenatal women (n=201) using 3 different polymerase chain reaction (PCR) assays. The prevalence of *C. trachomatis* in joint fluid ranged from 25% - 71%. The *C. trachomatis* strains found in joint fluid belonged to 2 serogroups and 4 genotypic groups. The majority of strains from joint fluid (24/26, 92%) belonged to serogroup B while serogroup C accounted for the remaining strains (2/26, 8%). The prevalence of *C. trachomatis* in urine specimens ranged from 25% - 69%. The *C. trachomatis* strains found in the urogenital tract belonged to 3 serogroups and 5 genotypic groups. The majority of strains from urine specimens (23/45, 49%) belonged to serogroup B and the remaining strains belonged to serogroup C (14/45; 30%), serogroup I (2/45, 4%) and serogroup BC (5/45; 17%). Genotypic group 1 accounted for the majority of *C. trachomatis* strains in both synovial fluid (15/25; 65%) and urine (92/125; 73%). Among reactive arthritis patients, *C. trachomatis* positivity was significantly associated with being female (25/44, 57% v. 20/56, 36%; p = 0.035) and 50 years of age and older (14/25, 56% v. 9/36, 25% v. 22/39, 56%; p = 0.011). No direct correlations were observed between serogroups and genotypic groups. A substantial proportion of reactive arthritis cases in Jamaica are Chlamydia induced and strains of serogroup B preferentially affect the joints. The results indicate that further work is needed to determine the relationships between *C. trachomatis* serogroups, genotypic groups and serovars and the selective distribution of serogroup B, compared to other *C. trachomatis* serogroups, in the joints of patients with ReA.

Author Disclosure Block:

T.R. Butterfield: None. **N. Christian:** None. **J.F. Lindo:** None. **D.A. Lyn:** None. **J. Igietseme:** None. **M. Smikle:** None.

Poster Board Number:

MONDAY-367

Publishing Title:

Transcription-mediated Amplification-based Screening of Male Sti Clinic Patients for *Mycoplasma genitalium*

Author Block:

E. Munson[1], D. Wenten[2], S. Jhansale[2], M. Schuknecht[2], N. Pantuso[2], M. Napierala[1], K. Munson[1], A. Steward[1], J. Gerritts[1], D. Hamer[1]; [1]Wheaton Franciscan Lab., Milwaukee, WI, [2]Holton Street Clinic, Milwaukee, WI

Abstract Body:

Background: Transcription-mediated amplification (TMA)-based methods have been shown to outperform culture and DNA-based amplification modalities for detection of *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, and *Trichomonas vaginalis* from rectal and/or pharyngeal sources. Recent data espouse the utility of TMA for detection of *Mycoplasma genitalium* from male demographics. We report on performance of TMA-based assays for detection of sexually-transmitted infection (STI) agents from urine and extra-urogenital sources, with particular focus on *M. genitalium*. **Methods:** Within a 22-month interval, 1494 male patient encounters at a Milwaukee STI clinic resulted in collection of 3732 specimens for *C. trachomatis*, *N. gonorrhoeae*, *T. vaginalis*, and *M. genitalium* TMA screening (Hologic). Positive *M. genitalium* results from extra-urogenital specimens were subject to confirmation via repeat analysis. **Results:** 66 patient encounters were managed with collection of one specimen, of which 18.2% were positive for at least one STI agent. This detection percentage increased to 22.2% with collection of specimens from two sources (618 patient encounters) and to 25.6% with collection of specimens from three sources (810 encounters). 270 encounters (18.1% of all encounters) were positive for detection of *C. trachomatis*, *N. gonorrhoeae*, and *T. vaginalis* in various combinations; addition of *M. genitalium* TMA to the screening panel resulted in 86 additional encounters with detection of at least one STI (31.9% increase in encounters positive for STI detection). 60 of these additional detections emanated from urine collections, while 21 resulted from rectal collections. Overall *M. genitalium* detection rates were 6.6% and 5.7% from urine and rectal specimens, respectively. 91.5% of initially-positive *M. genitalium* rectal specimens repeat tested as positive. 93.8% of males with a rectal specimen testing positive for *M. genitalium* indicated strict homosexual preference. **Conclusions:** Additional specimen source sampling contributes to comprehensive STI screening. Incorporation of extra-urogenital sources into assessment for *M. genitalium* may identify additional male carriers of the STI agent.

Author Disclosure Block:

E. Munson: None. **D. Wenten:** None. **S. Jhansale:** None. **M. Schuknecht:** None. **N. Pantuso:** None. **M. Napierala:** None. **K. Munson:** None. **A. Steward:** None. **J. Gerritts:** None. **D. Hamer:** None.

Poster Board Number:

MONDAY-368

Publishing Title:

Aetiological Pathogens of Male Urethritis Syndrome and Vaginal Discharge Syndrome in the Tshwane Area, South Africa

Author Block:

B. Osizigbo[1], M. Ehlers[2], **M. Kock**[2]; [1]Univ. of Pretoria, Pretoria, South Africa, [2]Univ. of Pretoria/Natl. Hlth.Lab. Service, Pretoria, South Africa

Abstract Body:

In South Africa, the burden of STI is high and the most common STI presentations to the public healthcare are male urethritis syndrome (MUS) and vaginal discharge syndrome (VDS). Male urethritis account for over 60% of STIs annually, reported at healthcare facilities in the Gauteng province of South Africa. The main STI pathogens responsible for these two syndromes are *Chlamydia trachomatis, Mycoplasma genitalium, Neisseria gonorrhoeae* and *Trichomonas vaginalis*. In South Africa, STI treatment services are provided by both the public and private sectors. In the former, treatment for STIs is reliant on syndromic management. The success of this depends on early detection and constant monitoring of the aetiological pathogens. The aim of this study was to determine the prevalence of *N. gonorrhoeae, T. vaginalis, C. trachomatis* and *M. genitalium* as aetiological pathogens of MUS and VDS using a multiplex real-time polymerase chain reaction assay. Furthermore, the antimicrobial resistance of *N. gonorrhoeae* clinical isolates to eight antibiotics (penicillin, cefoxitin, ceftriaxone, cefpodoxime, tetracycline, ciprofloxacin, ofloxacin and spectinomycin) and the corresponding minimum inhibitory concentrations (MICs) were determined by using the Kirby Bauer disc diffusion and E-test methods. Finally, *N. gonorrhoeae* multi-antigen sequence typing (NG-MAST) was used to determine the genetic relatedness of *N. gonorrhoeae* clinical isolates. The prevalence of MUS and VDS pathogens in this study was 57% (113/200). Pathogens responsible for urethritis were detected in 77% (48/62) of male participants while VDS were found in 47% (65/138) of the female participants. All isolates were susceptible to third-generation cephalosporins. The NG-MAST method revealed novel as well as previously described sequence types (STs). The NG-MAST ST 11715, 11716, 11717 and 11718 are novel STs. This study showed that *N. gonorrhoeae* is the most common cause of urethritis and vaginal discharge in the Tshwane region. The real-time multiplex PCR assay was rapid and suitable for the detection of all four pathogens at relatively low cost. The findings of this study further support the testing of more than one pathogen in a PCR assay for the laboratory diagnosis of MUS and VDS.

Author Disclosure Block:

B. Osizigbo: None. **M. Ehlers:** None. **M. Kock:** None.

Poster Board Number:

MONDAY-369

Publishing Title:

***Gardnerella vaginalis* Population Dynamics in Bacterial Vaginosis**

Author Block:

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Abstract Body:

Background: Bacterial vaginosis (BV) is the leading cause of vaginal discharge and *Gardnerella vaginalis* is thought to play a key role in disease pathogenesis. *G. vaginalis* population structure consists of four clades which differ with respect to virulence factors and antimicrobial resistance.**Methods:** We performed a longitudinal study, collecting vaginal specimens from women at initial BV diagnosis, after completion of treatment and at a 40-45 day follow-up visit. Women were evaluated for BV by Amsel criteria and Nugent scoring. DNA was extracted from these vaginal specimens and analyzed using quantitative real-time polymerase chain reactions (qPCRs) to determine the concentration of each of the four clades.**Results:** Overall, median concentrations of Clades 1 (1.6 E5) and 4 (1.5 E4) were far higher than those of Clades 2 (1.8 E2) and 3 (3.9 E1). Comparison by Nugent score found that Clade 1 and 4 concentrations significantly increased in a stepwise fashion from normal to intermediate to abnormal flora specimens ($P<0.05$). In contrast, the Clade 2 concentration did not differ between intermediate and abnormal flora specimens and the Clade 3 concentration did not differ between normal and intermediate flora specimens. Out of 78 women successfully treated for BV in the study, 32 (41%) would experience recurrent disease at the 40-45 day visit compared to 46 (59%) who remained cured. Both cured and recurrent BV subjects experienced a 2-3 log reduction in Clades 1 ($P<0.001$) and 4 ($P<0.05$) after treatment. The concentration of Clade 2 was reduced only in cured subjects ($P<0.01$) and Clade 3 only in recurrent BV subjects ($P<0.05$). Recurrent disease was characterized by post-treatment increases in Clades 1-3 ($P<0.05$) but not Clade 4.**Conclusions:** Clades 1 and 4 dominate the *G. vaginalis* niche in the vaginal microbiome, are associated with increasing degrees of abnormal vaginal flora and are suppressed similarly by antimicrobial treatment in both cured and recurrent BV patients. In contrast, Clades 2 and 3 were associated with specific vaginal flora transitions and their suppression by therapy with different patient outcomes.

Author Disclosure Block:

D.W. Hilbert: D. Employee; Self; Medical Diagnostic Laboratories. **J.A. Schuyler:** None. **M.E. Adelson:** D. Employee; Self; Medical Diagnostic Laboratories. **E. Mordechai:** D. Employee; Self; Medical Diagnostic Laboratories. **J.D. Sobel:** None. **S.E. Gygax:** D. Employee; Self; Medical Diagnostic Laboratories.

Poster Board Number:

MONDAY-370

Publishing Title:

Clinical Symptoms Associated with *Mycoplasma hominis* in Women Genital Tracts

Author Block:

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Abstract Body:

Mycoplasma hominis is a commensal bacterium in women's genital tracts but recently the role of this organism as a multi-potential pathogen has been suggested. Some physicians believe that this organism colonizes female's genital tract as normal flora with no detrimental effects. This study has compared the frequency of *M. hominis* in symptomatic and asymptomatic women. 226 women with at least one of the clinical symptoms including infertility, vaginosis, abortion or preterm labor and 42 women with none of mentioned manifestations referred to Kowsar University Hospital in Qazvin/Iran were evaluated for the presence of *M. hominis* in their cervix from November 2012 to December 2014. Conventional culture media including broth and agar were prepared manually and supplemented with yeast extract, horse serum, L-arginine, sucrose and antibiotics and also two separate real-time PCR assays targeting 16S rRNA and *yidC* were used for detection of genetically variable *M. hominis* in endocervical specimens.39 (17.2%) symptomatic patients were infected by *M. hominis* whereas among asymptomatic women only 1 (2.4%) person carried this organism in her cervix. Presence of *M. hominis* in women with infertility and vaginosis was significant ($p < 0.05$). Clinical and analytical sensitivity and specificity of real-time PCR targeting *yidC* as a recently used target for detection of *M. hominis* were 100%. Also clinical sensitivity and specificity of the culture were 91.5% and 100% respectively. To distinguish distribution of *M. hominis* between symptomatic and asymptomatic women most (if not all) of related symptoms should be considered, not just one or two of them. Culture method and molecular based assays for detection of such fastidious and heterogeneous bacteria should be design carefully. In this study distribution of bacteria among symptomatic and asymptomatic women was significantly different. *yidC* can be considered as a reliable candidate gene for detection of *M. hominis* although more investigations are necessary in the other populations. Molecular based techniques such as real-time PCR are high sensitive however culture method for detection of *Mycoplasma hominis* is still valuable and cost effective especially in developing countries.

Author Disclosure Block:

T. Naserpour Farivar: None. **S. Saadat:** None. **F. Samiee Rad:** None. **T. Dabbaghi Ghaleh:** None. **H. Jahanihashemi:** None. **H. Bahrami:** None. **F. Abbasi:** None.

Poster Board Number:

MONDAY-371

Publishing Title:

Age-Specific Prevalence of Cervical *Human papilloma virus* (HPV) Infection and Cytological Abnormalities During the Postpartum Period

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Abstract Body:

Background: In Korea, most women are screened for cervical cancer using cytology, but prevalence of human papilloma virus (HPV) infection is not well known. Therefore, this study was conducted to investigate the age-specific prevalence of HPV infection and cytological abnormalities during the postpartum period. **Methods:** Between January 2013 and December 2014, we conducted a cross-sectional study of 1927 patients who had given birth at Cheil General Hospital and Women's Healthcare Center. All participants were screened with liquid based Pap smear and cervical human papilloma virus infection test at one to three months after delivery. HPV testing was undertaken using the Seeplex HPV4A ACE screening kit(Seegene).**Results:** During the postpartum period, 6.7% of the patients (128/1927) had positive test results for HPV and 4.2% (81/1927) patients showed abnormal cytology. Among HPV-positive patients, high-risk HPV DNA prevalence was 97.6% (125/128). Among abnormal cytology group, prevalence of low and high-grade squamous intraepithelial lesions(LSIL, HSIL) were 13.6% and 4.9% (11/81 and 4/81). Total 2.5% of the patients (50/1927) showed abnormalities in both HPV and cervical cytology and 2.9% of the patients (58/1927) had positive HPV test with normal cervical cytology. Total 47 patients with abnormal cytology and/or positive HPV infection had further histological confirmation test by colposcopic biopsy and 17/47 patients (36.2%) revealed LSIL or HSIL. 38.5% of the patients (15/39) with both tests positive and 25.0% of the patients (2/8) with abnormal cytology but negative HPV tests were confirmed to have LSIL or HSIL in colposcopic biopsy. Age-specific prevalence of HPV and cytology were not statistically different and showed a plateau-shaped curve.**Conclusions:** The prevalence of HPV infection was lower than expected in Korean pregnant women. More proportion of the patients with both tests positive was confirmed to have histologic LSIL or HSIL than those with only abnormal cytology. Age-specific prevalence was not different between each age group in normal postpartum women.

Author Disclosure Block:

S. Lee: None.

Poster Board Number:

MONDAY-372

Publishing Title:

High Risk *Human papilloma virus* (HR-HPV) Infection Is Not Associated with Adverse Pregnancy Outcomes

Author Block:

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Abstract Body:

Background: Human papillomavirus (HPV) is one of the most common sexually-transmitted infections among women in their childbearing years. Recently, HPV is known to be associated with several adverse pregnancy outcomes, for example, preeclampsia and premature rupture of membranes. However, only a few studies have been reported in this topic. Therefore, the objective of this study was to determine the association between high-risk HPV (HR-HPV) and adverse pregnancy outcomes. **Methods:**Between January 2013 and December 2014, we conducted a retrospective cohort study of women with HR-HPV at postpartum compared with those with normal cervical cytology at Cheil General Hospital and Women's Healthcare Center. HPV testing was undertaken using the Seeplex HPV4A ACE screening kit(Seegene). We also compared HPV infection with other genital infections. **Results:**Hundred and twenty five women with HR-HPV were age-matched with 250 women with normal pap smears. Our sample included 53 cases of premature rupture of membranes (PPROM), 30 cases of preterm deliveries before 37 weeks, 43 cases of small for gestational age infant (SGA), 21 cases of gestational diabetes mellitus (GDM) and 3 cases of preeclampsia. In our study group, there were no differences in the prevalence preterm delivery, PPROM, SGA, GDM or preeclampsia. Exposed HR-HPV patients had lower body mass index at the beginning of pregnancy and the rate of emergency cesarean section was lower than normal control (16.8% vs 32.0%, p=0.003). Twelve patients were infected by *group B streptococcus* (GBS) and 11 patients were infected by other sexually transmitted diseases (*ureaplasma, mycoplasma, Chlamydia, E.coli* etc). However, co-infection with other types of bacteria or virus was not more prevalent in HR-HPV infected women.**Conclusions:**HR-HPV infection is known as a very important risk factor for cervical neoplasia, however, according to our study, HR-HPV infection was not associated with any adverse pregnancy outcomes including PPROM and preeclampsia.

Author Disclosure Block:

S. Lee: None.

Poster Board Number:

MONDAY-373

Publishing Title:

High Risk Human Papilloma Virus Subtypes in Women of Karachi Pakistan

Author Block:

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Abstract Body:

Introduction: Human Papilloma virus is the major cause of cervical cancer (CC) which is the second most common cancer among women worldwide. Presently, more than 100 HPV genotypes have been identified and about 20 of them have been strongly implicated in the development of ano-genital cancer. In developing country like Pakistan Population-based data for HPV-type distribution is insufficient. The need is to compose and present an up to date data that would be instrumental in improving the therapeutic strategies against HPV. **Objective:** To determine the prevalence of high-risk HPV types in healthy women of Sindh Pakistan. **Methodology:** 360 samples consisting of cervical scrapes were collected from women recruited. Following smearing slides for Pap test, remaining cervical cells were transferred to sterile vials containing phosphate buffered saline, and stored at -20^0C. DNA was extracted from samples and HPV specific PCR was carried out for subtypes 16, 18, 31, 33, 35, 39, 45, 52, 56, 58, 59 and 66. **Results:** Out of 360 normal sexually active women visiting the gynecology clinic evaluated for the presence of HPV 54 (15.42%) were found positive for HPV with general primers which subjected to types specific PCR and 15 (4.2%) of them were found to have high risk oncogenic strains of HPV including genotype 16, 18, 56, 45, 66, 31, 39 and 58. Among the high rich strains three were HPV type 56, three HPV type 18, two each type 45, 66 and 31 and one each was HPV 16, HPV 58 and HPV type 39. **Discussion:** This study was performed to determine the prevalence of high risk HPV among normal Pakistani women. The study will help to evaluate the available vaccine and guide the vaccine production in future that could be effective for the local population. In our study we found HPV subtypes other than 16 and 18. Therefore, only the use of available vaccine will not be sufficient to protect the spread of the local strains. There is no question that early detection will continue to be a key element even once a vaccine is available.

Author Disclosure Block:

W. Yousuf: None. **S. Khan:** None.

Poster Board Number:

MONDAY-374

Publishing Title:

High Rate of Colistin Dependence in *Acinetobacter baumannii* Isolates from South Korea

Author Block:

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Abstract Body:

Background: Colistin is currently considered the last option for multidrug-resistant *Acinetobacter baumannii* infection. However, colistin resistance in *A. baumannii* is increasing worldwide. We report the in vitro development of colistin dependence in not few colistin-susceptible *A. baumannii* isolates after exposure to colistin. **Methods:** We performed colistin susceptibility testing for 170 *A. baumannii* isolates collected from intensive care units of a Korean hospital in 2015 and examined colistin dependence among colistin-susceptible isolates by using the population analysis and colistin disc method. Multilocus sequence typing (MLST) was performed for the isolates developing colistin dependence. For 45 patients with *A. baumannii* infection, we compared 3-day and 7-day treatment failure in patients with and patients without colistin-dependent strains, 18 and 27 patients, respectively.**Results:** Among 170 *A. baumannii* isolates, 21 (12.4%) were resistant to colistin. We found that 49 of 149 colistin-susceptible *A. baumannii* isolates (32.9%) developed colistin dependence. The 49 isolates developing colistin dependence showed six STs in MLST. The ST208 (28 isolates, 57.1%) had the most common. Three-day and 7-day treatment failure in patients with and patients without colistin-dependent strains were 66.7% versus 37.0% (3-day; $p = 0.051$) and 44.4% versus 25.9% (7-day; $p = 0.197$).**Conclusions:** In vitro development of colistin dependence after exposure to colistin may not be rare phenomenon in *A. baumannii*. Fast substitution of antimicrobial agents may be required if colistin treatment is ineffective and colistin dependence is identified.

Author Disclosure Block:

Y. Hong: None. **J. Lee:** None. **Y. Wi:** None. **K. Ko:** None.

Poster Board Number:

MONDAY-375

Publishing Title:

Assessing The Efficacy Of Silver-Doped Antimicrobial Coatings On Prosthetic Liners

Author Block:

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Abstract Body:

Abstract: Approximately 185,000 patients undergo an amputation each year, with an estimated 50 percent increase in the next 35 years[1,2]. One of the leading issues facing amputee patients is late developing antibiotic-resistant bacterial infections in the stump of the patient. The enclosed design of the prosthetic socket often causes friction, sweat and debris build-up at the prosthetic-stump interface. Coupled with body heat, these factors result in a favorable condition for skin irritation, bacterial growth and eventual infection and chronic malodor. In this study, we characterized the efficacy of a silver-doped, titanium dioxide-polydimethylsiloxane coating at preventing bacterial growth on orthopaedic liners as a possible solution to the complex issue facing prosthesis wearers.**Methods:** This study utilized seven commonly encountered pathogens: Coagulase-Negative *Staphylococcus epidermidis*, Methicillin-sensitive *Staphylococcus aureus*, Methicillin-resistant *Staphylococcus aureus*, *Propionibacterium acnes*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, and Multi-Drug Resistant *Acinetobacter baumannii*.Kirby Bauer Assay was conducted using 4 different types of commercially available prosthetic liners and 9 different conditions of coating. Liner plugs were plated on a lawn of bacteria with a set concentration of $1E^6$ CFU/mL. Area inhibition was measured over 72 hours.Efficacy of the coating was determined via dose response curves against each pathogen independent of the prosthetic liners at aconcentration of $1E^7$ CFU/mL. Data was analyzed using ImageJ.**Results:** The silver-doped coating has antimicrobial effects on each of the seven pathogens assessed. On prosthetic liners, the coating is effective at preventing bacterial growth for at least 72 hours. The 95% 10X preparation of the coating was found to be the most effective at killing and preventing bacterial colonization.**Conclusion:** The coating conditions tested display a pattern of bacterial inhibition and continuous protection which point towards the 95%10X condition being the most promising as an antimicrobial coating in prosthetic liners. The identified conditions promise to be an effective tool towards mitigating infections and malodor found in the stump-liner interface.

Author Disclosure Block:

J.M. Haglin: None. **A.V. Hayda:** None. **D. Garcia:** C. Consultant; Self; Materials Science Associates, LLC. **J. Jarrell:** D. Employee; Self; BioIntraface, Inc., Materials Science Associates, LLC.. K. Shareholder (excluding diversitied mutual funds); Self; BioIntraface,

Inc.. **R. Hayda:** None. **C. Born:** C. Consultant; Self; BioIntraface, Inc.. K. Shareholder (excluding diversitied mutual funds); Self; BioIntraface, Inc..

Poster Board Number:

MONDAY-376

Publishing Title:

Microorganisms Recovered From Adult Patients With Cystic Fibrosis

Author Block:

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Abstract Body:

Chronic, pulmonary infections are the most prominent cause of the increased morbidity and mortality in cystic fibrosis (CF). *Pseudomonas aeruginosa* remains the main pathogen in adults, but other Gram-negative bacteria such as *Achromobacter xylosoxidans* and *Stenotrophomonas maltophilia* have been shown to play an important role in this lung disease. We reviewed the organisms identified from respiratory specimens from CF patients collected over a three year period to determine if there were shifts in the populations of microorganisms.Culture and identification of microorganism followed standard procedures. In brief a 10% solution of sputolysin was added to the respiratory sample to break down the mucus. The sample is then diluted to 1.0 x10-3 and plated onto three selective plates; Mannitol Salt Agar, Burkholderia cepacia Selective Agar, and Chocolate Agar with Bacitracin. Serial dilutions of the sample were plated on 5% sheep blood agar and MacConkey agar. After 16-18 hours of incubation in at 37^0C, 5% CO2, the culture was examined for bacterial growth and the organisms identified by standard biochemical assays or by commercial identification systems.Between 2012 through 2015, 4996 organisms were identified from CF cultures. Of this total, 30% of the cultures were positive for *Pseudomonas aeruginosa*, 23% were positive for *Staphylococcus aureus* and 9% were positive for methacillin-resistant *Staphylococcus aureus* (MRSA). Non-lactose fermenting gram negative rods were present in 8% of the cultures with *Stenotrophomonas maltophilia* and *Achromobacter (Alcaligenes) xylosoxidans* being the two predominating organisms. Mold was recovered in 7% of the cultures with the majority identified as *Aspergillus*. Other organisms isolated, each with a percent less than 7%, included *Pseudomonas* species, *Burkholderia cepacia*, *Haemophilus influenza*, group A streptococcus, yeast, and enteric gram negative rods .In our adult CF patient population *Pseudomonas aeruginosa* was the most predominant organism identified in CF respiratory cultures followed by *Staphylococcus aureus*. Interesting the percentage of MRSA, nonlactose fermenting gram negative rods, *Burkholderia cepacia* and group A strep remained unchanged while the other organism identified either increased or decreased in percentage from year to year.

Author Disclosure Block:

K. Lucey: None. **R. Harbeck:** None.

Poster Board Number:

MONDAY-377

Publishing Title:

Variation Of Biofilm Forming Ability Of Aggr-Positive *escherichia Coli* Strains

Author Block:

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Abstract Body:

Background: Enteroaggregative *Escherichia coli* (EAEC) cause acute or persistent diarrhea and their adherence to the intestinal mucosa is characterized by the formation of a thick biofilm. Although the gold standard method for EAEC identification is the HEp-2 cell adherence assay, the transcriptional regulator AggR is widely used as a genetic marker of typical EAEC for PCR detection. Recently, we have found that some *aggR*-positive *E. coli* strains did not form strong biofilm. Therefore, we examined the correlation between the biofilm forming ability and prevalence of virulence genes among the *aggR* gene-positive *E. coli* strains. **Methods:** A total of 58 *aggR*-positive *E. coli* strains were used, and they were isolated from diarrheal children since 2011 through 2013 in Kagoshima, Japan. Biofilm formation is assessed by microtiter plate assay, and strains were classified into two groups according to the biofilm index: the biofilm group ($OD_{570}\geq 0.2$) and the non-biofilm group ($OD_{570}<0.2$). They were also examined for virulence genes of EAEC (plasmid; *aatA*, *aap*, *aggA*, *aafA*, *agg3A*, *hdaA*, *aaf5*, *pet*, *sepA* and *astA*, chromosome; *sat*, *pic* and *aaiC*) and UPEC (*sfa*, *pap* and *afa*) by PCR. **Results:** Thirty (51.7%) strains belonged to the biofilm group (range, 0.209-2.53), while 28 strains (48.3%) belonged to the non-biofilm group (range, 0-0.098). In the biofilm group, *aap* (96.7%) was the most frequently detected gene followed by *aaiC* (90.0%), *aatA* (83.3%) and *pic* (80.0%). In contrast, *aaf3* (60.7%), *pap* (60.7%) and *sfa* (42.9%) were frequently found in the non-biofilm group. The biofilm group strains predominantly harbored both plasmid and chromosomal genes of EAEC, while the non-biofilm group strains mainly possessed UPEC virulence genes. **Conclusions:** Approximately half of *aggR*-positive *E. coli* strains did not form the characteristic biofilm of EAEC in our district. It was supposed that the *aggR* gene was horizontally transferred from EAEC to UPEC. Not only the *aggR* gene but also other EAEC virulence genes or biofilm formation are required to identify EAEC.

Author Disclosure Block:

N. Imuta: None. **T. Ooka:** None. **K. Yoshiie:** None. **J. Nishi:** None.

Poster Board Number:

MONDAY-378

Publishing Title:

Increased Nasopharyngeal Density And Concurrent Carriage Of *streptococcus Pneumoniae, Haemophilus Influenzae And Moraxella Catarrhalis* are Associated With Pneumonia In Febrile Children

Author Block:

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Abstract Body:

Background: We assessed nasopharyngeal (NP) carriage of five pathogens in febrile children with and without acute respiratory infection (ARI) of the upper (URTI) or lower tract, attending health facilities in Tanzania.**Methods:** NP swabs collected from children (N= 960) aged 2 months to 10 years, and with a temperature ≥38°C, were utilized to quantify bacterial density of *S. pneumoniae (Sp), H. influenzae (Hi), M. catarrhalis (Mc), S. aureus (Sa) and N. meningitidis (Nm)*. We determined associations between presence of individual species, densities, or concurrent carriage of all species combination with respiratory diseases including clinical pneumonia, pneumonia with normal chest radiography (CXR) and endpoint pneumonia.**Findings:** Individual carriage, and NP density, of *Sp*, *Hi* or *Mc*, but not *Sa or Nm*, was significantly associated with febrile ARI and clinical pneumonia when compared to febrile non-ARI episodes. Density was also significantly increased in severe pneumonia when compared to mild URTI (*Sp*, $p<0.002$; *Hi* $p<0.001$; *Mc*, $p=0.014$). Accordingly, concurrent carriage of Sp^+, Hi^+ and Mc^+, in the absence of Sa^- and Nm^-, was significantly more prevalent in children with ARI ($p=0.03$), or clinical pneumonia ($p<0.001$) than non-ARI, and in children with clinical pneumonia ($p=0.0007$) than URTI. Furthermore, Sp^+, Hi^+ and Mc^+ differentiated children with pneumonia with normal CXR, or endpoint pneumonia, from those with URTI and non-ARI cases.**Interpretation:** Concurrent NP carriage of *Sp, Hi* and *Mc* was a predictor of clinical pneumonia and identified children with pneumonia with normal CXR and endpoint pneumonia from those with febrile URTI or non-ARI episodes.

Author Disclosure Block:

S. Chochua: None. **V. D'Acremont:** None. **C.R. Hanke:** None. **D. Alfa:** None. **J. Shak:** None. **M. Kilowoko:** None. **E. Kyungu:** None. **L. Kaiser:** None. **B. Genton:** None. **K.P. Klugman:** None. **J.E. Vidal:** None.

Poster Board Number:

MONDAY-379

Publishing Title:

Increased Rate of *Gordonia* Species Identified from Clinical Isolates in New York State

Author Block:

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Abstract Body:

Gordonia species are gram-positive, partially modified acid-fast bacilli commonly found in the environment, which have recently been described as emerging pathogens, causing disease primarily in immunocompromised patients. *Gordonia* species are often difficult to identify at both a genus and species level by conventional culture and biochemical analyses and can be misidentified as *Rhodococcus, Corynebacterium,* or *Nocardia* species. Molecular identification of *Gordonia* species using 16S rDNA gene sequence analysis improves the rate of identification, however several of the species cannot be differentiated using this method. The Wadsworth Center (WC) serves as the diagnostic reference center for the detection and identification of bacteria for New York State. From 2001 to 2014 the average the number of specimens received and identified as *Gordonia* species annually was 3.57, however in 2015 the number of specimens identified quadrupled to 16. The 16 isolates originated from 11 different hospitals and from various specimen types including blood, sputum, bronchial wash, corneal scrapings, and wound biopsies. Patients ranged in age from 3 months to 83 years old and were equally distributed between males and females. Of the 16 isolates received, 31% were able to be definitively identified to the species level, while the other 69% were unable to be differentiated between different species using MALDI-TOF MS, biochemicals, and 16S rDNA sequence analysis. The *Gordonia* species received in 2015 by WC that were not readily distinguished by 16S rDNA sequence analysis were *G. sputi*, *G. aichiensis* and *G. otitidis*. Interestingly, amongst these three species the biochemical profiles are significantly different, however the WC isolates do not match the biochemical profiles of any of the type strains. This recent increase in *Gordonia* isolates received may be the result of new test systems in clinical laboratories such as the MALDI-TOF MS or indicate an increase in infections with *Gordonia* species. Studies are underway to identify new genetic targets which may be utilized for more rapid identification methods of these emerging pathogens.

Author Disclosure Block:

K. Mitchell: None. **L. Mingle:** None. **J. Cole:** None. **N. Dumas:** None. **K. Musser:** None.

Poster Board Number:

MONDAY-380

Publishing Title:

Comparison of Virulence Factors in Pigmented and Non-Pigmented *Pseudomonas aeruginosa* by Using phenotypic and Genotypic Tests

Author Block:

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Abstract Body:

Background: The incidence of *Pseudomonas aeruginosa* has been increased in recent years and its virulence factors are various. The aim of this study was to compare the differences in the virulence factors of pigmented and non-pigmented *P. aeruginosa* isolates. **Methods:** Isolates of patients that were identified as *P. aeruginosa* were included into the study. The isolated *P. aeruginosa* strains (n: 100) were divided into two main groups equally according to their pigment production. Antibiotic susceptibility tests of all strains were performed by disk diffusion method according to CLSI (Clinical and Laboratory Standards Institute) standards. DNase, protease, elastase, haemolysis and motility tests were performed as phenotypic tests. The presence of several virulence-associated genes encoding exotoxins T (*exo*T), S (*exo*S), U (*exo*U) and Y (*exo*Y) and quorum sensing mediators (*rhl*A and *rhl*B) were assessed by Real-time PCR method for genotypic identification. The relationship between pigment production, antibiotic resistance and virulence factors were examined.**Results:** There was no significant difference between pigmented and non-pigmented isolates when elastolytic activity and mucus production were compared. Pigmented isolates produced significantly more ($p \leq 0.05$) protease and haemolysis activity. Motility was present in pigmented isolates more frequently than in non-pigmented isolates. DNase activity was significant in pigmented isolates then non-pigmented isolates ($p \leq 0.05$). Antibiotic resistance was present more frequently in non-pigmented isolates than pigmented isolates. Pigmented isolates had more frequently and significant more ($p \leq 0.05$) virulence-associated genes rhlB, *exo*S, *exo*Y ($p \leq 0.05$). *Exo*T was present in pigmented isolates more frequently than in non-pigmented isolates.**Conclusions:** The results of this study suggest that both phenotypic and genotypic virulence factors are associated with the pigment production in *P. aeruginosa.* Pigment production is easy to determine, which might be a good starting point to identify the virulence status of an isolate.

Author Disclosure Block:

S. Macin: None. **Y. Akyon:** None.

Poster Board Number:

MONDAY-381

Publishing Title:

Clinical and Molecular Characterization of Panton-Valentine Leukocidin-Positive Invasive *Staphylococcus aureus* Infections in Korea

Author Block:

E. Kim[1], K-H. Song[1], M. Kim[1], J. Park[1], P. Choe[2], Y. Park[3], W. Park[2], J. Bang[2], K. Park[1], S. Park[2], N. Kim[2], M-d. Oh[2], H. Kim[1], Korea Infectious Diseases (KIND) Study Group; [1]Seoul Natl. Univ Bundang Hosp., Seongnam-si, Korea, Republic of, [2]Seoul Natl. Univ Coll. of Med., Seoul, Korea, Republic of, [3]Natl. Hlth.Insurance Corp. Ilsan Hosp., Goyang-si, Korea, Republic of

Abstract Body:

Background: Panton-Valentine leukocidin (PVL) is a virulent cytotoxin which is known as one of indicators of community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) infection. This study was performed to evaluate the prevalence and clinical and molecular characteristics of PVL (+) invasive *S. aureus* (ISA) infections in Korea. **Methods:** A total of 1,962 non-duplicate *S. aureus* isolates, which were obtained from 2,533 cases in two prospective multicenter Korean surveillance studies for ISA infections in 2009-2011 and in 2012, were screened for possession of *pvl* gene. Clinical data were collected and multilocus sequence typing (MLST), staphylococcal cassette chromosome *mec* (SCC*mec*) typing, accessory gene regulator (agr) typing, and PCR for arginine catabolic mobile element (ACME) was performed for PVL (+) *S. aureus* isolates. **Results:** Among 1,962 *S. aureus* isolates, 28 (1.4%) showed PVL (+). Of these, 19 (67.9%) were MRSA: 8 community-associated and 11 healthcare-associated. In 28 cases of PVL (+) ISA infection, 17 (60.7%) were male patients and median age was 63 years old (range 13-93). Sixteen patients (57.1%) had no underlying comorbidities. Primary sites of infection were skin and skin structure (9), respiratory tract (9), bone and joint (4), cardiovascular system (4), and unknown (2). Thirty day mortality was 41.7% (10/24). In PVL (+) MRSA, ST8 or ST8 variant (ST8v)-SCC*mec* IVa-agr type 1 strain was most prevalent (11/19, 57.9%), 9 of which possessed ACME, but some minor clones such as ST30-SCC*mec* IVc-agr 3 (2), ST938-SCC*mec* IV-agr 3 (2), ST59-SCC*mec* V-agr 1 (1), ST188-SCC*mec* V-agr 1 (1), ST234-SCC*mec* IV-agr 3 (1), ST1232-SCC*mec* V-agr 1 were also present. **Conclusions:** The PVL (+) ISA infection was very rare in Korea and the most common types of infection were skin and skin structure infection and pneumonia. The possession of PVL toxin was not a distinguishable characteristic in CA-MRSA infection and the PVL (+) MRSA strains showed diverse genetic background with a predominance of ST8/ST8v-SCC*mec* IVa-agr 1 strain in Korea.

Author Disclosure Block:

E. Kim: None. **K. Song:** None. **M. Kim:** None. **J. Park:** None. **P. Choe:** None. **Y. Park:** None. **W. Park:** None. **J. Bang:** None. **K. Park:** None. **S. Park:** None. **N. Kim:** None. **M. Oh:** None. **H. Kim:** None.

Poster Board Number:

MONDAY-382

Publishing Title:

***Staphylococcus aureus* Bacteremia (SAB) due to Soft Tissue Infection: Comparison between Monobacterial (M-SAB) and Polymicrobial (P-SAB) Cases**

Author Block:

R. Khatib, M. Sharma, K. Riederer; St John Hosp. & Med. Ctr., Grosse Pointe Woods, MI

Abstract Body:

Background: *S. aureus* causes a wide spectrum of soft tissue infections (STI), often as a single pathogen. The characteristics of patients with STI-associated P-SAB are not well defined.**Methods:** We reviewed SAB among adults between 2002 and 2015, selected patients with STI and compared cases with P-SAB and M-SAB.**Results:** We encountered 275 STI-associated SAB among 1674 SAB (16.4%). Other organisms were present in 27 instances, 16 (5.8%) were STI-associated P-SAB. Eleven cases where not counted as P-SAB including ten with a commensal organism regarded as a contaminant, and one with persistent SAB and an unrelated nosocomial *Acinetobacter* bacteremia infection acquired five days after admission. No significant differences were noted between P-SAB and M-SAB in patient characteristics including age (63.1 ±13.5 vs. 58.0 ±17.2 years), the frequency of diabetes (62.5% vs. 40.9%), and hemodialysis-dependence (6.3% vs. 8.1%) but the majority of P-SAB (13/16; 81.3%) were due to necrotizing infections. Among patients with necrotizing infections, P-SAB was noted in 7/30 (23.3%) sacral decubiti, 5/34 (14.7%) foot/leg foci, and 1/3 (33.3%) Fournier' gangrene. P-SAB tended to be more often due to MRSA (81.3% vs. 61.4%; p=0.2), and hVISA (9.1% vs. 4.7%; p=0.3) and was much more frequently caused by SCC*mec* type II (66.7% vs. 23.0% [M-SAB]; $p<0.001$). Overall mortality rate was much higher in P-SAB (68.8% vs. 22.4%; $p<0.001$).**Conclusions:** P-SAB is uncommon in STI-associated SAB, but accounts for a sizable portion of necrotizing infections and is associated with higher mortality. Additional studies of patients with necrotizing infections, including non-bacteremic cases, are needed to better determine the incidence of *S. aureus* and the benefit of including anti-staphylococcal antibiotics in the empirical management strategy.

Author Disclosure Block:

R. Khatib: None. **M. Sharma:** None. **K. Riederer:** None.

Poster Board Number:

MONDAY-383

Publishing Title:

Characterization of Colonizing Strains of Group B *Streptococcus* in Toronto, Canada

Author Block:

S. Teatero[1], I. Martin[2], W. Demczuk[2], A. McGeer[3], Toronto Invasive Bacterial Disease Network, N. Fittipaldi[1]; [1]Publ. Hlth.Ontario, Toronto, ON, Canada, [2]Natl. Microbiol. Lab., Winnipeg, MB, Canada, [3]Mount Sinai Hosp., Toronto, ON, Canada

Abstract Body:

Background: Group B *Streptococcus* (GBS) causes invasive disease in both adults and neonates. This opportunistic pathogen colonizes the intestinal and/or genital tract of approximately 20% of humans. The link between colonization and adult GBS disease is poorly understood. However, GBS-colonized mothers can transmit the organism vertically to newborns. Understanding the population structure of colonizing GBS strains may therefore reveal trends in invasive GBS infections. **Methods:** Our GBS sample comprised 102 GBS isolates recovered in December 2014 from vaginal/rectal swabs from healthy pregnant women in the metropolitan region of Toronto/Peel, Canada. Strains were serotyped by latex agglutination and PCR, and antimicrobial susceptibility to erythromycin, clindamycin and tetracycline was determined according to CLSI standards. We also performed multilocus sequence typing (MLST) on all strains, and used whole-genome sequencing and bioinformatics tools to study recombination leading to capsular switching in selected strains. **Results:** The most frequently identified serotypes among the GBS colonizing isolates were III (27%), V (25%), and Ia (21%). Notably, 6% of the carriage isolates were serotype IV, an emerging serotype that we recently identified as an important cause of invasive disease in Toronto. The most frequently found MLST types were ST17, ST23, ST19, and ST1. Interestingly, strains of four different serotypes belonged to MLST sequence type 1. Further examination revealed capsular switching in this group of organisms due to single-recombination events in four strains. We also found examples of large-scale genomic recombination among ST1 strains. Overall, we observed a high rate of tetracycline resistance (88%), while resistance to erythromycin and clindamycin was found in 36% and 25% of the strains, respectively. **Conclusions:** Our results provide a portrait of colonizing GBS strains circulating in Toronto, Canada. The serotyping and genotyping results closely mirror those we recently described among invasive GBS in the same area. Emergence of serotype IV strains and the occurrence of frequent capsule switching have implications for GBS vaccine developments.

Author Disclosure Block:

S. Teatero: None. **I. Martin:** None. **W. Demczuk:** None. **A. McGeer:** None. **N. Fittipaldi:** None.

Poster Board Number:

MONDAY-384

Publishing Title:

Xdr *Acinetobacter baumannii* and *Pseudomonas aeruginosa* Associated with Meningitis in Brazil

Author Block:

A. S. Ballaben[1], J. C. Ferreira[1], R. Galetti[1], D. O. Garcia[2], P. Silva[3], A. L. C. Darini[1]; [1]Sch. of Pharmaceutical Sci. of Ribeirão Preto - USP, Ribeirão Preto, Brazil, [2]Adolfo Lutz Inst., São Paulo, Brazil, [3]Adolfo Lutz Inst., Ribeirão Preto, Brazil

Abstract Body:

Non-fermenting gram-negative bacilli (NFGNB) have established themselves as an important nosocomial pathogen over the last decade and have been responsible for numerous complications such as meningitis. Carbapenemases resistance genes in NFGNB have been described both inserted into chromosome and plasmids. The aim of this study was to characterize molecularly and epidemiologically carbapenem resistant NFGNB isolated from cerebrospinal fluid and blood of patients with meningitis in different hospitals in São Paulo state, from 2007 to 2014. These bacteria were isolated at Adolfo Lutz Institute, a reference laboratory. Twenty *Acinetobacter baumannii* and 11 *Pseudomonas aeruginosa* were studied. The genes conferring resistance to carbapenems antibiotics and plasmid groups of *A. baumannii* (AB-PBRT) were investigated by PCR and the relationship was established by PFGE, using Tenover criteria. 80% of *A. baumannii* were extreme drug resistant (XDR) and 20% were multidrug resistant (MDR). All *A. baumannii* (confirmed by *rpo*B sequencing) produced the intrinsic $bla_{\text{OXA-51-like}}$ gene while 17/20 presented $bla_{\text{OXA-23-like}}$ gene which is spread worldwide among this microorganism. After PFGE analysis, was found a high clonal diversity. Plasmids of Groups 6 and 8 were found in two and three isolates, respectively. The two Group 6 isolates were not genetically related. Two isolates of Group 8 were from the same hospital and were genetically related while the other isolate was not. It is the first report about *A. baumannii* plasmids typing by AB-PBRT in Brazil; Group 6 has been reported in Europe countries, Australia and North Africa while Group 8 only in China. 82% of *P. aeruginosa* were XDR while 18% were classified as MDR. The isolates were clustered in 6 different pulsotypes. Only one isolate presented $bla_{\text{IMP-16}}$ which was classified in a separate pulsotype. Hence, the demonstrated results with clinical isolates of NFGNB reinforce the evidence that these bacteria are high and dangerous potential pathogens especially when associated with meningitis. This scenario is increasingly concerned by the ability of these pathogens acquires mobile genetic elements.

Author Disclosure Block:

A.S. Ballaben: None. **J.C. Ferreira:** None. **R. Galetti:** None. **D.O. Garcia:** None. **P. Silva:** None. **A.L.C. Darini:** None.

Poster Board Number:

MONDAY-385

Publishing Title:

***Globicatella Sanguinis*: A Rare Human Pathogen with an Expanding Spectrum of Disease**

Author Block:

A. O. Miller[1], M. W. Henry[1], B. Abraham[2], S. Whittier[3], B. J. Hartman[2], K. F. Maloney[4], F. Wu[3], B. D. Brause[1], P. Della-Latta[3], T. J. Walsh[2], A. N. Schuetz[2]; [1]Hosp. for Special Surgery, New York, NY, [2]Weill Cornell Med., New York, NY, [3]Columbia Univ. Coll. of Physicians and Surgeons, New York, NY, [4]Univ. at Buffalo, Buffalo, NY

Abstract Body:

Background: Commercial phenotypic methods may not consistently identify uncommon bacterial species. Two clinical cases, in which ceftriaxone-resistant, aerobic, alpha-hemolytic, *Streptococcus*-like pathogens were not able to be identified phenotypically, led to further molecular identification, and to a review of the clinical and microbiologic characteristics of the species, *Globicatella sanguinis*.**Methods:** Two ceftriaxone-resistant, aerobic, alpha-hemolytic clinical strains (from two patients with a prosthetic hip infection and a bacteremia) were analyzed with standard clinical automated and manual phenotypic techniques, and then with 16s rRNA and *sodA* PCR. The entire medical literature on *Globicatella* was surveyed.**Results:** Standard phenotypic methods failed to identify the isolates consistently. 16s PCR yielded sequences that were adequate for genus, not species, identification. *SodA* sequencing was required for species-level clarity. *G. sanguinis* is an uncommon cause of infections of the urine, meninges, and blood; no prior reports of orthopedic infection were found.**Conclusions:** Phenotypic methods fail to identify some uncommon pathogens. Advanced molecular techniques expand the spectrum of illness associated with rare organisms, such as *G. sanguinis*, which until now not a known cause of orthopedic foreign body infections. When assessing an alpha-hemolytic streptococcal pathogen with elevated MICs to third-generation cephalosporins, *G. sanguinis* should be considered. Microbiologists and clinicians alike should be alert to new, clinically-relevant findings as the increasing prevalence of advanced microbiologic identification techniques leads to better understanding of the role of uncommon pathogens in common diseases.

Author Disclosure Block:

A.O. Miller: None. **M.W. Henry:** None. **B. Abraham:** None. **S. Whittier:** None. **B.J. Hartman:** None. **K.F. Maloney:** None. **F. Wu:** None. **B.D. Brause:** None. **P. Della-Latta:** None. **T.J. Walsh:** None. **A.N. Schuetz:** None.

Poster Board Number:

MONDAY-386

Publishing Title:

Virulent Characteristics of Multidrug Resistant *E. coli* from Zaria, Nigeria

Author Block:

J. A. Onaolapo; Ahmadu Bello Univ., Zaria, Zaria, Nigeria

Abstract Body:

Most multidrug resistant *E. coli* isolates (resistant to more than 3 classes of antibiotics) exhibit co-virulent characteristics that contribute to mortality and morbidity as a result of resistance to commonly prescribed antibiotics in the clinics. This study evaluated phenotypically some virulent characteristics in *E. coli* that contribute to the expressed MDR properties of *E. coli* using standard microbiological methods. Eighty seven *E. coli* isolates were confirmed as *E. coli* from urinary tract infection and diarrhea patients in selected hospitals in Zaria Nigeria using Microgene identification kit, out of which 58.6% (51) were observed to be MDR. Significant number of the MDR isolates (70.6% (36)) were extended spectrum beta-lactamase producers, 45.1% (23) were resistant to cefoxitin and produce ampC. While further analysis on the isolates showed that 23.5% (12) were biofilm producers, 47.1% (24) were heteroresistant to cefoxitin while 5.9% (3) produced carbapenemase. This study showed that most MDR *E. coli* from UTI and diarrhea could exhibit more than one virulent characteristics. Hence, isolates with MDR should be subjected to various tests in other to validate the mechanisms of resistance. This will encourage better treatment options and good periodic surveillance in prescription and dispensing of antibiotics in clinical settings.

Author Disclosure Block:

J.A. Onaolapo: None.

Poster Board Number:

MONDAY-387

Publishing Title:

Impact of Outer Membrane Protein *ompc* and *ompf* on Antibiotics Resistance of *E. coli* Isolated from Uti and Diarrhea Patients in Zaria, Nigeria

Author Block:

J. C. Igwe; Natl. Biotechnology Developement Agency, Abuja, Nigeria, Abuja, Nigeria

Abstract Body:

Multidrug resistant (MDR) *E. coli* associated infections remains one of the most bacteria infections that have contributed significantly to increased morbidity and mortality in clinical settings. One of the known resistant mechanisms of MDR bacteria is reduced cell wall permeability, which is controlled by outer membrane protein OmpF and OmpC. This study evaluate the difference in molecular weight of outer membrane protein of MDR *E. coli* isolated from UTI and diarrhea patients in Zaria, Nigeria and antibiotic susceptible ATCC29522 strain using standard microbiological and molecular techniques. Eighty seven (87) confirmed *E. coli* isolates from UTI and diarrhea patients in selected hospitals in Zaria, Nigeria were evaluated for MDR using 15 antibiotics commonly prescribed for *E. coli* associated infections. The results showed that the 21 suspected multidrug isolates were 100% susceptible to Imipenem and Amikacin, and 28.6% susceptible to Nitrofurantoin but highly (100%) resistant to Amoxicillin, Ofloxacin, Ciprofloxacin, Cefpodoxime and Ceftaxime, 95.2% resistant to Cefpirome, 85.7% to Tetracycline and Sulphamethonidazole-Trimethroprim, 76.2% to Gentamicine, 66.7% to Chloramphenicol, 61.9% to Aztreonam and 57.1% to Ceftriaxone. Cell wall protein evaluation using SDS-PAGE showed that both the MDR isolates and susceptible strain had equal OmpC bands at 38kDa while the OmpF varied from one MDR isolate to another compared with the ATCC29522 used as control. This study contributes to other findings that a decrease in cell wall outer protein OmpF could contribute to high resistance to antibiotics.

Author Disclosure Block:

J.C. Igwe: None.

Poster Board Number:

MONDAY-388

Publishing Title:

Biofilm Formation among the Clinically Relevant *Pseudomonas aeroginosa*: An Evaluation of Two Different Screening Methods

Author Block:

S. Javed; Jinnah Univ. For Women, Karachi, Pakistan

Abstract Body:

Background: *Pseudomonas aeroginosa* is an opportunistic bacterial pathogen with an ability to produce biofilm. Biofilms are the matrices of microorganisms embedded in their own microbial originated extracellular polymeric substances (EPSs). Microorganisms growing in a biofilm are associated with various chronic infections in humans and they are highly resistant to antimicrobial agents and environmental stresses. **Aim:** The aim of the present study was to evaluate two methods for detection of biofilm development in *Ps. aeroginosa*. **Materials & Methods:** For the detection of biofilm formation, 112 clinical isolates of *Pseudomonas* spp. were screened by Tissue culture plate (TCP) and Tube method (TM) method. **Results:** Of the 112 *Pseudomonas* spp. 72(64.28%) displayed a biofilm-positive phenotype under the optimized conditions in the TCP method and strains were further classified as high 32 (28.57 %) and moderate 20 (17.8 %) while in 20 (17.8 %) isolates weak or no biofilm was detected. Though Tube Method associated well with the Tissue Culture Plate test for 20 (17.85 %) strongly bio-film producing strains. Although, weak producers were more difficult to distinguished from biofilm negative isolates. **Conclusion:** The TCP method was found to be most sensitive, precise and reproducible screening method for detection of bio-film formation by Pseudomonas and has the advantage of being a quantitative model to study the adherence of Pseudomonas on biomedical devices.

Author Disclosure Block:

S. Javed: None.

Poster Board Number:

MONDAY-389

Publishing Title:

The Role of Cornyeform-like Bacteria in Clinical Infections

Author Block:

S. M. Leal, Jr., P. Gilligan; Univ. of North Carolina, Chapel Hill, NC

Abstract Body:

Pleomorphic gram positive rods are part of the human skin microbiome. Often presumptively identified in clinical samples as diphtheroids using phenotypic characteristics, they frequently are dismissed as contaminants. The majority of diphtheroids belong to either the genus *Corynebacterium* or other coryneform-like genera. However, MALDI-TOF MS enables their speciation. In this study we performed a retrospective microbiological culture data review of patients from 2012-2015 growing gram positive rods with speciation by MALDI-TOF (98.2%) or 16S rRNA (1.8%) analysis. Our data set included 810 isolates from 15 genera constituting 45 bacterial species. The most frequent isolates were *Propionibacterium acnes* (35%), *C. striatum* (19%), and *Actinomyces neuii* (7%) followed by multiple *Corynebacterium* species. Site-specific laboratory criteria were utilized to determine true infection versus contaminant. Utilizing these criteria we show that 10% of all isolates met the criteria for true infection and 90% were contaminants. The percentage of isolates causing true infection were: anaerobic wounds (3%), aerobic wounds (2%), blood (11%), urine (41%), cerebrospinal fluid (31%), ophthalmologic cultures (20%), and sterile sites (33%). Speciation of these organisms revealed true infection with 23% of *C. kroppenstedtii* isolates from breast tissue, 56% of *C.striatum* isolates in the blood and 61% in the urine. Additionally, true infection was noted in 36% of *C. aurimucosum*, 75% of *C. urealyticum*, and 58% of *Actinomyces neuii* isolates from the urine. Lastly, 80% of *Propionibacterium acnes* isolates from CNS shunts were associated with true infection versus only 5% of isolates from lumbar punctures. Taken together these findings indicate that speciation of diphtheroids via MALDI-TOF is essential to accurately determine whether the isolate is a known or suspected pathogen versus contaminant and caution should be taken with presumptive identification of these organisms in routine laboratory practice.

Author Disclosure Block:

S.M. Leal: None. **P. Gilligan:** None.

Poster Board Number:

MONDAY-390

Publishing Title:

Group A Streptococcal (Gas) Carriage in Childhood Tic Disorder and Tourette'S

Author Block:

D. Patel[1], T. Hedderly[2], M. Woods[2], D. Martino[3], T. Murphy[4], I. Heyman[4], A. Schrag[5], G. Kapatai[1], A. Efstratiou[1]; [1]Publ. Hlth.England, London, United Kingdom, [2]Evelina London Children's Hosp., London, United Kingdom, [3]King's Coll. Hosp., London, United Kingdom, [4]Great Ormond Street Hosp., London, United Kingdom, [5]Univ. Coll. London, London, United Kingdom

Abstract Body:

Paediatric Acute-onset Neuropsychiatric Syndrome (PANS) includes acute onset OCD with the potential association of tics with clinical evidence of an infection, often GAS. However, the role of GAS in mediating symptoms is unknown. Our study investigated bacterial genetic factors which may play a role in mechanisms that create vulnerability for tic development through complex host-pathogen interactions and may aid development of targeted therapeutic interventions.Pan-European GAS strains were isolated as part of the European Multicentre Tics in Children Studies. Children with tic disorders were recruited to the COURSE cohort (ages 3-16) assessing symptom fluctuations and unaffected siblings were recruited to the ONSET cohort (ages 3-10) monitoring onset of tics. Whole-genome sequencing (WGS) was performed using an illumina HiSeq platform. In-house bioinformatics pipelines were used for mapping and determining multilocus sequence type (MLST) and *emm* type. Multiple alignments were performed on isolates between cohorts and within a cohort between children with and without an onset or exacerbation of tics.A GAS positivity of 31% was observed among all 42 ONSET children and among those with an onset of tics. Similarly a 12% GAS positivity was observed among the 63 COURSE children and among those with an exacerbation of tics. GAS isolates from ONSET or COURSE children were genetically similar; 7/8 *emm* types and 9/10 MLST types were found in both cohorts. Manual sequence variant analysis identified no variants exclusive to isolates from either cohort or children who experienced an onset or exacerbation of tics.A higher rate of GAS carriage was observed among the unaffected siblings than the tic-affected children which may be due to age or other unexplored factors. No genetic difference was observed between isolates from each cohort and between children with and without an onset or exacerbation of tics. Therefore the role of GAS in PANS remains elusive and requires in-depth exploration. Further investigation of WGS data is underway which may identify specific genes or virulence markers associated with tic disorders.

Author Disclosure Block:

D. Patel: None. **T. Hedderly:** None. **M. Woods:** None. **D. Martino:** None. **T. Murphy:** None. **I. Heyman:** None. **A. Schrag:** None. **G. Kapatai:** None. **A. Efstratiou:** None.

Poster Board Number:

MONDAY-391

Publishing Title:

The Genus *Eikenella* is Heterogenic, Based on Whole Genome Sequencing Analysis

Author Block:

A-M. Bernier[1], T. Burtz[2], D. Wiebe[2], V. Laminman[2], K. Bernard[2]; [1]Université de Saint-Boniface, Winnipeg, MB, Canada, [2]Natl. Microbiol. Lab., Winnipeg, MB, Canada

Abstract Body:

Background: The genus *Eikenella* has a single species, *E. corrodens;* this rare human pathogen is a facultatively anaerobic Gram-negative rod and member of the HACEK group. Eight strains consistent with *E. corrodens* biochemically and with >99% identity by 16S rRNA gene sequencing [16S] were compared with 13 isolates from sterile body fluids/sites closest to but discernable from (97.0%-98.9% by 16S) *E. corrodens.* All 21 *E. corrodens* or *Eikenella*-like isolates were studied by whole genome sequencing (WGS) to see if the 13 strains represented 1 or more new *Eikenella* species or unusual *E. corrodens* biotypes.**Methods:** 8 *E. corrodens* and 13 isolates close to but distinct from the *E.corrodens* TS were studied using standard biochemical and cellular fatty acid (CFA) methods. Comparative whole genome sequencing was performed in paired-end runs using the MiSeq Sequencer (Illumina1.9, 2x 300 cycles). Genomes were assembled using Spades (version 3.5.1) and JSpecies was used to calculate Average Nucleotide Identity values (ANIb and ANIm) and correlate indexes of tetra-nucleotide signatures (TETRA values).**Results:** ANib and ANIm values > 95% identified 4 distinct groups, with G+C values ranging from 55.5 to 58.5% and genome lengths of 1.8 to 2.5 Mb. The 8 isolates closest by 16S to the *E. corrodens* TS grew best in 5% CO2 and were consistent with that species biochemically, by CFAs and by WGS. The 13 other isolates had enhanced growth under strictly anaerobic conditions, grew poorly or not at all in air or 5% CO2, and were found by WGS to fall into 3 distinct groups/possible novel species. The 1st consisted of 10 strains, the 2nd was 2 strains/1 patient (130454/130455) and the 3rd a single isolate (02A017).**Conclusions:** 1) the genus *Eikenella* is heterogenic and additional aerotolerant or anaerobic species may exist; 16S relationships were a reasonable predictor for WGS comparisons. 2) ANIb values identified three new taxonomic groups related to but distinct from, *E. corrodens*.3) The 3 new *Eikenella* taxon groups had a range of G+C mol% of 55.5 to 58.5% and genome lengths from 1.8 to 2.54 Mb.

Author Disclosure Block:

A. Bernier: None. **T. Burtz:** None. **D. Wiebe:** None. **V. Laminman:** None. **K. Bernard:** None.

Poster Board Number:

MONDAY-392

Publishing Title:

Diphtheria: Forgotten But Not Gone

Author Block:

P. K. Cassiday[1], T. Tiwari[1], H. M. Soeters[1], M. J. Laraque[2], M. D. J. Baptiste[2], E. Rossignol[2], J. Buteau[2], J. Boncy[2], K. Bowden[1], M. Williams[1], A. Pruitt[1], R. Patel[3], C. Salomon[2], J. Francois[2], P. Adrien[2], S. Martin[1], M. L. Tondella[1]; [1]CDC, Atlanta, GA, [2]Haiti Ministere de la Sante Publique et Population, Port-au-Prince, Haiti, [3]CDC, Port-au-Prince, Haiti

Abstract Body:

Respiratory diphtheria is a vaccine preventable disease that is caused by toxigenic *Corynebacterium diphtheriae*. Haiti, a country with childhood DTP3 coverage <80% and endemic for diphtheria, experienced an increase in reported cases starting in December 2014. Between December 24, 2014 and December 11, 2015, 66 throat swabs and three isolates from 80 patients (42 % males; median age, 7 years (range: 1-50 years); 85% without documented DTP vaccination history with ≥1 dose; and from 7 of 10 departments) were tested at the Centers for Disease Control and Prevention. All 66 swabs were tested by real-time PCR for the presence of the A and B subunits of the diphtheria toxin gene (*tox*); 60 (91%) swabs were cultured on Tinsdale and sheep blood agars. Isolates were further tested for toxin production using the modified Elek assay and were genotyped using multi-locus sequence typing (MLST). Cases were considered confirmed upon recovery of toxigenic *C. diphtheriae*, and probable if culture was negative but had diphtheria-like symptoms and were PCR-positive for *tox*. In total, 34 swabs and all 3 isolates (54%) were PCR-positive for *tox*; 17 *tox*-positive swabs and the 3 isolates (29%) were culture-positive for *C. diphtheriae* biovar *mitis*. All 20 isolates produced toxin. Therefore, 20 (29%) of cases tested were confirmed and an additional 17 (25%) were classified as probable. By MLST, 4 of 20 isolates were sequence type (ST) 31, a ST seen in prior outbreak strains from Haiti and Dominican Republic dating back to 2004. Overall case fatality was 29%. Continued surveillance and testing are needed to inform vaccination policy to prevent outbreaks of this forgotten disease.

Author Disclosure Block:

P.K. Cassiday: None. **T. Tiwari:** None. **H.M. Soeters:** None. **M.J. Laraque:** None. **M.D.J. Baptiste:** None. **E. Rossignol:** None. **J. Buteau:** None. **J. Boncy:** None. **K. Bowden:** None. **M. Williams:** None. **A. Pruitt:** None. **R. Patel:** None. **C. Salomon:** None. **J. Francois:** None. **P. Adrien:** None. **S. Martin:** None. **M.L. Tondella:** None.

Poster Board Number:

MONDAY-393

Publishing Title:

Plant Natural Products Potentiate Antimicrobial Activity of Antibiotics Against *Pseudomonas aeruginosa*

Author Block:

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Abstract Body:

The problem associated with the use of antibiotics is the development of antibiotic resistant strains of bacteria. One way of reducing the development of antibiotic resistance is to reduce the amount of antibiotics used for treating infectious diseases. In other words, can we enhance the potency of antibiotics? In the traditional medicine a number of plant products have been used to treat bacterial infections. A study was conducted to test if natural compounds that occur in food and in traditional medicine enhance the activity of antibiotics. The antimicrobial effect of four natural compounds; caffeine, quercitin, curcumin and piperine were tested against *Pseudomonas aeruginosa* PA14. Curcumin and piperine exhibited antimicrobial effect at a low concentration of 25µg/ml. The antimicrobial effect of combination of antibiotics tetracycline and streptomycin at MIC_{25} and MIC_{50} and curcumin and piperine were tested. Both the compounds significantly enhanced the potency of antibiotics. Curcumin showed the most promising effect. Since curcumin showed a high synergetic activity with antibiotic, possible mechanism(s) by which curcumin caused this effect was investigated. Curcumin significantly reduced the *P. aeruginosa* ability to form biofilm. Lower concentration of tetracycline increased the expression of genes that play a role in biofilm formation, quorum sensing, and virulence suggesting that this may be one of the mechanisms bacteria become resistant to antibiotics. When lower concentration of tetracycline was mixed with curcumin the expression of quorum sensing genes were significantly reduced. The combination of lower concentrations of tetracycline (MIC_{25} and $MIC_{50)}$ and curcumin was used in the *P. aeruginosa* - *C. elegans* pathosystem model to test its effect on infection. The combination increased worm's survival against the bacterial infection. Curcumin (12 and 24µg/ml) when combined with tetracycline (MIC_{50}) significantly reduced the mortality of the worms. These treatments were much more effective than full strength tetracycline. In conclusion, curcumin showed synergistic effect with antibiotics and improved efficacy of antibiotics against *P. aeruginosa*.

Author Disclosure Block:

H. Prithiviraj: None. **G. Kulshreshtha:** None. **B. Prithiviraj:** None.

Poster Board Number:

MONDAY-394

Publishing Title:

Antibacterial Activity of Pomegranate Wastes to Control the Food Borne Pathogens

Author Block:

U. Farooq; Univ. of Sargodha, Sargodha, Pakistan

Abstract Body:

Food born infectious diseases due to pathogenic microorganism is a global issue in the current scenario. Among these diseases, the infections caused by *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus,* and *Enterococcus feacalis* are most prevalent in the societies. In such alarming situations, the scientists have focused their attentions to control these pathogens through natural ways as the use of antibiotics lead towards many complications. The present study was planned to explore natural and stronger mode of treatment against food borne infectious diseases, a comparative antibacterial study was conducted between aqueous extracts of pomegranate (*Punica granatum*) peel and seeds against above mentioned food-borne pathogens.High yield of aqueous extracts of pomegranate peel and seed was obtained by optimization of extraction conditions (temperature and time) using response surface methodology. The optimization study was based on highest yield of extracts. Antibacterial potentials of the extracts (pomegranate peel and seed) were examined by using disc inhibition zone technique and the results were also compared with commercial antibiotic (Ciprofloxacine).The studied extracts possessed strong antibacterial activity against the tested microorganisms. But the maximum antibacterial activity was shown by peel extract as compared to seed extract. The strong antibacterial activity of peel was shown against *Escherichia coli* with average zone of inhibition 19.5 ± 0.93mm followed by seeds 7.12 ± 0.99mm. The comparison with commercial antibiotic showed that the antibacterial activities of peel extract were comparable with commercial antibiotic (21.12 ± 0.23mm) as compared to seed extract. Results also showed that among all the pathogens *Enterococcus feacalis* was appeared to be the most resistant with inhibition induced by the extracts of peel 14.87 ± 0.13mm and seeds 7.12 ± 0.19mm.It may be concluded from the results that the pomegranate peel proved to be a stronger antibacterial agent as compared to pomegranate seed and also parallel to commercial antibiotic.Keywords: Pomegranate, antibacterial activity

Author Disclosure Block:

U. Farooq: None.

Poster Board Number:

MONDAY-395

Publishing Title:

Lipophilic Green Tea Polyphenols as Potential Inhibitor for Endospore Germination in *Bacillus* spp

Author Block:

B. Ali[1], L. H. Lee[1], T-C. Chu[2]; [1]Montclair State Univ., Montclair, NJ, [2]Seton Hall Univ., South Orange, NJ

Abstract Body:

Background: *Bacilli* possess the ability to form endospores when placed under unfavorable conditions. Due to their highly resistant characteristics, endospores allow the bacteria to survive for extended periods that create high concern in the food and medical industries. The preliminary study suggested that the tea polyphenols can inhibit endospore germination in *Bacillus* spp. *B. cereus* is one of the most prevalent bacterial species found in contaminating food and beverages. In this study, a time-kill experiment was carried out to determine the minimal treatment time resulting in 95-100% of germination inhibition. In addition, the antibacterial and antispore effects of the tea polyphenol in food and milk were determined. **Methods:** For the time kill-study; *B. cereus* endospores were treated with modified crude lipophilic green tea polyphenols (cLTP) and purified lipophilic green tea (pLTP) at 1% and 5% concentrations for 5, 10, 15, and 30 minutes, respectively. Colony-forming unit (CFU) was recorded after incubated at 37°C for 24 hours and the percent of inhibition was determined. The same methods were used for the food and milk study and were only treated with 5% pLTP for 60 minutes and 24 hours. Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) were used to study the internal and external structure of spores. **Results:** The time-kill result indicated that the minimal kill time was 15 minutes for both tea polyphenols. The average inhibition rate for the 15-minute treatment time was 98.7% for 1% cLTP, 99.6% for 1% pLTP, 99.9% for 5% cLTP and 100% for 5% pLTP, respectively. The EM results indicated that the endospore coat was significantly destructed after treatment. **Conclusion:** These results suggested that cLTP and pLTP play a vital role in inhibiting endospore germination. Therefore, these natural compounds may aid in preventing food and beverage spoilage caused by spore-forming bacteria.

Author Disclosure Block:

B. Ali: None. **L.H. Lee:** None. **T. Chu:** None.

Poster Board Number:

MONDAY-396

Publishing Title:

Inhibition of *Streptococcus mutans* Biofilm Formation by Propolis, Green Tea, and Miswak

Author Block:

J. E. Callahan, J. Bryan, N. Molineros, M. Salmon; Saint Peter's Univ., Jersey City, NJ

Abstract Body:

Background: Bacteria growing in biofilms demonstrate higher resistance to antimicrobial agents than their planktonic counterparts. *Streptococcus mutans* is among the earliest colonizers existing on the tooth surface biofilm known as dental plaque. Within this community, *S. mutans* functions by lowering the oral pH, leading to erosion of tooth enamel and resulting in dental caries. Previous studies in various cultures have demonstrated the antibacterial nature of natural plant-based remedies including propolis, green tea *(Camellia sinensis)*, and miswak *(Salvadora persica)*. We hypothesized a similar inhibitory effect on *S. mutans* and evaluated the role of these agents on its biofilm formation. **Methods:** Developing *S. mutans* biofilms were grown under microaerophilic conditions in a sucrose based-minimal media, using 24-well polystyrene plates. Biofilms were exposed to various concentrations of Propolis (0-20% v/v), green tea (0-20% v/v), and miswak (0-20% w/v). Following incubation, wells were examined for biofilm formation, using a standard crystal violet assay. Each condition was incubated in triplicate and repeated a minimum of 3 times. **Results:** Biofilms treated with propolis exhibited decreased biofilm mass (-0.33-fold, $P<0.0001$) when added at 20% w/v and (-0.50-fold, $P<0.0001$) when added at 30% as compared to the control. Treatment with green tea exhibited a (-0.95-fold $P<0.0001$) change at all values added (5-20% v/v) relative to the control. Addition of miswak to the developing biofilm demonstrated a (-0.95-fold $P<0.0001$) change in biofilm mass when added at concentrations (5-20% v/v). **Conclusions:** Our findings support the inhibition of cariogenic *S. mutans* biofilms by propolis, green tea and miswak. These easily accessible and natural plant-based remedies may provide necessary caries biofilm infection control in many areas of the world.

Author Disclosure Block:

J.E. Callahan: None. **J. Bryan:** None. **N. Molineros:** None. **M. Salmon:** None.

Poster Board Number:

MONDAY-397

Publishing Title:

Inhibition of Growth and Biofilm Formation of *Streptococcus pyogenes* by Herbal Extracts

Author Block:

N. M. Wijesundara[1], R. Davidson[2], H. P. V. Rupasinghe[1]; [1]Dalhousie Univ., Truro, NS, Canada, [2]Dalhousie Univ., Halifax, NS, Canada

Abstract Body:

Background: Streptococcal pharyngitis caused by *Streptococcus pyogenes*, remains an important public health concern. A renewed interest in alternative herbal medicines, particularly as potential antimicrobial agents, has become increasingly important given the lack of new drug development. **Methods:** In this study we evaluated the antibacterial and anti-biofilm formation activities of 32 phytochemical extracts of 12 selected Canadian medicinal plants against three strains of *S. pyogenes* (ATCC 19615, ATCC 49399) and a clinical isolate using broth dilution assay, kill-curve assay, anti-biofilm formation assay and scanning electron microscopy (SEM).**Results:** Ethanol extracts (EE) demonstrated a range of minimum inhibitory concentrations (MICs) between 62.5 and 1000 g/mL while minimum bactericidal concentrations (MBCs) ranged from 125-1000 µg/mL. Among aqueous extracts (AE), licorice root, oregano flowering shoots and thyme flowering shoots were the most active, with MICs ranging from 1560 to 12,500 g/mL. The MBC was 6250 g/mL. Essential oils (EO) prepared from oregano flowering shoot and sage leaves showed significantly lower MIC and MBC values, ranging from 250-500 g/mL and 500 g/mL, respectively. The comparator penicillin G remained the most active compound against planktonic *S. pyogenes* with a MIC and MBC of 0.0078 and 0.0156 µg/mL, respectively. Oregano EO was demonstrated rapid killing kinetics against *S. pyogenes* (5 min of exposure) whereas sage EE, sage EO, echinacea flower EE and licorice root EE were also eliminated of the initial bacterial inoculum after 3-4 hr of exposure. However, penicillin required 24 hours to achieve the same degree of kill. Anti-biofilm formation and SEM analyses of biofilms revealed that the minimum biofilm inhibitory concentrations (MBICs) of the extracts ranged from 31.5 to 6250 µg/mL.**Conclusions:** Overall, EO from oregano flowering shoots and sage leaves as well as EE of licorice root, echinacea flower and sage leaves demonstrated promise as potential new alternate agents in the management of streptococcal pharyngitis.

Author Disclosure Block:

N.M. Wijesundara: None. **R. Davidson:** None. **H.P.V. Rupasinghe:** None.

Poster Board Number:

MONDAY-398

Publishing Title:

Antibacterial and Anti-Hemolytic Effect of Proteins and Polyphenolic Compounds of Honey from *Melipona beecheii* against *Enterohemorrhagic e. coli*

Author Block:

L. Pool Yam, J. Ramon Sierra, J. Ruiz Ruiz, D. Magaña Ortiz, E. Ortiz Vazquez; Inst. Tecnológico de Mérida, Merida, Mexico

Abstract Body:

Background: Current studies, based on ethnographic knowledge, have reported that honey from *Melipona beecheii*, a stingless bee from Yucatan, has antibacterial activity against several pathogens including *E.coli* strains. At this respect, foodborne diseases are a worldwide problem and *E. coli* O157:H7 is the main serotype associated with outbreaks of hemorrhagic colitis and hemolytic uremic syndrome. This bacterium has different mechanisms of infection that are involved in the colonization of digestive tract cells. The main objective of this study was to evaluate the antibacterial and anti-hemolytic effect of proteins and polyphenolic compounds from honey of *Melipona beecheii* against *E. coli* O157: H7**Methods:** The proteins (P) were extracted using phosphate buffer and polyphenolic compounds (PC) were extracted using Amberlite X-2. The antimicrobial activity and minimum inhibitory concentration of *M. beecheii* honey, P and PC samples were determined by the disk diffusion and micro-dilutions methods. Strains *E. coli* 25922 and *E. coli* O157:H7 were used. Hemolytic activity of bacteria was evaluated employing red blood cell. The virulence genes of *E.coli* O157:H7 were detected by PCR. **Results:** *M. beecheii* honey, P and PC samples inhibited the growth of both *E.coli* strains. The MICs of honey, P and PC against for *E. coli* 25922 were 19% (v/v), 70 µg/mL, and 160 µg/mL, respectively. The values for *E. coli* O157:H7 were 17% (v/v), 55 µg/mL and 180 µg/mL. Remarkably, the hemolytic activity of the O157: H7 was 100%, but in the presence of honey this decreases to 5.7%. This phenomenon was also observed in *E. coli* 25922, in this case the hemolysis was reduced from 2.1% to 0.99%. Virulence genes were detected using PCR standard protocols to confirm that the hemorrhagic strain was employed in experiments. **Conclusions:** *Melipona beecheii* honey, protein extract and polyphenolic compounds extracts inhibit the growth of both *E. coli* strains. Honey decreased hemolytic activity of *E. coli* O157:H7, possibly proteins and polyphenols in honey inactivated the hemolysin and thereby protected blood cells. Further investigation is needed for the isolation and structure elucidation of bioactive proteins and polyphenols in *M. beecheii* honey.

Author Disclosure Block:

L. Pool Yam: None. **J. Ramon Sierra:** None. **J. Ruiz Ruiz:** None. **D. Magaña Ortiz:** None. **E. Ortiz Vazquez:** None.

Poster Board Number:

MONDAY-399

Publishing Title:

Efficacy of Trans-cinnamaldehyde and Eugenol in Reducing *A. baumannii* Adhesion to and Invasion of Human Keratinocytes and Controlling Wound Infections *In Vitro*

Author Block:

D. P. Karumathil, **M. Surendran Nair**, K. Venkitanarayanan; Univ. of Connecticut, Storrs, CT

Abstract Body:

Background: *Acinetobacter baumannii* is a multi-drug resistant, nosocomial pathogen causing a variety of disease conditions, especially wound infections in humans. After *A. baumannii* outbreaks in military combat personnel in Iraq and Afghanistan, reports of *A. baumannii* wound infections are increasingly recognized. *A. baumannii*'s ability to form biofilms and colonize epithelial cells potentially makes it difficult to treat skin and soft-tissue infections of this pathogen. Thus, in light of the multidrug resistance and biofilm producing capacity, new strategies for controlling *A. baumannii* wound infections are necessary.**Methods:** This study investigated the efficacy of two natural, plant-derived antimicrobials (PDAs), namely trans-cinnamaldehyde (TC) and eugenol (EG) for decreasing *A. baumannii* adhesion to and invasion of human keratinocytes (HEK001). Moreover, the efficacy of two PDAs for inhibiting *A. baumannii* biofilm formation was determined using an *in vitro* collagen matrix wound model. In addition, the effect of TC and EG on *A. baumannii* biofilm architecture was visualized using confocal scanning microscopy. Further the effect of both PDAs on *A. baumannii* genes critical for biofilm synthesis was determined using real-time quantitative PCR (RT-qPCR).**Results:** Both TC and EG significantly reduced *A. baumannii* adhesion to HEK001 by ~2 to 2.5 $\log_{10}$ CFU/ml ($P < 0.05$), and invasion by ~2 to 3 log CFU/ml, compared to the controls ($P < 0.05$). Further, after 24 and 48 h, TC inhibited biofilm formation by ~1.5 and ~2 to 3.5 $\log_{10}$ CFU/ml, while EG decreased biofilm-associated bacteria by ~2 and ~3.5 $\log_{10}$ CFU/ml, respectively, compared to controls ($P < 0.05$). Confocal microscopy revealed that TC and EG resulted in the death of biofilm-associated *A. baumannii*, and disrupted the biofilm architecture. RT-qPCR results indicated that the two phytochemicals significantly down-regulated the transcription of genes associated with *A. baumannii* biofilm production. The results suggest that both TC and EG could potentially be used to treat *A. baumannii* wound infections; however, their efficacy in *in vivo* models needs to be validated.

Author Disclosure Block:

D.P. Karumathil: None. **M. Surendran Nair:** None. **K. Venkitanarayanan:** None.

Poster Board Number:

MONDAY-400

Publishing Title:

Reciprocal Cooperation of Phytochemicals against *Borrelia burgdorferi* and *Borrelia garinii*

Author Block:

A. Goc, A. Niedzwiecki, M. Rath; Dr. Rath Res. Inst. BV, Santa Clara, CA

Abstract Body:

Background: Lyme borreliosis is a tick-borne disease caused by the spirochete *Borrelia burgdorferi* sensu lato. Throughout nature, phytochemicals represent a growing theme in antimicrobial defense, however, little is known about their anti-borrelaea reciprocal cooperation. To better understand this subject, we studied the efficacy of selected phytochemicals such as flavones and fatty acids to ascertain the type of their related cooperation against *Borrelia sp*. **Methods:** Phytochemicals such as baicalein, luteolin, 10-HAD, and glycerol monolaurate were tested in different "two-agent" combinations for their *in vitro* effectiveness against vegetative (spirochetes) and dormant (rounded bodies, biofilm) forms of *Borrelia burgdorferi* and *Borrelia garini,* using dark field and fluorescence microscope. Antibacterial effects of these agents' cooperation were evaluated at their MIC values and three dilutions above (2-6 X MIC) as well as three dilutions below their MICs (1/2-1/8 X MIC), according to checkerboard assays, and defined by fractional inhibitory concentration index. Additionally, a Student t-test statistical analysis was performed for further validation. All experiments were performed in triplicates. **Results:** The results showed that the combination of baicalein with luteolin exhibited synergistic anti-spirochetal as well as additive effects against rounded forms and biofilm of both studied *Borrelia sp,* whereas their respective cooperation with tested fatty acids showed to be additive against spirochetes and indifferent against other morphological forms. Moreover, additive anti-spirochetal and anti-biofilm effects were observed when monolaurin was used in combination with 10-HAD. Antagonism was not observed in any of the cases. **Conclusions:** The data obtained from this *in vitro* study revealed the intrinsic anti-borreliaea activity of tested combinations of flavones and fatty acids, respectively. Therefore, they might represent valuable adjuvants for antimicrobial chemotherapy.

Author Disclosure Block:

A. Goc: None.

Poster Board Number:

MONDAY-401

Publishing Title:

Characterizing the Antibacterial Properties and Transcriptional Alterations Induced by Lemongrass Oil in *Staphylococcus aureus*

Author Block:

C. J. Christensen, K. L. Anderson; Concordia Univ., Ann Arbor, MI

Abstract Body:

Essential oils have risen in popularity as "all natural" alternatives used to treat a myriad of conditions. However, the precise mechanisms by which essential oils are effective are not well understood. To begin to elucidate the antibacterial properties of essential oils, we tested the effectiveness of Lemongrass Oil (LGO), Tea Tree Oil (TTO), and Willow Bark Extract (WBE) against *Staphylococcus aureus* growth. To do so, a Methicillin-Resistant strain of *Staphylococcus aureus* (USA300) was exposed to each oil using Kirby-Bauer disk diffusion assays. Of the oils, LGO had the greatest zone of inhibition. Thus, further studies were conducted only with LGO and its chemical component Citral. The Minimum Inhibitory Concentration (MIC) of both LGO and Citral was determined in macro-broth cultures; exposure to increased concentrations of each resulted in dramatic cell death as determined by kill curve assays. To begin to determine the molecular mechanisms underlying the observed antibacterial effects, we exposed cells to a sub-inhibitory concentration Citral for 30 min and hybridized the RNA to Affymetrix GeneChips® in order to identify any transcriptional changes that occurred. Results indicated that 8 and 9 transcripts were up- or down-regulated (at least 2-fold), respectively, in Citral-treated cells when compared to mock-treated samples. These transcripts represent virulence factors, hypothetical proteins, and intergenic regions. Current studies are designed to elucidate the function that the affected intergenic regions may play in gene regulation. Taken together, these results demonstrate that LGO exhibits antibacterial properties against a highly pathogenic bacterial species that is exceedingly resistant to the currently available antibiotics. The underlying transcriptional changes induced by Lemongrass Oil are under further examination.

Author Disclosure Block:

C.J. Christensen: None. **K.L. Anderson:** None.

Poster Board Number:

MONDAY-403

Publishing Title:

Anti-halitosis Effects of *Amomum compactum* Essential Oil Against *Actinomyces viscosus*

Author Block:

Y. Yanti, M. Tjakra, B. W. Lay; Atma Jaya Catholic Univ., Jakarta, Indonesia

Abstract Body:

Background: Halitosis (oral malodour) has been a problem for many years. About 20-60% of the population has a chronic halitosis on regular basis. The microbial activities are the main cause of oral disease. The activities from the microbes also cause halitosis through the accumulation of volatile sulphur compounds (VSCs), diamines, and short-chain fatty acids. *Amomum compactum* (Java cardamom) is one of the tropical spices that also known as "the Queen of spices" because of its unique taste and aroma. In India, *A. compactum* has been traditionally used to fight bad breath naturally. This study was aimed to test the activity of essential oil from *A. compactum* toward *Actinomyces viscosus* by using VSCs, pH-stat, and biofilm assays.**Methods:** Several steps had been conducted, including essential oil extraction from *A. compactum*, quantitative analysis of essential oil inhibition effects toward pH-stat, quantitative analysis of essential oil inhibition effects toward *A. viscosus* biofilm prevention and eradication, qualitative analysis of VSCs producing oral bacteria and also essential oil inhibition effects toward VSCs.**Results:** For acid production system, *A. compactum* essential oil showed inhibition activity to acid production of *A. viscosus* by pH-stat assay. *A. viscosus* was able to produce acid rapidly in 20 minutes, resulting in pH 5.44 from neutral pH (7.00). At 100 µg/mL, *A. compactum* essential oil exerted the highest inhibition of acid production. Regarding its antibiofilm effect, *A. compactum* essential oil demonstrated both preventive and eradication effects against *A. viscosus* biofilm formation and the established biofilm compared to that of ampicillin standard. At 40 µg/mL, essential oil removed up to 50% of the established biofilm. In contrast, essential oil at higher concentration (80 µg/mL) prevented ~50% of biofilm growth. In terms of VSCs results, *A. compactum* essential oil showed similar effect with ampicillin on inhibiting VSCs production from *A. viscosus*.**Conclusions:** *A. compactum* essential oil exerted anti-halitosis activity toward *A. viscosus* by preventing and eradicating oral biofilm, inhibiting acid formation, and reducing VSCs production *in vitro*.

Author Disclosure Block:

Y. Yanti: None. **M. Tjakra:** None. **B.W. Lay:** None.

Poster Board Number:

MONDAY-404

Publishing Title:

Targeting Common Staphylococci Species in Prosthetic Joint Infections with Tea Polyphenol

Author Block:

C. Chen; Montclair State Univeristy, Egg Harbor Township, NJ

Abstract Body:

Background: Normal human microbial flora such as *Staphylococcus aureus (S. aureus)* and *Staphylococcus epidermidis (S. epidermidis)* may enter the system through wounds or surgical implants. Both species are biofilm forming and the most problematic pathogens in prosthetic joint infections (PJI). Studying the effects of different tea polyphenols on Gram-positive staphylococci species may provide a novel approach in inhibiting biofilm accumulation in prosthetic joint area.**Methods:** Crude lipophilic tea polyphenols (cLTP) and purified lipophilic tea polyphenols (pLTP) at different concentrations (200 ug/ml, 250ug/ml and 500ug/ml) were used to study their effects on biofilm inhibition. Congo red agar (CRA) method was developed to analyze biofilm inhibition qualitatively, in the presence of tea polyphenols. Crystal violet (CV) assays were used to quantitatively analyzed percent of biofilm inhibition in relation to the controls. Scanning electron microscopy (SEM) was used to observe biofilm and morphological changes of cells treated with cLTP and pLTP. Fluorescent microscopy was used to study cell viability. Colony forming units (CFU) was carried out to analyze overall population density and growth rate. **Results:** CRA analysis suggested biofilm formation was repressed in the presence of cLTP/pLTP, at concentrations of 250 ug/ml and 500 ug/ml in both Staphylococci species. CV results indicated that significant reduction with 97.4% biofilm inhibition at 250 ug/ml and 73.2% at 500 ug/ml for *S. aureus and S. epidermidis*, respectively. Fluorescent microscopy revealed population density and cell viability was significantly reduced for both species. SEM also showed population and biofilm reduction at 250ug/ml of pLTP. CFU test with 250 ug/ml of pLTP recorded significant inhibition of *S. aureus and S. epidermidis* growth with 100% and 99.7% inhibition respectively.**Conclusion:** These results suggested that pLTP might be potent in treating prosthetic joint infection as a novel alternative treatment to antibiotics. Studies showed that there was no recurring growth or biofilm formation after several days, thus suggested that pLTP could be a potential solution to reduce chronic infections in patients.

Author Disclosure Block:

C. Chen: None.

Poster Board Number:

MONDAY-405

Publishing Title:

Minimun Inhibitory Concentration of Proteolytic Fraction from Royal Jelly against *E. coli, s. aureus* and *V. cholerae*

Author Block:

E. Lopez Baños[1], D. Magaña Ortiz[2], J. Ruiz Ruiz[1], J. Ramon Sierra[1], **E. Ortiz-Vázquez**[1]; [1]Inst. Tecnológico de Mérida, Mérida, Yucatán, Mexico, [2]Inst. Tecnológico de Mérida, Mérida, Mexico

Abstract Body:

Background: Royal Jelly is a honeybee secretion that is used in the nutrition of larvae and adult queens; this food is composed of 12.5 % protein, 67-5% water, 11% carbohydrates and 5% lipids. Several reports have validated the relationship between continuous consumption of royal jelly and health, mainly attributed to its multiple biological activities.. The aim of this study was to determine the antibacterial potential of the proteolytic fraction of royal jelly and to evaluate its action mechanism. **Methods:** Royal jelly samples were collected directly from hives in the state of Yucatan, Mexico. Strains used were *E. coli* ATCC 25922, *S. aureus* ATCC 25923 and *V. cholerae*. The protein extraction was performed according to Schmitzová (1998). Protein extract was purified by size-exclusion chromatography (Sepharose-G200). The antibacterial effect of protein fractions was tested by disk diffusion method (Schwalbe et al., 2007). MICs were determined using CLSI Microdilution method. Proteins were analyzed by SDS-PAGE (Bizani et al., 2005). Proteolytic activity was determined using substrate-gel electrophoresis (Abreu-Payrol et al., 2005). Growth inhibition kinetics was performed according to Schneider et al. (2010).**Results:** Three peaks were obtained by size-exclusion chromatography from the royal jelly protein extract peak 1 and 2 had protein content. The peak 2 (RJP2) showed proteolytic activity and antimicrobial activity against the strains tested, with inhibition diameters of 1.10 cm (*E. coli*), 1.5 (*S. aureus*), 1.9 cm (*V. cholerae*). The SDS-PAGE gel of the RJP2 showed seven different bands from 10 to 72.8 kDa. The MICs of Royal Jelly, Protein extract and RJP2 were 212, 9.2 and 0.136 mg/mL for *E. coli*, 87, 1.4 and 0.086 mg/mL for *S. aureus* and 62, 4 and 0.091 mg/mL for *V. cholerae***Conclusions:** The proteolytic fraction of the Royal jelly is the main group of proteins that confer the antibacterial activity against gram positive and gram negative strains. The low values of MIC of the proteolytic fraction suggest the high potential of this kind of proteins obtained from Royal Jelly.

Author Disclosure Block:

E. Lopez Baños: None. **D. Magaña Ortiz:** None. **J. Ruiz Ruiz:** None. **J. Ramon Sierra:** None. **E. Ortiz-Vázquez:** None.

Poster Board Number:

MONDAY-406

Publishing Title:

Plasmid Profile of Vancomycin-Resistant *Staphylococcus aureus* and Their Antibacterial Susceptibility to Extracts of *Vernonia amygdalina*

Author Block:

J. O. Oluyege, D. Oguntoye; Ekiti State Univ., Ado-Ekiti, Nigeria

Abstract Body:

Susceptibility of vancomycin resistant Staphylococcus aureus (VRSA) strains isolated from clinical and environmental sources to the extracts of *Vernonia amygdalina*, a Nigerian medicinal plant and to conventional antibiotics was investigated. Three hundred (300) strains of *Staphylococcus aureus* were isolated from clinical and environmental sources out of which 20 vancomycin-resistant strains were obtained. The clinical/enviromental isolates exhibited antibiotic resistance patterns in the following order: ofloxacin (0%/0.7%), gentamicin (0.7%/0%), vancomycin (10.7%/8.0%), erythromycin (60.7%/48.7%), ceftriaxone (68%/60.7%), cefuroxime (91.3%/90.7%), ceftazidim (93.3%/93.3%), cloxacillin (100%/100%) and augmentin (100%/96.7%). The VRSA isolates from clinical and the environmental sources harbored 1-3 plasmid-DNAs with molecular sizes ranging from 250-2500 kilobase pairs. The zone of growth inhibition exhibited by the extracts of *Vernonia amygdalina* ranged from 10mm to 20mm at concentrations of 20-40mg/ml. It is noteworthy that community acquired VRSA can also be found outside clinical environments while gentamicin and ofloxacin could be effective for the treatment of VRSA infections. This piece of work also revealed that antibacterial phytochemicals may be elucidated from extracts of *Vernonia amygdalina* for the treatments VRSA infections.

Author Disclosure Block:

J.O. Oluyege: None. **D. Oguntoye:** None.

Poster Board Number:

MONDAY-407

Publishing Title:

Melanoidins of *Melipona beecheii* Honey Have Antibacterial Effects Against *E. coli* Atcc 25922 and *Staphylococcus aureus* Atcc 25923

Author Block:

J. Ramon Sierra, J. Ruiz Ruiz, D. Magaña Ortiz, E. Ortiz Vazquez; Inst. Tecnológico de Mérida, Merida, Mexico

Abstract Body:

Background: Melanoidins are the final products of Maillard reactions and they have remarkable properties as food preservatives, antioxidants and prebiotics. Interestingly, previous reports have demonstrated the presence of melanoidins in Apis mellifera honey. However, most of properties of honey melanoidins remain unknown. Since prehispanic times, honey of stingless bee Melipona beecheii has been employed for the treatment of many diseases. The main purpose of this work was to study the antibacterial effect of Melipona honey melanoidins against pathogenic bacteria such Escherichia coli and Staphylococcus aureus. **Methods:** TThe melanoidin extract of Melipona honey was obtained using phosphate buffer and concentrated with a Millipore membrane with cut-off 3 kDa. After this, the proteins present in this extract were quantified using Bradford method employing BSA as standard. In order to obtain the fractions of melanoidins, the extract was separated using acrilamide gels in non-reducing conditions and the fractions were directly excised from this gel. The antimicrobial activity and minimum inhibitory concentration of Melipona honey and the melanoidin fractions obtained were determined using the disk diffusion and micro-dilutions method against E. coli ATCC 25922 and S. aureus ATCC 25923. **Results:**Three fractions of melanoidins were observed in non-reducing gels and all of them were tested in order to determine its antibacterial effect. Only one fraction was able to inhibit the growth of the two pathogenic bacteria tested. Using only 100 µg/ml of protein in melanoidin extract antibacterial effect was detected in disk diffusion method. The MICs of Melipona honey and melanoidin fraction were 17%, 23 µg/ml and 19 µg/ml for *S. aureus* 25923 whereas for *E. coli* 25922 were 19%, 21 µg/ml, and 22 µg/ml respectively. **Conclusions:** Melipona honey and one of three fractions of melanoidins observed were able to inhibit the growth of both pathogenic strains. This is the first report of the antibacterial properties of melanoidins in Melipona honey and future research could establish if the fraction tested contain antibacterial proteins or biological active flavonoids.

Author Disclosure Block:

J. Ramon Sierra: None. **J. Ruiz Ruiz:** None. **D. Magaña Ortiz:** None. **E. Ortiz Vazquez:** None.

Poster Board Number:

MONDAY-408

Publishing Title:

Avidocin-CDs: Novel Engineered Bacteriocins Use Phage Receptor Binding Protein to Target S-Layer Protein a Variants on the *Clostridium difficile* Cell Surface

Author Block:

J. A. Kirk[1], D. Gebhart[2], A. Buckley[3], S. Lok[2], D. Scholl[2], G. Douce[3], R. Fagan[1], **G. Govoni**[2]; [1]Univ. of Sheffield, Sheffield, United Kingdom, [2]AvidBiotics Corp., South San Francisco, CA, [3]Univ. of Glasgow, Glasgow, United Kingdom

Abstract Body:

Background: *C. difficile* is an opportunistic pathogen that takes advantage of dysbiosis in the gut to proliferate and cause disease. There is a need for anti-*C. difficile* agents that can eliminate or block infection without off-target effects and disruption of the gut microbiota. We recently demonstrated that an engineered bacteriocin retargeted with the *ptsM* prophage gene specifically killed ribotype 027 strains *in vitro* while preventing colonization of susceptible mice and not altering healthy microbiota *in vivo* (ref 1).**Methods:** Homologues to PtsM with different predicted specificities were identified in the genomes of *C. difficile* bacteria and phage. New Avidocin-CDs were constructed using a selection of PtsM homologues and tested for killing on a panel of 78 *C. difficile* strains. R20291 (027) mutants insensitive to Av-CD291.2 were isolated and characterized for defects. Complementation of the mutations with wild-type allele validated association between mutations and defect phenotypes. Mutants heterologously expressing non-027 *slpA* alleles were tested for sensitivity to the different Avidocin-CDs.**Results:** Each Avidocin-CD tested killed a different subset of strains with Av-CD027. 2 having the broadest coverage (killed 50% of the strains tested). Rare R20291 mutants ($<10^{-9}$) insensitive to Av-CD291.2 had point mutations in the *slpA* gene that resulted in loss of SlpA protein on the cell surface. *In vitro* these mutants exhibited low spore production and increased sensitivity to lysozyme and anti-microbial peptides. *In vivo* the *slpA* mutants were avirulent (100% survival) in a hamster model of CDI. Expression of non-027 *slpA* alleles in the mutant resulted in a gain of sensitivity to other Avidocin-CDs. In total, Avidocin-CDs were constructed that killed strains expressing SlpA variants from 10 of the 12 known S-layer cassettes. A cocktail of Avidocin-CDs could effectively target >95% of the clinical *C. difficile* isolates with a known ribotype.**Conclusions:** Avidocin-CDs represent an anti-bacterial platform capable of producing targetable, precision anti-*C. difficile* agents that can prevent or treat CDIs without disrupting indigenous microbiota.

Author Disclosure Block:

J.A. Kirk: None. **D. Gebhart:** D. Employee; Self; AvidBiotics Corp.. **A. Buckley:** None. **S. Lok:** D. Employee; Self; AvidBiotics Corp. **D. Scholl:** D. Employee; Self; AvidBiotics Corp.. **G. Douce:** None. **R. Fagan:** None. **G. Govoni:** D. Employee; Self; AvidBiotics corp..

Poster Board Number:

MONDAY-409

Publishing Title:

Comparison of Cytomegalovirus (CMV) UL89 Gene Mutations Selected *In Vitro* after Exposure to 3 Terminase Inhibitor Compounds

Author Block:

S. Chou; Oregon Hlth.and Sci. Univ., Portland, OR

Abstract Body:

Background: Terminase inhibitors 275175X (175X, a benzimidazole) and tomeglovir (TGV, Bay38-4766) were studied in preliminary clinical trials, while letermovir (LMV) is in Phase III trial for prevention of CMV disease in stem cell recipients. The spectrum and overlap of CMV mutations conferring resistance to these compounds is not well defined, but UL56 gene mutations are the most common pathway of LMV resistance, and the UL89 gene mutation D344E is associated with resistance to benzimidazoles. This study seeks to compare the UL89 mutations elicited by the 3 inhibitors. **Methods:** Published procedures were used for serial culture passage of a cloned CMV exonuclease mutant under drug starting near 50% inhibitory concentration (EC50) and increasing as permitted by viral growth. Emerging UL56 and UL89 mutations were detected by DNA sequencing of culture extracts, and were evaluated by recombinant phenotyping for the degree of resistance and cross-resistance conferred. **Results:** 6 expts with 175X all selected for UL89 D344E at a median of 7.5 passages, with UL89 C347S later adding to or replacing D344E in 2 cases. 8 expts with TGV selected for UL89 H389N in 1 case at passage 8, and V362M in all cases at a median of 12 passages. UL89 mutation was found in 2 of 17 expts with LMV at passages 13-25, adding to published UL56 mutations that evolved within 5 passages in all cases. In 1 case, UL89 N320H and M359I were detected (on separate genomes); the other evolved UL89 D344E. Recombinant phenotyping showed that D344E conferred 10x increased EC50 for 175X, slightly (1.5-2x) increased EC50 for LMV and TGV, and it slightly increased the LMV EC50 of the UL56 mutant F261L when combined. C347S conferred 25x increased EC50 for 175X only. H389N and V362M conferred 15x and 50x increased EC50 respectively for TGV only. Low-grade (≤3x) increased EC50s were observed for 175X, LMV and TGV (N320H) or LMV and TGV (M359I). **Conclusion:** Distinct UL89 mutations are consistently selected to confer substantial resistance to 175X and TGV. UL89 mutations are newly revealed as an uncommon accessory pathway of LMV resistance, fractionally increasing the level of resistance arising from preferred UL56 mutations. The proximity of UL89 mutations selected by all 3 drugs, with some conferring low-level cross-resistance, suggests targeting of a similar terminase functional locus with interfacing UL89 and UL56 components.

Author Disclosure Block:

S. Chou: None.

Poster Board Number:

MONDAY-410

Publishing Title:

Letermovir Is Active Against the Four Major Cytomegalovirus (CMV) gB Subtypes

Author Block:

C. M. Douglas[1], X. D. Zhang[1], D. J. Holder[1], P. Lischka[2]; [1]Merck & Co., Inc, Kenilworth, NJ, [2]AiCuris GmbH & Co KG, Wuppertal, Germany

Abstract Body:

Letermovir, an inhibitor of CMV terminase, is being evaluated for prevention of clinically significant CMV infection in adult CMV seropositive allogeneic hematopoietic stem cell transplant recipients. There are four major glycoprotein B (gB) subtypes associated with CMV infections in immunocompromised patients (gB1, gB2, gB3, and gB4). It has been reported that there are significant differences in the incidence of death due to myelosuppression between gB subtypes in CMV-infected marrow transplant recipients (1997 Blood **90**: 2097-2102). We evaluated the impact of gB subtype on letermovir susceptibility for 66 low-passage clinical CMV isolates. The gB subtype was determined by genotyping ORF UL55 (bp 1319-1596) and phylogenetic tree analysis, and letermovir susceptibility was measured using GFP- or plaque reduction assays. Letermovir EC_{50} values ranged from 0.7 to 6.1 ng/mL, and the distribution of gB subtypes among the 66 isolates was 29/24/10/3 for gB 1/2/3/4, respectively. The F-Test from an ANOVA model for testing the null hypothesis that there is no mean difference in EC_{50} of letermovir susceptibility among any of the four gB subtypes yielded a p-value of 0.44. The largest estimated ratio of mean EC_{50} values was 1.56 for gB4/gB2, with a 95% confidence interval of (0.74, 3.31). All of the 95% confidence intervals for pairwise ratios between gB subtypes contain the null value of ratio = 1. We conclude that letermovir has potent in vitro antiviral activity against CMV irrespective of the gB subtype.

Author Disclosure Block:

C.M. Douglas: D. Employee; Self; Merck & Co., Inc.. K. Shareholder (excluding diversitied mutual funds); Self; Merck & Co., Inc. **X.D. Zhang:** D. Employee; Self; Merck & Co., Inc.. K. Shareholder (excluding diversitied mutual funds); Self; Merck & Co., Inc. **D.J. Holder:** D. Employee; Self; Merck & Co., Inc. **P. Lischka:** D. Employee; Self; AiCuris GmbH & Co KG.

Poster Board Number:

MONDAY-411

Publishing Title:

Mbx-400, Active Against Betaherpesviruses, Does Not Cause Genotoxicity in *In Vitro* and *In Vivo* Models

Author Block:

J. Brooks, T. Bowlin; Microbiotix, Inc., Worcester, MA

Abstract Body:

Background: MBX-400 is currently in clinical development for betaherpesvirus-related disease. MBX 400 has demonstrated good *in vitro* potency against cytomegalovirus (CMV) and human herpesviruses 6A and 6B (HHV6A/B), with EC_{50} values of 1.2, 1.3 and 2.5 µM, respectively (Prichard, 2013). Preclinical studies support the initial clinical program and have demonstrated a margin of safety that supports human use (Bowlin, 2009). A standard battery of genetic toxicology tests, suitable to support an Investigational New Drug Application (IND), were performed to evaluate the potential for genotoxicity. **Methods:** Genetic toxicity was evaluated using four assays that have been well described: an Ames assay, a rat micronucleus assay, a Chinese hamster ovary assay and a rat Comet assay. The Ames assay was conducted using MBX-400 concentrations of 33.3 to 5000 µg per plate (with and without S9). The rat micronucleus assay was evaluated in the bone marrow of Sprague Dawley rats 24 and 48 hours after a single dose of MBX-400 at 500 to 2000 mg/kg. In the Chinese hamster ovary assay, cultures were treated for 3 hours with and without S9 at concentrations ranging from 6.78 to 1000 µL. The Comet assay was conducted using liver and kidney cells from male Sprague Dawley rats following two daily doses of MBX-400 at 500 to 2000 mg/kg/day.**Results:** MBX-400 did not induce reverse mutations in an Ames assay. In a rat micronucleus assay, MBX-400 did not cause significant increases in polychromatic erythrocytes nor bone marrow toxicity. Exposure to MBX-400 did result in chromosomal aberrations both with and without metabolic activation in a Chinese hamster ovary assay. In order to better characterize the potential nature of the aberrations, a rat Comet study was performed, which evaluated DNA damage in the liver and kidney following exposure to MBX-400. MBX-400 did not induce DNA damage in this study.**Conclusions:** The overall genetic toxicology assessment indicates that MBX-400 does not have mutagenic potential. The Chinese hamster ovary assay is known to provide false positive results. The results from this assay could not be confirmed in a subsequent Comet assay. Therefore, unlike ganciclovir, the current standard treatment for CMV infection, MBX-400 has not demonstrated the potential for genotoxicity.

Author Disclosure Block:

J. Brooks: D. Employee; Self; Microbiotix, Inc. **T. Bowlin:** D. Employee; Self; Microbiotix, Inc..

Poster Board Number:

MONDAY-412

Publishing Title:

Prophylactic Efficacy of Quercetin-3-β-O-D-Glucoside Against Ebola Virus Infection

Author Block:

X. Qiu[1], R. Kozak[1], A. Kroeker[1], S. He[1], J. Audet[1], M. Mbikay[2], M. Chretien[2]; [1]Natl. Microbiol. Lab., Publ. Hlth.Agency of Canada, Winnipeg, MB, Canada, [2]Clinical Res. Inst. of Montreal, Montreal, QC, Canada

Abstract Body:

Background: Ebola outbreaks occur on a regular basis, with the current outbreak in West Africa being the largest ever recorded. It has resulted in over 11 000 deaths in four African countries and has received international attention and intervention. Although there currently are no approved therapies or vaccines, many promising candidates are undergoing clinical trials and several have had success in promoting recovery from Ebola. However, these prophylactics and therapeutics have only been designed and tested against the species of Ebola that causing the current outbreak. Future outbreaks involving other species would require reformulation and possibly redevelopment. Therefore a broad spectrum alternative is highly desirable. **Method and Results:** Using a variant of *Ebola virus* (EBOV) that expresses green fluorescent protein, a flavonoid derivative called Quercetin-3 β-O-D-glucoside (Q3G) has been identified to have antiviral activity in vitro. Similar results were also found with *Sudan virus* (SUDV). Furthermore, we have evaluated the protective efficacy of Q3G as prophylactic and/or post-exposure treatment agent in a mouse-adapted EBOV model. The results showed that Q3G not only can fully protect mice from EBOV infection when given 30 minutes prior to infection, but also can partially protect mice when the treatment was initiated at 24 hour or 48 hours post challenge. **Conclusion:** This study serves as a proof of principle that Quercetin-3-β-O-D-glucoside has potential as a prophylactic/treatment agent against Ebola virus infection.

Author Disclosure Block:

X. Qiu: None. **R. Kozak:** None. **A. Kroeker:** None. **S. He:** None. **J. Audet:** None. **M. Mbikay:** None. **M. Chretien:** None.

Poster Board Number:

MONDAY-413

Publishing Title:

Gold Nanorods Reduce Respiratory Syncytial Virus Infection in BALB/c Mice

Author Block:

S. Bawage[1], P. Tiwari[1], A. Singh[2], S. Dixit[1], S. Pillai[1], V. Dennis[1], S. Singh[1]; [1]Alabama State Univ., Montgomery, AL, [2]Univ. of South Alabama, Mobile, AL

Abstract Body:

Respiratory Syncytial virus (RSV) is one of the leading causes of respiratory illness in the infants, children and older adults. RSV causes severe pneumonia and bronchitis to which only symptomatic treatment is prescribed along with the antiviral drug ribavirin. There is no vaccine in the market for RSV specific treatment; therefore there is a need for new approach to address the problem. The field of nanomedicine against pathogenic viruses was unexplored until last two decades, however, their potential for application has gained interest in recent years. Metallic nanoparticles (silver and gold) have shown antiviral activities *in vitro* against respiratory viruses like influenza, parainfluenza and adenoviruses. Here, we show the antiviral activity of gold nanorods (GNRs) against RSV and try to understand the mechanism of inhibition. Our preliminary study has demonstrated RSV inhibition by GNRs *in vitro* by 82%, therefore we carried out mice experiments to demonstrate the efficacy *in vivo*. Mice treated with GNRs showed almost 50% reduction of RSV (plaque assay), when compared to the RSV infected mice. The histology report of lungs showed that GNRs did not cause any notable pathology. However, there was infiltration of leukocytes as the GNRs were present in the extracellular matrix. Transmission electron microscopy showed that GNRs were contained in the cytoplasmic vesicles resulting from the phagocytosis of extracellular GNRs. Antiviral gene response (84 genes) indicate the up-regulation of TLR, NLR and RLR pathways against GNRs and RSV treatment, particularly OAS2, Mx1, Cxcl9, Cxcl10 and Cxcl11. Cytokine analysis of lung homogenate, bronchoalveolar lavage and serum showed increased production of inflammatory cytokines (IL-12, IL-9, IL-13, IL-17) for the RSV and GNR-RSV mice group. Restimulation of splenocytes with RSV, GNR, GNR-RSV did not produce significant cytokines (IL-6, IL-8, IL-10, IFN-γ, TNF-α). Our study demonstrates inhibition of RSV in mice by GNR mediated innate immune response.

Author Disclosure Block:

S. Bawage: None. **P. Tiwari:** None. **A. Singh:** None. **S. Dixit:** None. **S. Pillai:** None. **V. Dennis:** None. **S. Singh:** None.

Poster Board Number:

MONDAY-414

Publishing Title:

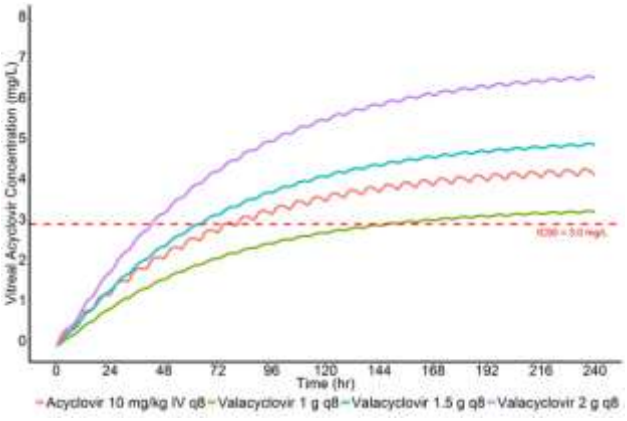
Valacyclovir as Initial Treatment for Acute Retinal Necrosis: A Pharmacokinetic Modeling and Simulation Study

Author Block:

A. Jain[1], T. Liu[2], M. W. Fung[3], V. Ivaturi[2], C. Vinnard[1]; [1]Drexel Univ. Coll. of Med., Philadelphia, PA, [2]Ctr. for Translational Med., Sch. of Pharmacy, Univ. of Maryland, Baltimore, MD, [3]Univ. of Pennsylvania Hlth.System, Philadelphia, PA

Abstract Body:

Background: Acute retinal necrosis (ARN) is a feared complication of infectious retinitis most commonly caused by varicella zoster virus (VZV), herpes simplex virus 1 (HSV-1), or herpes simplex virus 2 (HSV-2). We performed a pharmacokinetic modeling and simulation study using previously published data to evaluate the vitreal penetration of oral valacyclovir for the treatment of ARN, under various dosing scenarios. **Methods:** We compared different oral valacyclovir dosing regimens with intravenous acyclovir. The vitreous compartment was modeled as an effect compartment, and paired serum and vitreal acyclovir concentrations were obtained from previously published data of adult patients with ARN undergoing vitrectomy. The efficacy threshold for vitreal acyclovir concentrations was based on the previously reported IC_{50} values for VZV, HSV-1, and HSV-2. **Results:** Based on the time required to attain minimum vitreal acyclovir concentrations (C_{min}) greater than the highest reported IC_{50}, valacyclovir 1.5 g every 8 hours performed similarly to intravenous acyclovir 700 mg every 8 hours. In contrast, the standard dosing regimen for herpes zoster, valacyclovir 1 g every 8 hours, required 8 days for the C_{min} to exceed the target value. **Conclusion:** Modeling and simulation data support oral valacyclovir for the treatment of ARN, although the required dosing exceeds the recommended FDA dose size for herpes zoster. **Figure.** Mean simulation of vitreal acyclovir concentrations under different dosing scenarios of oral valacyclovir, compared with intravenous acyclovir.



Author Disclosure Block:

A. Jain: None. **T. Liu:** None. **M.W. Fung:** None. **V. Ivaturi:** None. **C. Vinnard:** None.

Poster Board Number:

MONDAY-415

Publishing Title:

Safety and Tolerability of High-Dose Oral Valacyclovir during a Shortage of Intravenous Acyclovir

Author Block:

M. M. McLaughlin[1], J. S. Esterly[2]; [1]Midwestern Univ., Downers Grove, IL, [2]Chicago State Univ. Coll. of Pharmacy, Chicago, IL

Abstract Body:

Background: Intravenous acyclovir (IVA) is the drug of choice for viral meningitis (VM) caused by herpes viruses. A national shortage of IVA from 11/20/12-09/03/13 mandated restricting this agent at Northwestern Memorial Hospital (NMH) for use only in patients with confirmed VM. Pharmacokinetic data suggests similar serum concentrations to IVA can be achieved with high-dose, oral valacyclovir (HDVA); however, there is a lack of data as to the safety and tolerability of HDVA. In certain cases, patients received HDVA empirically to conserve IVA for patients with confirmed VM. The objective of this study was to assess the safety and tolerability of HDVA as an alternative to IVA for patients with suspected VM during an IVA shortage. **Methods:** Subjects were considered for analysis in this retrospective study if they were ≥18 years of age, and received HDVA from 11/20/12-09/03/13 at NMH for suspected or proven VM. HDVA was defined as any dose exceeding the product label recommended doses for given indications and patient renal function (e.g. >6 gram/day for normal renal function). Pertinent patient variables were collected and outcome measures assessed included ADRs from HDVA and neurological sequelae. **Results:** Overall, 18 patients were included in the study. The average patient included in this study was a 49 year old (SD 17.3), immunocompromised, Caucasian male, modified APACHE II of 14.9 (SD 7.2), with a median hospital stay of 6.5 days (IQR 4-32). The most common presenting symptom suggestive of VM was altered mental status (n=15) followed by nuchal rigidity (n=7). 17 patients had a lumbar puncture and there were 4 definitive cases and 6 physician diagnosed cases of VM. Six (33.3%) patients experienced at least one ADR to HDVA (headache, n=1; nausea, n=1; rash, n=1; and thrombocytopenia, n=5). There were no discontinuations or any symptomatic therapy necessary to treat any of the ADRs. In total, 8 patients ultimately received IVA for treatment of VM and there were 2 patients that had neurological sequelae. **Conclusion:** HDVA appears to have been safe and well tolerated by patients with suspected VM and may be an option with IVA is unavailable. Future study is needed to elucidate treatment outcomes and the most appropriate dosing regimen for HDVA.

Author Disclosure Block:

M.M. McLaughlin: None. **J.S. Esterly:** None.

Poster Board Number:

MONDAY-416

Publishing Title:

Ribavirin for Respiratory Syncytial Virus: Mortality Rate and Risk Factors for Mortality Following Treatment

Author Block:

N. N. Pettit, Z. Han, J. delaCruz, A. Charnot-Katsikas, K. Beavis, J. Pisano; Univ. of Chicago Med., Chicago, IL

Abstract Body:

Background: Respiratory Syncytial Virus (RSV) is a common cause of viral pneumonia and is associated with high rates of mortality in immune-compromised (IC) patients. Ribavirin (RBV) is the mainstay of treatment with efficacy data available for oral and aerosolized administration. At our hospital, the rate of RSV positivity is 92-103 cases/yr, with 6-12% of these patients receiving RBV. To assess the clinical efficacy of RBV for RSV in IC patients, we sought determine the rate of and identify risk factors for mortality among those treated with RBV.**Methods:** All adult inpatients with RSV positive PCR and that received oral RBV (PORBV) or aerosolized RBV (aRBV) between 3/1/2013 - 3/1/2015 were included. The primary endpoint was 30 day all-cause mortality. A regression analysis was performed to identify risk factors for mortality.**Results:** A total of 26 patients received RBV (2 PORBV, 24 aRBV). Overall 30 day all-cause mortality was 19.2% (n=5). Mean duration of RBV was 5.1 $\pm$ 3 days. Most patients had a lower respiratory tract infection (LRTI) (n=18) at time of treatment. Risk factors assessed for mortality are shown in table 1.

Table 1: Risk Factor Analysis			
Risk factor	Survival 30d (n=21)	Non-survival 30d (n=5)	P value
Age (mean)	55.8	61	0.54
Stem-cell transplant (SCT)	8 (38)	4 (80)*	0.12
Solid organ transplant	8 (38)	1 (20)	0.42
LRTI	14 (67)	4 (80)*	0.50
IVIG	8 (38)	1 (20)	0.42
IgG <800 mg/dL	14 (67)	4 (80)*	0.50
Mixed Viral Infection	3 (14)	1 (20)	0.60
Duration RBV	4.5 $\pm$ 2.2	7.4 $\pm$ 4.9	0.17

SCT and duration RBV met a priori ($p<0.2$) for inclusion in multivariate regression (MV) analysis, no significant association with mortality was identified.
* 3 of 4 patients had all 3 risk factors: SCT and LRTI and IgG <800 mg/dL.

Conclusions: Our observed mortality rate is similar to available literature showing a rate of 18.5% among IC patients with RSV treated with RBV. None of the risk factors assessed were statistically associated with mortality, however the small sample size precludes definitive conclusions. A larger proportion of patients with the mortality outcome were SCT patients, had a an IgG <800 and LRTI at baseline, suggesting these characteristics may place a patient at greater risk for mortality even with RBV treatment. Additionally, adjunctive use of IVIG was more commonly implemented in the surviving group, indicating a potential role for IgG repletion to reduce mortality risk.

Author Disclosure Block:

N.N. Pettit: None. **Z. Han:** None. **J. delaCruz:** None. **A. Charnot-Katsikas:** None. **K. Beavis:** None. **J. Pisano:** None.

Poster Board Number:

MONDAY-417

Publishing Title:

Effectiveness & Safety of New Direct-Acting Antivirals for the Treatment of Hepatitis C Infection in Coinfected Hiv/Hcv Population: Results in Routine Clinical Practice

Author Block:

E. Chamorro-de-Vega, C. Rodriguez-Gonzalez, A. Gimenez-Manzorro, A. Herranz-Alonso, M. Sanjurjo-Saez, GRUVIC Study Group; Gregorio Maranon Univ. Hosp. - Hlth.Res. Inst. Gregorio Maranon, Madrid, Spain

Abstract Body:

Background: The new Direct-Acting Antivirals (DAAs) for Hepatitis C Virus (HCV) infection have dramatically changed the therapeutic landscape. However, data on effectiveness and safety of these combinations in coinfected HIV/HCV patients population are lacking. The objective was to provide preliminary data on effectiveness and safety of DAAs for the treatment of HCV infection in the HIV/HCV coinfected population when used under routine clinical practice.**Methods:** Study design: descriptive, prospective. Inclusion criteria: all HIV/HCV coinfected patients who finished their treatment with DAAs before December 31st 2015. The primary effectiveness end point was a Sustained Virologic Response 12 weeks after the end of treatment (SVR12). Safety was evaluated by the incidence of Adverse Drug Events (ADEs), including laboratory abnormalities. These data were collected at baseline and every 4-week visits during treatment.**Results:** Of the 175 patients enrolled, 61.7% had genotype 1 infection (66.7% genotype 1a), 21.1% genotype 4, 16.6% genotype 3 and 0.6% genotype 2. Overall, 73.1% were men, 50.9% had been previously treated for HCV, and 63.4% had cirrhosis (12.0% decompensated). 56.6% of the patients received SOF/LPV, 18.9% OBV/PTV/r+DSV and 13.7% SOF+DCV. Ribavirin was added in 50.0% of the patients. The duration of treatment was 12 weeks in 45.1% of the patients. SVR12 was achieved by 78/86 of the patients (90.7%; IC95% 84.0-97.4) (no data for SVR12 available for the remaining patients yet). Eight patients did not achieve SVR (3 virologic relapses, 1 virologic failure, 2 deaths -not drug-related-, 1 interruption due to ADEs and 1 desertion). Only 1 ADE of grade 3 was identified (insomnia). The three most common ADEs were: anemia (38.9%), asthenia (29.1%) and headache (26.9%). The haemoglobin level was less than 10 g/dL in 5.1% of patients. **Conclusions:** Preliminary data corroborates high effectiveness and a good safety profile of DAAs regimens in HIV/HCV coinfected population when used under routine clinical practice.

Author Disclosure Block:

E. Chamorro-de-Vega: None. **C. Rodriguez-Gonzalez:** None. **A. Gimenez-Manzorro:** None. **A. Herranz-Alonso:** None. **M. Sanjurjo-Saez:** None.

Poster Board Number:

MONDAY-418

Publishing Title:

Outcomes of Veterans Treated for Hepatitis C Infection with Interferon-Free Regimens

Author Block:

M. Segarra-Newnham, G. A. Fox-Seaman, T. J. Church; VA Med. Ctr., West Palm Beach, FL

Abstract Body:

Background: Five percent of veterans have hepatitis C virus (HCV). Interferon-free regimens have higher response rates and improved adherence. Our Veterans Affairs Medical Center (VAMC) has treated HCV infection in a nurse practitioner (NP)-based clinic for over 15 years. Treatment is selected by the NP in consultation with a clinical pharmacy specialist. Direct acting antivirals (DAA) such as ledipasvir/sofosbuvir (LDV/SOF) and ombitasvir/paritaprevir/ritonavir plus dasabuvir (PrOD) increase sustained virological response (SVR) particularly for patients with genotype (GT) 1 infection and in both treatment-naïve and treatment-experienced patients. **Purpose:** Determine outcomes including SVR 12 weeks after DAA treatment completion with viral load (VL). **Methods:** A retrospective chart review of patients treated with a DAA from January 2015 to December 2015 was conducted. Data collected included medication prescribed, hepatitis C GT, FIB4 score, cirrhosis, previous HCV therapy, SVR12 and discontinuation rates. **Results:** A total of 322 patients (avg age 62; 97% men) and median FIB4 score of 2.15 were treated during 2015. Twenty-one patients had GT2 with 4 patients having SVR12 evaluations (3/4 negative VL). One had GT4 and is still on treatment. Nineteen GT3 patients were treated with 8 having SVR12 evaluation. Four were treatment failures; all with cirrhosis and 2/4 treatment-experienced. The other 11 patients are pending SVR12 check. A total of 281 GT1 patients started treatment; 91 (32%) with cirrhosis. Of 281 GT1 patients, 208 (74%) were treatment-naïve and 56 (27% of naïve patients) had cirrhosis. Of 73 treatment-experienced patients, 35 (48%) had cirrhosis. Outcomes for GT1 patients are listed in table 1.

Treatment given	Started[a]	Rx complete/Pending SVR12	On treatment	SVR12 (neg VL)
LDV/SOF-based	250[b]	104	69	69/75 (92%)[c]
PrOD-based	31	20	5	3/5 (60%)

[a]3pts d/c rx due to side effects (2 LDV/SOF and 1 PrOD). [b]84 prescribed 8wks due to VL < 6M at baseline [c]15/17 (88%) 8 wks rx had neg VL 16wks post rx **Conclusions:** HCV infected patients treated with LDV-SOF at our clinic had high rates of response, similar to those seen in clinical trials. Response to PrOD regimen was lower than anticipated but with small number of patients evaluated.

Author Disclosure Block:

M. Segarra-Newnham: None. **G.A. Fox-Seaman:** None. **T.J. Church:** None.

Poster Board Number:

MONDAY-419

Publishing Title:

Identification of a Novel Antiviral Compound Targeting the Main Protease of Middle East Respiratory Syndrome Coronavirus

Author Block:

H. Shuai, S. Yuan, B-j. Zheng; The Univ. of Hong Kong, Hong Kong, Hong Kong

Abstract Body:

Middle East Respiratory Syndrome Coronavirus (MERS-CoV) is a newly discovered positive single stranded RNA virus which can cause severe pneumonia.MERS-CoV has caused a higher fatality rate (up to 35%). Besides, MERS patients might experience more severe clinical symptoms, such as renal failure. Importantly, the risk of pandemic caused by human adapted MERS-CoV mutant can not be excluded. Currently, no specific or effective antiviral therapy against MERS-CoV is available yet. Main protease (nsp5), which is highly conservative among the *Coronaviridea* family, mainly functions to release the essential viral nonstructural proteins for forming replication/transcription complex (RTC), thus is crucial to the replication of coronavirus, making it an ideal druggable target for development of antivirals. We screened compounds library to target MERS-CoV nsp5. Recombinant nsp5 was expressed using the E.coli system and its biological activity was verified by fluorescent resonance energy transfer (FRET) assay. A total of 879 compounds, previously enriched against SARS-CoV , were screened and we found a novel compound with >50% inhibition of enzyme activity at the concentration of 50μg/mL. The compound showed reduced cytopathic effect (CPE) on Vero-E6 cells at 48h post-infection (p.i.) and 50% inhibitory concentration (IC_{50}) was 3μM. Plaque assay further confirmed that viral titer in supernatant of Vero-E6 cell culture decreased about 4 logs at 24h p.i. at concentration of 12.5μM. Cell viability assay showed that 50% cytotoxicity concentration (CC_{50}) was 75μM at 48h after inoculation. The selection index (SI) of the compound is 25. Western blotting showed that nucleotide protein (NP) decreases in a dose-dependent manner in MERS-infected Vero-E6 cells at 24h p.i., indicating virus replication is reduced. Moreover, the compound showed cross-protection towards SARS-CoV. In conclusion, our work has provided valuable in developing viral protease inhibitor based antivirals for prophylaxis and therapy of infections by coronaviruses such as MERS-CoV and SARS-CoV.

Author Disclosure Block:

H. Shuai: None. **S. Yuan:** None. **B. Zheng:** None.

Poster Board Number:

MONDAY-420

Publishing Title:

A Sensitive Fluorescence Polarization Screen to Identify Rna Virus Replication Inhibitors

Author Block:

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Abstract Body:

Background: Ebola hemorrhagic fever is a highly lethal emerging infectious disease caused by Ebola virus (EBOV), which resulted in 15,215 confirmed cases and 11,300 deaths in 2014-2015. To address the acute medical need for effective therapeutics, we devised and executed a novel high throughput screen to identify small-molecule inhibitors of the essential interaction between EBOV nucleoprotein (NP) and its polymerase co-factor, eVP35. A 29 amino acid peptide at the N-terminus of eVP35 (NP binding peptide, NPBP) is sufficient for interaction with NP (Leung et al., 2015) and is able to suppress EBOV replication, suggesting the NPBP binding pocket as a potential drug target. Conservation of the essentiality of the interaction between the viral nucleoprotein (N or NP) and the polymerase (L) co-factor protein (P or VP35) for replication of paramyxoviruses and filoviruses indicates that this class of targets might be broadly useful for antiviral screening. **Methods:** A fluorescence polarization assay (FPA) was developed to detect inhibition of the interaction between EBOV NP and FITC-labeled VP35-NPBP and used to screen 200,000 compounds. Secondary assays included an unrelated FPA counter-screen to detect inhibitors of a FITC-labeled peptide containing an ETGE domain with purified human Keap1 protein containing a Kelch domain, an orthogonal NP/VP35-NPBP biolayer interferometry (BLI) assay, a cytotoxicity evaluation, and a minigenome assay for EBOV RNA synthesis (Edwards et al., 2015). **Results:** A total of 1,065 compounds were primary hits (0.5%), 498 (0.25%) were confirmed in the primary and counter screens, and 39 (0.02%) were reordered and tested in FPA dose responses. A total of 7 compounds (0.004%) displayed significant inhibition (IC_{50} values ranging from 2-35 μM) of the NP/VP35-NPBP interaction and were validated in all secondary assays. Evaluation in the minigenome assay demonstrated that two of the inhibitors reduced RNA replication significantly at non-cytotoxic concentrations. **Conclusions:** The EBOV NP/VP35-NPBP FPA exhibited excellent sensitivity, reproducibility, and ease of execution. The successful identification of selective NP/VP35 inhibitors supports using this approach for additional RNA viruses.

Author Disclosure Block:

P.J. Nash: D. Employee; Self; Microbiotix, Inc. **G. Liu:** D. Employee; Self; Microbiotix, Inc.. **D.W. Leung:** None. **T.L. Bowlin:** D. Employee; Self; Microbiotix, Inc.. **C.F. Basler:** None. **G.K. Amarasinghe:** None. **D.T. Moir:** D. Employee; Self; Microbiotix, Inc..

Poster Board Number:

MONDAY-421

Publishing Title:

In Vitro* Activity of Wck 5999, a Carbapenem/β-Lactamase Inhibitor Combination Tested Against Contemporary Kpc-Producing *Enterobacteriaceae

Author Block:

M. Castanheira, P. R. Rhomberg, B. A. Schaefer, R. N. Jones, H. S. Sader; JMI Lab., North Liberty, IA

Abstract Body:

Background: KPC serine-carbapenemases are prevalent worldwide and are usually resistant to all β-lactams and often resistant to other antimicrobial classes. WCK 5999 is a combination of meropenem (MEM) and WCK 4234, a β-lactamase inhibitor with enhanced inhibitory activity against class D oxacillinases (including carbapenem hydrolyzing) as well as activity against class A and C. We tested MEM-WCK 4234 against 137 Enterobacteriaceae isolates producing KPC enzymes of six bacterial species. **Methods:** 137 clinical KPC-producing isolates collected during 2013-2015 from USA hospitals were susceptibility tested against MEM-WCK 4234 (WCK 4234 at fixed 4 and 8 µg/mL), MEM and WCK 4234 alone according to CLSI guidelines. The presence of bla_{KPC} was determined by PCR/sequencing.**Results:** MEM alone ($MIC_{50/90}$, 8/>64 µg/mL) had a very limited activity based on CLSI interpretative criteria (≤1 µg/mL). MIC_{50} values for MEM-WCK 4234 were ≤0.03 µg/mL for both inhibitor concentrations and MIC_{90} was 0.25 and 0.12 µg/mL for fixed 4 and 8 µg/mL, respectively. MEM-WCK 4234 inhibited 97.8 and 99.3% of the isolates at ≤1 µg/mL using fixed 4 and 8 µg/mL of inhibitor, respectively. Highest MEM-WCK 4234 MIC value among KPC-producing *K. pneumoniae* (n=111, $MIC_{50/90}$, ≤0.03/0.25 µg/mL) was 4 µg/mL (only one isolate) when tested at fixed 4 µg/mL of inhibitor and 2 µg/mL using fixed 8 µg/mL of WCK 4234. All four KPC-producing *E. coli* and one *C. freundii* isolates tested were inhibited by MEM-WCK 4234 at ≤0.03 µg/mL and 5 *K. oxytoca* and 12 *E. cloacae* tested were inhibited at ≤0.5 and ≤1 µg/mL, respectively. MEM-WCK 4234 MIC results for two *S. marcescens* tested were ≤0.03 and 2 µg/mL and ≤0.03 and 4 µg/mL when using WCK4234 at fixed 4 and 8 µg/mL, respectively. WCK 4234 had no activity against the isolates tested (MIC_{50}, >32 µg/mL).**Conclusions:** MEM-WCK 4234 displayed promising activity against a collection of very recent (2013-2015) KPC-producing isolates collected from USA hospitals, regardless of the inhibitor concentration used. This combination (WCK 5999) was eight- to >256-fold (mode, 128-fold) more active against KPC-producing isolates when compared to MEM alone. The emergence of pan-drug resistant KPC-producing isolates highlight the need for new therapeutic options for these isolates and the further development of WCK 5999 is warranted.

Author Disclosure Block:

M. Castanheira: H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Wockhardt. **P.R. Rhomberg:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Wockhardt. **B.A. Schaefer:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Wockhardt. **R.N. Jones:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Wockhardt. **H.S. Sader:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Wockhardt.

Poster Board Number:

MONDAY-422

Publishing Title:

Activity of the New Carbapenem/β-Lactamase Inhibitor Combination Wck 5999 Against Gram-negative Isolates Producing Oxacillinases (Oxas)

Author Block:

M. Castanheira, P. R. Rhomberg, J. M. Lindley, R. N. Jones, H. S. Sader; JMI Lab., North Liberty, IA

Abstract Body:

Background: OXAs are class D serine β-lactamases that can display hydrolytic activity against cephalosporins and/or carbapenems. OXAs with extended- (ESBL) or narrow-spectrum (NS) are detected among Enterobacteriaceae (ENT) and *P. aeruginosa* (PSA). OXAs with carbapenemase (carb) activity were common among *Acinetobacter* spp. (ASP) and ENT. We tested WCK 5999, a combination of meropenem (MEM) with the β-lactamase inhibitor WCK 4234, against 118 OXA-producing isolates. **Methods:** 82 isolates producing OXA-carb (55 ASP and 27 ENT) and 36 isolates producing OXA-ESBL/NS (29 ENT and 7 PSA) were tested. Susceptibility (S) testing was performed according to the CLSI guidelines for MEM-WCK 4234 (WCK 4234 at fixed 4 and 8 μg/mL), MEM and WCK 4234. The presence of OXA-encoding genes was previously determined by PCR/sequencing.**Results:** Overall, MEM-WCK 4234 inhibited all ENT producing OXAs at ≤0.5 and ≤0.25 μg/mL using WCK 4234 at fixed 4 and 8 μg/mL, respectively. MEM alone inhibited only 64.3% of these isolates at the CLSI S breakpoint. All OXA-ESBL/NS were inhibited by MEM-WCK 4234 at ≤0.12 μg/mL, regardless of the inhibitor concentration and 89.7% of these strains were MEM S. MEM inhibited only 37.0% of the ENT isolates producing OXA-carb (OXA-48-like); however, MEM-WCK 4234 inhibited all OXA-Carb ENT isolates at ≤0.5 or ≤0.25 μg/mL (WCK 4234 at fixed 4 and 8 μg/mL, respectively). All OXA-producing ASP were resistant to MEM (MIC_{90}, >64 μg/mL), but MEM-WCK 4234 at fixed 8 μg/mL inhibited 65.5 and 90.9% of these isolates at ≤2 and ≤4 μg/mL, respectively. MEM-WCK 4234 had higher activity against more prevalent OXA-23-producing ASP when compared to isolates producing OXA-24. MEM-WCK 4234 (fixed 8 μg/mL) inhibited 84.4 and 96.9% of the OXA-23 isolates and 29.4 and 76.5% of the OXA-24 isolates at ≤2 and ≤4 μg/mL, respectively. Based on a small set of isolates (n=7), the activity of MEM-WCK 4234 was limited against PSA isolates producing OXA-ESBL/NS.**Conclusions:** MEM-WCK 4234 displayed enhanced activity against ENT and ASP isolates producing OXAs with carbapenemase activity when compared to MEM. The activity of MEM-WCK 4234 was very good against ENT producing OXA-ESBL/NS, but showed limited activity against OXA-ESBL/NS-producing PSA since the MEM resistance mechanism in these isolates is not mediated by β-lactamases.

Author Disclosure Block:

M. Castanheira: H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Wockhardt. **P.R. Rhomberg:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Wockhardt. **J.M. Lindley:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Wockhardt. **R.N. Jones:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Wockhardt. **H.S. Sader:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Wockhardt.

Poster Board Number:

MONDAY-423

Publishing Title:

Wck 5999 (Carbapenem-Wck 4234): *In Vitro* and *In Vivo* Activity of Novel β-Lactam-β-Lactamase Inhibitor (Bl-Bli) against Oxa β-Lactamase-Producing *Klebsiella* (Kp) and *Pseudomonas* (Pa)

Author Block:

H. N. Khande, S. R. Palwe, S. S. Takalkar, K. V. Umarkar, J. S. Satav, P. R. Joshi, S. S. Biniwale, S. S. Bhagwat, **M. V. Patel**; Wockhardt Res. Ctr., Aurangabad, India

Abstract Body:

Background: WCK 4234 is a novel BLI with expanded activity against Class D β-lactamases including OXA carbapenamases associated with *Acinetobacter*. KP harbouring OXA 48/181 and PA harbouring OXA 1/2 in conjunction with VEB or GES ESBLs may show resistance towards carbapenems and/or cephalosporins. Herein, we describe the *in vitro* and *in vivo* activity of WCK 4234 in combination with carbapenems.**Method:** BLI activity of WCK 4234 (IC_{50}) was determined against OXA 48/181and OXA 1/2 +VEB β-lactamases using nitrocefin assay. Combination MICs were determined as per CLSI, against KP (n=14) and PA (n=27) strains collected from Indian hospitals. Combination time-kill studies (initial inoculum: 6.6-7.23 $\log_{10}$ CFU/mL) were performed and periodic CFU counts recorded. $ED_{50/90}$, was determined using systemic infection model. Mice were intraperitonealy infected (KP S465 and S468) with 6.30–6.47 $\log_{10}$ CFU/mouse and treatment initiated 2h post-infection (BID, 3h apart for 1 day) and survival was monitored till day 7.**Results:** IC_{50} for WCK 4234 against KP OXA-48, -181 and PA OXA-1/2+VEB was 0.067, 0.09 and 0.043 µM respectively. In OXA KP strains, addition of WCK 4234 (4 µg/mL) lowered the standalone $MIC_{50/90}$ of imipenem (IPM) and meropenem (MEM) from 4/8 and 16/32 µg/mL to ≤0.12/0.25 µg/mL respectively [$MIC_{50/90}$: ceftazidime (CAZ)-avibactam (AVI) - 1/1 µg/mL; IPM-relebactam (REL) - 4/4 µg/mL]. For PA strains, IPM and MEM combined with WCK 4234 (8 µg/mL) provided $MIC_{50/90}$ of 0.5/32 µg/mL, 75% of PA strains were inhibited at 4 and 2 µg/mL, respectively [$MIC_{50/90}$: CAZ-AVI4 - 16/64 µg/mL; IPM-REL4 - 0.5/32 µg/mL]. Against KP S465 and S468, time-kill studies employing WCK 4234 (1 and 2 µg/mL) with either IPM or MEM (1 and 2 µg/mL) showed >2 $\log_{10}$ CFU/mL kill, while standalone IPM (8 µg/mL) failed. In PA Q125, a 1.7 $\log_{10}$ CFU/mL kill was achieved with IPM+WCK 4234 (4+4 µg/mL) while IPM and CAZ-AVI (4+4 µg/mL) failed. ED_{50} for KP S465 and S468: IPM (12.5 mg/kg)-WCK 4234 (6.40 mg/kg) and ED_{90}: IPM (12.5 mg/kg)-WCK 4234 (12.44 mg/kg). Standalone IPM even at higher doses failed to provide protection.**Conclusion**: Carbapenem-WCK 4234 combination provided useful *in vitro* and *in vivo* activity against OXA expressing KP and OXA+VEB expressing PA.

Author Disclosure Block:

H.N. Khande: D. Employee; Self; Wockhardt Research Center. **S.R. Palwe:** D. Employee; Self; Wockhardt Research Center. **S.S. Takalkar:** D. Employee; Self; Wockhardt Research Center. K. Shareholder (excluding diversitied mutual funds); Self; Wockhardt Ltd.,. **K.V. Umarkar:** D. Employee; Self; Wockhardt Research Center. **J.S. Satav:** D. Employee; Self; Wockhardt Research Center. **P.R. Joshi:** D. Employee; Self; Wockhardt Research Center. **S.S. Biniwale:** D. Employee; Self; Wockhardt Research Center. **S.S. Bhagwat:** D. Employee; Self; Wockhardt Research Center. K. Shareholder (excluding diversitied mutual funds); Self; Wockhardt Ltd.,. **M.V. Patel:** D. Employee; Self; Wockhardt Research Center. K. Shareholder (excluding diversitied mutual funds); Self; Wockhardt Ltd.,.

Poster Board Number:

MONDAY-424

Publishing Title:

Wck 5999 (Carbapenem-Wck 4234): *In Vitro* and *In Vivo* activity of Novel Broader-Spectrum β-Lactam-β-Lactamase Inhibitor (Bl-Bli) against Indian Oxa Carbapenemase (Oxa-Carb) Expressing *Acinetobacter* (Ab)

Author Block:

H. N. Khande, S. S. Takalkar, K. V. Umarkar, A. M. Kulkarni , P. R. Joshi, S. R. Palwe, S. S. Biniwale, **S. S. Bhagwat**, M. V. Patel; Wockhardt Res. Ctr., Aurangabad, India

Abstract Body:

Background: OXA-cabapenamases are largely responsible for pan β-lactam resistance in AB. Neither the approved β-lactamase inhibitors (BLIs) nor those under development extend antibacterial coverage to OXA-CARB-expressing AB. Herein, we describe a novel broader-spectrum BLI WCK 4234, that inhibits Class D OXA-CARBs besides Class A and C β-lactamases. **Method:** Inhibitory activity (IC_{50}) of WCK 4234 against diverse OXA-CARBs was determined by employing nitrocefin assay. MICs were determined as per CLSI recommended agar dilution method against 208 OXA-CARB AB strains collected from various Indian tertiary-care centers. Time-kill studies were conducted to assess the cidality of carbapenem-WCK 4234 (WCK 4234 at 4 and 8 µg/mL, initial inoculums: 6.5-6.75 $\log_{10}$ CFU/mL) in AB NCTC 13301 (OXA 23) and 13303 (OXA 26). Viable counts were enumerated by plating aliquots of serially diluted culture. *In vivo,* $ED_{50/90}$ was determined using systemic infection models. Mice were intraperitonially infected with 6.87-7.0 $\log_{10}$ CFU/mouse and treatment (BID for 1 day) was initiated 1h (AB NCTC 13301) or 2h (AB NCTC 13303) post-infection. **Results:** IC_{50} for WCK 4234 against OXA-23, -24, -25, -26 and OXA-27 β-lactamases was 0.70, 0.59, 0.92 and 0.51 µM respectively. $MIC_{50/90}$ of unprotected imipenem (IPM) was 16/64 µg/mL. Addition of 4 and 8 µg/mL of WCK 4234 lowered the $MIC_{50/90}$ of IPM to 2/8 µg/mL and 1/4 µg/mL, respectively. $MIC_{50/90}$ of ceftazidime (CAZ)-avibactam (AVI) was 64/128 µg/mL. Time-kill studies revealed that carbapenem-WCK 4234 (4-8 µg/mL) showed 2 $\log_{10}$ CFU/mL kill, while IPM and CAZ-AVI failed. Efficacy studies showed that ED_{50} was achieved at IPM (50 mg/kg)-WCK 4234 (6.40-23.23 mg/kg) while ED_{90} was achieved at IPM (50 mg/kg)-WCK 4234 (12.44-52.30mg/kg). Standalone IPM even at higher doses failed to provide protection. **Conclusion:** Carbapenem-WCK 4234 combination provided useful therapeutic option for the treatment of OXA-CARB expressing AB.

Author Disclosure Block:

H.N. Khande: D. Employee; Self; Wockhardt Research Center. **S.S. Takalkar:** D. Employee; Self; Wockhardt Research Center. K. Shareholder (excluding diversitied mutual funds); Self; Wockhardt Ltd.,. **K.V. Umarkar:** D. Employee; Self; Wockhardt Research Center. **A.M.**

Kulkarni : D. Employee; Self; Wockhardt Research Center. **P.R. Joshi:** D. Employee; Self; Wockhardt Research Center. **S.R. Palwe:** D. Employee; Self; Wockhardt Research Center. **S.S. Biniwale:** D. Employee; Self; Wockhardt Research Center. **S.S. Bhagwat:** D. Employee; Self; Wockhardt Research Center. K. Shareholder (excluding diversitied mutual funds); Self; Wockhardt Ltd.,. **M.V. Patel:** D. Employee; Self; Wockhardt Research Center. K. Shareholder (excluding diversitied mutual funds); Self; Wockhardt Ltd.,.

Poster Board Number:

MONDAY-425

Publishing Title:

***In Vitro* Antibacterial Activity of WCK 5999: A New Carbapenem/β-Lactamase Inhibitor Combination against ESBL-Phenotype and Carbapenemase-Producing *Enterobacteriaceae* from a Worldwide Surveillance Program (2015)**

Author Block:

M. D. Huband, M. Castanheira, D. J. Farrell, R. K. Flamm, R. N. Jones, H. S. Sader; JMI Lab., North Liberty, IA

Abstract Body:

Background: WCK 5999 is a new carbapenem/β-lactamase inhibitor combination comprising meropenem (MEM) and the novel broader-spectrum β-lactamase inhibitor, WCK 4234, with enhanced activity against Class D carbapenemases. The *in vitro* antibacterial activity of MEM-WCK 4234 using both fixed 4 (F4) and fixed 8 (F8) µg/mL of WCK 4234 was evaluated against Enterobacteriaceae (ENT) subgroups expressing resistance to β-lactams including ESBL-phenotype, ceftazidime (CAZ) non-susceptible (NS), MEM NS and carbapenem-resistant ENT (CRE).**Methods:** MEM-WCK 4234 (F4 and F8) and comparator compound MIC values were determined using a reference broth microdilution method against ENT subgroups collected during a 2015 worldwide surveillance program.**Results:** MEM-WCK 4234 (F4 and F8) displayed potent activity (MIC_{50}/MIC_{90} values of ≤0.06/≤0.25 µg/mL) against 369 ENT isolates displaying either an ESBL-phenotype, CAZ NS, MEM NS or CRE (Table). The highest MEM-WCK 4234 MIC (F4 and F8) was 0.5 µg/mL compared to >32 µg/mL for MEM alone. Applying CLSI breakpoint interpretive criteria, S rates against ESBL-phenotype *E. coli* (EC) and *Klebsiella pneumoniae* (KPN) ranged 9.2-18.3% for CAZ, 80.0-98.3% for MEM and 41.7-81.7% for piperacillin-tazobactam (P/T). CAZ and P/T S rates against MEM NS KPN and CRE were very low (3.1-4.3%) whereas MEM-WCK 4234 (F4 and F8) combinations retained activity (100% S) with MIC_{90} values of 0.25 and 0.12 µg/mL, respectively.**Conclusions:** WCK 5999 is a potent new antibacterial combination against ENT displaying and ESBL phenotype, CAZ NS, MEM NS and CRE. These data support the continued development of this promising antibacterial combination.

	MIC50/MIC90 µg/mL (%Susceptible[a])				
Organism / Phenotype (n)	**MEM-WCK 4234 (F4)**	**MEM-WCK 4234 (F8)**	**CAZ**	**MEM**	**P/T**
EC / ESBL-phenotype (120)	≤0.015/0.03 (100%)[b]	≤0.015/0.03 (100%)[b]	32/>32 (18.3%)	0.03/0.06 (98.3%)	4/64 (81.7%)

KPN / ESBL-phenotype (120)	0.03/0.06 (100%)[b]	0.03/0.06 (100%)[b]	>32/>32 (9.2%)	0.06/8 (80.0%)	32/>128 (41.7%)
Enterobacter spp. / CAZ NS (74)	≤0.015/0.03 (100%)[b]	≤0.015/0.03 (100%)[b]	>32/>32 (0.0%)	0.06/2 (89.2%)	32/>128 (35.1%)
KPN / MEM NS (23)	0.06/0.25 (100%)[b]	0.06/0.12 (100%)[b]	>32/>32 (4.3%)	8/>32 (0.0%)	>128/>128 (4.3%)
ENT / CRE (32)	0.03/0.25 (100%)[b]	0.03/0.12 (100%)[b]	>32/>32 (3.1%)	8/>32 (0.0%)	>128/>128 (3.1%)
a. According to CLSI breakpoints; b. % inhibited at ≤1 μg/mL MEM.					

Author Disclosure Block:

M.D. Huband: H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Wockhardt. **M. Castanheira:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Wockhardt. **D.J. Farrell:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Wockhardt. **R.K. Flamm:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Wockhardt. **R.N. Jones:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Wockhardt. **H.S. Sader:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Wockhardt.

Poster Board Number:

MONDAY-426

Publishing Title:

Wck 5999 (Carbapenem-Wck 4234): *In Vitro* Activity against Esbls, Class C, Kpc-Expressing *Enterobacteriaceae* (Ent) Isolates

Author Block:

P. R. Joshi, H. N. Khande, S. R. Palwe, S. S. Biniwale, S. S. Bhagwat, **M. V. Patel**; Wockhardt Res. Ctr., Aurangabad, India

Abstract Body:

Background: WCK 4234 is a novel β-lactamase inhibitor (BLI) with expanded activity against ENT ESBLs, Class C, KPCs and Class D β-lactamases including OXA carbapenamses associated with *Acinetobacter*. Herein, we describe the *in vitro* activity of WCK 4234 in combination with carbapenems against diverse MDR ENT.**Methods:** BLI activity of WCK 4234 (IC_{50}) was determined by employing nitrocefin assay. Combination ENT MICs were determined (as per CLSI) against ESBLs (n=135), Class C (n=58) and KPCs (n=23) and ENT outer membrane porin underexpression (OMP)+ESBL (n=6) collected from Indian hospitals. Combination time-kill studies employed initial bacterial count of 6.5-7.0 $\log_{10}$ CFU/mL and periodic CFU was determined.**Results:** WCK 4234 IC_{50} range against ESBLs, Class C and KPC were 0.023-0.10 µM, 0.027-0.033 µM, 0.020-0.054 µM, respectively. For ESBL ENT, $MIC_{50/90}$ for imipenem (IPM) and meropenem (MEM) were 0.12/0.5 and 0.03/0.06 µg/mL respectively that lowered to 0.12/0.25 and 0.015/0.03 µg/mL, respectively upon addition of WCK 4234 (4µg/mL). IPM-relebactam (REL 4 µg/mL) $MIC_{50/90}$ was 0.12/0.25 µg/mL. OMP ENT MIC range for IPM and MEM were 1-4 µg/mL and 2-8µg/mL, respectively that lowered to 0.12-0.25µg/mL, when combined with WCK4234 at 4 µg/mL. IPM-REL MIC range was 0.25-2 µg/mL. For Class C harboring strains, $MIC_{50/90}$ for IPM and MEM were 0.25/1 and 0.06/0.5 µg/mL, respectively. In combination with WCK 4234 (4 µg/mL) $MIC_{50/90}$ were 0.12/0.5 and 0.03/0.25 µg/mL, respectively. IPM-REL showed $MIC_{50/90}$ of 0.12/0.5 µg/mL. For, KPC, $MIC_{50/90}$ for IPM and MEM (32/32 and 32/>32 µg/mL, respectively) lowered to 0.5/0.5 and 0.25/0.25 µg/mL, respectively by WCK 4234. Against KPC *K. pneumoniae* (KP) H521 and KP H 524, time-kill studies employing WCK 4234 (1 and 2 µg/mL) with either IPM or MEM (1 and 2 µg/mL) showed >2 $\log_{10}$ CFU/mL kill, while standalone IPM or MEM (8 µg/mL) failed to do so. Similarly, for KP OMP+ESBL, KP J101, >3 log10 CFU/mL kill was achieved with IPM - WCK 4234 (1+4 µg/mL).**Conclusions:** WCK 4234 activity in combination with IPM and MEM shows promising *in vitro* features against widely prevalent ENT expressing diverse β-lactamases and KPC.

Author Disclosure Block:

P.R. Joshi: D. Employee; Self; Wockhardt Research Center. **H.N. Khande:** D. Employee; Self; Wockhardt Research Center. **S.R. Palwe:** D. Employee; Self; Wockhardt Research Center. **S.S. Biniwale:** D. Employee; Self; Wockhardt Research Center. **S.S. Bhagwat:** D. Employee; Self; Wockhardt Research Center. K. Shareholder (excluding diversitied mutual funds); Self; Wockhardt Ltd.,. **M.V. Patel:** D. Employee; Self; Wockhardt Research Center. K. Shareholder (excluding diversitied mutual funds); Self; Wockhardt Ltd.,.

Poster Board Number:

MONDAY-427

Publishing Title:

Enhanced Activity of Wck 4282 (Cefepime-Tazobactam) Against Kpc-producing Enterobacteriaceae Collected Worldwide When Tested in Physiological Conditions

Author Block:

M. Castanheira, P. R. Rhomberg, B. A. Schaefer, R. N. Jones, H. S. Sader; JMI Lab., North Liberty, IA

Abstract Body:

Background: KPC-producers are often resistant to several or all available antimicrobials. WCK 4282 (cefepime-tazobactam [high dose]; FEP-TAZ) displayed in vivo efficacy against KPC expressing strains despite elevated FEP-TAZ MICs. In vitro activity of FEP-TAZ was evaluated in physiological conditions against KPC-producers.**Methods**: KPC-producing Enterobacteriaceae (n=191) were susceptibility (S) tested against FEP-TAZ (TAZ at fixed 8 µg/mL) and FEP by CLSI guidelines using Mueller-Hinton broth (MHB) ± 50% human serum or 0.85% sodium chloride (NaCl). *bla*$_{KPC}$ presence was determined by PCR/sequencing.**Results:** FEP-TAZ activity against KPC-producing *K. pneumoniae* (KPN; n=156) was enhanced by at least 2- to 4-fold with the addition of serum ($MIC_{50/90}$, 8/64 µg/mL) or NaCl ($MIC_{50/90}$, 8/64 µg/mL) when compared to results for FEP-TAZ tested under standard conditions ($MIC_{50/90}$, 32/>64 µg/mL). FEP-TAZ inhibited 75.0 and 73.1% of the isolates at ≤16 µg/mL (high-dose FEP-TAZ PK-PD tentative breakpoint; Table) in the presence of added NaCl or serum, respectively. FEP exhibited limited activity against KPC-producing KPN ($MIC_{50/90}$, 32/>64 µg/mL) with only 17.9% of the isolates S at ≤8 µg/mL. FEP-TAZ activity against KPC-producing KPN was similar to that of FEP alone and inhibited 29.5% of these isolates at ≤8 µg/mL when tested under standard conditions. Against non-KPN, the addition of serum ($MIC_{50/90}$, 4/16 µg/mL) or NaCl ($MIC_{50/90}$, 4/32 µg/mL) lowered the MICs for FEP-TAZ when compared to the reference method (n=35; 5 species/complexes) and 88.6-94.3% of these isolates were inhibited by FEP-TAZ at ≤16 µg/mL in the presence of serum or NaCl. The activity of FEP-TAZ (MIC_{50}, 8 µg/mL) and FEP (MIC_{50}, 16 µg/mL) tested in standard conditions was limited against non-KP KPC-producers.**Conclusions:** FEP-TAZ MIC results for all KPC-producing isolates were consistently lower in MHB supplemented with human serum or NaCl (in vivo conditions) and 73.1 to 94.3% of the isolates were inhibited by FEP-TAZ at 16 µg/mL.

Organism (no. tested)/ Antimicrobial agent	No. of isolates at MIC (µg/mL; cumulative %):					
	1	2	4	8	16	32
K. pneumoniae (n=156)						
FEP-TAZ	3 (3.2)	6 (7.1)	9 (12.8)	26 (29.5)	31 (49.4)	35 (71.8)

FEP-TAZ + NaCl	6 (6.4)	17 (17.3)	22 (31.4)	39 (56.4)	29 (75.0)	21 (88.5)
FEP-TAZ + Serum	11 (12.2)	12 (19.9)	26 (36.5)	30 (55.8)	27 (73.1)	22 (87.2)
FEP alone	4 (2.6)	4 (5.1)	5 (8.3)	15 (17.9)	31 (37.8)	41 (64.1)
Non-*K. pneumoniae* (n=35)						
FEP-TAZ	1 (8.6)	2 (14.3)	8 (37.1)	7 (57.1)	5 (71.4)	6 (88.6)
FEP-TAZ + NaCl	2 (14.3)	7 (34.3)	8 (57.1)	6 (74.3)	5 (88.6)	2 (94.3)
FEP-TAZ + Serum	1 (28.6)	2 (34.3)	12 (68.6)	2 (74.3)	7 (94.3)	1 (97.1)
FEP alone	1 (2.9)	0 (2.9)	3 (11.4)	10 (40.0)	5 (54.3)	8 (77.1)

Author Disclosure Block:

M. Castanheira: H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Wockhardt. **P.R. Rhomberg:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Wockhardt. **B.A. Schaefer:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Wockhardt. **R.N. Jones:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Wockhardt. **H.S. Sader:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Wockhardt.

Poster Board Number:

MONDAY-428

Publishing Title:

***In Vitro* Activity of Cefepime Combined with Tazobactam (Wck 4282) Against a Challenge Set of Esbl and Carbapenemase Producing Gram Negative Species**

Author Block:

M. R. Jacobs[1], S. Bajaksouzian[1], K. M. Papp-Wallace[2], R. A. Bonomo[2]; [1]CWRU,UHCMC, Cleveland, OH, [2]Cleveland VAMC, CWRU, Cleveland, OH

Abstract Body:

Background: WCK 4282 is a high dose combination of cefepime (FEP) with tazobactam (TAZO) in Phase I clinical trials. This combination targets a broader spectrum of activity than piperacillin-tazobactam (PIP-TAZO), particularly against PIP-TAZO resistant ESBL-producing Enterobacteriaceae. **Methods:** In vitro susceptibilities of FEP-TAZO (WCK 4282, Wockhardt Research Center, India) and comparator agents including PIP-TAZO, FEP, imipenem (IMI) and meropenem (MER) were tested by CLSI broth microdilution. PIP was tested with TAZO at a fixed concentration of 4 mg/L and FEP with TAZO at fixed concentrations of 4 and 8 mg/L. Isolates tested were from a genetically well-characterized collection of strains that included Enterobacteriaceae and non-fermenters expressing i) class A ESBLs (CTX-M, SHV and TEM types; L2), ii) KPC, OXA 23/24, NDM, VIM and L1 β-lactamases and iii) OMP deletions (Omp35, -36 and OprD). PIP-TAZO resistant ESBL-producing Enterobacteriaceae were specifically selected for inclusion in this study.**Results:** PIP-TAZO and FEP were only active against the NSBL group, with PIP-TAZO only active against 18.9% of the ESBL group. FEP-TAZO (WCK 4282), was active against 73.0% and 81.1% of the ESBL group at TAZO concentration of 4 mg/L and 8 mg/L, respectively, compared to 91.9% for IMI and MER. None of the agents tested showed good activity against carbapenemase producing groups, although FEP-TAZO was active against 45.3-50.9% of KPC strains tested.**Conclusions:** In this study FEP-TAZO (WCK 4282) demonstrated nearly 4-fold greater in vitro activity against many ESBL producing strains of Enterobacteriaceae compared to PIP-TAZO, with improved activity with TAZO at 8 mg/L compared to 4 mg/L.

Percent inhibited at breakpoint							
Agent		PIP-TAZO(TAZO at 4 mg/L)	FEP	FEP-TAZO(TAZO at 4 mg/L)	FEP-TAZO (TAZO at 8 mg/L)	IMI	MER
Breakpoint (mg/L)		16	8	8	8	4	4
Resistance mechanism	Number						

Narrow spectrum β-lactamase (NSBL)	17	88.2	94.1	100.0	100.0	100.0	100.0
Extended spectrum β-lactamase (ESBL)	37	18.9	43.2	73.0	81.1	91.9	91.9
ESBL/OMP	10	10.0	10.0	20.0	20.0	70.0	20.0
KPC	53	0.0	20.8	45.3	50.9	26.4	22.6
NDM	57	10.5	0.0	21.1	22.8	22.8	21.1
OMP	8	12.5	12.5	25.0	25.0	25.0	25.0
OXA	18	5.6	5.6	5.6	22.2	16.7	16.7
L1	5	20.0	0.0	40.0	80.0	0.0	0.0
VIM	10	0.0	0.0	0.0	0.0	0.0	0.0
All	215	14.9	21.4	40.5	46.0	41.9	38.1

Author Disclosure Block:

M.R. Jacobs: E. Grant Investigator; Self; Actavis, Wockhardt. **S. Bajaksouzian:** None. **K.M. Papp-Wallace:** E. Grant Investigator; Self; Actavis, AstraZeneca, Merck, Wockhardt. **R.A. Bonomo:** E. Grant Investigator; Self; Actavis, AstraZeneca, Merck, Wockhardt, GSK.

Poster Board Number:

MONDAY-429

Publishing Title:

Wck 4234: Synthesis and Structure-Activity Relationship (Sar) Identifying a Novel β-Lactamase Inhibitor Active against *Acinetobacter* Expressing Oxa-Carbapenemases (Ab-Oxa)

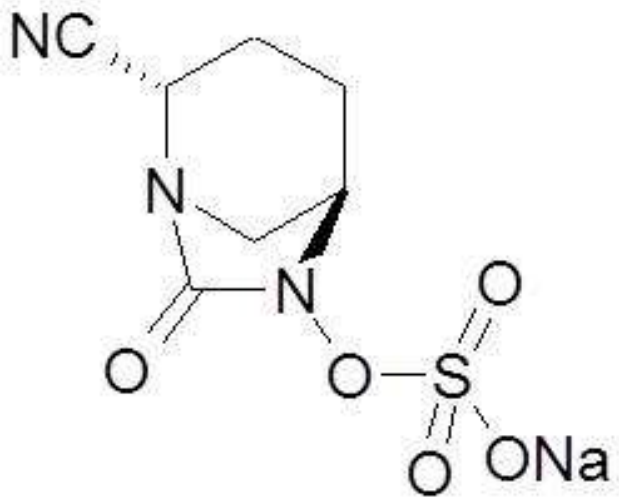
Author Block:

V. J. Patil, R. Tadiparthi, S. S. Birajdar, B. D. Dond, M. U. Shaikh, D. V. Dekhane, M. J. Pawar, S. S. Bhagwat, M. V. Patel; Wockhardt Res. Ctr., Aurangabad, India

Abstract Body:

Background:To date, none of the clinically employed β-lactamase inhibitors are effective against carbapenamases and Class D β-lactamases produced by *Pseudomonas*, *Enterobacteriaceae* and *Acinetobacter*. Thus treatment of these infections poses significant therapeutic challenge in a clinical setting. Here, we present the SAR of a novel β-lactamase inhibitor WCK 4234, that inhibits Class D enzymes, in addition to the Class A, C and KPC. **Methods:** A novel series of DBO test compounds were synthesized by modification of the functional group at C-5 of the 1,6-diazabicyclo octane core. IC_{50} was determined using Nitrocefin as the substrate. Antibacterial synergistic effect of these compounds was evaluated with carbapenem combinations employing CLSI broth micro dilution method. **Results:** Several compounds had good enzyme inhibition (IC_{50}) against Class A and Class C β-lactamases (0.0137->10 µM). Out of these WCK 4234 had the best IC_{50} values (Class A: 0.023-0.10 µM; Class C: 0.027-0.033 µM; KPC: 0.020-0.054 µM). For Class D CHDLs, IC_{50} for WCK 4234 against OXA-23, -24, -25, -26 and OXA-27 β-lactamases were 0.702, 0.591, 0.915 and 0.512 µM respectively. Other C-5 cyano derivatives had poor Class D inhibition (IC_{50} range 1.59->10 µM). AB-OXA (n=197) MICs of imipenem and meropenem were lowered by 4-8 times in combination with WCK 4234 at 4/8 µg/mL indicating good permeation features. **Conclusions:** Of all the DBO compounds synthesized, the cyano DBO derivative WCK 4234 showed promising inhibitory activity for Class A, C, KPC and D β-lactamases. Importantly, WCK 4234 demonstrated activity in whole-cell Acinetobacter MIC tests.

WCK 4234



Author Disclosure Block:

V.J. Patil: D. Employee; Self; Wockhardt Research Center. K. Shareholder (excluding diversitied mutual funds); Self; Wockhardt Ltd.,. **R. Tadiparthi:** D. Employee; Self; Wockhardt Research Center. K. Shareholder (excluding diversitied mutual funds); Self; Wockhardt Ltd.,. **S.S. Birajdar:** D. Employee; Self; Wockhardt Research Center. **B.D. Dond:** D. Employee; Self; Wockhardt Research Center. **M.U. Shaikh:** D. Employee; Self; Wockhardt Research Center. **D.V. Dekhane:** D. Employee; Self; Wockhardt Research Center. **M.J. Pawar:** D. Employee; Self; Wockhardt Research Center. **S.S. Bhagwat:** D. Employee; Self; Wockhardt Research Center. K. Shareholder (excluding diversitied mutual funds); Self; Wockhardt Ltd.,. **M.V. Patel:** D. Employee; Self; Wockhardt Research Center. K. Shareholder (excluding diversitied mutual funds); Self; Wockhardt Ltd.,.

Poster Board Number:

MONDAY-430

Publishing Title:

Activity of Ceftolozane-Tazobactam (TOL/TAZ) against Drug-Resistant Gram-Negative Pathogens Collected from USA Medical Centers in 2015

Author Block:

M. D. Huband, R. K. Flamm, R. N. Jones, H. S. Sader, D. J. Farrell; JMI Lab., North Liberty, IA

Abstract Body:

Background: TOL/TAZ is an antipseudomonal cephalosporin/β-lactamase inhibitor combination that received USA FDA approval for the treatment of complicated intra-abdominal and complicated urinary tract infections including pyelonephritis in December 2014.**Methods:** Susceptible (S) and multidrug resistant (MDR) *Pseudomonas aeruginosa* (PSA) and Enterobacteriaceae (ENT) isolates collected from medical centers in the USA during 2015 were tested using a reference broth microdilution method against TOL/TAZ (TAZ at fixed 4 µg/mL) and comparator compounds including ceftazidime (CAZ), cefepime (FEP), meropenem (MEM), and piperacillin-tazobactam (P/T). USA-FDA S breakpoints were applied for TOL/TAZ (ENT, ≤2 µg/mL; PSA, ≤4 µg/mL); CLSI breakpoints were applied for comparator compounds.**Results:** Most CAZ non-S (NS), MEM NS, and MDR PSA isolates were S to TOL/TAZ ($MIC_{50/90}$, 1/8 µg/mL; 81.0-88.5% S) whereas S to comparators including CAZ (0.0-57.3% S), FEP (41.4-64.6% S), MEM (0.0-46.8% S) and P/T (10.3-45.8% S) was substantially lower (Table). TOL/TAZ was less active against extensively drug-resistant (XDR) PSA ($MIC_{50/90}$, 2/32 µg/mL; 69.0% S), however, comparator compounds including CAZ, MEM and P/T were inactive (0.0-6.9% S) against XDR PSA. Only colistin (100.0% S) was more active than TOL/TAZ against XDR PSA. TOL/TAZ was also very active against ESBL-phenotype *E. coli* ($MIC_{50/90}$, 0.25/2 µg/mL) with 93.7% S and was more active than CAZ, FEP and P/T (21.4, 23.2, and 88.2% S, respectively). Compared to CAZ (8.6-10.4% S), FEP (15.7-24.4% S), and P/T (38.8-42.9% S), TOL/TAZ ($MIC_{50/90}$, 2/>32 µg/mL; 51.6-54.3% S) was more active against MDR Enterobacteriaceae and ESBL-phenotype *Klebsiella pneumoniae* (KPN).**Conclusions:** TOL/TAZ was highly active against CAZ NS, MEM NS and MDR PSA and ESBL-phenotype isolates collected from patients in USA medical centers during 2015. The potent *in vitro* activity of TOL/TAZ against these drug-resistant isolates support continued development studies with this antibacterial combination.

	MIC_{50}/MIC_{90} µg/mL (%Susceptible[a])				
Organism / Phenotype (# tested)	**TOL/TAZ**	**CAZ**	**FEP**	**MEM**	**P/T**

P. aeruginosa CAZ-NS (77)	1/8 (84.4%)[b]	32/>32 (0.0%)	16/>16 (45.5%)	4/16 (46.8%)	64/>64 (13.0%)
P. aeruginosa MEM-NS (96)	1/8 (88.5%)[b]	8/>32 (57.3%)	8/>16 (64.6%)	8/16 (0.0)	32/>64 (45.8%)
P. aeruginosa MDR (58)	1/8 (81.0%)[b]	32/>32 (20.7%)	16/>16 (41.4%)	8/16 (12.1%)	64/>64 (10.3%)
P. aeruginosa XDR (29)	2/32 (69.0%)[b]	32/>32 (6.9%)	16/>16 (34.5%)	16/32 (0.0%)	>64/>64 (6.9%)
Enterobacteriaceae MDR (250)	2/>32 (51.6%)[b]	>32/>32 (10.4%)	32/>64 (24.4%)	0.06/16 (74.4%)	32>128 (38.8%)
E. coli ESBL-phenotype (238)	0.25/2 (93.7%)[b]	32/>32 (21.4%)	64/>64 (23.2%)	≤0.015/0.03 (99.6%)	4/32 (88.2%)
K. pneumoniae ESBL-phenotype (140)	2/>32 (54.3%)[b]	>32/>32 (8.6%)	32/>64 (15.7%)	0.03/32 (69.3%)	64/>128 (42.9%)
a. According to CLSI breakpoints; b. Breakpoints from USA FDA package insert					

Author Disclosure Block:

M.D. Huband: H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Merck & Co. **R.K. Flamm:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Merck & Co. **R.N. Jones:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Merck & Co. **H.S. Sader:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Merck & Co. **D.J. Farrell:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Merck & Co..

Poster Board Number:

MONDAY-431

Publishing Title:

***In Vitro* Activity of Ceftolozane-Tazobactam against *Pseudomonas aeruginosa* and *Enterobacteriaceae* Isolates Collected from Medical Centers in the USA (2015)**

Author Block:

M. D. Huband, R. K. Flamm, R. N. Jones, H. S. Sader, D. J. Farrell; JMI Lab., North Liberty, IA

Abstract Body:

Background: Ceftolozane-tazobactam (TOL/TAZ) is an antipseudomonal cephalosporin combined with a β-lactamase inhibitor approved for the treatment of complicated intra-abdominal infections (cIAI; in combination with metronidazole) and complicated urinary tract infections (cUTI), including pyelonephritis in the United States (USA).**Methods:** *Pseudomonas aeruginosa* (PSA) and Enterobacteriaceae (ENT) isolates collected from USA medical centers during 2015 were tested using a reference broth microdilution method for susceptibility (S) to TOL/TAZ (TAZ at fixed 4 µg/mL) and comparator compounds including ceftazidime (CAZ), cefepime (FEP), meropenem (MEM), and piperacillin-tazobactam (P/T). USA-FDA breakpoints for S were applied for TOL/TAZ (≤2 µg/mL for ENT; ≤4 µg/mL for PSA); CLSI breakpoints were applied for comparator compounds.**Results:** TOL/TAZ was highly active against contemporary PSA isolates (n=507; 97.6% S) with $MIC_{50/90}$ values of 0.5/1 µg/mL (Table). Against PSA, TOL/TAZ was more active than CAZ, FEP, MEM, and P/T (81.1-88.3% S) and comparable in activity to colistin ($MIC_{50/90}$, ≤0.5/1 µg/mL; 99.8% S) and amikacin ($MIC_{50/90}$, 4/8 µg/mL; 95.3% S). TOL/TAZ was very active against ENT (n=3,800; $MIC_{50/90}$, 0.12/0.5 µg/mL) with 94.9% S and was more active than CAZ, FEP and P/T (87.0-92.6% S). Against *E. coli* (43.6% of ENT), TOL/TAZ was highly active ($MIC_{50/90}$, 0.12/0.25 µg/mL) with 99.1% S. Similarly, against *Klebsiella pneumoniae* (KPN, 22.5% of ENT), TOL/TAZ ($MIC_{50/90}$, 0.25/1 µg/mL; 92.5% S) was more active than CAZ, FEP and P/T (85.0-89.6% S). Slightly reduced S was observed for TOL/TAZ against *Citrobacter* spp. (89.4% S) and *Enterobacter* spp. (85.5% S) compared to FEP (92.5-97.7% S) and MEM (97.3-100.0%).**Conclusions:** TOL/TAZ demonstrated potent *in vitro* activity against PSA and ENT isolates from patients in USA medical centers during 2015. The activity of TOL/TAZ against these contemporary isolates support the continued development of this antibacterial combination.

	MIC50/MIC90 µg/mL (%Susceptible[a])				
Organism (#)	**TOL/TAZ**	**CAZ**	**CAZ**	**MEM**	**P/T**
P. aeruginosa (507)	0.5/1 (97.6%)[b]	2/32 (84.8%)	2/16 (88.3%)	0.5/8 (81.1%)	4/64 (81.9%)

Enterobacteriaceae (3,800)	0.12/0.5 (94.9%)[b]	≤0.25/32 (87.0%)	≤0.5/2 (90.5%)	≤0.015/0.06 (98.3)	2/16 (92.6%)
Citrobacter spp. (217)	0.12/4 (89.4%)[b]	0.25/>32 (86.2%)	≤0.03/0.5 (97.7%)	≤0.015/0.03 (100.0%)	2/32 (88.5%)
Enterobacter spp. (523)	0.25/4(85.5%)[b]	0.25/>32 (76.9%)	0.06/2 (92.5%)	0.03/0.06 (97.3%)	2/64 (82.8%)
E. coli (1,658)	0.12/0.25 (99.1%)[b]	0.12/16 (88.7%)	≤0.03/8 (89.0%)	≤0.015/0.03 (99.9%)	2/4 (96.3%)
K. pneumoniae (855)	0.25/1 (92.5%)[b]	≤0.25/32 (85.0%)	≤0.5/16 (86.2%)	0.03/0.03 (95.0%)	2/32 (89.6%)
Indole positive *Proteus* spp. (132)	0.25/0.5 (96.2%)[b]	0.12/8 (87.9%)	≤0.03/0.12 (97.7%)	0.06/0.12 (100.0%)	0.5/2 (98.5%)
Serratia spp. (192)	0.5/1 (97.9%)[b]	0.25/0.5 (97.9%)	0.06/0.25 (97.4%)	0.03/0.06 (97.9%)	2/4 (96.9%)
a. According to CLSI breakpoints; b. Breakpoints from USA FDA package insert					

Author Disclosure Block:

M.D. Huband: H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Merck & Co. **R.K. Flamm:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Merck & Co. **R.N. Jones:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Merck & Co. **H.S. Sader:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Merck & Co. **D.J. Farrell:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Merck & Co..

Poster Board Number:

MONDAY-432

Publishing Title:

Antibacterial Activity of Avibactam Combinations Against Recent Carbapenemase-producing *Enterobacteriaceae* Clinical Isolates

Author Block:

Y. Zhang, A. Kashikar, C. Brown, G. Denys, **K. Bush**; Indiana Univ., Bloomington, IN

Abstract Body:

Background: Avibactam is a novel non-beta-lactam beta-lactamase inhibitor recently approved in combination with ceftazidime to treat serious, multidrug-resistant nosocomial infections. In this study avibactam was combined with either ceftazidime (CAZ), ceftaroline (CPT) or aztreonam (ATM) to determine its ability to potentiate their activity against a recent collection of carbapenem-resistant *Enterobacteriaceae* (CRE).**Materials:** CRE isolates collected from central Indiana Health Care Centers from 2010 - 2013 (n=110) were characterized for carbapenemase genes by PCR and sequencing. MICs for avibactam (AVI) combinations, meropenem (MEM) and piperacillin-tazobactam (PTZ) were determined in broth microdilution assays according to CLSI standards, with AVI and tazobactam tested at a fixed 4 μg/ml.**Results:** Serine carbapenemases from 96 *Klebsiella pneumoniae*, 6 *Escherichia coli*, 3 *Serratia marcescens* and 2 *Enterobacter cloacae* isolates included 16 KPC-2- and 91 KPC-3-producing strains. SME-1 was in 3 additional *S. marcescens* isolates. Metallo-beta-lactamases (MBLs) were stably co-produced with KPC-3 in *E. cloacae* (VIM-1, n=2), *E. coli* (VIM-1, n=1) and *K. pneumoniae* (NDM-1, n=1). All isolates were resistant to MEM (MIC >=4 μg/ml); only two isolates (SME-1/*S. marcescens*) had PTZ MICs <128 μg/ml. At least 98% of the isolates were resistant to CAZ, CPT or ATM. The addition of AVI to CAZ and CPT lowered CAZ and CPT MICs to 8 μg/ml for all isolates, except for the 4 MBL producing strains. ATM MICs with AVI were <=8 μg/ml for all isolates, including those with MBLs; 96% (106/110) of all isolates had an ATM MIC <=0.5μg/ml.**Conclusions:** Avibactam together with ceftazidime, ceftaroline or aztreonam lowered MICs of the partner beta-lactams to 4 μg/ml or less for at least 95% of the CRE tested. The added stability of aztreonam to MBL hydrolysis provided a lowering of all ATM MICs to at least 8 μg/ml, indicating the potential utility of these combinations to treat infections caused by carbapenemase-producing bacteria.

Agent	MIC range in μg/ml	MIC_{50} in μg/ml	MIC_{90} in μg/ml
Ceftazidime	1 - >128	>128	>128
Ceftazidime-Avibactam	0.12 - >128	1	2
Ceftaroline	32 - >128	>128	>128

Ceftaroline-Avibactam	<=0.06 - >128	0.5	2
Aztreonam	8 - 128	64	128
Aztreonam-Avibactam	0.06 - 8	0.25	0.5
Meropenem	4- >16	>16	>16
Piperacillin-Tazobactam	<=2 - >128	>128	>128

Author Disclosure Block:

Y. Zhang: None. **A. Kashikar:** None. **C. Brown:** None. **G. Denys:** C. Consultant; Self; Exoxemis, Inc. F. Investigator; Self; Becton Dickinson. I. Research Relationship; Self; Exoxemis, Inc, Great Basin Scientific. **K. Bush:** C. Consultant; Self; Achaogen, Naeja. H. Research Contractor; Self; Achaogen, Actavis, Tetraphase. J. Scientific Advisor (Review Panel or Advisory Committee); Self; Achaogen, Allecra, Entasis, Gladius, Melinta, Roche, Tetraphase, WarpDrive. K. Shareholder (excluding diversitied mutual funds); Self; Fedora, Johnson & Johnson. N. Other; Self; Bristol-Myers Squibb, Johnson & Johnson, Pfizer.

Poster Board Number:

MONDAY-433

Publishing Title:

Coumarin Phosphonates Behave as Inhibitors of the Kpc-2 Carbapenemase

Author Block:

O. Pemberton, Y. Chen; Univ. of South Florida, Tampa, FL

Abstract Body:

Background: Each year in the United States an estimated 2 million people are infected with antibiotic-resistant bacteria, with at least 23,000 people dying as a direct result of those infections. Among all of the bacterial resistance problems, Gram-negative bacterial pathogens pose the greatest threat since they have acquired resistance to many antibiotics, including the beta-lactam antibiotics. The most widespread mode of beta-lactam resistance involves the expression of enzymes called beta-lactamases. Disclosed herein are a series of novel derivatives of coumarin phosphonates, which act as inhibitors of the class A KPC-2 carbapenemase, with the best compound displaying a K_i of 1.5 μM. The complex crystal structure of six compounds in the KPC-2 active site have been solved. **Methods**: The gene encoding KPC-2 was cloned into the pET-GST vector containing an N-terminal hexa-histidine tag. KPC-2 was purified to homogeneity using nickel affinity and gel-filtration chromatography. Steady-state kinetic parameters were determined by using a Biotek Cytation Multi-Mode Reader. Compounds were tested for synergy with the carbapenem antibiotic, imipenem, against BL21 (DE3) *E. coli* expressing KPC-2. MIC values were determined with the Mueller-Hinton broth microdilution method. Crystallization trials were carried out by using Qiagen crystallization kits. After an initial crystallization hit was found, conditions were optimized and crystals were used for compound soaking trials. X-ray data was processed with iMOSFLM, Phenix suite, and WinCoot. **Results**: Steady-state kinetic analysis demonstrated that all the coumarin phosphonate derivatives inhibited KPC-2 with micromolar potency, with the best two compounds possessing a K_i of 1.5 μM and 5.7 μM. The 1.5 μM inhibitor was able to reduce the MIC of imipenem eight-fold in BL21 (DE3) *E. coli* expressing KPC-2. The complex crystal structures demonstrated that the inhibitors form hydrogen bonds with Ser70, Ser130, Thr235, and Thr237. The inhibitors also form a stacking interaction with Trp105. **Conclusions**: The emerging health threat posed by antibiotic resistance is an issue that needs to be addressed. Use of a coumarin phosphonate compound could be useful in potentiating the action of beta-lactam antibiotics in the treatment of multi-drug resistant bacterial infections.

Author Disclosure Block:

O. Pemberton: None. **Y. Chen:** None.

Poster Board Number:

MONDAY-434

Publishing Title:

Ceftazidime-Avibactam Antimicrobial Activity When Tested Against Gram-Negative Bacteria Isolated from Intensive Care Unit (ICU) Patients with Pneumonia (2012-2014)

Author Block:

H. S. Sader, M. Castanheira, R. K. Flamm, D. J. Farrell, R. N. Jones; JMI Lab., Inc., North Liberty, IA

Abstract Body:

Background: Ceftazidime-avibactam (CAZ-AVI) consists of CAZ combined with the novel non-β-lactam β-lactamase (BL) inhibitor AVI, which inhibits extended-spectrum BLs (ESBLs), KPCs, AmpCs and some OXA enzymes. CAZ-AVI is under clinical development for treatment of nosocomial pneumonia.**Methods**: Clinical isolates were consecutively collected from 51 United States (USA) medical centers in 2012-2014 as part of the INFORM Surveillance Program and tested for susceptibility (S) at a central laboratory by reference broth microdilution methods. Results for 1,428 Gram-negative (GN) isolates from ICU patients with pneumonia were evaluated. BL encoding genes were evaluated for all *Klebsiella* spp. (KSP) and *E. coli* (EC) with an ESBL-phenotype (n=98) by microarray-based assay.**Results**: The most frequent GN organisms isolated were *P. aeruginosa* (PSA; 28.5% of GN isolates), followed by KSP (23.8%), *Enterobacter* spp. (EBS; 12.0%) and EC (11.7%). CAZ-AVI was the most active β-lactam tested against PSA (96.1% S) and exhibited good activity against multidrug-resistant (MDR; 82.3% S) and extensively drug-resistant (XDR; 76.3% S) PSA (Table). All Enterobacteriaceae (ENT) strains were S to CAZ-AVI except for one EBS with a CAZ-AVI MIC of 16 μg/mL (99.9% S), which had negative results for all BLs tested. ENT S rates for piperacillin/tazobactam (P/T) and meropenem (MEM) were 84.9 and 96.4%, respectively. MEM exhibited limited activity against ESBL-phenotype KSP (59.2% S) and MDR-ENT (63.5% S), whereas CAZ-AVI was very active against these resistant subsets (98.8-100.0% S). CAZ-AVI, colistin and tigecycline were the only agents active against XDR-ENT and carbapenem-resistant ENT (CRE). *Acinetobacter* spp. showed low S rates for all agents except colistin (94.7% S).**Conclusions**: CAZ-AVI demonstrated potent activity against a large collection of GN isolates from ICU patients with pneumonia. These *in vitro* results support further development of CAZ-AVI for treatment of nosocomial pneumonia in the USA.

Organism (no. tested)	**$MIC_{50/90}$ in μg/mL (% susceptible [CLSI and USA-FDA])**				
	CAZ-AVI	**P/T**	**Meropenem**	**Gentamicin**	**Levofloxacin**
P. aeruginosa (407)	2/4 (96.1)	8/>64 (76.7)	0.5/8 (78.1)	2/>8 (87.7)	0.5/>4 (74.9)

MDR (79)	4/16 (82.3)	64/>64 (17.7)	8/>8 (20.3)	8/>8 (48.1)	>4/>4 (19.0)
XDR (38)	4/>32 (76.3)	>64/>64 (2.6)	8/>8 (7.9)	>8/>8 (34.2)	>4/>4 (0.0)
Enterobacteriaceae (851)	0.12/0.5 (99.9)	2/64 (84.9)	≤0.06/≤0.06 (96.4)	≤1/2 (91.8)	≤0.12/>4 (82.7)
Klebsiella spp. (340)	0.12/0.5 (100.0)	4/>64 (84.7)	≤0.06/≤0.06 (91.5)	≤1/2 (91.5)	≤0.12/>4 (85.9)
ESBL-phenotype KSP (71)	0.5/1 (100.0)	>64/>64 (26.8)	≤0.06/>8 (59.2)	2/>8 (60.6)	>4/>4 (36.6)
Enterobacter spp. (171)	0.12/0.5 (99.4)	4/64 (74.7)	≤0.06/≤0.06 (98.8)	≤1/≤1 (100.0)	≤0.12/0.25 (98.2)
CAZ-non-S EBS (49)	0.25/1 (98.0)	64/>64 (12.2)	≤0.06/0.25 (95.9)	≤1/≤1 (100.0)	≤0.12/0.25 (93.9)
MDR ENT (85)	0.5/2 (98.8)	>64/>64 (21.4)	≤0.06/>8 (63.5)	8/>8 (49.4)	>4/>4 (11.8)
XDR ENT (11)	0.5/1 (100.0)	>64/>64 (0.0)	>8/>8 (0.0)	>8/>8 (0.0)	>4/>4 (0.0)
CRE (29)	1/2 (100.0)	>64/>64 (0.0)	>8/>8 (0.0)	2/>8 (62.1)	>4/>4 (13.8)

Author Disclosure Block:

H.S. Sader: H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Allergan. **M. Castanheira:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Allergan. **R.K. Flamm:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Allergan. **D.J. Farrell:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Allergan. **R.N. Jones:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Allergan..

Poster Board Number:

MONDAY-435

Publishing Title:

Antimicrobial Activity of Ceftazidime-Avibactam and Comparator Agents When Tested against Bacterial Isolates Causing Infection in Cancer Patients (2012-2014)

Author Block:

H. S. Sader, D. J. Farrell, R. K. Flamm, M. Castanheira, R. N. Jones; JMI Lab., Inc., North Liberty, IA

Abstract Body:

Background: Avibactam (AVI) inhibits classes A (including ESBLs and KPCs), C (AmpC) and some D β-lactamases (BL), restoring ceftazidime (CAZ) activity against Gram-negative (GN) organisms producing these enzymes.**Methods:** A total of 623 Gram-negative (GN) isolates were collected from 52 medical centers over three years (2012-2014) from patients with cancer as part of the International Network for Optimal Resistance Monitoring (INFORM) program in the United States. Isolates were tested for susceptibility (S) against CAZ-AVI (AVI at fixed 4 μg/mL) and comparator agents at a central laboratory by a reference broth microdilution method. BL encoding genes were evaluated for all *E. coli* (EC) and *Klebsiella* spp. (KSP) with an extended-spectrum BL (ESBL) phenotype by microarray-based assay.**Results:** The isolates were predominantly from bloodstream (33.2%) and skin/soft tissue (26.0%), and the most frequent GN organisms were EC (31.5%), KSP (20.9%), *P. aeruginosa* (PSA; 14.1%) and *Enterobacter* spp. (EBS; 12.7%). ESBL-phenotype was observed among 17.3 and 11.2% of EC and KSP, respectively; and 21.5% of EBS were ceftazidime (CAZ)-non-S. All Enterobacteriaceae (ENT; n=486) were S to CAZ-AVI with the highest MIC value at 1 μg/mL. Meropenem (MEM) was also very active against ENT overall (99.6% S); but showed more limited activity against ESBL-phenotype KSP (84.6% S) and multidrug-resistant (MDR) ENT (93.3% S). ESBL-phenotype KSP and MDR-ENT exhibited low S to piperacillin/tazobactam (P/T; 46.2 and 51.7%, respectively), gentamicin (GEN; 46.2 and 26.7%), levofloxacin (LEV; 53.8 and 10.0%) and colistin (83.3 and 86.4%). The most active agents tested against PSA were colistin (100.0% S), amikacin (97.7% S) and CAZ-AVI (96.6% S).**Conclusions:** GN organisms isolated from cancer patients hospitalized in USA medical centers were highly S to CAZ-AVI, including PSA and MDR and/or carbapenem-resistant ENT. The role of CAZ-AVI for treatment of cancer patients should be further evaluated.

Organism (no. tested)	**$MIC_{50/90}$ in μg/mL (% susceptible [CLSI and USA-FDA])**				
	CAZ-AVI	**P/T**	**MEM**	**GEN**	**LEV**
Enterobacteriaceae (486)	0.12/0.25 (100.0)	2/16 (93.6)	≤0.06/≤0.06 (99.6)	≤1/2 (90.9)	≤0.12/>4 (81.3)

E. coli (196)	0.06/0.12 (100.0)	2/8 (94.9)	≤0.06/≤0.06 (100.0)	≤1/>8 (85.7)	≤0.12/>4 (63.3)
ESBL phenotype (34)	0.12/0.25 (100.0)	8/32 (82.4)	≤0.06/≤0.06 (100.0)	≤1/>8 (58.8)	>4/>4 (8.8)
Klebsiella spp. (129)	0.06/0.25 (100.0)	2/16 (92.9)	≤0.06/≤0.06 (98.4)	≤1/≤1 (94.6)	≤0.12/0.5 (95.3)
ESBL-phenotype (13)	0.25/1 (100.0)	32/>64 (46.2)	≤0.06/4 (84.6)	8/>8 (46.2)	1/>4 (53.8)
Enterobacter spp. (79)	0.25/0.5 (100.0)	4/32 (87.2)	≤0.06/≤0.06 (100.0)	≤1/≤1 (97.5)	≤0.12/0.5 (92.4)
CAZ-non-S EBS (17)	0.5/1 (100.0)	32/>64(37.5)	≤0.060.25 (100.0)	≤1/>8 (88.2)	≤0.12/>4 (70.6)
MDR ENT (30)	0.12/0.5 (100.0)	16/>64 (51.7)	≤0.06/0.12 (93.3)	>8/>8 (26.7)	>4/>4 (10.0)
P. aeruginosa (88)	2/8 (96.6)	4/64 (84.1)	0.5/8 (79.5)	≤1/4 (93.2)	0.5/>4 (73.9)
MDR (12)	8/>32 (75.0)	>64/>64 (0.0)	>8/>8 (0.0)	4/>8 (50.0)	>4/>4 (16.7)

Author Disclosure Block:

H.S. Sader: H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Allergan. **D.J. Farrell:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Allergan. **R.K. Flamm:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Allergan. **M. Castanheira:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Allergan. **R.N. Jones:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Allergan..

Poster Board Number:

MONDAY-436

Publishing Title:

Activity of ceftazidime-avibactam tested against clinical isolates of antimicrobial resistant *Pseudomonas aeruginosa* (PSA) isolates from United States (USA) medical centers (2012-2014)

Author Block:

H. S. Sader, D. J. Farrell, R. K. Flamm, R. N. Jones; JMI Lab., Inc., North Liberty, IA

Abstract Body:

Background: Avibactam (AVI) is a non-β-lactam β-lactamase (BL) inhibitor that inhibits ESBLs, KPCs, AmpCs and some OXA enzymes. Ceftazidime (CAZ)-AVI was approved by the USA-FDA for treatment of intra-abdominal and urinary tract infections in 2015, and is under clinical development for treatment of pneumonia.**Methods**: 5,643 PSA isolates (one per patient) were consecutively collected in 2012-2014 from 74 USA medical centers (37 states from all 9 census regions) by the INFORM Surveillance Program. CAZ-AVI (AVI, fixed 4 μg/mL) and comparators were tested for susceptibility (S) by CLSI broth microdilution methods. The isolates were predominantly from pneumonia (39.8%) and skin/soft tissue (23.7%).**Results**: CAZ-AVI was active against 96.7% of strains at the USA-FDA S breakpoint of ≤8 μg/mL (Table), while S rates for CAZ, cefepime, piperacillin/tazobactam (P/T) and meropenem (MEM), were 83.8, 84.5, 79.9 and 82.3%, respectively. Colistin (COL) and amikacin (AMK) and were active against 99.2 and 97.2% of PSA strains, respectively. High rates of cross-resistance was observed among CAZ, P/T and MEM, whereas CAZ-AVI exhibited good activity against isolates non-S to CAZ (79.6% S), P/T (84.3% S), MEM (84.8%), as well as isolates non-S to CAZ, P/T and MEM (n=610; $MIC_{50/90}$, 8/32 μg/mL; 76.6% S). Multidrug-resistant (MDR) and extensively drug-resistant (XDR) phenotypes were observed in 14.9 and 8.6% of strains, respectively. Among MDR and XDR PSA, 79.8 and 71.5% were CAZ-AVI-S, respectively, while S rates for CAZ, P/T and MEM were ≤21.8% for MDR and ≤9.2% for XDR strains. The most active compounds tested against MDR/XDR strains were COL (98.9/99.0% S), AMK (88.0/83.8% S) and CAZ-AVI (78.9/71.5% S). CAZ-AVI activity remained stable during the study period.**Conclusions**: CAZ-AVI exhibited potent *in vitro* activity and spectrum when tested against a large collection (n=5,643) of recent USA PSA clinical strains, and retained activity against isolates non-S to other anti-PSA β-lactams, as well as MDR and XDR strains.

	MIC_{50}/MIC_{90} in μg/mL (% S)				
Subset (no.)	**CAZ-AVI**	**CAZ**	**P/T**	**MEM**	**AMK**
All (5,643)	2 / 4 (96.7)	2 / 32 (83.8)	4 / >64 (79.9)	0.5 / 8 (82.3)	2 / 8 (97.2)
CAZ-NS (912)	4 / 16 (79.6)	--	>64 / >64 (7.8)	4 / >8 (44.1)	4 / 16 (90.9)
P/T-NS (1,133)	4 / 16 (84.3)	32 / >32 (25.8)	--	4 / >8 (46.0)	4 / 16 (92.3)

MEM-NS (996)	4 / 16 (84.8)	16 / >32 (48.9)	32 / >64 (38.8)	--	4 / 16 (91.3)
MDR (841)	4 / 16 (79.8)	32 / >32 (21.8)	>64 / >64 (11.1)	8 / >8 (21.2)	4 / 32 (88.0)
XDR (488)	8 / 32 (71.5)	32 / >32 (9.2)	>64 / >64 (3.7)	8 / >8 (6.4)	8 / >32 (83.8)
2012 (1,966)	2 / 4 (96.9)	2 / 32 (83.2)	8 / >64 (78.3)	0.5 / 8 (82.0)	2 / 8 (97.5)
2013 (1935)	2 / 4 (96.8)	2 / 32 (84.3)	8 / >64 (78.7)	0.5 / 8 (81.9)	2 / 8 (97.3)
2014 (1,742)	2 / 4 (96.3)	2 / 32 (84.0)	4 / 64 (83.0)	0.5 / 8 (83.1)	2 / 8 (96.8)

Author Disclosure Block:

H.S. Sader: H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Allergan. **D.J. Farrell:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Allergan. **R.K. Flamm:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Allergan. **R.N. Jones:** H. Research Contractor; Self; This study and abstract presentation were funded by a research grant from Allergan..

Poster Board Number:

MONDAY-437

Publishing Title:

***In Vitro* Activity of Ceftazidime-Avibactam (CAZ-AVI) and Comparators against Gram-Negative Pathogens Isolated from Patients in Canadian Hospitals in 2009-2015: CANWARD Surveillance Study**

Author Block:

P. Lagacé-Wiens[1], H. Adam[1], A. Denisuik[2], M. Baxter[2], J. Karlowsky[1], A. Walkty[1], D. Hoban[1], G. G. Zhanel[2], Canadian Antimicrobial Resistance Alliance; [1]Diagnostic Services Manitoba, Winnipeg, MB, Canada, [2]Univ. of Manitoba, Winnipeg, MB, Canada

Abstract Body:

Background: Avibactam, a β-lactamase inhibitor of Ambler class A, C and some class D enzymes in combination with ceftazidime, is approved for the treatment of complicated urinary tract (cUTI) and intra-abdominal infections (IAI). We determined the in vitro activity of ceftazidime (CAZ) with avibactam (4 μg/mL concentration) and comparators versus Gram-negative pathogens, including extended-spectrum β-lactamase producing (ESBL) and cephalosporin-resistant, non-ESBL-producing *Enterbacteriaceae*, and *Pseudomonas aeruginosa* isolates recovered from October 2009 to December 2015 from patients in medical and surgical wards, intensive care units, clinics, and emergency rooms at 15 Canadian hospitals.**Methods:** Antimicrobial susceptibility testing was performed using broth microdilution panels following CLSI recommendations (M07-A10). Susceptibility was defined in accordance with CLSI, except for CAZ-AVI, where FDA breakpoints were used. Cephalosporin-resistant *Escherichia coli* and *Klebsiella* spp. isolates were genetically characterized for ESBL-production using PCR and sequence analysis.**Results:** The activity of CAZ-AVI and comparators is summarized in the table.

MIC_{90}/% susceptible				
	CAZ-AVI	Ceftazidime	Meropenem	TZP
Escherichia coli (4757)	0.25/100	1/93.8	≤0.03/100	4/97.8
E. coli CRO-R (400)	0.5/99.8	>32/30.8	≤0.03/99.8	16/91.8
E. coli ESBL (295)	0.5/99.7	>32/34.2	≤0.03/99.7	16/93.2
Pseudomonas aeruginosa (2337)	8/94.3	32/81.8	8/79.5	64/84.3
P. aeruginosa (CAZ-R) (271)	>16/67.2	>32/0	32/44.3	512/10.3
P. aeruginosa (TZP-R) (170)	>16/68.2	>32/1.8	32/39.4	512/0
P. aeruginosa (MER-R) (300)	16/75.3	>32/40.0	>32/0	256/46.0

Klebsiella pneumoniae (1541) *K. pneumoniae* CRO-R (71) *K. pneumoniae* ESBL (62)	0.5/100 2/100 2/100	1/96.0 >32/18.3 >32/25.8	≤0.03/99.7 0.25/93.0 0.12/96.8	8/97.3 512/64.5 >512/64.5
Enterobacter cloacae (626) *E. cloacae* CRO-R (154) *E. cloacae* ERT-R (22)	1/99.7 2/98.7 4/90.9	>32/77.3 >32/9.7 >32/4.5	0.12/99.2 0.25/96.8 2/77.3	64/85.6 128/41.6 256/27.3
Serratia marcescens (391)	0.5/100	1/99.5	0.06/99.5	4/95.9
Klebsiella oxytoca (402)	0.5/100	0.5/98.5	≤0.03/100	128/88.1
Proteus mirabilis (381)	0.12/100	≤0.25/99.0	0.12/100	≤1/100
Enterobacter aerogenes (176)	0.5/99.4	>32/76.1	0.12/99.4	32/88.6
Acinetobacter baumannii (108)	>16/--‡	32/79.6	1/95.4	64/85.2
Stenotrophomonas maltophilia (402)	>32/--*	>16/23.8	>32/--	>512/--
CAZ-AVI: ceftazidime-avibactam, CRO-R: ceftriaxone-resistant; CAZ-R: ceftazidime-resistant; TZP: piperacillin-tazobactam, ERT-R: ertapenem-resistant, ESBL: extended spectrum β-lactamase-producing. ‡63.0% of isolates had MIC ≤ 8 μg/mL *31.1% of isolates had MIC ≤ 8μg/mL				

Conclusions: CAZ-AVI demonstrated potent in vitro activity against clinical isolates of *Enterobacteriaceae*, including those with resistance to oximinocephalosporins by a variety of mechanisms. *P. aeruginosa* were highly susceptible to CAZ-AVI overall, while CAZ, MER and TZP-resistant *P. aeruginosa* were moderately susceptible to CAZ-AVI. Activity against *A. baumannii* was not improved compared to CAZ alone. Activity against *S. maltophilia* was poor but somewhat better than CAZ alone. CAZ-AVI may be useful for the treatment of cUTI and IAI caused by β-lactam-resistant *Enterobacteriaceae* and *P. aeruginosa*.

Author Disclosure Block:

P. Lagacé-Wiens: None. **H. Adam:** None. **A. Denisuik:** None. **M. Baxter:** None. **J. Karlowsky:** None. **A. Walkty:** None. **D. Hoban:** I. Research Relationship; Self; Abbott, Astellas, Pharmascience, Sunovion, Tetraphase, The Medicines Company, Cubist, Merck, Basilea. **G.G. Zhanel:** I. Research Relationship; Self; Astellas, Basilea, Merck, Pharmascience, Sunovion, Tetraphase, The Medicines Company, Cubist.

Poster Board Number:

MONDAY-438

Publishing Title:

Efficacy of OP0595, a Novel Serine-β-Lactamase Inhibitor, in a Mouse Pneumonia Model Caused by ESBL-Producing *K. pneumoniae*

Author Block:

N. Kaku, K. Kosai, K. Takeda, Y. Morinaga, T. Miyazaki, K. Izumikawa, H. Mukae, **K. Yanagihara**; Nagasaki Univ., Nagasaki, Japan

Abstract Body:

Background: OP0595 is a novel serine beta-lactamase inhibitor. Since it acts at PBP2 and shows an enhancer effect with beta-lactams, which inhibit PBP3, the combination of OP0595 and beta-lactams such as cefepime (FEP), showed good *in-vitro* antimicrobial activities. In this study, we investigated the antimicrobial effect of the combination of OP0595 and FEP in a pneumonia mouse model. **Methods:** A pneumonia mouse model was generated by the transtracheal-inoculation of ESBL-Kpn at 10^6 CFU/mouse. We treated the mice by intraperitoneal with normal saline (control), 20 mg / kg of OP0595, 100mg / kg of FEP or combination of OP0595 and FEP at an interval of every 12 hours (from 12 hours post-inoculation).**Results:** In the survival study, only combination therapy improved the survival rates, and the survival rate was significantly higher in combination therapy than the others ($P < 0.001$, log-rank test) **(Fig.A)**. The bacterial counts in the lungs had significantly decreased in combination, compared with the others ($P < 0.001$ versus the others) **(Fig.B)**. In the combination therapy, the efficacies in survival rate and bacterial counts were concentration dependent **(Fig.C and D)**. On the basis of the MICs against the ESBL-Kpn and pharmacokinetic analysis, the %T>MIC for 4, 20 or 100 mg/kg of FEP and 20 mg/kg of OP0595 at an interval of every 12 hours were calculated as 0, 3.1, 9.6 and 6.5% in alone, and all 14.1% in combinations.**Conclusions:** The combination of OP0595 and FEP showed good *in-vivo* antimicrobial effect against ESBL-Kpn. Since the %T>MIC for all doses of FEP in combination with OP0595 was the same, OP0595 might act as a β-lactamase-independent β-lactam enhancer to FEP.

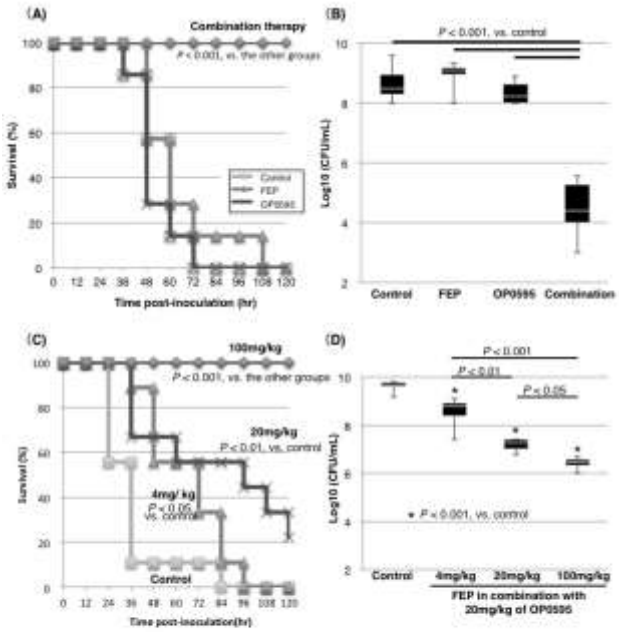


Figure. The survival rate (A and C) and bacterial count in the lungs (B and D).
We treated the mice by intraperitoneal with normal saline (control), 20 mg/kg of OP0595, 100 mg/kg of FEP or combination of OP0595 and FEP (combination) (A and B). We treated the mice with intraperitoneal with normal saline (control) or 4, 20, or 100 mg/kg of FEP in combination with 20 mg/kg of OP0595 (C and D). The survival rate until 120 hours post-inoculation (A and C) and the bacterial count in the lungs at 36 hours post-inoculation were evaluated (B and D). n=6 in each groups (D)

Author Disclosure Block:

N. Kaku: I. Research Relationship; Self; Meiji Seika Pharma Co., Ltd. **K. Kosai:** I. Research Relationship; Self; Meiji Seika Pharma CO., Ltd.. **K. Takeda:** None. **Y. Morinaga:** None. **T. Miyazaki:** None. **K. Izumikawa:** None. **H. Mukae:** None. **K. Yanagihara:** I. Research Relationship; Self; Meiji Seika Pharma Co., Ltd..

Poster Board Number:

MONDAY-439

Publishing Title:

Antimicrobial Susceptibility and Molecular Epidemiology of *Clostridium difficile* Isolates from US Patients with *C. difficile*-Associated Diarrhea (CDAD) from LCD-CDAD-11-06

Author Block:

D. Devaris[1], L. Chesnel[1], S. E. Dale[2], **J. Nary**[2], S. Johnson[3], S. P. Sambol[3], D. M. Citron[4], R. V. Goering[5]; [1]Merck & Co., Inc., Kenilworth, NJ, [2]ACM Med. Lab./ACM Global Central Lab., Rochester, NY, [3]Edward Hines, Jr., Veterans Admin. Hosp., Hines, IL, [4]R.M. Alden Res. Lab., Culver City, CA, [5]Creighton Univ. Sch. of Med., Omaha, NE

Abstract Body:

Background: This study determined susceptibility and epidemiology of *Clostridium difficile* isolates from North America collected from 2012-2015 during the conduct of LCD-CDAD-11-06 (NCT01598311), a phase 3, randomized, double-blind, multi-center trial of surotomycin vs vancomycin in adults with CDAD.**Methods:** *C. difficile* isolates were recovered from baseline fecal samples of microbiological modified intent-to-treat patients from clinical centers in the US (57) and Canada (8). Susceptibility testing was performed by broth microdilution and CLSI-recommended agar dilution methods. Isolates were typed by ribotyping and restriction endonuclease analysis (REA).**Results: Results:** Isolates (N=424) were evaluated from 328 patients in the US and 96 in Canada. In the US fecal samples, 15.9% of strains vs 19.8% of strains from Canadian centers were identified as the epidemic strain BI/NAP1/027 (RT-027). The REA type Y/ribotype 014/020 strain was the most common type found in US patients at 22.0%. Isolates of REA type BK/ribotype 078 were also recovered from US patients. Against RT-027 strains, the *in vitro* activities of surotomycin and vancomycin were similar ($MIC_{50/90}$=0.25/0.25 µg/mL by broth microdilution; $MIC_{50/90}$=1/2 µg/mL by agar dilution). Against non-RT-027 strains, the *in vitro* activity of surotomycin and vancomycin were similar ($MIC_{50/90}$=0.5/2 µg/mL by agar dilution; $MIC_{50/90}$=0.25/0.25 and 0.25/0.5 µg/mL by broth microdilution for surotomycin and vancomycin respectively).**Conclusions:** In this study, the Y/014/020 strain of *C. difficile* was the most prevalent type isolated from US centers. The epidemic BI/NAP1/027 strains represented 15.9% and 19.8% of the US and Canadian isolates, respectively. $MIC_{50/90}$ values and ranges from this study are in agreement with those determined previously from other clinical trials and surveillance studies. From these data, epidemiological cut-off values of 4 µg/mL for agar dilution and 2 µg/mL for broth microdilution can be proposed.

Author Disclosure Block:

D. Devaris: None. **L. Chesnel:** D. Employee; Self; Merck. K. Shareholder (excluding diversitied mutual funds); Self; Merck RSUs. **S.E. Dale:** D. Employee; Self; ACM Global Central Laboratory, which was contracted to perform portions of this study. **J. Nary:** None. **S.**

Johnson: E. Grant Investigator; Self; Lab PI for study. **S.P. Sambol:** None. **D.M. Citron:** D. Employee; Self; RM Alden Research Lab and did the testing. **R.V. Goering:** None.

Poster Board Number:

MONDAY-440

Publishing Title:

Ridinilazole (Rdz) for *Clostridium difficile* Infection (Cdi) - Further Data from the Codify Phase 2 Clinical Trial

Author Block:

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Abstract Body:

Background: CDI is the most common hospital acquired infection in the USA. RDZ is a novel antimicrobial for CDI with a highly targeted spectrum of activity expected to reduce collateral damage to gut microbiota during therapy. Here we report additional safety and efficacy data from the CoDIFy proof-of-concept Phase 2 clinical trial.**Methods:** This multi-centre, double-blind, study randomized 100 patients 1:1 to 10 days RDZ 200 mg BID or vancomycin (VAN) 125 mg QID treatment. Clinical response was assessed 2 days after end of therapy (EOT). Primary endpoint was non-inferiority on sustained clinical response (SCR), defined as clinical response at EOT with an absence of recurrent disease for the next 30 days. The primary analysis population was the modified intent-to-treat (MITT) which included all randomized subjects with a diagnosis confirmed by presence of free toxin in stool.**Results:** The study exceeded its primary endpoint, with RDZ shown to be superior on SCR to VAN with rates of 66.7% and 42.4% respectively (difference in treatment proportions 21.1%; 90% CI 3.1, 39.1). Rates of clinical cure at EOT were 77.8% and 69.7% for RDZ and VAN, respectively (difference in treatment proportions 8.3%; 90% CI -9.3, 25.8). A marked reduction in recurrent CDI for RDZ (14.3%) compared with VAN (34.8%) accounted for superior SCR. When SCR for the MITT was analysed across subgroups at high risk of recurrence, RDZ was favoured over VAN with estimated improvements (90% CI) for patients >75 years of age of 42.7% (9.7, 75.7), for severe disease of 15.9% (-29.8, 61.6) and for prior episodes of CDI of 19.9% (-22.8, 62.5). There were no clinically important differences in overall adverse events (AEs) or serious adverse events (SAEs) between groups. 41 vs 40 AEs and 8 vs 9 SAEs were recorded for RDZ and VAN, respectively. RDZ was associated with reduced gastrointestinal AEs (40% vs 56%). Two deaths on the study were both in the VAN arm. Administration of RDZ resulted in day 5 faecal concentrations markedly above MIC (mean 1298 µg/g) and low systemic exposure (mean day 5 plasma concentrations 0.16 ng/mL).**Conclusions:** RDZ has been shown in an RCT to be highly effective at reducing recurrent CDI which is likely due to its microbiome sparing characteristics. Further clinical development in Phase 3 studies is warranted.

Author Disclosure Block:

R.J. Vickers: E. Grant Investigator; Self; Wellcome Trust. K. Shareholder (excluding diversitied mutual funds); Self; Summit. **M.H. Wilcox:** C. Consultant; Self; Actelion, Astellas, The Medicines Company, Merck, Novartis, Pfizer, Sanofi-Pasteur, Synthetic Biologics, VH Squared. E. Grant Investigator; Self; Actelion, Astellas, bioMerieux, Da Volterra, Merck, Summit. **D.N. Gerding:** C. Consultant; Self; Merck, Roche, Novartis, Cangene, Sanofi Pasteur, Actelion.

Poster Board Number:

MONDAY-441

Publishing Title:

Ridinilazole For *Clostridium difficile* Infection (CDI). Reductions in Calprotectin and Lactoferrin During Therapy

Author Block:

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Abstract Body:

Background: CDI is characterised by inflammation of the intestine due to secretion of toxins by *C. difficile*. Ridinilazole (RDZ) is a novel antimicrobial for CDI that has been shown in a Phase 2 clinical trial to be effective a reducing rates of recurrent CDI when compared with vancomycin (VAN). Here we report data on reductions in the key inflammatory markers calprotectin and lactoferrin monitored in faecal samples collected during the course of therapy in the Phase 2 clinical trial.**Methods:** Subjects were randomised to 10 days of either ridinilazole or vancomycin treatment. Faecal samples were collected at the time of randomisation (prior to initiation of therapy) and at day 5 and day 10. Concentrations of lactoferrin and calprotectin were assessed by validated methods, following FDA and EMA guidelines, using commercially available ELISA kits from ALPCO (Salem, NH, USA) and BioLegend (San Diego, CA, USA) respectively. Only those subjects with a diagnosis of CDI confirmed by the presence of free toxin in stool were considered for this analysis.**Results:** A total of 69 subjects (36 RDZ; 33 VAN) were analysed. Median baseline (BL) faecal concentrations of lactoferrin for subjects receiving RDZ or VAN were 183.95 µg/g and 263.37 µg/g respectively. For calprotectin median BL concentrations were 1948.10 µg/g and 2446.13 µg/g for RDZ and VAN treated subjects respectively. Over the course of therapy, 1.34 and 1.30 log10 reductions in median lactoferrin concentrations were recorded for RDZ and VAN respectively for all subjects. When considering only those subjects with severe CDI, greater reductions in lactoferrin were observed with RDZ therapy with median log10 reductions of 1.93 compared with only a 0.62 recorded for VAN treated subjects. Similar results were observed with calprotectin with 0.89 and 0.92 log10 reductions for all patients receiving either RDZ or VAN, respectively, whereas for those patients with severe CDI 1.70 and 0.22 Log10 reductions with RDZ or VAN were recorded.**Conclusions:** These data indicate that RDZ therapy is associated with more pronounced reductions in key markers of intestinal inflammation for subjects with severe CDI when compared to subjects receiving VAN.

Author Disclosure Block:

Y. Ke: None. **R. Vickers:** D. Employee; Self; Summit. K. Shareholder (excluding diversitied mutual funds); Self; Summit. **Y. Li:** None.

Poster Board Number:

MONDAY-442

Publishing Title:

Analysis of *C. difficile* Isolated from Stools of Patients Enrolled in a Phase II Clinical Trial of Ridinilazole (Smt 19969)

Author Block:

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Abstract Body:

Background: Ridinilazole is a novel agent being developed for treatment of *C. difficile*-associated diarrhea (CDAD). A phase II randomized trial comparing ridinilazole orally to vancomycin in patients with moderate and some severe CDAD has recently been successfully completed. As part of the clinical trial analysis, stools from patients enrolled in the trial were evaluated for quantity of *C. difficile, C. difficile* ribotype, toxin gene profile and *in-vitro* susceptibility to a panel of antimicrobial agents. **Methods:** Stools from patient enrolled in the phase II clinical trial of patients with CDAD were sent to Tufts Medical Center. Following isolation and confirmation of *C. difficile,* MICs for ridinilazole and a panel of antimicrobial agents were measured by agar dilution using CLSI M11-A8 methods. Toxin gene profiling was performed using the method of Persson et al. and ribotyping was determined by capillary gel electrophoresis.**Results:** Of 100 patients enrolled in the phase 2 trial, 91 had day 1 samples, 90 (98.9%) of whom had isolates of *C. difficile* recovered. One patient had 2 different morphologies of *C. difficile* on day 1; these proved to be 2 different strains for a total of 109 strains evaluated. The median colony count was 1.2×10^4 (range 2.5×10^2 - 7.0×10^6). There were 18 patients with recurrence, all grew *C. difficile*. Ribotypes of patients with recurrence matched initial isolates, except in one instance. All isolates were inhibited by ridinilazole with a median MIC of 0.12 mcg/ml (range 0.06-0.5), including all isolates from patients with recurrence. The median vancomycin MIC was 1 mcg/ml (range 0.5-4 mcg/ml). The most common ribotypes seen in this trial were 014-20 (14), 027 (13), 311 (8), and 002 (7). Toxin gene profiling was able to be performed on 99.1% of isolates. Binary toxin genes (*cdtA/cdtB)* were present in 33% of strains where toxin was detectable.**Conclusions:** Ridinilazole was active against *C. difficile* isolates from CDAD patients enrolled in this clinical trial. Recurrence was not associated with a change in susceptibility. A relatively small proportion of isolates in this trial were ribotype 027.

Author Disclosure Block:

D.R. Snydman: C. Consultant; Self; Merck, Cubist, Summit PLC, BioK+, MedImmune, Chimerix. E. Grant Investigator; Self; Merck, Cubist, Actelion, Summit PLC, Tetraphase. L.

Speaker's Bureau; Self; Cubist. **L.A. McDermott:** None. **C.M. Thorpe:** None. **J. Chang:** None. **J. Wick:** None. **S.T. Walk:** None. **R. Vickers:** D. Employee; Self; Summit Plc.

Poster Board Number:

MONDAY-443

Publishing Title:

Resistance Development Studies with Ridinilazole (RDZ) and *Clostridium difficile*

Author Block:

D. Hall[1], C. Pillar[1], R. Vickers[2], **D. Shinabarger**[1]; [1]Micromyx, LLC, Kalamazoo, MI, [2]Summit Therapeutics, Oxford, United Kingdom

Abstract Body:

Background: *C. difficile* infection is a major burden on healthcare resources. RDZ (SMT19969) is a novel antibiotic with good *C. difficile* activity but sparing of the gut microbiota. Resistance development of *C. difficile* to RDZ and comparators was evaluated through spontaneous mutation frequency (FREQ) and serial passage studies.**Methods:** Susceptibility testing of RDZ and comparators was determined by standard agar and broth methods (CLSI M11) against *C. difficile* isolates. FREQ testing was conducted with agar plates containing 4 and 8-fold the MIC of RDZ or vancomycin (VAN) for 2 *C. difficile* isolates. Serial passage studies were conducted with RDZ, fidaxomicin (FDX), VAN, or ciprofloxacin (CIP) using the broth macrodilution method and Brain Heart Infusion broth. Growth from sub-MIC tubes at each passage was used as the inoculum for each subsequent passage. A total of 15 passages were completed with RDZ and the comparator agents.**Results:** The 2 isolates for the FREQ study had initial RDZ agar dilution MIC values of 0.25 μg/mL, and FREQ values are shown in the table below.

Organism	Spontaneous Mutation Frequency			
	Ridinilazole		Vancomycin	
	4X MIC	8X MIC	4X MIC	8X MIC
C. difficile ATCC 700057	7.0×10^{-8}	5.6×10^{-8}	$\leq 7.0 \times 10^{-9}$	$\leq 7.0 \times 10^{-9}$
C. difficile NCTC 13307 (toxin AB)	1.4×10^{-8}	$\leq 7.0 \times 10^{-9}$	$\leq 7.0 \times 10^{-9}$	$\leq 7.0 \times 10^{-9}$

At 4-fold the MIC, RDZ yielded FREQ values of $1.4\text{-}7.0 \times 10^{-8}$, and at 8-fold the MIC the FREQ values ranged from $\leq 7.0 \times 10^{-9}$ to 5.6×10^{-8}. Agar dilution susceptibility testing of RDZ spontaneous mutants revealed that most isolates did not have elevated RDZ MIC values. No spontaneous mutants were obtained with VAN. For serial passage studies, no resistance development occurred after 15 passages with RDZ or FDX for the 2 evaluated isolates, and low level resistance detected with VAN and CIP was generally lost after transfer over drug-free

agar.**Conclusions:** Very few spontaneous mutants were selected in the presence of RDZ, and confirmatory susceptibility testing with mutants revealed a 4-fold increase in the RDZ MIC for a few of the *C. difficile* ATCC 700057 spontaneous mutants only. No resistance development occurred for RDZ after 15 serial passages with 2 *C. difficile* isolates.

Author Disclosure Block:

D. Hall: H. Research Contractor; Self; Summit Therapeutics. **C. Pillar:** H. Research Contractor; Self; Summit Therapeutics. **R. Vickers:** B. Collaborator; Self; Summit Therapeutics shareholder. **D. Shinabarger:** H. Research Contractor; Self; Summit Therapeutics.

Poster Board Number:

MONDAY-444

Publishing Title:

***In Vivo* Efficacy of Dual-Action Molecule Tnp-2092 in Hamster and Mouse *Clostridium difficile* Infection Models**

Author Block:

X. XU[1], W. J. WEISS[2], G. T. ROBERTSON[3], F. GUO[4], C. LI[4], J. ZHANG[4], L. JIANG[4], M. YANG[4], D. XU[4], X. WANG[1], Z. MA[1]; [1]TENNOR THERAPEUTICS LTD., SUZHOU, China, [2]Univ. OF NORTH TEXAS HEALTH Sci. CENTER, FORT WORTH, TX, [3]COLORADO STATE Univ., Fort Collins, CO, [4]WuXi AppTec (Shanghai) Co., Ltd, SHANGHAI, China

Abstract Body:

Background: TNP-2092 is a dual-acting molecule in development for the treatment of diseases associated with GI tract infections. TNP-2092 is GI restricted following oral administration and is efficacious in a mouse *H. pylori* infection model. Current studies sought to evaluate the *in vivo* efficacy of TNP-2092 in hamster and mouse *C. difficile* infection models.**Methods:** *In vitro* MICs were determined by the agar dilution method (M11-A6) using a panel of isogenic *C. difficile* strains bearing specific resistance mutations. For the hamster model, animals were pretreated with 10 mg/kg clindamycin on Day -1 and infected with 2-5 x 10^5 CFU *C. difficile* ATCC #43255 by oral gavage on Day 0. Drug treatment (20 mg/kg, PO) started 4 hours after infection and was given once daily for 5 days. Hamsters were monitored for morbidity and mortality during the 5 days of treatment and an additional 16 days for potential relapse. For the mouse model, all animals were administered cefoperazone in drinking water for 10 days followed by a single injection of clindamycin. The mice were then infected with ~5 x 10^6 CFU *C. difficile* ATCC #43255 by oral gavage. Drug treatments began 4 hours later and were given once daily for 7 days by oral gavage. TNP-2092 was given at 6.67, 20 and 60 mg/kg, vancomycin and metronidazole were given at 20 mg/kg doses. The animals were monitored for morbidity and mortality during the 7 days of treatment and an additional 16 days for relapse.**Results:** TNP-2092 treatment protected 100% hamsters from death during both the treatment and relapse period, while vancomycin only partially protected animals from death during the treatment period with all animals ultimately succumbing to infection during relapse. In the mouse model, all 3 doses of TNP-2092 rescued 100% of the infected mice and prevent relapse, while vancomycin only prevented animals from death during the treatment period but failed to provide any protection during the relapse periods.**Conclusions:** TNP-2092 is highly efficacious against lethal *C. difficile* infection in both hamster and mouse infection models.

Author Disclosure Block:

X. Xu: None. **W.J. Weiss:** None. **G.T. Robertson:** None. **F. Guo:** None. **C. Li:** None. **J. Zhang:** None. **L. Jiang:** None. **M. Yang:** None. **D. Xu:** None. **X. Wang:** None. **Z. Ma:** None.

Poster Board Number:

MONDAY-445

Publishing Title:

Impact of Surotomycin and Vancomycin Exposure on the Gut Microbiota in Correlation with Clinical Outcomes in the Phase 3 LCD-CDAD-11-06 Clinical Trial

Author Block:

L. Chesnel[1], D. Devaris[1], J. Nary[2], **S. E. Dale**[2]; [1]Merck & Co., Inc., Kenilworth, NJ, [2]ACM Med. Lab./ACM Global Central Lab., Rochester, NY

Abstract Body:

Background: The impact of surotomycin and vancomycin on gut microbiota and correlation with clinical outcomes were analyzed in patients with *Clostridium difficile*-associated diarrhea (CDAD) from LCD-CDAD-11-06 (NCT01598311), a phase 3, randomized, active-controlled, double-blind trial.**Methods:** Stool specimens were recovered from the microbiological intent-to-treat population (N=577) [surotomycin 250 mg/BID (n=285); vancomycin 125 mg/QID (n=292)] at baseline, end of treatment (EOT; Day 10-13), mid-follow-up (Day 24±3), and any recurrence through Day 40-50. Analyses by standard methods included isolation of *C. difficile* and vancomycin-resistant enterococci (VRE), quantitation of *C. difficile* spores and VRE, and quantitative polymerase chain reaction for *Bacteroides fragilis* and *Bacteroides/Prevotella* groups.**Results:** Surotomycin resulted in minimal disruption of *B. fragilis* and *Bacteroides/Prevotella* groups from baseline compared with vancomycin [$p<0.001$ for difference in $\log_{10}$ CFU/mL counts between treatment groups at EOT and Day 24]. Compared with vancomycin, a decrease in VRE count was seen in surotomycin-treated patients [$p<0.001$ for difference in $\log_{10}$ CFU/mL counts between treatment groups at EOT and p=0.004 at Day 24]. *C. difficile* spore counts (mean [standard deviation]) decreased from baseline (4.68 [1.84]/4.66 [1.84] surotomycin/vancomycin) to EOT (0.94 [1.73]/0.85 [1.67]), and then increased by Day 24 to 3.41 [2.60]/3.09 [2.71]. For both treatment groups, the change from baseline to EOT or to Day 24 in $\log_{10}$ CFU/mL counts of *B. fragilis*, *Bacteroides/Prevotella*, *C. difficile* spores, and VRE was not significantly different between patients with recurrence vs those with sustained clinical response.**Conclusions:** Surotomycin treatment resulted in minimal disruption of *B. fragilis* and *Bacteroides/Prevotella* groups and decreased VRE counts compared with vancomycin. For both treatments, change from baseline to EOT and to Day 24 in *Bacteroides/Prevotella*, *C. difficile* spore, and VRE counts was not predictive of clinical outcome. These results highlight differences in the impact of surotomycin, a narrower-spectrum agent than vancomycin, on fecal microbial composition and a lack of correlation with clinical outcomes.

Author Disclosure Block:

L. Chesnel: D. Employee; Self; Merck. K. Shareholder (excluding diversitied mutual funds); Self; Merck RSUs. **D. Devaris:** None. **J. Nary:** None. **S.E. Dale:** D. Employee; Self; ACM Global Central Laboratory, which was contracted to perform portions of this study.

Poster Board Number:

MONDAY-446

Publishing Title:

Surotomycin vs. Vancomycin in Adults with *Clostridium difficile*-Associated Diarrhea (CDAD): Primary Clinical Outcomes from Study LCD-CDAD-11-06

Author Block:

T. Louie[1], P. Daley[2], J. E. Lutz[3], U. Stoutenburgh[4], M. Jin[4], A. Adedoyin[4], K. Larson[4], D. Guris[4], L. Chesnel[4], Y. Murata[4]; [1]Univ. of Calgary, Calgary, AB, Canada, [2]Mem. Univ., St. John's, NL, Canada, [3]St. Charles Hlth.System, Bend, OR, [4]Merck & Co., Inc., Kenilworth, NJ

Abstract Body:

Background: Surotomycin has potent *in vitro* activity against *Clostridium difficile* and was studied in LCD-CDAD-11-06 (MK-4261 P006; NCT01598311), a phase 3, randomized, double-blind study of surotomycin vs vancomycin in adults with CDAD.**Methods:** Eligible patients (N=608) were randomized 1:1 to surotomycin 250 mg/BID po or vancomycin 125 mg/QID po for 10 days. Clinical response was defined as resolution of diarrhea at end of therapy (EOT) + 2 days and no need for additional antibiotic treatment after EOT. The primary efficacy endpoint was non-inferiority of surotomycin vs vancomycin based on difference in clinical response rates at EOT in the microbiological MITT population with a 10% non-inferiority margin. Two key secondary endpoints were superiority of surotomycin vs vancomycin clinical response over time (clinical response through EOT and sustained clinical response EOT-Day 40) and superiority of surotomycin vs vancomycin sustained clinical response at end of study (EOS; Day 40-50). Adverse events (AEs) were monitored and patients with cure at EOT were evaluated for CDAD recurrence until EOS.**Results:** Primary efficacy endpoint was met (difference, 1.4% [95% CI: -4.9, 7.6] between cure rates of 83.4%/82.1% with surotomycin/vancomycin). The first key secondary efficacy endpoint was not met (p=0.281). The other key secondary efficacy endpoint was not met (cure rates of 63.3%/59.0% for surotomycin/vancomycin; difference, 4.3% [95% CI: -3.6, 12.2]). Of surotomycin patients, 52.4% experienced ≥1 treatment-emergent AE vs 60.1% of those on vancomycin. Discontinuation of study drug due to AEs was seen in 4.4%/2.3% of the surotomycin/vancomycin groups. Serious AEs (SAEs) and deaths were reported in 11.2% and 3.1% of surotomycin vs 13.0% and 3.7% of vancomycin recipients, respectively. No deaths were deemed treatment related; 1 drug-related SAE occurred in the vancomycin group.**Conclusions:** Surotomycin demonstrated non-inferiority to vancomycin for primary endpoint but neither key secondary superiority endpoints were met. Surotomycin was generally well tolerated.

Author Disclosure Block:

T. Louie: H. Research Contractor; Self; Actelion; Synthetic Biologics, Cubist (Merck). J. Scientific Advisor (Review Panel or Advisory Committee); Self; Actelion, Pfizer, Sankyo Daiichi, BioK. **P. Daley:** None. **J.E. Lutz:** None. **U. Stoutenburgh:** D. Employee; Self; Merck.

M. Jin: D. Employee; Self; Merck. **A. Adedoyin:** D. Employee; Self; Merck. **K. Larson:** D. Employee; Self; Merck. **D. Guris:** D. Employee; Self; Full-time employee of Merck and own stock options. **L. Chesnel:** D. Employee; Self; Merck. K. Shareholder (excluding diversitied mutual funds); Self; Merck RSUs. **Y. Murata:** D. Employee; Self; Merck and hold Merck stock options.

Poster Board Number:

MONDAY-447

Publishing Title:

A Randomized Controlled Trial of Oral Vancomycin Followed by Fecal Transplantation versus Tapering Oral Vancomycin Treatment for Recurrent *Clostridium difficile* Infection

Author Block:

S. S. Hota[1], V. Sales[2], G. Tomlinson[1], M-J. Salpeter[1], A. McGeer[3], D. E. Low[3], **S. M. Poutanen**[3]; [1]Univ. Hlth.Network, Univ. of Toronto, Toronto, ON, Canada, [2]Markham-Stouffville Hosp., Markham, ON, Canada, [3]Univ. Hlth.Network, Mount Sinai Hosp., Univ. of Toronto, Toronto, ON, Canada

Abstract Body:

Background: Recurrent Clostridium difficile infection (RCDI) is a debilitating problem affecting 20-47% of patients with Clostridium difficile infection (CDI). Fecal transplantation (FT) is a promising treatment for RCDI, but its true effectiveness remains unknown. We compared FT with standard of care oral vancomycin taper, in adult patients with RCDI. **Methods:** In a phase 2/3, single center, open-label trial, participants experiencing recurrence of CDI were randomly assigned in a 1:1 ratio to 14 days of oral vancomycin (125 mg orally four times per day) followed by a single FT of 500 mL volume by enema or a six week taper of oral vancomycin. The primary endpoint was recurrence of CDI within 16 weeks. Crossover to the alternate treatment was offered to those with recurrences during follow-up.**Results:** The study was terminated at the interim analysis, after randomization of 30 patients. Baseline characteristics were similar in both randomization groups. Nine of 16 (56.2%) patients who received FT and 5/12 (41.7%) in the vancomycin taper group experienced recurrence of CDI. The Bayesian 95% interval for the change in risk of CDI recurrence with FT ranged from a 2.8% reduction to a 47.3% increase. There was a posterior probability of 22.2% that the FT reduced recurrences at all and only a 2.8% probability that risk was reduced by 20% or more. A futility analysis did not support continuing the study. Adverse events were similar in both groups. Four serious adverse events were reported in three patients. None were deemed related to the study interventions.**Conclusions:** A single FT by enema using fresh donor stools was not significantly different from oral vancomycin taper in reducing RCDI. Further research is needed to explore optimal donor selection, FT preparation and administration, including number of administrations.

Author Disclosure Block:

S.S. Hota: None. **V. Sales:** None. **G. Tomlinson:** None. **M. Salpeter:** None. **A. McGeer:** None. **D.E. Low:** None. **S.M. Poutanen:** None.

Poster Board Number:

MONDAY-448

Publishing Title:

Recurrent *Clostridium difficile* infection (rCDI) and colonization in the 12 months following administration of Bezlotoxumab (BEZ) alone and in combination with Actoxumab (ACT)

Author Block:

E. Goldstein[1], D. Citron[1], D. Gerding[2], M. Wilcox[3], R. Tipping[4], M. Dorr[4], L. Gabryelski[4], K. Eves[4], N. Kartsonis[4], A. Pedley[4]; [1]RM Alden Res Lab, Santa Monica, CA, [2]Hines VA Hosp, Hines, VA, [3]Univ of Leeds, Leeds, United Kingdom, [4]Merck, Kenilworth, NJ

Abstract Body:

Background: MODIFY II was a global, randomized, double-blind, placebo-controlled trial of ACT & BEZ, monoclonal antibodies which neutralize *C. difficile* toxins A & B, respectively, in adults receiving standard of care antibiotics for primary or rCDI. The trial showed that a single 10 mg/kg IV dose of ACT+BEZ (14.9%) or BEZ alone (15.7%) is superior to placebo (PBO, 25.7%) in prevention of rCDI through 12 weeks (both $p \leq 0.0003$). This abstract summarizes the results from the subset of ~300 subjects enrolled in an extension sub-study & followed for up to 12 months. **Methods:** Loose stool counts & sampling for toxigenic *C. difficile* testing were carried out for subjects with a new diarrheal episode. Also, stool samples were collected at Month 6, 9 & 12 visits to assess for *C. difficile* colonization using culture with toxin testing. **Results:** Only 3 subjects experienced rCDI during the extension phase of the study (ACT+BEZ: n=2; PBO: n=1) among those who resolved their initial episode & had no rCDI during the 12 weeks of the main study. *C. difficile* colonization rates among subjects who provided a stool sample were similar across treatment groups at each follow-up time point & ranged between 16.3% & 32.8%. **Conclusion:** In this extension cohort, rCDI rates increased only marginally when follow up time was extended from 12 weeks to 12 months. *C. difficile* colonization rates were not different in antibody versus in PBO recipients. These findings suggest that the efficacy of ACT+BEZ & BEZ seen in the main study is due to prevention of CDI recurrence rather than a delay in onset of a recurrent CDI episode.

	ACT+BEZ % (n/N)	BEZ % (n/N)	PBO % (n/N)
Main Study, rCDI			
12 Weeks	14.9 (58/390)	15.7 (62/395)	25.7 (97/378)
Extension Study			
rCDI			
12 Weeks	19.6 (22/112)	16.2 (16/99)	41.5 (34/82)
12 Months	21.4 (24/112)	16.2 (16/99)	42.7 (35/82)
Colonization			
6 Months	23.6 (21/89)	24.4 (20/82)	32.8 (23/71)
9 Months	18.0 (16/89)	16.3 (13/80)	18.8 (13/69)
12 Months	24.7 (22/89)	16.9 (13/77)	21.2 (14/66)

Author Disclosure Block:

E. Goldstein: F. Investigator; Self; Merck. **D. Citron:** F. Investigator; Self; Merck. **D. Gerding:** F. Investigator; Self; Merck. **M. Wilcox:** F. Investigator; Self; Merck. **R. Tipping:** D. Employee; Self; Merck. **M. Dorr:** D. Employee; Self; Merck. **L. Gabryelski:** D. Employee; Self; Merck. **K. Eves:** D. Employee; Self; Merck. **N. Kartsonis:** D. Employee; Self; Merck. **A. Pedley:** D. Employee; Self; Merck.

Poster Board Number:

MONDAY-449

Publishing Title:

Bezlotoxumab (Bzo) Decreases Recurrence and is Associated with a Reduction in 30-day Cdi-associated Readmissions in Hospitalized Patients with *Clostridium difficile* Infection (Cdi)

Author Block:

Y. Golan[1], E. Dubberke[2], M. Hanson[3], J. Liao[3], A. Pedley[3], M. Dorr[3], S. Marcella[3], V. Prabhu[3]; [1]Tufts, Boston, MA, [2]Washington Univ. Sch. of Med., St. Louis, MO, [3]Merck, Kenilworth, NJ

Abstract Body:

Background: MODIFY I & II demonstrated that BZO and standard of care antibiotics (SOC) prevent rCDI significantly better than placebo (PBO) + SOC when given to patients with initial or rCDI. In a *post hoc* analysis we used pooled data from the MODIFY trials to estimate 30-day all-cause and CDI-associated hospital readmission rates in hospitalized patients at high risk of rCDI. **Methods:** rCDI was defined as a new episode of diarrhea and positive stool test for toxigenic C *difficile* after resolution of baseline CDI episode. All-cause 30-day readmission was defined as % of patients hospitalized at the time of medication infusion that had a readmission within 30-days of discharge. Readmissions with a CDI-related discharge diagnosis were classified as CDI associated readmissions. Confidence intervals (CI) were estimated using Miettinen and Nurminen's method. **Results:** At baseline, 68% of patients were in a healthcare facility. The % of patients with 30-day all-cause and CDI-associated readmission & the difference between treatment arms with 95% CI are shown in the table for inpatients and by risk group. The % of inpatients with rCDI was lower with BZO + SOC [73/530 (13.8)] vs placebo (PBO) + SOC [120/520 (23.1); difference (95% CI): -9.3%, -14.0,-4.6)], consistent with the overall study population. Treatment with BZO + SOC vs PBO + SOC resulted in fewer 30-day CDI associated hospital readmissions for inpatients and for all high-risk groups assessed. **Conclusion:** Among inpatients, including those at high risk for rCDI, BZO + SOC was associated with a reduction in rCDI and 30-day CDI-associated hospital readmissions vs PBO + SOC.

	BZO + SOC, n/N (%)	PBO + SOC, n/N (%)	Difference (95% CI)
30-day all-cause readmissions	123/530 (23.2)	140/520 (26.9)	-3.7% (-9.0, 1.5)
30-day CDI-associated readmissions	21/530 (4.0)	50/520 (9.6)	-5.7% (-8.8, -2.7)
Age ≥ 65 years	11/298 (3.7)	37/308 (12.0)	-8.3% (-12.8, -4.2)
CDI prior 6 months	8/127 (6.3)	18/122 (14.8)	-8.5% (-16.6, -0.9)
Immunocompromised	5/131 (3.8)	9/112 (8.0)	-4.2% (-11.2, 1.8)
Severe CDI	2/113 (1.8)	12/116 (10.3)	-8.6% (-15.7, -2.7)
027 ribotype	7/67 (10.4)	14/81 (17.3)	-6.8% (-18.2, 4.9)

Author Disclosure Block:

Y. Golan: E. Grant Investigator; Self; Merck, Actavis. J. Scientific Advisor (Review Panel or Advisory Committee); Self; Merck, Actavis, Pfizer. L. Speaker's Bureau; Self; Merck, Actavis,

Pfizer, The Medicines Company, Theravance. **E. Dubberke:** F. Investigator; Self; Merck. **M. Hanson:** D. Employee; Self; Merck. **J. Liao:** D. Employee; Self; Merck. **A. Pedley:** D. Employee; Self; Merck. **M. Dorr:** D. Employee; Self; Merck. **S. Marcella:** D. Employee; Self; Merck. **V. Prabhu:** D. Employee; Self; Merck.

Poster Board Number:

MONDAY-450

Publishing Title:

Inactivation of Vegetative Bacteria During Production of Ser-109, a Microbiome-Based Therapeutic for *Clostridium difficile* Infection

Author Block:

S. A. ALMOMANI, J. E. BUTTON, B. M. SCHUSTER, J. G. AUNINS, M-J. LOMBARDO, **G. J. MCKENZIE**; SERES THERAPEUTICS, CAMBRIDGE, MA

Abstract Body:

Background: Fecal microbiota transplant (FMT) from healthy screened stool donors has been shown to be effective in preventing *Clostridium difficile* infection (CDI) in patients with multiply recurrent disease. FMT is minimally processed, so potential transmission of infectious agents remains a concern. Screened donors may be asymptomatic carriers or be colonized with pathogens below the limit of detection of standard assays. An alternative treatment for recurrent CDI with improved safety is needed. SER-109 is a purified ecology of bacterial spores, which was shown to be highly efficacious in the treatment of recurrent CDI in a Phase 1/2b study. SER-109 is derived from stool of healthy screened donors, but production includes processing steps such as treatment with ethanol, a well-known bactericide, parasiticide, and virucide that does not affect bacterial spores. We present spike recovery studies demonstrating that a model of the SER-109 manufacturing process kills vegetative cells.**Methods:** Model Gram-positive (*Listeria innocua, Enterococcus faecalis* and *Staphylococcus aureus*) and Gram-negative (*Salmonella enterica*) bacteria were spiked into SER-109 process intermediates to simulate contamination. Ethanol was then added to simulate process inactivation. Titers of the spiked bacteria were determined by plating on selective media.To assess whether non-spore-forming organisms were in SER-109, bacteria in SER-109 lots were cultured by standard anaerobic culture methods and 10,000 colonies were randomly picked and identified by Sanger 16S rDNA sequencing.**Results:** Ethanol treatment reduced the viable titers of spiked vegetative bacteria to below the limit of detection in less than a minute. Extrapolation of linear inactivation kinetics to process duration demonstrates a very high level of inactivation. Characterization of more than 10,000 cultivatable bacteria in SER-109 after ethanol treatment revealed only spore-forming bacteria from the phylum Firmicutes.**Conclusions:** The SER-109 manufacturing process leads to a purified fraction of bacterial spores. Ethanol processing is lethal to vegetative bacterial cells and thus reduces the risk of pathogen transmission to a level that cannot be achieved through donor screening alone.

Author Disclosure Block:

S.A. Almomani: D. Employee; Self; SERES THERAPEUTICS. **J.E. Button:** D. Employee; Self; SERES THERAPEUTICS. **B.M. Schuster:** D. Employee; Self; SERES THERAPEUTICS.

J.G. Aunins: D. Employee; Self; SERES THERAPEUTICS. **M. Lombardo:** D. Employee; Self; SERES THERAPEUTICS. **G.J. Mckenzie:** D. Employee; Self; SERES THERAPEUTICS.

Poster Board Number:

MONDAY-451

Publishing Title:

Differences in Characteristics and Outcomes among Veterans with *Clostridium difficile* Infection (Cdi) by Treatment

Author Block:

H. Morrill, J. Morton, Y. Wang, K. LaPlante, A. Caffrey; Providence VAMC, Providence, RI

Abstract Body:

Background: Clinical characteristics and outcomes by treatment among Veterans with first episode CDI are largely unknown. **Methods:** This was a retrospective study in a national cohort of adult Veterans with a first CDI episode between 2010-2014, defined as a positive stool sample for *C. diff* toxin(s) or an ICD-9 code for CDI (008.45), and receipt of ≥2 days of CDI treatment (IV or PO metronidazole [MTZ], PO or PR vancomycin [VAN|, or fidaxomicin [FID]) and no CDI episodes in prior year. Recurrence was a CDI episode within 30 days of the end of treatment. CDI admission/re-admission were defined as admission (for outpts) /re-admission (for inpts) to an acute care facility and a primary CDI diagnosis. Differences between patients treated with MTZ vs. VAN, MTZ+VAN, and FID alone or in combination with VAN +/- MTZ (FID/+) were assessed using Fisher's exact or χ^2 tests and Wilcoxon Rank Sum test.**Results:** The table below includes characteristics and crude outcomes by treatment. Data presented as n (%) or median (IQR) (*p<0.05).

	MTZ	**VAN**	**MTZ+VAN**	**FID/+**
	43,904 (81.1)	4368 (8.1)	5779 (10.7)	93 (0.2)
Median Age (IQR)	65 (58-76)	69 (63-81)*	68 (62-80)*	72 (65-82)*
Gender, male	40,958 (93.3)	4139 (94.8)*	5541 (95.9)*	89 (95.7)
White Race	32,546 (74.1)	3330 (76.2)*	4221 (73.0)	81 (87.1)*
Inpatient treatment	12,232 (27.9)	1913 (43.8)*	4320 (74.8)*	61 (65.6)*
Comorbidities				
Solid Organ Cancer	2856 (6.5)	461 (10.6)*	901 (15.6)*	19 (20.4)*
COPD	2989 (6.8)	455 (10.4)*	918 (15.9)*	11 (11.8)
CKD	3216 (7.3)	601 (13.8)*	1046 (18.1)*	23 (24.7)*
DM	3602 (8.2)	644 (14.7)*	933 (16.1)*	20 (21.5)*
30-Day CDI Recurrence	6175 (14.1)	920 (21.1)*	1534 (26.5)*	15 (16.1)

30-Day All Cause Mortality	3783 (8.6)	488 (11.2)*	1194 (20.7)*	15 (16.1)*
30-Day CDI Readmission	869/10,799 (8.1)	164/1645 (10.0)*	370/4022 (9.2)*	<5/59
30-Day CDI Hospital Admission	447/32,653 (1.4)	73/2658 (2.8)*	57/1657 (3.4)*	<5/33*

Conclusions: Patients that received VAN, MTZ+VAN, or FID/+ were sicker, older and had higher mortality rates compared to those that received MTZ. Higher recurrence rates were observed in those that received VAN and MTZ+VAN compared to MTZ. Comparative effectiveness studies are needed to identify optimal CDI treatment.

Author Disclosure Block:

H. Morrill: N. Other; Self; Supported in part by a VA VISN-1 CDA and has received research funding from Merck.. **J. Morton:** None. **Y. Wang:** N. Other; Self; Supported in part by Merck. **K. LaPlante:** N. Other; Self; Research funding or acted as an advisor, or consultant for Cubist, Davol, Forest, and Pfizer Inc. **A. Caffrey:** N. Other; Self; Received research funding from Merck and Pfizer Inc..

Poster Board Number:

MONDAY-452

Publishing Title:

Tapering Courses of Oral Vancomycin Induce Persistent Disruption of the Microbiota That Provide Colonization Resistance to *Clostridium difficile* and Vancomycin-Resistant Enterococci in Mice

Author Block:

M. E. Tomas[1], T. S. C. Mana[2], M. A. Retuerto[2], B. M. Wilson[1], P. K. Mukherjee[2], M. A. Ghannoum[2], C. J. Donskey[1]; [1]Cleveland VA Med. Ctr., Cleveland, OH, [2]Case Western Reserve Univ., Cleveland, OH

Abstract Body:

Background: Vancomycin taper regimens are recommended for the treatment of recurrent *Clostridium difficile* infections. One rationale for tapering and pulsing of the dose at the end of therapy is to reduce vancomycin selective pressure on the normal intestinal microbiota. However, it is not known if the indigenous microbiota that provide colonization resistance against *C. difficile* is re-populated during tapering courses of vancomycin.**Methods:** Mice were treated orally with fidaxomicin (30 mg/kg/d) for 10 days, vancomycin (37.5 mg/kg/d) for 10 days, vancomycin taper for 39 days (37.5 mg/kg/d for 10 days, 18.75 mg/kg/d for 7 days, 9.38 mg/kg/d for 7 days, 9.38 mg/kg/d every other day for 3 doses, and 9.38 mg/kg/d every three days for 3 doses), or saline. To assess recovery of colonization resistance, subsets of mice were challenged with 10^4 colony-forming units (CFU) of an epidemic NAP1/027 *C. difficile* strain and vancomycin resistant Enterococci (VRE) at multiple time points during and after completion of the antibiotic courses. 16S rRNA sequencing was used to analyze the fecal microbiota.**Results:** Vancomycin taper-treated mice developed disruption of colonization resistance that persisted 18 days after discontinuation of treatment, whereas mice treated with a 10 day course of vancomycin exhibited recovery of colonization resistance by 15 days after discontinuation of treatment. Fidaxomicin and saline-treated mice maintained intact colonization resistance to *C. difficile* and VRE. Firmicutes were markedly suppressed during and after completion of the vancomycin-taper, whereas Firmicutes recovered to pre-treatment levels within 2 weeks in mice receiving the 10 day course of vancomycin and remained intact in fidaxomicin-treated mice.**Conclusions:** In mice treated with 39-day tapering course of vancomycin, disruption of the indigenous microbiota responsible for colonization resistance to *C. difficile* and VRE persisted up to 2 weeks after completion of therapy. In contrast, colonization resistance recovered within 2 weeks after completion of a 10 day course of vancomycin and was not altered by fidaxomicin.

Author Disclosure Block:

M.E. Tomas: None. **T.S.C. Mana:** None. **M.A. Retuerto:** None. **B.M. Wilson:** None. **P.K. Mukherjee:** None. **M.A. Ghannoum:** None. **C.J. Donskey:** F. Investigator; Self; Merck,

Cepheid, Ecolab, Clorox. J. Scientific Advisor (Review Panel or Advisory Committee); Self; SERES, 3M.

Poster Board Number:

MONDAY-453

Publishing Title:

Impact of Oral Fidaxomicin Administration on the Intestinal Microbiota and Susceptibility to *C. difficile* Infection

Author Block:

N. Ajami[1], C. Buffie[2], E. Pamer[2], M. Wong[3], J. Petrosino[1], **L. Chesnel[4]**; [1]Diversigen, Houston, TX, [2]Sloan-Kettering, New York, NY, [3]Alkek, Houston, TX, [4]Merck, Kenilworth, NJ

Abstract Body:

Background: Antibiotic (ABX) administration disrupts the intestinal microbiota, increasing susceptibility to pathogens such as *Clostridium difficile*. We used a mouse model of *C. difficile* infection (CDI) to determine susceptibility to CDI following P.O. administration of fidaxomicin (FDX) or vancomycin (VAN) and characterized the impact of drug exposure on the intestinal microbiota. **Methods:** Samples were collected over 24 days from mice administered VAN (5.625 mg/ml), or FDX (30 mg/ml) by oral gavage once daily for 3 days. At 1,3,6,10,14, and 20 days after the administration of the third antibiotic dose, mice were challenged with *C. difficile* strain VPI 10463 by oral gavage of 1,000 spores. Fecal and intestinal samples were collected and subjected to 16S rDNA sequencing (v4v5, Illumina MiSeq). **Results:** One mouse remained susceptible to CDI 6 days after exposure to FDX. Mice remained susceptible to CDI 3, 6, and 10 days after exposure to VAN. All mice were resistant to CDI after day 10. Fecal bacterial diversity was similar for all treatment groups before ABX administration and decreased significantly 3 days after. Treatment with FDX resulted in a reduced loss of bacterial community evenness as evidenced by the Shannon Diversity Index scores. Although treatment with FDX resulted in a shift in the bacterial community structure, the loss of bacterial community was lower and recovery was faster compared to VAN treatment. The relative abundance of Enterobacteriaceae species was 9.04% compared to 70.26% in the vancomycin group across all intestinal sites 1 day after cessation. Four days after cessation of ABX, the FDX group had 25% of the initial relative abundance of Clostridiales species compared to only 10% in the VAN treated group. Recovery was marked by a lack of expansion of *Enterococcus* spp., and rapid expansion of species of Erysipelotrichaceae, Lactobacillus spp., Bifidiobacterium spp., and Turicibacter spp. across all sites. **Conclusion:** Both ABX treatments resulted in loss of bacterial diversity and a shift in the bacterial community structure and composition. All FDX exposed mice (except for 1) remained resistant to CDI post exposure, which correlated with reduced disruption and rapid recovery of the intestinal microbiome.

Author Disclosure Block:

N. Ajami: F. Investigator; Self; Merck. **C. Buffie:** F. Investigator; Self; Merck. **E. Pamer:** F. Investigator; Self; Merck. **M. Wong:** D. Employee; Self; Merck. **J. Petrosino:** F. Investigator; Self; Merck. **L. Chesnel:** D. Employee; Self; Merck.

Poster Board Number:

MONDAY-454

Publishing Title:

***Clostridium difficile* Infection Recurrence Using Vancomycin vs. Fidaxomicin in High Risk Patients**

Author Block:

K. Beaulac, J. Wick, S. Doron; Tufts Med. Ctr., Boston, MA

Abstract Body:

Background: *Clostridium difficile* infection (CDI) is a common healthcare-acquired infection with high rates of recurrence. A novel antimicrobial, fidaxomicin (F), has been associated with a lower rate of recurrence in clinical trials compared with vancomycin (V). Our institutional protocol recommends the use of fidaxomicin in patients at high risk for recurrent CDI (rCDI), which we define as age >70, creatinine clearance (CrCl) <60 mL/min, or history of CDI in the past year. We aimed to validate the clinical trial findings in a real-life cohort of patients at high risk for rCDI. **Methods:** A prospective observational study was performed from 2012-2015. Patients were included in the analysis if CDI positive, received V or F as an inpatient for at least 2 days, and considered high risk of rCDI per our protocol. The primary outcome was 30 day (30D) rCDI, but demographic data, length of stay, readmission, and mortality were compared as well. Patients were excluded if they were lost to follow up before 30D, except for the 12 month endpoint in which patients were excluded if lost before 12 months.**Results:** Of 126 patients enrolled, 58 received F as their predominant therapy, and 68 received V. There were 7 excluded in each arm for loss to follow up before 30D. Five patients experienced 30D rCDI, 4/51 F (7.8%) and 1/61 V (1.6%) (p=0.11). Fourteen patients developed rCDI within 12 months, 11/33 F (33.3%) and 3/26 V (11.5%) (p=0.034). Patients in both arms had an average of 1.6 of the 3 risk factors (p=0.46), but F patients were more likely to have a history of CDI (p=0.006) and a higher Charlson Comorbidity Index (5.2 vs. 4.2, p=0.048). In patients with history of CDI, 30D rCDI occurred in 4/28 F and 1/18 V patients (p=0.35). There were 13 instances of 30D readmission in each arm (p=0.60), with 30D mortality occurring in 9F and 4V patients (p=0.68).**Conclusions:** We were not able to demonstrate the reduction in recurrence with F seen in clinical trials. Our observed rate of rCDI was lower than expected, potentially resulting in inadequate power. Furthermore, the observational nature of the study assigned choice of therapy to the clinician, thus potential unmeasured factors led to decision to use F in otherwise higher risk patients. With history of CDI being the strongest known predictor of recurrence, the heterogeneity of this risk factor in the two arms potentially confounds the rCDI rate.

Author Disclosure Block:

K. Beaulac: C. Consultant; Self; Cubist. **J. Wick:** None. **S. Doron:** L. Speaker's Bureau; Self; Merck.

Poster Board Number:

MONDAY-455

Publishing Title:

Vancomycin-Resistant *Enterococcus* (VRE) Colonization and *C. difficile* Epidemiology: Results From the Phase 3b Deflect-1 Study

Author Block:

L. Chesnel[1], D. Devaris[1], D. Citron[2], S. Sambol[3], D. Hecht[3], P. Sears[1]; [1]Merck, Kenilworth, NJ, [2]Rm Aldan Res, Culver City, CA, [3]Hines VA Hosp, Hines, IL

Abstract Body:

BACKGROUND: This multicenter study used a randomized, placebo-controlled, double-blind design to evaluate the safety and efficacy of fidaxomicin (FDX) vs. placebo (PLC) as *Clostridium difficile*-associated diarrhea (CDAD) prophylaxis in individuals undergoing hematopoietic stem cell transplantation (HSCT). • *C. difficile* susceptibility, typing and VRE colonization were analyzed and compared across both arms. **METHODS:** Stool samples collected at Screening and End of Treatment/Early Termination (EOT/ET) for all subjects were submitted to the central microbiology laboratory and tested for colonization by VRE. • At any Unscheduled Visit (USV) where CDAD was suspected, a stool sample was collected. An aliquot was assayed locally for the presence of toxigenic *C. difficile*. For positive samples, an aliquot was shipped to the central microbiology laboratory for isolation and culture, restriction endonuclease analysis (REA), antibiotic susceptibility testing. **RESULTS:** *C. difficile* was isolated after baseline from 44 (7.3%) subjects in the mITT population, 16 subjects in the FDX arm and 28 subjects in the PLC arm. The most common REA group was non-specific REA (16 subjects), followed by Y group (10 subjects). • For all strains tested, rifaximin had the lowest MIC_{50}, MIC_{90}, and geometric mean, followed by FDX. Overall, the types and susceptibilities of isolates were similar in both treatment arms. • At Screening, 13.2% and 12.3% of subjects in the FDX and PLC arms were colonized with VRE. At follow-up (EOT/ET or USV), the incidence of VRE colonization was significantly lower in the FDX arm (15.6%) vs. the PLC arm (26.4%; p=0.0058). The proportion of subjects negative at Screening but positive at EOT/ET or USV was 2-fold higher in the PLC arm (17.7%) as compared to the FDX arm (8.4%; p=0.0090). **CONCLUSION:** All C. difficile strains were susceptible to FDX in both treatment groups with minimum inhibitory concentration distribution within the wild type range. • No changes in susceptibility were observed upon the duration of FDX exposure. • The incidence of VRE colonization was significantly lower in the FDX arm (15.6%) than in the PLC arm (26.4%) at follow-up (p=0.0058). • The proportion of subjects who developed VRE posttransplant was 2-fold higher in the PLC arm (17.7%) versus the FDX arm (8.4%; p=0.0090).

Author Disclosure Block:

L. Chesnel: D. Employee; Self; Merck. **D. Devaris:** D. Employee; Self; Merck. **D. Citron:** F. Investigator; Self; Merck. **S. Sambol:** F. Investigator; Self; Merck. **D. Hecht:** A. Board Member; Self; CSLI. F. Investigator; Self; Merck. **P. Sears:** D. Employee; Self; Merck.

Poster Board Number:

MONDAY-456

Publishing Title:

Fidaxomicin for the Management of *Clostridium difficile* Infection Non-Responsive to Oral Vancomycin Based Therapy or Recurrent/Relapsed Disease

Author Block:

N. N. Pettit, K. Mullane, Z. Han, J. Pisano; Univ. of Chicago Med., Chicago, IL

Abstract Body:

Background: *Clostridium difficile* infection (CDI) is among the most common healthcare associated infections. Optimal management of CDI non-responsive to first line therapy or those with relapsed or recurrent infection presents a clinical challenge. Fidaxomicin (FDX) is a treatment option for managing severe or recurrent CDI. At our hospital, FDX is commonly reserved for patients failing to respond to oral vancomycin (POVAN) or in the setting of relapsed/recurrent (R/R) CDI. The efficacy of FDX in the setting of persistent or R/R disease is not well established.**Methods:** All adult inpatients receiving FDX for CDI non-responsive to POVAN or R/R CDI between 1/1/2012-1/31/2015 were included. Patients were evaluated to determine incidence of relapse at <=8weeks or recurrence at >8weeks following FDX, all-cause 30-day mortality, and length of stay (LOS). Also assessed were potential risk factors for R/R following FDX.**Results:** 27 patients received FDX for the included indications, of which 6 (22%) patients had R/R (n=3 relapse, n=3 recurrence) following FDX therapy. Average LOS was 34 and 23 days in the R/R versus non-R/R group. No patients died 30 days following FDX. Risk factors assessed are shown in table 1.

TABLE 1: Risk Factors for R/R following FDX salvage therapy			
Risk Factor	R/R (n=6)	Non-R/R (n=21)	p-value
Age >65	1 (16.7)	10 (46.7)	0.2
Hematologic malignancy	2 (33.3)	6 (28.5)	0.5
WBC >15,000	1 (16.7)	6 (28.5)	0.5
SCr >1.5x baseline	2 (33.3)	4 (20)	0.4
Albumin <3.5	6 (100)	14 (70)	0.1
Concomitant PPI	5 (83.3)	10 (50)	0.1
Fluoroquinolone (FQ) w/in 24 hrs	1 (16.7)	1 (5)	0.25
Concomitant cephalosporin	3 (50)	8 (40)	0.5
History of 1 or more recurrences	1 (16.7)	2 (10)	0.4

IgG <800	4 (66.7)	10 (50)	0.094
IVIG	3 (50)	3 (15)	0.1
FDX followed by taper	2 (33.3)	10 (50)	0.4
Change to FDX after poor POVAN response	6 (100)	13 (65)	0.09

Risk factors meeting a priori of <0.20 on univariate analysis were included in multivariate regression, no statistically significant association identified.			

Conclusions: FDX is a reasonable option for CDI non-responsive to POVAN or for R/R CDI, with only 22% of patients with R/R disease following FDX for the included indications. None of the risk factors assessed were significantly associated with R/R, however the small sample size precludes definitive conclusions. A larger proportion of patients in the R/R group had recent FQ exposure, received concomitant cephalosporins, and had markers of more severe disease at baseline and a history of 1 more recurrences.

Author Disclosure Block:

N.N. Pettit: None. **K. Mullane:** F. Investigator; Self; Actelion, Astellas, Chimerix, Merck, Novartis, Optimer, ViroPharma. J. Scientific Advisor (Review Panel or Advisory Committee); Self; Optimer, Merck. **Z. Han:** None. **J. Pisano:** None.

Poster Board Number:

MONDAY-457

Publishing Title:

Transcriptomic Profiling of *Escherichia coli* Following Exposure to Smp24 and Smp43, Alpha-Helical Antimicrobial Peptides from the Egyptian Scorpion, *Scorpio maurus palmatus*

Author Block:

M. M. Tawfik[1], P. R. Heath[2], M. A. Abdel-Rahman[3], P. Strong[1], K. Miller[1]; [1]Biomolecular Sci. Res. Ctr., Sheffield, United Kingdom, [2]Sheffield Inst. for Translational NeuroSci., Sheffield, United Kingdom, [3]Suez Canal Univ., Ismailia, Egypt

Abstract Body:

Background: Scorpion venoms consist of diverse mixtures of peptides and proteins with varying activities and offer an attractive source of novel therapeutics. Smp24 (24 aa) and Smp43 (43 aa) are antimicrobial peptides (AMPs) that were identified from the venom gland of the scorpion *Scorpio maurus palmatus*. The main objective of this study was to analyse the transcriptomic response of *E.coli* following exposure to Smp peptides.**Methods:** Bacteria were cultured overnight at 37°C in MH broth, MICs were determined by serial microdilution. Using DNA micoarray, we examined the transcriptomic responses of *E. coli* to sub-inhibitory doses of Smp24 and Smp43 peptides following 5 hrs incubation. Knockout strains for differentially regulated genes were screened to assess susceptibility to Smp peptides.**Results:** Smp peptides showed activity against Gram-positive and Gram-negative bacteria with MICs ranging from 4 - 128 µg/ml. Genes were identified by microarray where there was a greater than 2-fold change ($p<0.05$) in expression compared with cells in the absence of peptides. 72 genes were down-regulated by Smp24 and 79 genes were down-regulated by Smp43. Of these, 14 genes were down-regulated in common and were associated with bacterial respiration. 52 genes were up-regulated by Smp24 alone; these genes were predominantly related to cation transport, particularly iron transport. 3 unrelated genes were specifically up-regulated by Smp43. No genes were up-regulated by both Smp24 and Smp43. 10 mutants in the knockout library increased resistance to Smp24; these genes were associated with iron transport and binding. 2 mutants increased resistance to Smp43 but these mutants differed from those affected by Smp24. 5 mutants decreased resistance to Smp24 and 7 mutants decreased resistance to Smp43. Of these, the resistance of 1 gene (fdnG, formate dehydrogenase) was decreased in common.**Conclusions:** These peptides offer a promising starting point for the development of new antimicrobial agents and transcriptomic analysis can help identify metabolic processes affected by AMPs which may be beneficial in understanding their mechanism of action.

Author Disclosure Block:

M.M. Tawfik: None. **P.R. Heath:** None. **M.A. Abdel-Rahman:** None. **P. Strong:** None. **K. Miller:** None.

Poster Board Number:

MONDAY-458

Publishing Title:

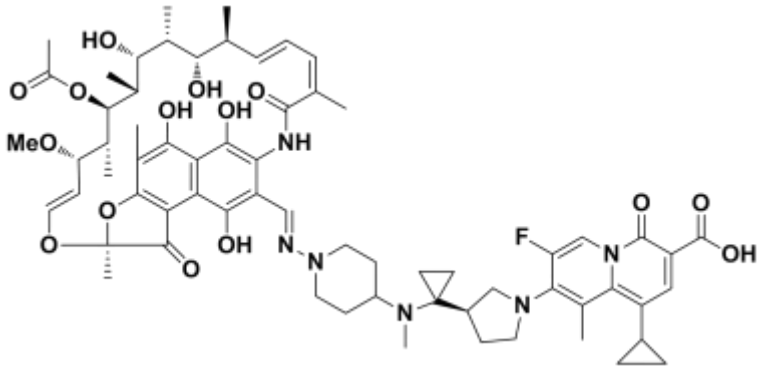
In Vitro* Evaluation of Dual-Action Molecule Tnp-2092: Studies of the Mode of Action in Gastrointestinal Pathogen *Helicobacter pylori

Author Block:

G. T. ROBERTSON[1], J. Ding[2], Z. MA[2]; [1]COLORADO STATE Univ., Fort Collins, CO, [2]TENNOR THERAPEUTICS LTD., SUZHOU, China

Abstract Body:

Background: TNP-2092 is a dual-acting molecule in development for the treatment of diseases associated with GI tract infections. It is a potent inhibitor of RNA polymerase, DNA gyrase and topoisomerase IV and exhibits a low propensity for resistance development in *S. aureus*. In the present study, the mode of action of TNP-2092 was further assessed in *H. pylori* by testing against a panel of isogenic strains with defined resistance mutations in each of its proposed cellular targets. **Methods**: TNP-2092 was tested against a panel of 14 isogenic strains derived from *H. pylori* ATCC #700392 bearing defined resistance mutations. MICs were measured on Mueller Hinton agar with 5% aged sheep blood in accordance with CLSI guideline M7-A9. All assays were conducted in a microaerophilic environment at 35-37°C for 72 h. **Results**: The MIC for TNP-2092 ranged 0.25-0.5 g/ml against *H. pylori* ATCC #700392, similar to rifampin (0.25 g/ml) and levofloxacin (0.5 g/ml). The MIC was not altered in strains bearing only *rpoB* mutations, as compared to 64 to >256-fold increase in MIC for rifampin. The MIC for TNP-2092 increased 2 to 16-fold in strains bearing *gyrA* mutations, as compared to 8 to >128-fold increase in MIC for levofloxacin. TNP-2092 showed a further 2-fold increase in MIC against a strain bearing both *rpoB* and *gyrA* mutations, relative to an otherwise isogenic strain bearing the *gyrA* allele alone. **Conclusions**: The activity of TNP-2092 against *H. pylori* is derived primarily from its quinolizinone functionality, but it also retains significant secondary antimicrobial activity attributed to the rifamycin pharmacophore.



Author Disclosure Block:

G.T. Robertson: None. **J. Ding:** None. **Z. Ma:** None.

Poster Board Number:

MONDAY-459

Publishing Title:

***In Vivo* Efficacy Of Dual-Action Molecule Tnp-2092 in Mouse *H. pylori* Infection Model: Dose Relationship and Impact of Proton Pump Inhibitor**

Author Block:

M. E. PULSE[1], W. J. WEISS[1], P. NGUYEN[1], Z. MA[2]; [1]Univ. OF NORTH TEXAS HEALTH Sci. CENTER, FORT WORTH, TX, [2]TENNOR THERAPEUTICS LTD., SUZHOU, China

Abstract Body:

Background: TNP-2092 is a dual-action molecule in development for the treatment of digestive diseases associated with GI tract infections. Previous studies indicated that TNP-2092 is highly active against *H. pylori* clinical isolates, including multidrug resistant strains. It is efficacious as a monotherapy in a mouse *H. pylori* infection model. TNP-2092 has high and prolonged exposure in the gastric mucosal layer following oral administration. Current studies further evaluate the dose relationships of TNP-2092 and impact of a proton pump inhibitor in the mouse *H. pylori* infection model.**Methods:** C57/BL6 mice were infected by *H. pylori* SS1 (CagA+, VacA+) and treatment initiated 7 days later. Mice were euthanized and their stomachs isolated, homogenized, serially diluted and plated for colony counts under microaerophilic conditions at 37°C. In the first experiment, TNP-2092 was administered orally at 5, 15 and 45 mg/kg, BID for 7 days. In the second experiment, TNP-2092 was administered orally or subcutaneously, with or without omeprazole, once or twice per day and continued for 7 or 14 days.**Results:** In the first experiment, administration of TNP-2092 at 45 mg/kg reduced stomach bacterial titers to 2.56 $\log_{10}$ CFU after 7 days of treatment. The 15 mg/kg, 5 mg/kg and vehicle control groups resulted in 4.67, 4.85 and 5.05 $\log_{10}$ CFU stomach titers respectively. In the second experiment, TNP-2092 (45 mg/kg, PO) alone reduced bacterial titers to 4.27 and 4.01 $\log_{10}$ CFU after 7 and 14 days of treatment as compared to 6.51 and 6.49 $\log_{10}$ CFU for the vehicle control group respectively. The bacterial titers for TNP-2092 when administered together with 1 mg/kg omeprazole at 7 and 14 days were 4.48 and 3.51 $\log_{10}$ CFU. TNP-2092 administered subcutaneously at 45 mg/kg did not show efficacy as compared to the untreated controls on Day 7.**Conclusions:** The effective dose for TNP-2092 in mouse *H. pylori* infection model was 45 mg/kg. Oral administration was more efficacious than subcutaneous administration and twice per day dosing was more efficacious than once per day administration. However, extension of treatment duration from 7 to 14 days and addition of omeprazole did not significantly improve overall efficacy.

Author Disclosure Block:

M.E. Pulse: None. **W.J. Weiss:** None. **P. Nguyen:** None. **Z. Ma:** None.

Poster Board Number:

MONDAY-460

Publishing Title:

***In Vivo* Efficacy of Dual-Action Molecule Tnp-2092 in Mouse *H. pylori* infection Model as Compared to Triple Therapies and Distribution within the Gastric Mucosal Layer**

Author Block:

W. J. WEISS[1], M. E. PULSE[1], P. NGUYEN[1], Z. MA[2]; [1]Univ. OF NORTH TEXAS HEALTH Sci. CENTER, FORT WORTH, TX, [2]TENNOR THERAPEUTICS LTD., SUZHOU, China

Abstract Body:

Background: TNP-2092 is a dual-action molecule in development for the treatment of diseases associated with GI tract infections. Previous studies indicated that TNP-2092 is highly active against *H. pylori* clinical isolates, including multidrug resistant strains. TNP-2092 is locally active in the GI tract after oral administration. Current studies sought to evaluate the *in vivo* efficacy of TNP-2092 in a mouse *H. pylori* infection model as compared to PrevPac (omeprazole + clarithromycin + amoxicillin) and Helidac (Bismuth salicylate + metronidazole + tetracycline) and study its distribution into gastric mucosal layer.**Methods:** C57/BL6 mice were infected by *H. pylori* SS1 (CagA+, VacA+) and treated with TNP-2092 and comparators orally, BID for 7 days. Mice were euthanized and their stomachs isolated, homogenized, serially diluted and plated for colony counts after incubation under microaerophilic conditions at 37°C. For PK studies, mice were euthanized at selected time points on Days 1 and 7 for collection of blood and stomach mucosal samples.**Results:** Low plasma exposure was observed for TNP-2092 with C_{max} of 1.2-1.5 ug/mL and $AUC_{0\text{-}inf}$ of 2.8-3.4 ug•hr/mL for Day 1 and Day 7. Exposure in the gastric mucin was high and prolonged with peak levels of 82.9-85.5 ug/g and exposures of 466.4-540.5 ug•hr/g for Days 1 and 7. At 30 mg/kg, TNP-2092 treatment resulted in stomach bacterial titers of 3.41 $\log_{10}$ CFU at 24 hours after the last dose. PrevPac exhibited comparable efficacy with bacterial titers of 3.47 $\log_{10}$ CFU. Helidac was less active with 4.42 $\log_{10}$ CFU. Neither rifampin nor rifalazil demonstrated efficacy. At 45 mg/kg, TNP-2092 reduced stomach bacterial titers to below the detection limit ($< 2.35 \log_{10}$ CFU).**Conclusions:** TNP-2092 exhibited high exposures in the gastric mucosal layer with drug levels in excess of its MIC against *H. pylori* 24-36 hours after oral administration at 45 mg/kg. This high exposure resulted in the reduction of the *H. pylori* bacterial titers to below the limit of detection after 7 days of treatment. TNP-2092 as a monotherapy appeared to be equally efficacious as PrevPac and superior to Helidac in mouse *H. pylori* infection model.

Author Disclosure Block:

W.J. Weiss: None. **M.E. Pulse:** None. **P. Nguyen:** None. **Z. Ma:** None.

Poster Board Number:

MONDAY-461

Publishing Title:

Activity of Dual-Action Molecule Tnp-2092 against *Helicobacter pylori* Clinical Isolates Collected from Three Hospitals in China

Author Block:

X. WANG[1], W. WANG[2], G. TENG[2], Y. CHU[2], Y. XIE[3], B. WANG[3], H. LU[4], D. XU[5], M. YANG[5], H. WANG[5], Y. LU[5], Z. MA[1]; [1]TENNOR THERAPEUTICS LTD., SUZHOU, China, [2]PEKING Univ. FIRST Hosp., BEIJING, China, [3]THE FIRST AFFLILIATED Hosp. OF NANCHANG Univ., NANCHANG, China, [4]Renji Hosp. of Shanghai Jiao Tong Univ. Sch. of Med., SHANGHAI, China, [5]WuXi App Tec (Shanghai) Co., Ltd, SHANGHAI, China

Abstract Body:

Background: *H. pylori* colonizes gastric mucosal layer and causes chronic gastritis, peptic ulcers, MALT lymphoma and gastric cancer. In China, *H. pylori* infects ~58% of adults and is associated with ~300,000 gastric cancer deaths per year. The eradication rate for standard triple therapy is declining due to development of antibiotic resistance, particularly to clarithromycin. Bismuth-containing quadruple therapy is now recommended as the first-line treatment in China. TNP-2092 has demonstrated potent activity against *H. pylori* in *vitro* and in *vivo*. The current studies report the antibacterial activity of TNP-2092 against *H. pylori* clinical isolates collected from three major hospitals in China. **Methods:** 239 *H. pylori* isolates were collected, including100 from primary patients admitted to The First Affiliated Hospital of Nanchang University, 78 from retreated patients admitted to Peking University First Hospital and 61 from Renji Hospital of Shanghai Jiao Tong University. MICs were measured using two-fold serial agar dilution method according to CLSI and EUCAST guidelines. *H. pylori* ATCC #43504 was used as QA strain. **Results:** See table below. **Conclusions:** TNP-2092 exhibited appreciably lower MIC_{50} and MIC_{90} values relative to comparator agents against both susceptible and drug-resistant *H. pylori* clinical isolates from China.

Source	Indicators	MIC（μg/mL）			
		TNP-2092	Clarithromycin	Levofloxacin*/Ciprofloxacin**	Metronidazole
NanChang Univ. 1st Hosp.（N=100）	MIC range	≤0.0625 - 128	≤0.0625 - 16	≤0.0625 - >64*	-
	MIC_{50}	0.125	≤0.0625	0.25*	-
	MIC_{90}	0.5	2	8*	-
Peking Univ. 1st Hosp.（N=78）	MIC range	0.016 - 16	0.016 - 256	≤0.25 - 64*	0.125 - 256
	MIC_{50}	0.5	8	8*	16
	MIC_{90}	4	16	32*	32
Renji Hosp.（N=61）	MIC range	≤0.0625 - 8	≤0.0625 - >8	≤0.0625 - >8**	0.25 - >8
	MIC_{50}	0.25	≤0.0625	2**	>8
	MIC_{90}	2	8	8**	>8

Author Disclosure Block:

X. Wang: None. **W. Wang:** None. **G. Teng:** None. **Y. Chu:** None. **Y. Xie:** None. **B. Wang:** None. **H. Lu:** None. **D. Xu:** None. **M. Yang:** None. **H. Wang:** None. **Y. Lu:** None. **Z. Ma:** None.

Poster Board Number:

MONDAY-462

Publishing Title:

Dual-Action Molecule Tnp-2092: *In Vivo* Pharmacokinetics, Excretion and Metabolic Profiles Following Intravenous and Oral Administration in Rats and Dogs

Author Block:

M. WU[1], G. QIN[1], Q. CUI[1], Y. ZHU[1], T. ZHANG[2], **Z. MA**[2]; [1]WuXi AppTec Co., Ltd, SHANGHAI, China, [2]TENNOR THERAPEUTICS LTD., SUZHOU, China

Abstract Body:

Background: TNP-2092 is a dual-acting molecule in development for the treatment of diseases associated with gastrointestinal tract infections. TNP-2092 is chemically and metabolically stable in liver microsomes and human simulated gastric juice. Current studies sought to investigate the *in vivo* pharmacokinetics, excretion and metabolic profiles of TNP-2092 following intravenous and oral administrations in rats and dogs.**Methods:** Male SD rats were administered with TNP-2092 by single intravenous bolus at 25 mg/kg and oral gavage at 100 mg/kg. Blood samples were collected for up to 24 hours, and feces, urine and bile samples were collected for up to 72 hours post dosing. Male beagle dogs were dosed with TNP-2092 by single intravenous bolus at 5 mg/kg and oral gavage at 30 mg/kg. Blood samples were collected for up to 24 hours and the feces were collected for up to 72 hours post dosing. Concentrations of TNP-2092 in plasma, urine, feces and bile samples were analyzed by using LC-MS/MS.**Results:** TNP-2092 showed low bioavailability of 1.81% in rats after single oral administration. The majority of the dose (86.7%) was recovered in feces as unabsorbed parent with low levels excretion in bile (1.22%) and in urine (0.143%). Four metabolites were identified in samples from rats as M1: dehydrogenation followed by glucuronidation; M2: glucuronidation; M3: dehydrogenation; M4: deacetylation followed by S-glutathione conjugation. M1, M2 and M3 were observed as metabolites in rat plasma and bile following oral administration. Only M3 was identified in feces and no metabolite was detected in urine samples. Similarly, TNP-2092 showed a low bioavailability of 0.315% after a single oral administration in dogs and 93.5% of dose was recovered in feces after 72 hours. Three metabolites were found in samples from dogs as M2, M3 and M5 (oxygenation). M2 was the only metabolite in plasma and no metabolites were detected in feces.**Conclusions:** TNP-2092 is a locally active drug in gastrointestinal tract. It is mainly excreted through feces as unchanged form after oral administration in rats and dogs and no linker cleavage products have been detected.

Author Disclosure Block:

M. Wu: None. **G. Qin:** None. **Q. Cui:** None. **Y. Zhu:** None. **T. Zhang:** None. **Z. Ma:** None.

Poster Board Number:

MONDAY-463

Publishing Title:

Dual-Action Molecule Tnp-2092: Caco-2 Cell Permeability, Stability and Metabolism in Liver Microsomes and Human Simulated Gastric Fluid

Author Block:

M. WU[1], G. QIN[1], S. CHEN[1], Y. ZHOU[1], T. ZHANG[2], **Z. MA**[2]; [1]WuXi AppTec Co., Ltd, SHANGHAI, China, [2]TENNOR THERAPEUTICS LTD., SUZHOU, China

Abstract Body:

Background: TNP-2092 is a dual-acting molecule in development for the treatment of diseases associated with gastrointestinal tract infections. Current studies evaluate its Caco-2 cell permeability, and its stability and metabolism in mouse, rat, dog, monkey and human liver microsomes and in human simulated gastric fluid pertaining to oral drug delivery.**Methods:** Caco-2 cell monolayer assay was performed according to an established method. Metabolic stability and metabolism of TNP-2092 in CD-1 mouse, SD rat, beagle dog, cynomolgus monkey and human liver microsomes were assessed by incubating with microsomes at 37°C for 0, 5, 10, 20, 30 and 60 min in the absence and presence of the NADPH regeneration system and analyzed by LC-MS/MS. Stability and metabolism in human simulated gastric fluid were performed by incubating TNP-2092 (10 μM) in human simulated gastric fluid for 4 hours. Potential metabolites were identified by LC-MS/MS.**Results:** The P_{app} (A-B) and P_{app} (B-A) values were 0.05×10^{-6} cm/s and 0.48×10^{-6} cm/s respectively for TNP-2092 at 10 μM. The P_{app} (B-A) value decreased to 0.03×10^{-6} cm/s in the presence of a P-gp inhibitor. After incubation with mouse, rat, dog, monkey and human liver microsomes for 60 min, there were 98.2%, 83.5%, 79.0%, 98.4% and 89.4% of TNP-2092 remaining respectively. No detectable metabolites of TNP-2092 were found. After incubating with human simulated gastric juice, only a dehydrogenation product of TNP-2092 was observed which could be a result of air oxidation. No linker cleavage products or other breakdown products were detected.**Conclusions:** TNP-2092 showed low permeability across the Caco-2 cell monolayer and might be a substrate of active transport mediated by P-gp. TNP-2092 was stable in mouse, rat, dog, monkey and human liver microsomes with or without NADPH cofactor. TNP-2092 was stable in human simulated gastric fluid and no linker cleavage products could be detected.

Author Disclosure Block:

M. Wu: None. **G. Qin:** None. **S. Chen:** None. **Y. Zhou:** None. **T. Zhang:** None. **Z. Ma:** None.

Poster Board Number:

MONDAY-464

Publishing Title:

In Vitro* Activity of FAB001, a Novel FabI Inhibitor against Antibiotic-Resistant *Neisseria gonorrhoeae

Author Block:

C. Mouze[1], S. Magnet[2], S. Hawser[2], I. Morrissey[2]; [1]FAB Pharma, Paris, France, [2]IHMA Europe, Epalinges, Switzerland

Abstract Body:

Background: FAB001 (formerly MUT056399), a novel antibacterial fatty acid biosynthesis inhibitor targeting FabI enzyme, is under clinical development by FAB Pharma for the treatment of severe human infections. A Phase I trial has already been conducted. *In vivo* results to date may have important clinical implications as FAB001 has the potential to treat *Staphylococcus aureus* infections and *Acinetobacter baumannii* infections in combination with carbapenems. The objective of this study was to investigate the activity of FAB001 against other Gram negative bacteria, such as *Neisseria gonorrhoeae*.**Methods:** A total of 76 recent antibiotic-resistant *N. gonorrhoeae* isolates with different resistance phenotypes and from various body locations (urethra, vagina, rectum, urine, wound) were tested to determine FAB001 MIC by CLSI agar dilution method.**Results:** Results are presented in the Table below. FAB001 exhibited good activity against all *N. gonorrhoeae* strains (N=76) with narrow MIC distribution and overall MIC range of ≤0.03 µg/mL to 0.5 µg/mL. MIC_{50} and MIC_{90} of FAB001 were 0.12 µg/mL and 0.25 µg/mL respectively. The activity of FAB001 was not influenced by resistance to other antimicrobials agents.**Conclusions:** FAB001 demonstrated promising *in vitro* activity against antibiotic-resistant *Neisseria gonorrhoeae.* Further studies are merited.

Antibacterial agent	*Neisseria gonorrhoeae* subset	N	MIC (µg/mL)		
			range	MIC_{50}	MIC_{90}
FAB001	All	76	≤0.03 - 0.5	0.12	0.25
	Ciprofloxacin-resistant	60	≤0.03 - 0.5	0.12	0.25
	Azithromycin-resistant	8	0.12 - 0.5	0.25	0.5
	Penicillin-resistant	43	≤0.03 - 0.25	0.12	0.12
	Tetracycline-resistant	33	0.06 - 0.5	0.25	0.25
Ciprofloxacin		76	0.001 - 64	4	32
Ceftriaxone		76	≤0.002 - 0.12	0.03	0.12
Azithromycin		76	≤0.03 - 16	0.25	1

Gentamicin		76	1 - 16	8	16
Penicillin		76	0.06 - >16	2	16
Tetracycline		76	0.06 - >16	1	16

Author Disclosure Block:

C. Mouze: None. **S. Magnet:** None. **S. Hawser:** None. **I. Morrissey:** None.

Poster Board Number:

MONDAY-465

Publishing Title:

***In Vitro* Activity of Pexiganan (Px) and Ten Comparator Antimicrobials Against Anaerobic Bacteria Recovered from Skin and Skin Structure Infections (SSSI)**

Author Block:

D. M. Citron, K. L. Tyrrell, E. J. C. Goldstein; R.M. Alden Res. Lab., Culver City, CA

Abstract Body:

Background: Pexiganan, a 22-amino acid synthetic cationic analogue of peptide magainin II, acts by selectively damaging bacterial cell membranes. PX is in Ph3 clinical development as a topical cream (0.8%) for treatment of mild infections of diabetic foot ulcer and has potential for other skin and skin structure infections. The aim of this study was to evaluate the MICs of recent anaerobic isolates associated with SSSI. **Methods:** Most of the strains were recovered during the past 3 years, although some of the unusual species were older. About 20% were from patients in Europe and Canada. PX was tested using Brucella broth supplemented with vitamin K and hemin, with and without 5% lysed horse blood (CLSI M11-A8). Lysed horse blood has the potential for increased binding of pexiganan. Agar is not suitable for testing PX due to interference by the calcium concentration in agar. Other antimicrobials including penicillin (PCN), amoxicillin-clavulanate (A-C), piperacillin-tazobactam (P-T), meropenem (MR), clindamycin (CM), doxycycline (DX), moxifloxacin (MX), metronidazole (MT), linezolid (LN) and vancomycin (VA) were tested by the agar dilution method (CLSI M11-A8). **Results:** For Gram-negative species, the no. of isolates and MIC90%s were as follows: *B. fragilis* (103), 16 µg/ml; other *Bacteroides fragilis* group spp. (67), 4 µg/ml; *Prevotella* and *Fusobacterium* spp., (82), 32 µg/ml; and *Porphyromonas* spp., (40), 64 µg/ml. For Gram-positive species the MIC 90%s were as follows: *P. acnes*, (20), 4 µg/ml; *E. lenta* and *P. anaerobius*, (20), 32 µg/ml; other Gram-positive rods and cocci, (129), 4 µg/ml; *Clostridium perfringens*, (10), 128 µg/ml; and other clostridia, (19), 256 µg/ml. None of the higher PX MICs was related to resistance in any of the other antimicrobial agents. Some of the fastidious anaerobes failed to grow in Brucella broth without lysed horse blood, thus the PX MICs reported here were all from the blood containing broth. **Conclusions:** PX showed a high level of activity against this diverse group of organisms. The concentration of PX in the cream is 8,000 µg/ml, more than 30 times the highest MIC obtained for a few of the clostridia and 500 times greater than 90% of *B. fragilis* isolates. PX shows great potential for treating SSSI involving anaerobes.

Author Disclosure Block:

D.M. Citron: None. **K.L. Tyrrell:** None. **E.J.C. Goldstein:** H. Research Contractor; Self; Dipexium.

Poster Board Number:

MONDAY-466

Publishing Title:

Sequence Optimization and Computational Design of Synthetic Antibiofilm Peptides

Author Block:

E. F. Haney, Y. Brito-Sánchez, M. J. Trimble, S. C. Mansour, A. Cherkasov, R. E. W. Hancock; Univ. of British Columbia, Vancouver, BC, Canada

Abstract Body:

Biofilms are aggregates of bacterial cells that are resistant to conventional antibiotics and are associated with many bacterial diseases and chronic infections. Specifically targeting the cells within a biofilm represents an attractive drug target and our group recently recognized that short synthetic peptides possessed potent antibiofilm activity (*PLoS Pathogens* 2014. 10(5):e1004152). While many of these antibiofilm peptides share characteristics with the well-studied antimicrobial peptides (such as positive charge and amphipathicity), the sequence requirements that govern their antibiofilm potency remain unclear. Using our most active peptide, 1018 (VRLIVAVRIWRR-NH_2), as a sequence template, a substitution library of 96 single amino acid substitution variants was SPOT-synthesized on cellulose membranes and their antibiofilm activity was assessed against biofilms formed by methicillin resistant *Staphylococcus aureus* (MRSA). The data from this *in vitro* screen was then used to establish, for the first time, quantitative structure activity relationship (QSAR) models that identified molecular descriptors of antibiofilm peptides and accurately predicted (up to 90%) the activity of antibiofilm peptides. These accurate QSAR models were subsequently used to predict the activity of 100,000 virtual peptides *in silico*. A subset of these virtual peptides were SPOT-synthesized and screened against MRSA biofilms to evaluate their activity and to further assess the quality of the QSAR models. The results revealed that the QSAR models could correctly predict ~85% of the optimized antibiofilm peptides, demonstrating the robustness of the initial models. The most active peptides were then chemically synthesized to high purity and their activity against MRSA biofilms was assessed and found to be largely comparable to 1018. Importantly, one peptide exhibited up to an 8-fold increase in antibiofilm potency compared to 1018 and eradicated MRSA biofilms grown in flow cells at a peptide concentration as low as 0.125 µM. This optimized antibiofilm sequence demonstrates that this methodology could be used to design novel antibiofilm peptides for therapeutic applications to treat biofilm associated infections.

Author Disclosure Block:

E.F. Haney: None. **Y. Brito-Sánchez:** None. **M.J. Trimble:** None. **S.C. Mansour:** None. **A. Cherkasov:** None. **R.E.W. Hancock:** None.

Poster Board Number:

MONDAY-467

Publishing Title:

Identification of *Staphylococcus pseudintermedius* Sortase A Inhibitors

Author Block:

M. Balachandran, J. Baudry, D. Bemis, S. Kania; Univ. of Tennessee, Knoxville, TN

Abstract Body:

Background: Conventional antibiotic-based therapies against *S.pseudintermedius* infections have failed in recent years due to the increased prevalence of methicillin resistance and multidrug resistance. Therefore, alternate strategies for treatment of infection have to be explored. Anti-infective therapy based on sortase inhibition holds potential to address this shortcoming. Sortase A (srtA) is a transpeptidase commonly produced by Gram-positive bacteria. It has specificity for proteins that harbor the LPXTG motif and cleaves between the threonine (T) and glycine (G) residues. These proteins are subsequently incorporated into the peptidoglycan cell wall and displayed on the surface. They include numerous virulence factors and proteins that may overcome the host immune response. The objectives of the present study were (a) to clone and express the *srtA* gene from *S.pseudintermedius* in *E.coli* and (b) to use the recombinant protein to screen for potential inhibitors against sortase A.**Methods and Result:** A *srt A* gene from *S.pseudintermedius* optimized for *E.coli* codon usage was synthesized, inserted into an expression vector and expressed successfully in *E.coli*. The size and purity of the protein were verified by SDS-PAGE and Western Blot. A FRET-based assay using a synthetic substrate, Abz-LPETG-K(Dnp)-NH_2, showed that the protein was functional and had a higher specific activity than commercially obtained *S.aureus* sortase A. Using molecular dynamic simulations, a 3-D model of sortase A was created to screen a library of compounds from the National Cancer Institute (NCI) for potential inhibitors. From the top twenty most active inhibitors, ten were selected and tested for their ability to inhibit sortase *in-vitro*. Four of these compounds showed ~50% inhibition of sortase A in the FRET-based functional assay. The effect of these compounds in an *in-vivo* model is yet to be investigated.**Conclusion:** Recent advancements in computational biology have made it possible for structure-based molecular discovery of novel compounds that could serve as potential inhibitors of sortase A. Therefore, these results indicate the possibility of sortase A inhibition as a potential therapeutic strategy in the treatment of staphylococcal infection in both humans and animals.

Author Disclosure Block:

M. Balachandran: None. **J. Baudry:** None. **D. Bemis:** None. **S. Kania:** None.

Poster Board Number:

MONDAY-468

Publishing Title:

Investigation of Antibacterial and Antifungal Activity of New Keton and Ketoxime Derivatives Containing Benzofuran, Naphthofuran or Cyclobutane Ring

Author Block:

H. Yigit, E. Demiray, M. Koca; Adiyaman Univ., Adiyaman, Turkey

Abstract Body:

In this study new ketone and benzofurane, naphthofurane or cyclobutane ring containing ketoxime derivatives were investigated for Gram (+) and Gram (-) antibacterial and antifungal activities for 4 *Candida* specieses. Minimum Inhibition Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the compounds were determined and time kill studies were carried out for the active compounds by using the CLSI methods. Compounds 4,, 21, 41 and 45 were active against some of the bacteria while compound 18 was active on some of the bacteria as well as the yeasts tested in this study. The results from the MBC and time kill studies suggested that compounds number 18 and 21 are bacteriocidal. MIC and MBC results for the *Candida* specieses were the same indicating sidal activity. MIC values of compound 18 were as follow: *Staphylococcus aureus* ATCC 29213 32-64 µg/mL, Methicillin Resistant *Staphylococcus aureus* (MRSA) 16-32 µg/mL, *Enterococcus faecalis* ATCC 29212 32-64 µg/mL, Vancomycin Resistant *Enterococcus faecalis* (VRE) 16-32 µg/mL. The MIC values of compound 18 for yeasts were: *Candida krusei* ATCC 6258 32 µg/mL, *Candida albicans* ATCC 10231 64 µg/mL, *Candida glabrata* ATCC 90030 128 µg/mL and *Candida parapsilosis* ATCC 22019 128 µg/mL. MIC values for compound 21 were: *Enterococcus faecalis* ATCC 29212 32-64 µg/mL and VRE 32-64 µg/mL. The results showed that some of the compounds containing benzofurane, naphthofurane or cyclobutane ring have anti-bacterial and/or antifungal activity. Interestingly, compounds like 18 and 21 demontsrated both anti-fungal and anti-bacterial activity. The antibacterial activity of compounds 18 and 21 included MRSA and VRE.

Author Disclosure Block:

H. Yigit: None. **E. Demiray:** None. **M. Koca:** None.

Poster Board Number:

MONDAY-469

Publishing Title:

Cell Membrane Interactions with Antimicrobial Alginate Oligog Cf-5/20

Author Block:

M. F. Pritchard[1], K. Beck[1], P. C. Griffiths[2], L. C. Powell[1], S. Khan[1], E. Onsøyen[3], P. D. Rye[3], K. E. Hill[1], D. W. Thomas[1], **E. L. Ferguson**[1]; [1]Cardiff Univ., Cardiff, United Kingdom, [2]Univ. of Greenwich, Gillingham, United Kingdom, [3]AlgiPharma AS, Sandvika, Norway

Abstract Body:

Background: Cystic Fibrosis is a chronic, autosomal-recessive disease, characterized by chronic pseudomonal lung infection and formation of antibiotic-resistant biofilms. We have shown that a low molecular weight alginate oligosaccharide therapeutic, OligoG CF-5/20, potentiates the activity of conventional antibiotics against a range of Gram-negative, multi-drug resistant pathogens including *Pseudomonas aeruginosa*. This study sought to determine whether this potentiation was the result of direct interaction with, and modulation of, the Gram-negative lipopolysaccharide (LPS) membrane. **Methods:** Cell membrane permeabilization was assessed by measuring the internalization of nitrocefin, propidium iodide and 1-N-phenylnaphthylamine (NPN) dye by *P. aeruginosa* strains and release of carboxyfluorescein from single lamellar liposomes in the presence of OligoG (0-20 mg/mL). A chromogenic endotoxin assay was used to measure the gel-clot reaction of *Limulus* amebocyte lysate (LAL) in the presence of LPS, following pre-incubation with OligoG. LPS from *P. aeruginosa* (10 mg/mL) was incubated with OligoG (0-20 mg/mL) at various pHs and NaCl concentrations and measured by electrophoretic light scattering (ELS), small-angle neutron scattering (SANS; ILL, Grenoble) and circular dichroism (CD) spectroscopy.**Results:** OligoG did not alter the surface charge of pseudomonal LPS, nor did it neutralize the biological activity or induce aggregation of LPS *in vitro*. Antibiotic potentiation by OligoG (of up to 512-fold) was independent of any significant membrane perturbation, observed in antimicrobial peptide (RTA3 or polymyxins) controls. Whilst OligoG induced subtle structural changes in LPS solution conformation at higher salt concentrations (0.1 > 0.01 > 0.001 M NaCl), these effects were pH-independent. Likewise, the CD spectra for LPS remained unaltered in the presence of OligoG. The effects seen with OligoG contrasted with the distinct conformational changes induced by colistin, which has high affinity for LPS.**Conclusions:** These data demonstrate that the novel potentiation of antimicrobial activity by OligoG does not simply reflect permeabilization of the cell membrane or structural changes to LPS.

Author Disclosure Block:

M.F. Pritchard: I. Research Relationship; Self; AlgiPharma AS Research support. **K. Beck:** I. Research Relationship; Self; AlgiPharma AS Research support. **P.C. Griffiths:** I. Research

Relationship; Self; AlgiPharma AS Research support. **L.C. Powell:** I. Research Relationship; Self; AlgiPharma AS Research support. **S. Khan:** I. Research Relationship; Self; AlgiPharma AS Research support. **E. Onsøyen:** D. Employee; Self; AlgiPharma AS. **P.D. Rye:** D. Employee; Self; AlgiPharma AS. **K.E. Hill:** I. Research Relationship; Self; AlgiPharma AS Research support. **D.W. Thomas:** I. Research Relationship; Self; AlgiPharma AS Research support. **E.L. Ferguson:** I. Research Relationship; Self; AlgiPharma AS Research support.

Poster Board Number:

MONDAY-470

Publishing Title:

MIC Reproducibility of Iron Depleted Cation Adjusted-Mueller Hinton Broth (ID-CAMHB) for Microdilution Testing of S-649266, a Novel Siderophore Cephalosporin

Author Block:

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Abstract Body:

Background: S-649266, a novel parenteral catechol-substituted siderophore cephalosporin, is active against carbapenem-resistant Gram-negative bacteria. Accurate *in vitro* testing of S-649266 by broth microdilution requires the use of iron-depleted conditions to mimic the condition in mammalian hosts. Previous MIC testing of S-649266 has used apo-transferrin containing medium and Chelex-treated Isosensitest broth (ISB) to suppress the trailing phenomenon which was observed in *Acinetobacter baumannii*. In this study, we evaluated MIC reproducibility using iron depleted Mueller Hinton Broth (ID-CAMHB) as an alternative medium for the *in vitro* microbiological assessment tests to establish the MICs for S-649266. **Methods**: Broth microdilution testing was done according to CLSI guidelines except that CAMHB was made iron deficient by pretreatment with Chelex-resin (Bio-Rad Laboratories) with subsequent replenishment of Ca, Mg and Zn. ID-CAMHB did not significantly affect the growth control. MIC endpoints were defined as the first drug well in which growth is significantly reduced (i.e. a button of < 1 mm or light/faint turbidity) relative to growth control. MIC reproducibility was determined against 20 clinical isolates (3 *Escherichia coli* including KPC and NDM strains), 4 *Pseudomonas aeruginosa* including VIM, OprD-deficient strains, 3 *Klebsiella pneumoniae* including KPC and NDM strains, 10 *A. baumannii* including OXA-23 and -24 strains. Reproducibility of S-649266 was calculated as the ratio of total number of results that fell within 1 or 2 wells of the mode results divided by total number of results. **Results**: Trailing phenomenon was observed for *A. baumannii* using ID-CAMHB although not observed in apo-transferrin containing or Chelex treated ISB. However, by using the established guidelines for reading MICs, ID-CAMHB was considered to be the appropriate medium for the MIC determination of S-649266. For the 5 of 20 test strains showing trailing phenomenon, it was possible to assign a reproducible MIC value to 471/500 (94.2%) of the isolates. **Conclusions:** Based on the good reproducibility of *in vitro* MICs obtained in ID-CAMHB, ID-CAMHB is the effective and reliable broth medium for assessing the *in vitro* activity of S-649266.

Author Disclosure Block:

M. Tsuji: D. Employee; Self; Shionogi. CO., LTD. **M. Hackel:** D. Employee; Self; IHMA Inc. **R. Echols:** D. Employee; Self; Shionogi Inc. **D. Sahm:** D. Employee; Self; IHMA Inc. **Y. Yamano:** D. Employee; Self; Shionogi. CO., LTD..

Poster Board Number:

MONDAY-471

Publishing Title:

Skin Deep - Novel Thiazole Compounds Exhibit Potent Antibacterial Activity *In Vitro* and *In Vivo* in a Methicillin-Resistant *Staphylococcus aureus* (MRSA) Skin Infection Mouse Model

Author Block:

H. Mohammad, M. Cushman, M. N. Seleem; Purdue Unversity, West Lafayette, IN

Abstract Body:

Bacterial resistance to antibiotics is a significant public health challenge that is currently affecting every geographic region of the world. One of the most problematic bacterial pathogens worldwide, methicillin-resistant *Staphylococcus aureus* (MRSA) is a significant source of skin and wound infections in humans. Many first-line antibiotics, such as mupirocin, and drugs of last resort, such as vancomycin, are losing their effectiveness as treatment options. This highlights the urgent need for identification and development of novel antibacterial agents to address this public health crisis. The present study demonstrates the ability of five thiazole compounds, synthesized by our interdisciplinary research group, to effectively and rapidly inhibit the growth of clinically-relevant strains of MRSA responsible for skin infections (at concentrations as low as 1.3 μg/mL). Interestingly, utilizing the checkerboard assay, the three most potent compounds *in vitro* exhibited a synergistic relationship when combined with mupirocin (at subinhibitory concentrations) against MRSA. Additionally, the MTS assay revealed four compounds are not toxic to human keratinocytes at concentrations up to 20 μg/mL. When tested in a murine model of MRSA skin infection, four compounds mimicked the effect of mupirocin in significantly reducing the bacterial load present in infected wounds. A preliminary mechanism of action study (using a target overexpression experiment with *Bacillus subtilis*) indicates these thiazole compounds may disrupt a pathway involved in cell wall synthesis in bacteria. Collectively, the results demonstrate these thiazole compounds warrant further investigation as a novel treatment option (alone and in combination with mupirocin) for drug-resistant staphylococcal skin infections.

Author Disclosure Block:

H. Mohammad: None. **M. Cushman:** None. **M.N. Seleem:** None.

Poster Board Number:

MONDAY-472

Publishing Title:

Phase 2 Study of Relebactam (Rel) + Imipenem/Cilastatin (Imi) vs Imi Alone in Subjects with Complicated Urinary Tract Infection (Cuti)

Author Block:

M. D. Sims[1], V. Mariyanovski[2], P. McLeroth[3], W. Akers[3], Y-C. Lee[3], M. Brown[4], J. Du[4], A. Pedley[4], N. Kartsonis[4], A. Paschke[4]; [1]Beaumont Hlth., Royal Oak, MI, [2]Univ. Hosp. N. I. Pirogov, Sofia, Bulgaria, [3]Covance Inc, Princeton, NJ, [4]Merck Sharp & Dohme Corp., North Wales, PA

Abstract Body:

Background: REL (MK-7655) is a novel Class A/C ß-lactamase inhibitor intended for use in combination with IMI for the treatment of Gram-negative bacterial infections. REL restores imipenem activity against resistant Enterobacteriaceae and *Pseudomonas* strains.**Methods:** In this multicenter double-blind study, subjects ≥18 years of age with cUTI or acute pyelonephritis were randomized (1:1:1) to IMI + REL 250 mg, IMI + REL 125 mg, or IMI + placebo, each given IV every 6 hours for 4-14 days. Efficacy was evaluated at discontinuation of IV therapy (DCIV), early follow-up (EFU), and late follow-up (LFU). The primary endpoint was the proportion of microbiologically evaluable (ME) subjects with a favorable microbiological response (MR) at DCIV, assessed by non-inferiority testing with a 15% margin.**Results:** Of 302 randomized subjects, 298 were treated and 230 (76%) were ME at DCIV (52% female, mean age 56 years; 51.7% with cUTI, 48.3% with pyelonephritis). 25 subjects (11% of the ME population) had imipenem-resistant Gram-negative infections at baseline. Efficacy and safety outcomes were similar across treatment groups, as shown below (see table). Both doses of REL + IMI were non-inferior to IMI alone: the favorable MR rate at DCIV was 95.5% for IMI + REL 250 mg, 98.6% for IMI + REL 125 mg, and 98.7% for IMI + placebo.**Conclusions:** In subjects with cUTI, both doses of REL + IMI were at least as effective as IMI alone, with respect to MR at DCIV, and were generally well tolerated.

Proportion of subjects with favorable microbiological response (ME population[†])

	Treatment Group			REL vs Placebo Comparison	
	IMI + REL 250mg % (n/m)	IMI + REL 125mg % (n/m)	IMI + Placebo % (n/m)	IMI + REL 250mg Difference (95% CI)[‡]	IMI + REL 125mg Difference (95% CI)[‡]
DCIV	95.5 (64/67)	98.6 (70/71)	98.7 (74/75)	-3.3 (-11.2, 3.2)	-0.1 (-6.4, 5.9)
EFU	61.5 (40/65)	68.1 (49/72)	70.4 (50/71)	-8.9 (-24.6, 7.1)	-2.4 (-17.4, 12.8)
LFU	68.3 (43/63)	65.2 (45/69)	62.5 (45/72)	5.8 (-10.4, 21.5)	2.7 (-13.1, 18.4)

Proportion of subjects with adverse events (APaT population[§])

	Treatment Group			REL vs Placebo Comparison	
	IMI + REL 250 (N=99) % (n)	IMI + REL 125 (N=99) % (n)	IMI + Placebo (N=100) % (n)	IMI + REL 250 Diff (95% CI)[‡]	IMI + REL 125 Diff (95% CI)[‡]
One or more AEs	28.3 (28)	29.3 (29)	30.0 (30)	-1.7 (-14.3, 10.9)	-0.7 (-13.4, 12.0)
Drug-related AEs	10.1 (10)	9.1 (9)	9.0 (9)	1.1 (-7.5, 9.8)	0.1 (-8.4, 8.6)
Serious AEs	3.0 (3)	1.0 (1)	3.0 (3)	0.0 (-5.8, 5.9)	-2.0 (-7.6, 2.8)
Discontinued due to AE	3.0 (3)	1.0 (1)	2.0 (2)	1.0 (-4.4, 6.8)	-1.0 (-6.1, 3.7)
Most common AEs					
Diarrhea	5.1 (5)	2.0 (2)	4.0 (4)	—	—
Nausea	4.0 (4)	6.1 (6)	4.0 (4)	—	—
Headache	7.1 (7)	3.0 (3)	4.0 (4)	—	—

CL confidence interval; EFU (early follow-up), 5-9 days post therapy; LFU (late follow-up), 28-42 days post therapy.
† ME population excludes subjects without gram-negative and/or anaerobic, pathogenic organism in prestudy culture; subjects who received < 96 hours of IV study therapy; and subjects with significant violations of inclusion/exclusion criteria. In addition, subjects with indeterminate or missing response at a particular time point were excluded from the analysis (17 at DCIV, 20 at EFU, and 23 at LFU).
‡ Difference (95% CI) based on unconditional asymptotic Miettinen and Nurminen method without stratification.
§ APaT (All Patients as Treated) population includes all randomized subjects who received at least one dose of IV study therapy; subjects are presented according to the study treatment actually received.

Author Disclosure Block:

M.D. Sims: E. Grant Investigator; Self; Merck. H. Research Contractor; Self; Merck, Abbott, Cempra, Curetis AG, Bayer Healthcare AG, Theravance, Debiopharm, AstraZenica, Aradigm, Sanofi Pasteur, Gilead, Rempex, Pfizer, Seres Health, Synthetic Biologics. **V. Mariyanovski:** H. Research Contractor; Self; Merck. **P. McLeroth:** H. Research Contractor; Self; Merck. **W. Akers:** H. Research Contractor; Self; Merck. **Y. Lee:** H. Research Contractor; Self; Merck. **M. Brown:** D. Employee; Self; Merck Sharp & Dohme Corp. **J. Du:** D. Employee; Self; Merck Sharp & Dohme Corp. **A. Pedley:** D. Employee; Self; Merck Sharp & Dohme Corp. **N. Kartsonis:** D. Employee; Self; Merck Sharp & Dohme Corp. **A. Paschke:** D. Employee; Self; Merck Sharp & Dohme Corp..

Poster Board Number:

MONDAY-473

Publishing Title:

Minimum Inhibitory Concentration (MIC) of New Heteroarotinoids Drugs for *Mycobacterium tuberculosis* (MTB)

Author Block:

S. N. M. Hanif[1], D. Benbrook[2], L. Garcia-Contreras[2]; [1]Bastyr Univ., San Diego, CA, [2]Oklahoma Hlth.Sci. Ctr., Oklahoma City, OK

Abstract Body:

Background: New drug entities are desperately needed to improve tuberculosis (TB) treatment and the threat of drug resistant strains. Evaluation of new drug candidates require accurate drug susceptibility testing (DST) for MTB. Heteroarotinoid compounds that have demonstrated efficacy against cancer share same structural characteristics with some existing anti-TB compounds. In this study, we performed DST of MTB to these compounds using two different methods. **Methods:** Middlebrook 7H11 medium supplemented with OADC was used to prepare DST plates with different concentrations of drugs SA, SB, SC or isoxyl (a known anti-TB drug used as control). 100 µl of MTB ($1x10^5$) were inoculated on the plates. Plates were incubated (37^oC, 5% CO_2) for 3 weeks. Colonies were counted visually. For Alamar Blue test, drugs were incubated with Middlebrook 7H9 (OADC) and MTB ($1x10^5$) for 8 days and MIC determined visually by color change from blue to pink after 24h of incubation with Alamar Blue. **Results:** Tested concentrations of 1, 2.5, 3, 5, 7.5, 10, 20 and 30 µg/ml for all drugs in agar diffusion test showed that MIC for SC, Isoxyl, SB and SA were 1, 5, 5, 10 µg/ml, respectively. Whereas, Alamar Blue assay resulted in MIC of 1.25, 5, 1.25, 5 µg/ml for SC, Isoxyl, SB and SA, respectively. The slight difference in the MIC obtained with Alamar Blue assay could be because of different in the media as both Middlebrook 7H9 and 7H11 media are different in their composition. **Conclusions:** Heteroarotinoids exhibit promising anti-TB activity with similar or greater efficacy compared to isoxyl. Particularly, SA could be a leading candidate since formal toxicological studies show no adverse effect to this drug. The Alamar Blue assay showed similar results to the agar plate method, thus it could be reliable for DST and save testing time.

Author Disclosure Block:

S.N.M. Hanif: None. **D. Benbrook:** None. **L. Garcia-Contreras:** None.

Poster Board Number:

MONDAY-474

Publishing Title:

Study of Anti-Bacterial Activity of Baicalin on Mycobacterium tuberculosis

Author Block:

H-L. Eng[1], J-Y. Chen[2], Y-F. Wang[1], T-M. Lin[3]; [1]Kaohsiung Chang Gung Mem. Hosp. and Chang Gung Univ. Coll. of Med.,, Kaohsiung., Taiwan, [2]Kaohsiung Med. Univ. Hosp., Kaohsiung., Taiwan, [3]E-DA Hosp./I-Shou Univ., Kaohsiung., Taiwan

Abstract Body:

Tuberculosis (TB) is a major worldwide health problem in part due the emergence of drug resistant strains that are threatening and impairing the control of this disease. Baicalin is a flavonoid compound extracted from Scutellaria roots that has been reported to possess antibacterial, anti-inflammatory, and antiviral activities. However, the antimycobacterial effect of baicalin is still unknown. In this study, the efficacy of baicalin on *mycobacterium tuberculosis* (*MTB*) was investigated *in vitro*. The bacteriostatic action of baicalin on *MTB* was detected by Bactec MGIT 960 automated drug susceptibility testing system. We established the standard growth curves of reference strain of *MTB*, H37Rv (ATCC27294) with time to detection (TTD) from MGIT 960 system. MIC50 was calculated from TTD of MGIT 960 system with the following concentrations: 0.625, 1.25 and 2.0 mM of baicalin. The minimum inhibitory concentration (MIC) of baicalin on H37Rv was 2.0 mM, and MIC50 was 0.6 mM, accompanied with minimal cytotoxity against THP-1 cells. The therapeutic dose was showed within an acceptable range. Of the total 36 clinical isolated *MTB* strains, the MIC of baicalin on 8 strains (8/10) of drug sensitive, 2 strains (2/8) of multiple-drug resistant(MDR), 1 strain (1/10) of INH resistant and 6 strains (6/8) of streptomycin resistant were < 2.0 mM. In comparison to the reference strain, the MIC50 of baicalin was higher (>0.6 mM) in 3 strains (30%) of drug sensitive, 7 strains (87.5%) of MDR, 10 strains (100%) of INH resistant and 1 strain (12.5%) of streptomycin resistant *MTB.* In conclusion, we demonstrated the anti-mycobacterial effect of baicalin on clinical *MTB isolates in vitro,* with minimal cytotoxicity of baicalin toward THP-1 cells. However, the MIC was significantly higher in INH resistant strains.

Author Disclosure Block:

H. Eng: None. **J. Chen:** None. **Y. Wang:** None. **T. Lin:** None.

Poster Board Number:

MONDAY-475

Publishing Title:

A Diversity Oriented Synthesis Compound Selectively Inhibits *M. tuberculosis* Tryptophan Synthase

Author Block:

S. Wellington[1], P. P. Nag[1], S. Johnston[1], S. L. Schreiber[2], D. T. Hung[3]; [1]Broad Inst., Cambridge, MA, [2]Harvard Univ., Cambridge, MA, [3]Harvard Med. Sch., Boston, MA

Abstract Body:

Mycobacterium tuberculosis (Mtb) has now surpassed HIV as the leading cause of deaths due to an infectious agent. Though the current regimen of antibiotics is effective against drug-sensitive strains of Mtb, it is also slow, requiring 6-9 months of treatment. The lengthy treatment time often results in patient non-compliance, fueling the development of extensive drug resistance. This rise in resistance necessitates the development of new therapeutics with novel mechanisms for which there will not be cross-resistance to current therapies.Standard screening methods are failing to identify these types of compounds. In a unique approach, whole-cell screening of new small molecules generated through diversity oriented synthesis has allowed us to identify new types of inhibitors. This research explores the mechanisms of action of these compounds.One such compound, BRD-4592, is stereoselective and non-toxic to human cells and to mice, yet is bactericidal and effective against clinical isolates of Mtb. Resistance to BRD-4592 can arise due to mutations in tryptophan synthase and work with recombinant protein demonstrates that BRD-4592 binds tryptophan synthase and inhibits enzyme activity through a completely novel mechanism. No inhibitors of Mtb tryptophan synthase have been previously described, and recent work in the field has demonstrated that host immune responses deprive bacteria of tryptophan making tryptophan synthesis essential *in vivo.* BRD-4592 is a promising therapeutic candidate and we ultimately aim to fully characterize mode of inhibition as well as *in vivo* efficacy.

Author Disclosure Block:

S. Wellington: None. **P.P. Nag:** None. **S. Johnston:** None. **S.L. Schreiber:** None. **D.T. Hung:** None.

Poster Board Number:

MONDAY-476

Publishing Title:

Elucidating the Mechanism of Action of Cb81, a Novel Anti-mycobacterial Agent

Author Block:

J. S. Aneke, L. A. Weiss, C. L. Stallings; Washington Univ. in St. Louis, St. Louis, MO

Abstract Body:

Each year, an estimated 9.6 million people become infected with *Mycobacterium tuberculosis* (*Mtb*). With the rising number of multidrug resistant strains, this presents a large public health threat. To combat this threat, there is a need to introduce new antibiotics into the drug pipeline. CB81 was identified in a screen for compounds that inhibit the growth of *Mycobacterium smegmatis*, a non-pathogenic model organism for *Mtb*, in culture. When we tested CB81 for activity in other bacteria, we found that CB81 has no effect on other Gram positive or Gram negative bacteria, but is specifically a potent killer of mycobacteria, with a minimum inhibitory concentration (MIC) between 100 and 500 nM in *Mycobacterium tuberculosis*. To gain insight into the mechanism of action of CB81 in *M. tuberculosis*, we are performing a detailed analysis of the effects of the drug on the bacteria. We have shown that in liquid culture, CB81 is bactericidal in *M. tuberculosis*. CB81 also inhibits biofilm formation, a physiological process that recapitulates some aspects of chronic infection, like drug tolerance and reduced replication, which may suggest that CB81 affects these pathways. Experiments are underway to determine the changes in expression profiles, metabolism, and lipid content that result from treatment of *M. tuberculosis* with CB81. Additionally, we are seeking to discover the target(s) of CB81 by generating resistant mutants. Finally, we show that a closely related compound, CB82 (MIC in *M. tuberculosis* between 1 and 10 uM), is non-toxic in mouse bone marrow-derived macrophages and inhibits replication of *M. tuberculosis* in macrophages at 50 uM. Future experiments include measuring the survival of drug sensitive and multidrug resistant *Mtb* during infection of murine macrophages and the effect on the efficacy of other standard of care antibiotics during macrophage infection. We have identified a novel and potent mycobacterium-specific antibiotic that kills *Mtb* at sub-micromolar concentrations, and is non-toxic in macrophages. CB81 presents the field with a promising new therapeutic to add to the pipeline of drugs available to treat *M. tuberculosis* infections.

Author Disclosure Block:

J.S. Aneke: None. **L.A. Weiss:** None. **C.L. Stallings:** None.

Poster Board Number:

MONDAY-477

Publishing Title:

Ht-03 - a Novel Anti-mycobacterial Imidazo[2,1-*b*]thiazole-5-Carboxamide - Demonstrates Favorable Safety and Pharmacokinetics

Author Block:

G. C. Moraski[1], Y. Cheng[2], M. Goodwin[3], M. Zimmerman[4], H. I. Boshoff[3], S. Cho[5], J. R. Anderson[5], S. G. Mulugeta[5], S. G. Franzblau[5], J. S. Schorey[2], M. J. Miller[2]; [1]Montana State Univ., Bozeman, MT, [2]Univ. of Notre Dame, Notre Dame, IN, [3]NIH, Bethesda, MD, [4]New Jersey Med. Sch. - Rutgers, Newark, NJ, [5]Univ. of Illinois at Chicago, Chicago, IL

Abstract Body:

Background: Recently, we have disclosed the *in vitro* profiling of various imidazo[2,1-*b*]thiazole-5-carboxamides that target QcrB and potently inhibit growth of both *Mycobacterium tuberculosis* (*Mtb*) and *Mycobacterium avium complex* (MAC). Herein we report the *in vivo* safety and lung tissue distribution of HT-03 being developed for the treatment of *Mtb* as well as MAC. **Methods:** *Synthesis* - Synthesis followed our previously published methods. HT-03 was purified and characterized by mp, NMR and LC/HRMS.*In vitro activity vs. Mtb* - H37Rv was evaluated in both GAS and 7H12 media with metabolic activity measured using a Microplate Alamar Blue Assay. Activity against non-replicating *Mtb* was evaluated with a luminescence readout using the low oxygen recovery assay. MIC determined in 7H9/ADC/Tween to lab adapted, hypersensitive and *qcrB* mutant strains.*In vitro activity vs.* MAC - 11 strains of *M. avium* were exposed to compounds for 3 days and metabolic activity and bacteriostatic/bactericidal activity was measured by the colorimetric resazurin assay and colony forming units, respectively.*In vivo* tolerability - 6 to 8 week old female Balb/c mice were dosed by oral gavage with 250 mg/kg and 500 mg/kg of HT-03 in DMSO. Animals were dosed once daily 6 days a week for two weeks at 250 mg/kg and 6 days a week for one week at 500 mg/kg.**Results:** HT-03 (MW 533, clogP 5.4) had MICs of 2 ng/mL against H37Rv-*Mtb* and MIC against MAC (clinical isolates from AIDS patients) serotypes from 31 to >100 ng/mL. HT-03 at steady state dosing of 100 mg/kg had a lung to plasma concentration ratio of 1.6 (8 hr) and 6.5 (12 hr) with drug levels above the MIC in the lung. HT-03 was well tolerated in mice with no deaths, weight loss or signs of distress observed.**Conclusion:** Novel imidazo[2,1-*b*]thiazole-5-carboxamide, HT-03, is potent inhibitor of growth of both *Mtb* and MAC strains. It has good *in vivo* safety, tolerability, pharmacokinetics and appropriate tissue distribution to warrant further development and evaluation within *Mtb* and MAC infection models.

Author Disclosure Block:

G.C. Moraski: K. Shareholder (excluding diversitied mutual funds); Self; Hsiri Therapeutics. **Y. Cheng:** None. **M. Goodwin:** None. **M. Zimmerman:** None. **H.I. Boshoff:**

None. **S. Cho:** None. **J.R. Anderson:** None. **S.G. Mulugeta:** None. **S.G. Franzblau:** None. **J.S. Schorey:** None. **M.J. Miller:** J. Scientific Advisor (Review Panel or Advisory Committee); Self; Hsiri Therapeutics. K. Shareholder (excluding diversitied mutual funds); Self; Hsiri Therapeutics.

Poster Board Number:

MONDAY-478

Publishing Title:

A Whole Cell Screen Targeting a Novel Methylthioadenosine Nucleosidase (Rv1422) of *Mycobacterium tuberculosis*

Author Block:

J. Zeng[1], K. Hartland[2], K. Coser[2], B. P. Selbach[3], J. Bittker[2], K. Rhee[3], R. Husson[1]; [1]Boston Children's Hosp., Harvard Med. Sch., Boston, MA, [2]Broad Inst. of Harvard and Massachusetts Inst. of Technology, Cambridge, MA, [3]Weill Cornell Med. Coll., New York, NY

Abstract Body:

There is an urgent need for new drugs to treat tuberculosis (TB), both to combat the increasing number of drug-resistant strains of *Mycobacterium tuberculosis* (Mtb) and to shorten the duration of treatment required to cure drug-susceptible TB. Genes that are required for virulence or growth during infection constitute a potentially rich, but un-mined, source of highly attractive drug targets. The *M. tuberculosis* gene Rv1422 encodes a protein of unknown function we previously showed to be essential for optimal *M. tuberculosis* growth on several carbon sources including cholesterol. Cholesterol is a critical carbon source for this pathogen during infection, particularly for intracellular bacteria within macrophages. This gene is also required for *M. tuberculosis* virulence and persistence in a mouse model of infection. We previously determined that a strain of *M. smegmatis* in which the Rv1422 homologue (MSMEG3080) has been deleted is hypersusceptible to β-lactam antibiotics, and that the *M. tuberculosis* Rv1422 gene fully complements this susceptibility phenotype. Based on these findings, we used the microplate alamarBlue assay (MABA) to perform a whole cell screen in the *M. smegmatis* MSMEG 3080 deletion strain complemented by Mtb Rv1422 to identify inhibitors of the *M. tuberculosis* Rv1422 protein. In a screen of ~10,000 compounds, we found 177 compounds that inhibit growth at 30 uM and showed an IC50 at least 2-fold greater in the presence versus the absence of cefotaxime. In recent work, we have shown that the Rv1422 protein catalyzes the glycosidic bond cleavage of 5'-methylthioadenosine. Using recombinant Rv1422 protein we have developed an *in vitro* microplate enzyme assay to measure the 5'-methylthioadenosine nucleosidase activity of Rv1422. We will use this assay to identify the compounds from the screen that directly inhibit Rv1422. In the future, we will further validate and optimize inhibitors of Rv1422, which will be valuable as probes to determine the role of Rv1422 in the Mtb cell and potentially as leads for the development of novel anti-TB drugs.

Author Disclosure Block:

J. Zeng: None. **K. Hartland:** None. **K. Coser:** None. **B.P. Selbach:** None. **J. Bittker:** None. **K. Rhee:** None. **R. Husson:** None.

Poster Board Number:

MONDAY-479

Publishing Title:

Type and Frequency of Mutations in *kat*G and *rpo*B Genes in Multidrug-Resistant Strains of *Mycobacterium tuberculosis* Complex

Author Block:

M. Munir; PMRC TB Res. Ctr., Lahore, Pakistan

Abstract Body:

Background: Tuberculosis remains a major cause of morbidity and mortality in several parts of world. Use of polymerase chain reaction amplification and ***Mycobacterium tuberculosis*** DNA sequencing analysis detects mutations in different genes and is a predictive of drug resistance with the potential to provide rapid detection of isoniazid, rifampicin and or multi drug resistant strains of *Mycobacterium tuberculosis*. Genetic basis and mutations in *kat*G and *rpo*B genes are responsible for isoniazid and rifampicin resistance respectively in most of the cases of ***Mycobacterium tuberculosis***. However these mutations are geographically distributed and different mutations confer different degree of drug resistance.**Objectives:** To determine the type and site of mutation in ***kat*G** and ***rpo*B** genes in confirmed MDR-TB isolates and to find the frequency of various mutations with respect to the site of mutation.**Methodology:** The study was carried out in Pakistan Medical research Council TB Research Centre and Department of Chest Medicine, Mayo Hospital Lahore. 100 AFB Smear positive specimens of MDR TB suspects and Rifampicin resistant on GeneXpert were collected. Drug susceptibility of isoniazid and rifampicin was carried out by standard drug proportion method. DNA isolation of ***Mycobacterium tuberculosis*** was done by boiling the bacterium at 80C° for 20 minutes and supernatant was used as template for sequencing, PCR Single Strand Conformation Polymorphism Analysis and restriction fragment polymorphism.**Results:** A total of 100 smear positive, rifampicin resistant on GeneXpert were included; consist of 53% females and 47% males with male to female ratio of 1:1.13 in the present study. Most of the study subjects (87%) lie in the age ≤45 years of age, 84% having low socio-economic status and 30/47 (63.8%) males were addicts. Mutations in ***rpo*B** Gene were found to be 98% of rifampicin resistant cases and in ***kat*G** 76.7% of isoniazid resistant cases. Most of the mutations (60%) in ***rpo*B** Gene were observed on codon 531 while all the mutations in ***kat*G** Gene were observed on codon 315. No novel mutation was found in the present study.**Conclusion:** Mutation pattern of ***rpo*B** gene that confers rifampicin resistance is different to a little extent from other national and international studies while pattern is same for ***kat*G** gene that confers isoniazid resistance. No novel mutation was observed in present study.

Author Disclosure Block:

M. Munir: None.

Poster Board Number:

MONDAY-480

Publishing Title:

Efflux Pump Gene Expression in Brazilian Clinical Isolates of *Mycobacterium tuberculosis*

Author Block:

L. B. Marino, C. Q. F. Leite, **F. R. Pavan, FRP**; São Paulo State Univ., Araraquara, Brazil

Abstract Body:

Background: Absence of mutations within the genes encoding drug targets in some drug resistant strains of *Mycobacterium tuberculosis* suggests possible involvement of alternative mechanisms such as overexpression of efflux pumps. In this study five putative multidrug efflux pumps genes expression were evaluated in 13 clinical isolates and H_{37}Rv (ATCC 27294) after treatment with rifampicin in four times, 0, 24 h, 48 h and 72 h: three members of Major Facilitator Superfamily (*Rv1258c, Rv1410c* and *Rv2459*) and two members of ATP-binding cassette Superfamily (*Rv1217c* and *Rv1218c*). **Methods:** MIC determination: The rifampicin MIC was determined by REMA technique. RT-qPCR: To evaluate the efflux pump expression profiles the isolates were grown in 7H9 medium until OD_{600nm}=0.6. Rifampicin was added in the concentration of 1/4 of the MIC. Samples for RNA extraction were collected in times of 0h, 24h, 48h and 72h after the treatment. RNA was extracted using RNeasy Mini Kit (QIAGEN) and cDNA was synthesized with High Capacity cDNA Reverse Transcription Kit (Applied Biosystems®). The reactions were run in Applied Biosystems 7500 Real-Time PCR System and the results interpreted in Biogazelle qbase+ software by ΔΔCt method, using *groEL2* and *sigA* as reference genes. **Results:** The treatment with rifampicin for 24 h was not enough to allow the expression of the genes studied. We observed 72 h as the best time for analysis of efflux pumps overexpression. 11 out 13 isolates were classified as MDR with MIC rangin from 2 to 125 μg/mL and 2 susceptible strains showed overexpression in all efflux pumps with similar results (±3 times fold) different than H_{37}Rv with down regulation. Two MDR isolates showed high overexpression in all genes highlighting the *Rv1258c* gene with an increase of 16 fold expression. Interestingly, we observed two MDR isolates with down regulation of expression in all genes overall the time-points. **Conclusions:** Likewise the literature our results showed overexpression in all efflux pumps and the *Rv1258c* was the most overexpressed between the strains. We have not seen any correlation between the MIC and the overexpressed genes. However, the results strengthen the role of efflux pumps in determining antimicrobial resistance for Brazilian clinical isolates of *M. tuberculosis.*

Author Disclosure Block:

L.B. Marino: None. **C.Q.F. Leite:** None. **F.R. Pavan:** None.

Poster Board Number:

MONDAY-481

Publishing Title:

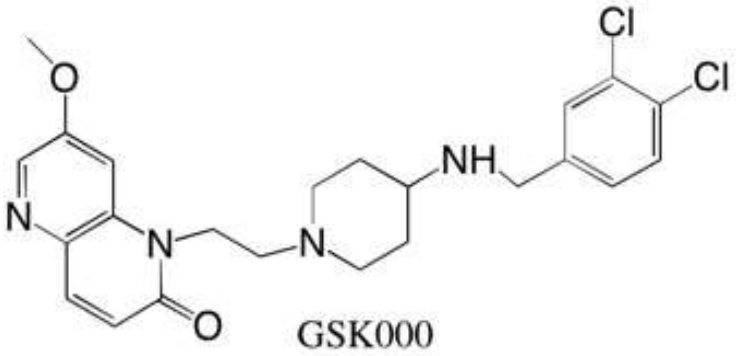
Characterization of "*Mycobacterium* Gyrase Inhibitors" (MGIs): A Novel Class of Gyrase Poisons

Author Block:

E. G. Gibson[1], T. Blower[2], M. Cacho[3], J. Berger[4], N. Osheroff[1]; [1]Vanderbilt Univ., Nashville, TN, [2]Durham Univ., Durham, United Kingdom, [3]GSK, Madrid, Spain, [4]Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract Body:

Tuberculosis is a leading cause of mortality worldwide. The current standard treatment is the RIPE regimen: rifampin, isoniazid, pyrazinamide, and ethambutol. Because resistance is developing against these drugs and the second-line treatment, fluoroquinolones (FQs), there is an urgent need for the development of novel antitubercular drugs to combat resistance. Therefore, we characterized a new class of "*Mycobacterium* Gyrase Inhibitors" (MGIs) that display activity against *Mycobacterium tuberculosis* (Mtb).[1] Three MGIs obtained from GlaxoSmithKline: GSK000, GSK325, and GSK126, displayed activity against Mtb gyrase, with GSK000 being the most efficacious. In marked contrast to FQs (which induce double-stranded DNA breaks), MGIs induce gyrase-mediated single-stranded DNA breaks, even at high drug concentrations or long cleavage time courses. While increasing single-stranded cleavage, MGIs appear to suppress double-strand DNA breaks. Like FQs, MGIs act by inhibiting religation of cleaved DNA. GSK000 displays a strong preference for Mtb gyrase over *Bacillus anthracis* topoisomerase IV or gyrase and *Escherichia coli* topoisomerase IV, suggesting specificity for Mtb. Finally, MGIs retain activity against common FQ resistant mutant gyrase enzymes (GyrA A90V, D94H, and D94G) and displayed no significant activity against recombinant human topoisomerase IIα. Our results suggest that MGIs are a novel class of gyrase poisons that have potential as antitubercular drugs.



GSK000

Author Disclosure Block:

E.G. Gibson: None. **T. Blower:** None. **M. Cacho:** None. **J. Berger:** None. **N. Osheroff:** None.

Poster Board Number:

MONDAY-483

Publishing Title:

Pravastatin Therapy Augments Bactericidal Activity of the First-line Tuberculosis Regimen *In Vivo*

Author Block:

N. K. Dutta[1], N. Bruiners[2], M. L. Pinn[1], M. D. Zimmerman[2], L. Klinkenberg[1], V. Dartois[2], M. L. Gennaro[2], P. C. Karakousis[1]; [1]Johns Hopkins Univ. Sch. of Med., Baltimore, MD, [2]Publ. Hlth.Res. Inst., New Jersey Med. Sch., Newark, NJ

Abstract Body:

Mycobacterium tuberculosis (Mtb) is known to subvert immune responses to establish infection and cause disease. Thus, host-directed therapy (HDT), as adjunctive treatment to traditional antitubercular regimens, is an attractive strategy. Statins are a class of drugs used to lower cholesterol levels by inhibiting the 3-hydroxy-3-methylglutaryl coenzyme A reductase, a crucial enzyme in the cholesterol biosynthesis pathway. Recently, we reported that simvastatin, when added to the first-line antitubercular regimen, shortens the duration of tuberculosis treatment in mice. Here, we conducted a preclinical animal study aimed at comparing the bactericidal activities of the standard TB regimen (rifampin, isoniazid, pyrazinamide and ethambutol; RHZE) with or without escalating doses of pravastatin against chronic TB in BALB/c mice. Antibiotics were given five times weekly for 8 weeks (continuous phase) beginning 6 weeks after infection. To better understand the in vivo outcomes, we also studied single-dose and steady-state pharmacokinetics of the drug in uninfected mice, as well as its efficacy in macrophages. Pravastatin at 180 mg/kg once daily was well tolerated for up to 8 weeks of treatment. Treatment with RHZE plus pravastatin at doses ranging from 30 to 180 mg/kg demonstrated a dose-dependent increase in bactericidal activity, reducing lung bacillary counts by 0.2-0.6 $\log_{10}$, 0.3-0.6 $\log_{10}$ and 0.3-0.8 $\log_{10}$ compared to RHZE alone at weeks 2, 4 and 8, respectively. After 8 weeks of treatment, the degree of lung inflammation correlated with the bactericidal activity of each drug regimen. Also, there was a distinct pravastatin dose-dependent effect observed between 5 μM and 50 μM against Mtb in THP-1 cells. The pharmacokinetics of pravastatin was dose proportional from 15 to 60mg/kg, and the drug is very soluble. Results of the current study raise the possibility of a potentially important class effect against TB. The goal of future studies will be to elucidate the mechanism(s) by which pravastatin exerts its antimycobacterial effect and to determine its contribution to the sterilizing activity of combination regimens against drug-susceptible and drug-resistant TB.

Author Disclosure Block:

N.K. Dutta: None. **N. Bruiners:** None. **M.L. Pinn:** None. **M.D. Zimmerman:** None. **L. Klinkenberg:** None. **V. Dartois:** None. **M.L. Gennaro:** None. **P.C. Karakousis:** None.

Poster Board Number:

MONDAY-484

Publishing Title:

Cd271+ Bone Marrow Mesenchymal Stem Cell Type Is a Targetable Hypoxic Niche for Dormant *Mycobacterium tuberculosis*

Author Block:

J. Garhyan[1], S. Gayan[2], S. Sandhya[2], J. Talkudar[2], R. Bhatnagar[1], B. Das[3]; [1]Jawaharlal Nehru Univ., New Delhi, India, [2]Kavikkrisna Lab., Guwahati, India, [3]The Forsyth Inst., Cambridge, MA

Abstract Body:

Background: The protective niche that shelters dormant *Mycobacterium tuberculosis* (Mtb) is not yet clearly known, hampering our efforts to eradicate latent Mtb infection. We have successfully isolated viable Mtb from the hypoxic CD271+/CD133+ bone marrow mesenchymal stem cells (CD271+ BM-MSCs) of subjects treated for pulmonary tuberculosis (1,2). We then characterized the CD271+ BM-MSC hypoxic niche of dormant Mtb-H37Rv in the Cornell model of Mtb dormancy in mice (2). Our studies identify a novel stem cell niche that shelters dormant Mtb. Here, we investigate the potential targeting of the dormant Mtb stem cell niche by hypoxia-activated agents including tirapazamine (TPZ), a ROS inducing hypoxia-activated agent and ursolic acid, an inhibitor of HIF-1α (3). **Method:** GFP labeled Mtb-H37Rv and streptomycin dependent mutant strain of 18b (m18b), an unique strain of non-replicating dormant Mtb (1) were used for the study. The CD271+ BM-MSCs were infected with these two Mtb strains in vitro/in vivo and then treated with TPZ or Ursolic acid to study potential ROS-induced apoptosis of the Mtb harboring stem cell population. **Results:** In vitro, the TPZ treatment led to increased ROS production, p53 expression, and apoptosis of the Mtb infected CD271+ BM-MSCs, which could be reversed by treating the BM-MSCs with pifithrin α treatment (10 μM for 3 days), a small molecular inhibitor of p53. We found similar results with Ursolic acid (15 μM for 3 days) treatment. The uninfected CD271+ BM-MSCs did not undergo apoptosis following treatment with TPZ or Ursolic acid. In vivo, TPZ treated group showed complete loss of CD271+ BM-MSCs in the Mtb-m18b treated mice and the TPZ effect was reduced by pifithrin-α treatment. We found similar results from ursolic acid group. **Conclusion:** Our work suggest that the dormant Mtb stem cell niche could be targeted by hypoxia-mimetic agents like TPZ and HIF-1α inhibitors like the naturally occurring agent, ursolic acid. Moreover, we speculate that HIF-1α could be working upstream of p53 for survival of Mtb in the host bm niche which could further researched as therapeutic intervention for dormant Mtb in stem cell niche.

Author Disclosure Block:

J. Garhyan: None. **S. Gayan:** None. **S. Sandhya:** None. **J. Talkudar:** None. **R. Bhatnagar:** None. **B. Das:** None.

Poster Board Number:

MONDAY-485

Publishing Title:

Analysis of *Mycobacterium smegmatis* Glutamate Racemase Mutants Suggests a New Pathway of D-Glutamate Biosynthesis

Author Block:

G. Rathnaiah, F. Barnawi, D. K. Zinniel, R. G. Barletta; Univ. of Nebraska, Lincoln, NE

Abstract Body:

Glutamate racemase (MurI) is the main enzyme for D-glutamate biosynthesis in eubacteria and a potential target for drug design. Unfortunately, there are contradictory reports on the essentiality of *murI* in *Mycobacterium*. For example, a recent study indicated that *M. smegmatis murI* is essential in the absence of D-glutamate. However, the corresponding *murI* mutants displayed unusually abundant growth in high osmolarity media without D-glutamate.To reexamine the issue of *murI* essentiality, we generated new *M. smegmatis murI* allelic exchange deletion mutants in low osmolarity Middlebrook 7H9 media (MADC) with 2% sucrose. We confirmed the deletion by PCR, Southern blotting and RT-qPCR analyses; and characterized the mutant strains by growth curves, drug-susceptibility assays and negative staining electron microscopy.These mutants were able to grow on the low osmolality MADC without D-glutamate, though with a significant lag time of about 48 hours as compared to the wild type. However after passage on the same media without D-glutamate, the mutant strain grew without this lag time. Moreover, complementation of the mutant strains restored wild type properties. In contrast to the previous study mentioned above, mutants and the wild type grew very poorly in high osmolarity conditions (MADC with 5% sodium chloride). Furthermore, in low osmolality MADC in the presence or absence of D-glutamate, both wild type and mutant strains displayed similar morphologies when analyzed by electron microscopy. The *murI* mutants were hypersusceptible to D-cycloserine (MIC $\leq$ 3.0 vs. 75.0 μg/ml for the wild type and complemented strains; $P < 0.05$).The viability of *murI* mutants in the absence of D-glutamate in media with low osmolarity indicates that the inactivation of *murI* does not impose an auxotrophic requirement for D-glutamate in *M. smegmatis*. Thus, *murI* is not an essential gene in the absence of D-glutamate. Moreover, these results suggest the existence of a redundant pathway for the biosynthesis of D-glutamate, one of the key amino acids involved in peptidoglycan crosslinking. Understanding this mechanism would be of utmost importance to design new peptidoglycan synthesis inhibitors that could be developed as antimicrobial agents against pathogenic mycobacteria.

Author Disclosure Block:

G. Rathnaiah: None. **F. Barnawi:** None. **D.K. Zinniel:** None. **R.G. Barletta:** None.

Poster Board Number:

MONDAY-486

Publishing Title:

Potential Involvement of RuvAB in the Resistance of Mycobacteria to Ethacridine

Author Block:

P. K. Garcia[1], R. Perera[2], Y-C. Tse-Dinh[1]; [1]Florida Intl. Univ., Miami, FL, [2]Sanford-Burnham Med. Res. Inst., Orlando, FL

Abstract Body:

Topoisomerases are essential enzymes for solving all topological problems that are related to the physical structure of the double helix of the DNA. These enzymes are required for the processes of DNA replication, transcription and influence virulence gene expression in many pathogenic bacteria. Topoisomerases generate transient protein-DNA complexes on DNA strand (covalent intermediate). Type I topoisomerase cleaves one DNA strand, while type II topoisomerases cleaves both DNA strands. The transient formation of these covalent intermediates is critical and also dangerous for the cell. Stabilization of any covalent intermediate would induce SOS response and may cause cell death. Bacteria do not possess any enzyme capable of directly hydrolyzing the tyrosyl-DNA phosphodiester bond in the covalent intermediate; instead these complexes are processed by mechanisms of DSB (Double Strand Break) repair. The RuvABC and RecG proteins play significant role in DSB repair mechanisms by resolving the Holliday junctions following DSB formation in the later stages of homologous recombination. In this study whole cell phenotypic profiling coupled with the whole genome sequencing (WGS) of spontaneous resistant mutants was employed in the identification of cellular targets in the growth inhibition of *Mycobacterium smegmatis* by ethacridine. The WGS revealed mutations in *ruvB* gene, which could be a potential target of ethacridine. Alternatively, RuvB may be involved in the ethacridine resistance mechanism because of its role in the processing of topoisomerase covalent intermediate.

Author Disclosure Block:

P.K. Garcia: None. **R. Perera:** None. **Y. Tse-Dinh:** None.

Poster Board Number:

MONDAY-487

Publishing Title:

Oral Encochleated Amikacin Demonstrates Reduced Toxicity Compared to Intravenous Amikacin

Author Block:

R. Lu[1], J. C. Craft[1], C. Lambros[2], R. Doppalapudi[3], L. Iyer[3], J. C. Mirsalis[3], E. C. Tramont[4], **R. J. Mannino**[1]; [1]Matinas BioPharma, Inc., Bedminster, NJ, [2]NIH/NIAID, Rockville, MD, [3]SRI Intl., Menlo Park, CA, [4]NIH, Bethesda, MD

Abstract Body:

Background: Amikacin (AMK) is a broad spectrum aminoglycoside commonly administered parenterally. Treatment with AMK is frequently associated with oto- and nephrotoxicity, therefore, careful monitoring of blood levels is required. To overcome issues associated with administration and toxicity of AMK, a lipid-crystal, nano-particle forumulation (CAMK) has been developed for oral delivery. Previously CAMK has been shown to be efficacious in mouse models of disseminated and pulmonary *Mycobacterium avium*.**Methods:** Rats were administered 50 mg/kg/day or 200 mg/kg/day oral dose of CAMK in 0.66 M NaCl, in 1mM bile salts, or in 2 mM bile salts for 7-days. Free AMK was administered at 200 mg/kg/day oral and at 50 mg/kg/day intravenously (iv). Rats were euthanized on Day 8. Parameters that were evaluated include mortality/morbidity, clinical observations, body weights, plasma drug levels, toxicokinetic analysis, clinical pathology, necropsy observations, organ weights, and histopathology.**Results:** All animals survived to scheduled sacrifice. Across dose groups oral CAMK provided 100-fold lower plasma levels of AMK than the iv dose. There was a trend toward a lower exposure on Day 7 versus Day 1 in several dose groups. The amikacin prescribing information states that the C_{max} of amikacin should not exceed 35 µg/mL. The C_{max} of all CAMK formulations did not exceed 2 µg/mL (2000 ng/mL) but in the *Mycobacterium avium* lung and disseminated model 100 mg/kg doses showed efficacy. The various changes seen in all treatment groups were of minimal toxicologic significance.**Conclusions:** CAMK in three different vehicle formulations was well-tolerated at 50 and 200 mg/kg for 7 days. Based on changes in organ weights, the NOAEL is considered to be less than 50 mg/kg for CAMK in NaCl and 200 mg/kg for CAMK in 1 mM and 2 mM bile salts. This compares favorably with the published NOAEL for free AMK in rats which is 100 mg/kg.

Author Disclosure Block:

R. Lu: D. Employee; Self; Matinas BioPharma, Inc.. K. Shareholder (excluding diversitied mutual funds); Self; Matinas BioPharma, Inc. **J.C. Craft:** J. Scientific Advisor (Review Panel or Advisory Committee); Self; Matinas BioPharma, Inc., Cempra, Inc., Anacor Pharmaceuticals, Inc., DNDI. K. Shareholder (excluding diversitied mutual funds); Self; Matinas BioPharma, Inc.,

Cempra, Inc., Abbott Labs, AbbVie. **C. Lambros:** None. **R. Doppalapudi:** None. **L. Iyer:** None. **J.C. Mirsalis:** None. **E.C. Tramont:** None. **R.J. Mannino:** D. Employee; Self; Matinas BioPharma, Inc.. K. Shareholder (excluding diversitied mutual funds); Self; Matinas BioPharma, Inc..

Poster Board Number:

MONDAY-488

Publishing Title:

Magnitude of Gene Mutations Conferring Drug Resistant in *Mycobacterium tuberculosis* Strains in Southwest Ethiopia

Author Block:

M. Tadesse; Jimma Univ., Jimma, Ethiopia

Abstract Body:

The nature and frequency of mutations in rifampicin (RIF) and isoniazid (INH) resistance *M. tuberculosis* isolates vary considerably according to the geographic locations. However, information regarding specific mutational patterns in Ethiopia remains limited. Mutations associated with RIF and INH resistance were studied by GenoType MTBDRplus line probe assay in 112 *M. tuberculosis* isolates. Culture (MGIT960) and identification tests were performed at Mycobacteriology Research Center of Jimma University, Ethiopia. Mutations conferring resistance to INH, RIF and MDR were detected in 36.6% (41/112), 30.4% (34/112) and 27.7% (31/112) of *M. tuberculosis* isolates respectively. The retreatment category 'treatment failure' is associated with a high rate of mutations associated with drug resistance (p-value <0.05). Among 34 rifampicin resistant isolates, 82.4% (28/34) had *rpoB* gene mutations at codon 531, 2.9% (1/34) at codon 526 and 5 had mutations only at wild type probes. The later isolates were depicted as unknown. Of 41 INH resistant strains, 87.8% (36/41) had mutations in the *katG* gene at Ser315Thr1 and 9.8% (4/41) of strains had mutation in the *inhA* gene at C15T. One INH resistant strain had mutation only at *KatG* wild type probe. Mutations in *inhA* promoter region were strongly associated with INH monoresistance. Monoresistance to INH (10 isolates) was frequently observed as compared to RIF monoresistance (3 isolates). High rate of drug resistance, including MDR, was commonly observed among failure cases. The most frequent gene mutations associated with the resistance to INH and RIF were observed in the codon 315 of the *katG* gene and codon 531 of the *rpoB* gene, respectively. Further studies on mutations in different geographic regions using DNA sequencing techniques are warranted to improve the kit by including more specific mutation probes in the kit.

Author Disclosure Block:

M. Tadesse: None.

Poster Board Number:

MONDAY-489

Publishing Title:

Inhibition of *Mycobacterium tuberculosis C*holesterol Catabolism Through Camp Production

Author Block:

K. L. Brown[1], I. Casabon[1], L. Ballell-Pages[2], L. D. Eltis[1]; [1]Univ. of British Columbia, Vancouver, BC, Canada, [2]Tres Cantos Open Lab Fndn., Madrid, Spain

Abstract Body:

Mycobacterium tuberculosis, the causative agent of TB, now outranks HIV as the world's leading cause of mortality from an infectious disease with 1.5 million deaths in 2014 alone. The rise of antibiotic resistant strains has led to an urgent need for novel therapeutics for the treatment of TB. The ability of *M. tuberculosis* to catabolize host-derived cholesterol during infection has emerged as a promising target for the development of new antimycobacterial agents as it has been shown repeatedly to be essential for pathogenesis. In a screen designed to identify compounds that inhibit growth of *M. tuberculosis* both intracellularly and *in vitro* with cholesterol as a sole carbon source, an intriguing candidate that inhibits growth on cholesterol with an IC_{50} of 0.2 μM was identified. The compound acts indirectly to inhibit cholesterol catabolism through stimulation of cAMP production by interaction with the adenylate cyclase Rv1625c. Treatment of *M. tuberculosis* with our compound resulted in a ~50x increase in intracellular [cAMP]. Using LC-MS, we found that the compound also leads to a decrease in the ratio of acetyl-CoA to propionyl-CoA from 4:1 to 1:1, indicating that the compound has profound effects on downstream central metabolism when *M. tuberculosis* is grown on cholesterol as a sole carbon source. Growth on cholesterol in the presence of the compound can be rescued with the addition of vitamin B12, enabling *M. tuberculosis* to shunt excess propionyl-CoA through the vitamin B12-dependent methylmalonyl pathway. These data suggest that the mode of action involves excess propionyl-CoA accumulation, which is toxic to *M. tuberculosis* and other microorganisms. This toxicity was shown to be mediated by stimulation of Rv1625c activity, as the effect is not observed in an Rv1625c transposon mutant. Treatment of *Rhodococcus jostii* RHA1 expressing full length but not truncated Rv1625c results in compound-dependent production of cAMP, indicating the compound likely interacts with the transmembrane domain of the protein. Rv1625c is a promising drug target and the downstream effects of the compound-mediated cAMP production are yielding intriguing insights into the metabolic regulation of *M. tuberculosis.*

Author Disclosure Block:

K.L. Brown: None. **I. Casabon:** None. **L. Ballell-Pages:** None. **L.D. Eltis:** None.

Poster Board Number:

MONDAY-490

Publishing Title:

Pharmacokinetic Parameters of Daptomycin According to Renal Function in Japanese Patients with Skin and Soft Tissue Infections: Subset Analysis of a Phase III Study

Author Block:

T. Ueda[1], Y. Takesue[1], H. Mikamo[2], S. Kusachi[3], K. Takahashi[4], N. Aikawa[5]; [1]Hyogo Coll. of Med., Hyogo, Japan, [2]Aichi Med. Univ., Aichi, Japan, [3]Toho Univ. Med. Ctr. Ohashi Hosp., Tokyo, Japan, [4]MSD K.K, Tokyo, Japan, [5]Sch. of Med., Keio Univ., Tokyo, Japan

Abstract Body:

Background: The clinical outcomes of a phase III study of patients with skin and soft tissue infections (SSTI) that were treated with 4 mg/kg of daptomycin were reported by Aikawa et al. (2013). Using data for a subgroup of the study population, we investigated the pharmacokinetic (PK) parameters of daptomycin according to renal function. **Methods**:We analyzed the PK parameters of 82 patients with creatinine clearance (CL_{CR}) rates of ≥30 ml/min in whom SSTI caused by Gram-positive cocci were treated with 4 mg/kg/day daptomycin. Blood samples (5 time points) were collected at 4 days after the initiation of treatment. The PK parameters were calculated for three renal function groups (CL_{CR} values: ≥80, 40-80, 30-40 ml/min). **Results**: There were significant correlations between renal function and the PK parameters including the clearance and half-life of daptomycin (Table, [a]: Significantly different from the >80 ml/min group, Scheffe adjusted p-value <0.05). Although the patients with CL_{CR} rates of 40-80 or 30-40 mL/min exhibited significantly higher minimum daptomycin concentrations (C_{min}) than those with CL_{CR} rates of ≥80 ml/min, both of these groups exhibited clinically low C_{min} values (7.81 and 7.49 μg•hr/mL, respectively). The $AUC_{0\text{-}24}$ was 407.0 μg•hr/mL in the patients with CL_{CR} rates of 40-80 ml/min and 323.0 μg•hr/mL in those with CL_{CR} rates of ≥80 ml/min. **Conclusions**:The PK values of each CL_{CR} group were similar to the values reported for phase II/III subjects by Dvorchik B et al. (2004). Renal function contributed to interindividual variations in daptomycin clearance in patients with SSTI who were administered 4 mg/kg/day daptomycin. In addition, we confirmed that dosage adjustment was not required in patients with CL_{CR} rates of 30-80 ml/min.

Summary of pharmacokinetic parameters sorted by estimated CLCR and obtained by Bayesian estimation

	Value for each CL_{CR} group: median (range)		
PK parameter	≥80 ml/min (n=38)	40-80 ml/min (n=37)	30-40 ml/min (n=7)
CL (L/hr)	0.75 (0.45-3.28)	0.53 (0.27-0.95)a	0.47 (0.26-0.78)a
V1 (L)	5.24 (3.42-14.00)	4.81 (2.06-11.30)	5.27 (3.25-15.00)
Vss (L)	9.84 (6.10-19.30)	8.97 (4.60-14.10)	9.05 (6.23-18.60)

T1/2 (hr)	9.16 (4.54-12.80)	11.90 (6.28-18.20)a	14.95 (10.80-20.10)a
Cmin (µg/mL)	4.75 (0.31-14.00)	7.81 (1.29-19.50)a	7.49 (5.37-17.70)a
Cmax (µg/mL)	45.65 (18.30-72.10)	45.30 (12.80-66.50)	40.80 (17.80-67.40)
AUC0-24 (µg•hr/mL)	323.0 (85.5-696.0)	407.0 (124.0-769.0)	368.0 (256.0-737.0)

Author Disclosure Block:

T. Ueda: None. **Y. Takesue:** None. **H. Mikamo:** None. **S. Kusachi:** None. **K. Takahashi:** D. Employee; Self; MSD K.K.. **N. Aikawa:** None.

Poster Board Number:

MONDAY-491

Publishing Title:

Pharmacokinetics of Meropenem During Initial Severe Sepsis

Author Block:

K. Trerayapiwat, S. Jaruratanasirikul; Prince of Songkla Univ., Faculty of Med., Hat Yai, Songkla, Thailand

Abstract Body:

Background: Pathophysiological alterations during severe sepsis can affect pharmacokinetics (PK), resulting in the therapeutic success of antimicrobial agents. The objective of this study was to determine the PK of meropenem during the initial period of severe sepsis. **Methods:** The study was conducted during the first 8 h of life-threatening severe sepsis in 12 patients. The severity of the critically illness of the patients were assessed by the Acute Physiology and Chronic Health Evaluation (APACHE) II scores and the Sepsis-related Organ Failure Assessment (SOFA) score. The PK studies were performed during the 1st dose of 1 g every 8 h of a 1-h infusion of meropenem and compared to PK parameters of a 3-h infusion of a single 1-g dose of meropenem in healthy volunteers from pervious study.**Results:** The mean PK parameters of meropenem in this patient population compared to healthy volunteers are shown in Table.

PK Parameter	Patients with severe sepsis	Healthy Volunteers	*p* value
C_{max} (mg/L)	46.24 ± 17.69	24.95 ± 6.85	< 0.01
C_{min} (mg/L)	6.47 ± 6.95	0.47 ± 0.23	< 0.01
$AUC_{0-\infty}$ (mg*h/L)	195.10 ± 163.62	80.06 ± 21.86	< 0.05
$t_{1/2}$ (h)	4.19 ± 4.04	0.61 ± 0.14	< 0.01
k_e (h^{-1})	0.30 ± 0.18	1.21 ± 0.38	< 0.01
V (L)	28.77 ± 16.18	11.72 ± 2.22	< 0.05
CL (L/h)	9.02 ± 7.45	14.46 ± 5.88	0.08

Conclusions: 1) The PK parameters in the patients with severe sepsis exhibited a high range of variability, and 2) the *V* and $t_{1/2}$ of meropenem were greater, but the CL lower, in the patients with severe sepsis than in the healthy volunteers.

Author Disclosure Block:

K. Trerayapiwat: None. **S. Jaruratanasirikul:** None.

Poster Board Number:

MONDAY-492

Publishing Title:

Dosage Modification of Linezolid in Patients Undergoing Continuous Venovenous Hemodiafiltration

Author Block:

T. Ueda[1], Y. Takesue[1], K. Nakajima[1], T. Ide[1], S. Nishi[1], H. Kohama[1], K. Ikawa[2], N. Morikawa[2]; [1]Hyogo Coll. of Med., Hyogo, Japan, [2]Hiroshima Univ., Hiroshima, Japan

Abstract Body:

Background: Linezolid (LZD) dosage adjustment is not recommended for patients undergoing continuous venovenous hemodiafiltration (CVVHDF). We reported that LZD clearance (CL) was decreased in patients that underwent low-flow-rate CVVHDF, which is popular in Japan (Ide T et al., ICAAC 2011). We propose a revised LZD regimen for such patients and investigate the pharmacokinetics (PK) of this protocol.**Methods:** Patients on CVVHDF received 600 mg LZD intravenously once a day (with/without a 600 mg loading dose twice daily for the first 2 days). Blood samples were collected at 0, 1, 1.5, 2, 3, and 5h after the infusion on days 5-8 of therapy. The maximum and minimum concentrations (C_{max}, C_{min}), $T_{1/2}$, concentration time curve (AUC_{0-24}), CL, and volume distribution (Vd) were evaluated. Thrombocytopenia was defined as a $\geq 10 \times 10^4$ cells/mm³ decrease or ≥30% reduction in the platelet count from the baseline.**Results:** Nine patients were included. No. 8 and 9 did not receive the twice-daily loading dose. The mean blood, dialysate, and filtration flow rates were 83.3±10.0 mL/min, 13.3±5.3 mL/hr, and 16.2±4.1 mL/min, respectively. Thus, the CVVHDF was performed at a low flow rate. The patients exhibited slower LZD CL than patients with normal renal function (Stalker D. J et al., LZD CL: 7.4 L/h). The target C_{min} value (≤7 μg/mL; Pea F et al.; chosen to prevent adverse effects) was only achieved in 5 patients (55.6%), but the target AUC_{0-24} value (≥200 μg h/mL; Rayner CR, et al.; chosen to increase clinical efficacy) was achieved in 8 patients (88.9%). Of the 4 patients without leukemia, No. 1, 5, and 7, who exhibited higher C_{min} values than patient 6, developed thrombocytopenia. Clinical success was achieved in No. 3, 7, and 8, and no correlation with AUC was seen.**Conclusions:** Although the target AUC value was achieved during the daily administration of 600 mg LZD, significant LZD accumulation was observed in patients that underwent low-flow-rate CVVHDF. Even lower doses might be required in this setting.

Table: The PK of LZD during the daily administration of 600 mg LZD

	C_{min}(μg/mL)	C_{max}(μg/mL)	T1/2(h)	AUC_{0-24}(μg*h/mL)	CL (L/h)	Vd (L)
No.1	8.3	29.6	19.3	420.9	1.4	39.8
No.2	1.9	37.3	4.4	279.0	2.2	13.7
No.3	2.0	22.9	5.9	181.6	3.3	28.1

No.4	6.5	21.2	8.6	308.8	1.9	24.1
No.5	12.2	30.8	13.3	409.5	1.5	28.2
No.6	5.3	17.3	10.1	221.4	2.7	39.6
No.7	11.2	31.6	13.0	425.9	1.4	26.5
No.8	3.2	23.8	6.3	220.0	2.7	25.0
No.9	7.2	27.2	7.1	302.4	2.0	20.3
Mean±S.D	6.4±3.7	26.9±6.2	9.8±4.7	307.7±92.9	2.1±0.7	27.2±8.4

Author Disclosure Block:

T. Ueda: None. **Y. Takesue:** None. **K. Nakajima:** None. **T. Ide:** None. **S. Nishi:** None. **H. Kohama:** None. **K. Ikawa:** None. **N. Morikawa:** None.

Poster Board Number:

MONDAY-493

Publishing Title:

Intraperitoneal Penetration of Piperacillin-Tazobactam in Patients Undergoing Abdominal Surgery

Author Block:

H. Ohge, N. Murao, K. Ikawa, Y. Watadani, S. Uegami, N. Shigemoto, N. Shimada, T. Kajihara, R. Yano, N. Morikawa, T. Sueda; Hiroshima Univ., Hiroshima, Japan

Abstract Body:

Background: While piperacillin-tazobactam (PIPC/TAZ) is widely used for the treatment of intra-abdominal infections, limited data on intraperitoneal penetration are available. This study was conducted to define the pharmacokinetics and pharmacodynamics of PIPC/TAZ in the peritoneal cavity.**Methods:** PIPC/TAZ (4.0-0.5 g) was intravenously administered to 10 abdominal surgery patients with inflammatory bowel disease. Plasma, peritoneal fluid, and peritoneum samples were obtained at the end of the infusion (0.5 h) and 1.5, 2.5, 3.5, and 4.5 h post dosing. Drug concentration data were analyzed non-compartmentally and were fit to a multi-compartment model to estimate the amount of time the drug concentration exceeded the minimum inhibitory concentration (T > MIC).**Results:** The mean maximum PIPC and TAZ concentrations were 192.7 and 23.6 mg/L in peritoneal fluid and 131.3 and 14.9 mg/kg in the peritoneum, respectively. The mean area under the PIPC concentration-time curve from 0 to infinity (AUC) and the ratio to plasma were 498.1 mg•h/L and 0.75 for peritoneal fluid and 327.6 mg•h/kg and 0.49 for the peritoneum. The mean PIPC/TAZ ratio was 8.1 in the two sites (**Table**). Using the mean pharmacokinetic model parameters, site-specific pharmacodynamic-derived breakpoints (the highest MIC at which T > MIC for PIPC in both peritoneal fluid and the peritoneum was >50%) were 16 mg/L for dosing both 4.5 g every 8 h and 3.375 g every 6 h (13.5 g/day) and 32 mg/L for dosing 4.5 g every 6 h (18 g/day).**Conclusions:** PIPC-TAZ penetrated well into the peritoneal cavity, maintaining the drug combination ratio of 8:1. Dosages of 13.5 g/day and 18 g/day achieved the bactericidal T > MIC target in the sites with MICs of 16 and 32 mg/L, respectively.

Mean ± SD (n = 10)	Peritoneal fluid AUC (mg•h/L)	Peritoneum AUC (mg•h/kg)	Peritoneal fluid/ plasma ratio	Peritoneum/ plasma ratio
PIPC (4 g)	498.1 ± 161.4	327.6 ± 84.0	0.75 ± 0.21	0.49 ± 0.12
TAZ (0.5 g)	61.4 ± 19.1	41.2 ± 12.4	0.79 ± 0.19	0.53 ± 0.14
PIPC/TAZ ratio	8.1 ± 0.6	8.1 ± 0.8		

Author Disclosure Block:

H. Ohge: None. **N. Murao:** None. **K. Ikawa:** None. **Y. Watadani:** None. **S. Uegami:** None. **N. Shigemoto:** None. **N. Shimada:** None. **T. Kajihara:** None. **R. Yano:** None. **N. Morikawa:** None. **T. Sueda:** None.

Poster Board Number:

MONDAY-494

Publishing Title:

Population Pharmacokinetics (Poppk) of Cefazolin (Cfz) in Serum and Adipose Tissue (At) from Obese Women Undergoing Cesarean Section

Author Block:

M. Grupper[1], J. L. Kuti[1], M. L. Swank[2], L. Maggio[3], D. P. Nicolau[1]; [1]Ctr. for Anti-Infective Res. & Dev., Hartford Hosp., Hartford, CT, [2]Miller Children's & Women's Hosp., Long Beach, CA, [3]Univ. of South Florida, Tampa, FL

Abstract Body:

Background: CFZ is often used as antibiotic prophylaxis for cesarean delivery. The optimal dosing regimen for obese women is unknown. We determined the variability of CFZ AT exposure in this population and evaluated 1, 2, and 3 gram (g) doses.**Methods:** PopPK analyses were performed using the non-parametric adaptive grid algorithm within Pmetrics for R to co-model serum and AT concentrations from patients enrolled in 2 previous pharmacokinetic studies. Patients received CFZ as a 5 min infusion followed by 1-3 serum and AT samples collected during surgery. AT penetration was calculated as the $AUC_{0\text{-}8AT}$ over the free $AUC_{0\text{-}8serum}$. Serum protein binding was assumed to be 85%. A 5000 patient Monte Carlo simulation was conducted for 1, 2 and 3g doses to calculate probability of target attainment (PTA) for AT concentrations remaining above the minimum inhibitory concentration (MIC_{90}, 1 mcg/mL) of methicillin susceptible *Staphylococcus aureus* (MSSA) at 2 and 4 h after the dose. **Results:** 68 patients (mean body weight (BW): 102 kg; range: 54-152 kg; mean body mass index (BMI): 38.5 kg/m^2; range: 24.3-55.8 kg/m^2) provided 102 serum and 136 AT samples for inclusion. The mean±SD time interval from CFZ administration to fascia closure was 76.5±21 min (max: 146 min). A 2 compartment model, with one of the compartments representing AT concentration, fit the data best. BW and BMI covariates were explored, and although significant, did not improve the model. The final model resulted in the following parameter values: clearance = 7.4±5.3 L/h, volume of central compartment = 11.7±9.3 L, and AT volume of distribution = 80.6±54.6 L. The mean±SD (median) penetration ratios into AT were 1.1±3.1 (0.73). At 2 h after the dose, PTA for the 1, 2, and 3 g doses was 80.6, 89.6, and 92.6%, respectively. At 4 h, PTA for the 1, 2, and 3 g doses was 57.3, 71.8, and 77.5%, respectively. **Conclusion:** Considering the surgery duration (~1-2 h) for most patients undergoing cesarean delivery, these data suggest that a CFZ 2 g dose will provide AT concentrations above the MIC of MSSA for the majority of obese females up to 150 kg. A second dose may be considered if surgery extends beyond 2 h.

Author Disclosure Block:

M. Grupper: None. **J.L. Kuti:** None. **M.L. Swank:** None. **L. Maggio:** None. **D.P. Nicolau:** None.

Poster Board Number:

MONDAY-495

Publishing Title:

Population Pharmacokinetics (Poppk) and Safety of Ceftolozane/Tazobactam (C/T) in Adult Cystic Fibrosis (Cf) Patients Admitted with Acute Pulmonary Exacerbation (Ape)

Author Block:

M. L. Monogue[1], R. S. Pettit[2], J. J. Cies[3], M. Muhlebach[4], D. P. Nicolau[1], J. L. Kuti[1]; [1]Ctr. for Anti-Infect. Res. & Dev., Hartford Hosp., Hartford, CT, [2]Riley Hosp. for Children, Indianapolis, IN, [3]St. Christopher's Hosp. for Children, Philadelphia, PA, [4]UNC, Chapel Hill, NC

Abstract Body:

Background: C/T has potent activity against *Pseudomonas aeruginosa*, a pathogen associated with CF APE. Due to the rapid elimination of many antibiotics, CF patients frequently have altered PK. Herein, we describe the PopPK and safety of C/T in CF patients admitted with an APE.**Methods:** In this multicenter, open-label PK study, adult CF patients admitted with APE received 3g of C/T q8h. After 4-6 doses, blood was collected at 6 time-points for measuring ceftolozane and tazobactam concentrations by HPLC. PopPK was performed by Non-Parametric Adaptive Grid in Pmetrics for R. A 5,000 patient Monte Carlo simulation was performed to determine the probability of target attainment (PTA) for a 3g q8h (1 h infusion) regimen across a range of MICs using a ceftolozane target of 39% free time above the MIC, assuming 20% protein binding.**Results:** 11 adults (3 male, 8 female) with mean (range) age, weight, and height of 21 (18-30) years, 51.3 (41.6-58.7) kg, and 159.6 (149.7-175.3) cm, respectively, were included. Ceftolozane and tazobactam concentration data were best described by 2-compartment models. Final parameter estimates for ceftolozane were: Clearance (CL) = 4.5±1.0 L/h, Volume of Central Compartment (Vc) = 6.9±2.9 L, and intercompartment transfer constants (Kcp) = 1.5±2.5 h^{-1} and (Kpc) = 0.8±1.2 h^{-1}. Final PopPK parameters for tazobactam were: CL = 19.7±5.0 L/h, Vc = 13.0±8.6 L, Kcp = 1.3±1.3 h^{-1}, and Kpc = 1.7±2.7 h^{-1}. The R^2, bias, and imprecision for the final individual predicted versus observed concentrations were 0.99, -0.13, and 1.75, respectively, for ceftolozane and 0.99, -0.09, and 1.01, respectively, for tazobactam. PTA for a 3g q8h regimen was 99.8, 99.7, 99.6 and 92.3% at MICs of 4, 8, 16, and 32 µg/mL, respectively. No clinically significant adverse events were observed.**Conclusions:** In these 11 CF patients with APE, C/T 3g q8h was well tolerated, PopPK estimates were in accordance with published data in healthy volunteers and non-CF patients, and this regimen achieved target PD exposure at MICs up to 32 µg/ml. These observations support additional studies of C/T for *Pseudomonas aeruginosa* APE in CF patients.

Author Disclosure Block:

M.L. Monogue: None. **R.S. Pettit:** None. **J.J. Cies:** None. **M. Muhlebach:** None. **D.P. Nicolau:** J. Scientific Advisor (Review Panel or Advisory Committee); Self; Merck & Co., Inc.

L. Speaker's Bureau; Self; Merck & Co., Inc. **J.L. Kuti:** E. Grant Investigator; Self; Merck & Co., Inc, Theravance Biopharma. J. Scientific Advisor (Review Panel or Advisory Committee); Self; Merck & Co., Inc, Theravance Biopharma, Actavis Inc. L. Speaker's Bureau; Self; Actavis Inc.

Poster Board Number:

MONDAY-496

Publishing Title:

Pharmacokinetics of Daptomycin (Dap) in Patients Undergoing Continuous Veno-Venous Hemodiafiltration (Cvvhdf)

Author Block:

T. Ide[1], S. Nishi[1], Y. Takesue[1], K. Ikawa[2], N. Morikawa[2]; [1]Hyogo Coll. of Med., Nishinomiya City, Hyogo, Japan, [2]Hiroshima Univ., Hiroshima City, Japan

Abstract Body:

Background: Eight mg/kg DAP every 48 h is recommended for critically ill patients on CVVHDF[1)] . Investigating effects of flow rate on DAP withdrawal during CVVHDF is essential for adjusting therapeutic dosage. **Methods:** DAP 6 mg/kg was administered intravenously every 48 h to CVVHDF patients. Blood and filtrate samples were collected at 0, 1, 1.5, 2, 5, 12, 24, and 48 h after infusion. All collected samples were analyzed using HPLC and pharmacokinetics evaluated. Patient characteristics and CVVHDF parameters including blood, dialysate, and filtration flow rates were recorded.**Results:** Seven patients were included in the study. Mean blood, dialysate, and filtration flow rates were 85.7 ± 9.8 mL/min, 7.7 ± 1.6 mL/min, and 7.7 ± 1.6 mL/min, respectively, confirming that CVVHDF was performed under low-flow setting. The types of infection were was bacteremia (3), intraabdominal infections (2), necrotizing fasciitis (1), and pyogenic spondylitis (1). The types of pathogen were methicillin-resistant *Staphylococcus aureus* (3), *Enterococcus faecium* (3), and empiric therapy (1). Maximum concentration was 50.5 ± 15.9 mg/L; trough serum concentration (C_{min}), 20.0 ± 16.5 mg/L; elimination half-life (T-1/2), 34.8 ± 26.0 h; area under concentration-time curve (AUC), 1060 ± 659 mg h/L; volume of distribution, 18.9 ± 10.5 L; clearance (CL), 7.63 ± 4.57 mL/min; and fraction unbound, 7.2 ± 2.6 %. Sieving coefficient and CL of dialyzer were 0.09 ± 0.04 and 1.34 ± 0.44 mL/min, respectively. No patient met the creatine phosphokinase (CPK) elevation criteria. The clinical response rate was 71.4% (5/7 patients).**Conclusions:** DAP, > 666 to 1061 mg h/L of AUC/MIC was required to obtain clinical success[2)], and C_{min} cutoff point of 24.3 g/mL was associated with CPK elevation[3)]. Because the MIC of DAP in almost all isolates was <1 μg/mL[2),4)], an adequate AUC was obtained in our study. However, because of the low-flow rate setting in our study, T-1/2 was relatively higher than in a previous report[5)], and resulted in higher C_{min}. Although target AUC was achieved, 6 mg/kg DAP every 48 h increased the risk of accumulation in patients on CVVHDF in a low-flow setting.

Author Disclosure Block:

T. Ide: None. **S. Nishi:** None. **Y. Takesue:** None. **K. Ikawa:** None. **N. Morikawa:** None.

Poster Board Number:

MONDAY-497

Publishing Title:

Ampicillin (AMP) Pharmacokinetics (PK) in Neonates Receiving Extra-Corporal Life Support (ECLS)

Author Block:

J. Cies[1], W. Moore, II[1], M. Young[2], V. Bains[2], D. Carella[2], A. Enache[3], A. Chopra[4]; [1]The Ctr. for Pediatric Pharmacotherapy, Pottstown, PA, [2]St. Christopher's Hosp. for Children, Philadelphia, PA, [3]Atlantic Diagnotic Lab., Ben Salen, PA, [4]NYU Langone Med. Ctr., New York, NY

Abstract Body:

Background: Many factors can affect PK in the neonatal ICU including sepsis and ECLS. Currently, no AMP PK data exists for children on ECLS with the current ECLS equipment. The purpose of this study was to describe AMP PK in neonates with a contemporary ECLS operation, including the Quadrox-ID Pediatric (HMOD 30000) diffusion membrane.**Methods:** This was a PK study of hospitalized neonates receiving IV AMP 100 mg/kg/dose q8 hr infused over 0.5 hr for suspected sepsis/meningitis between January-December 2015. Each patient contributed at least 2 blood samples over the dosing interval for AMP concentration determination. AMP concentrations were determined by liquid chromatography/tandem mass spectroscopy. AMP concentrations were modeled using Pmetrics, a nonparametric pharmacometric modeling and simulation package for R. Multiple compartmental and covariate (body weight) models were explored. The % of the dosing interval with free drug concentrations above the MIC (*f*T>MIC) was calculated using an MIC of 8 and 16 mcg/mL. Bactericidal exposure was defined as ≥ 50% *f*T>MIC. The probability of target attainment (PTA) > 90% for each MIC was defined as optimal.**Results:** 4 neonates contributed 9 AMP concentrations. The median gestational age was 37.5 weeks (range 36-39 weeks). Mean ± SD birth weight was 3.14 ± 0.3 kg. AMP concentration data best fit a 2-compartment model using weight as a covariate for clearance (CL) and volume of the central compartment (Vc). Population estimates for CL, Vc, and intercompartment transfer constants were 0.74 ± 0.52 mL/min/kg, 0.05 ± 0.03 L/kg, 2 ± 0.16 h^{-1} and 3.2 ± 2 h^{-1}, respectively. R^2, bias, and precision for the individual predicted versus observed fit were 0.6, -1.74 and 1.05 µg/mL, respectively. The mean ± SD k_e was 0.4 ± 0.17 hr^{-1}. The mean ± SD $t_{1/2}$ was 1.94 ± 0.83 hrs. At the MICs of 8 and 16 mcg/mL, the PTA for 100 mg/kg/dose q8 hr was 100%.**Conclusions:** These are the first AMP PK data in neonates with a contemporary ECLS circuit utilizing the Quadrox-ID Pediatric (HMOD 30000) diffusion membrane demonstrating higher CL values with a smaller Vd.

Author Disclosure Block:

J. Cies: C. Consultant; Self; Atlantic Diagnostic Laboratories. **W. Moore:** None. **M. Young:** None. **V. Bains:** None. **D. Carella:** None. **A. Enache:** None. **A. Chopra:** None.

Poster Board Number:

MONDAY-498

Publishing Title:

Gentamicin Pharmacokinetics in Neonates with Hypoxic Ischemic Encephalopathy in the Setting of Controlled Hypothermia

Author Block:

T. Habib[1], O. Menkiti[1], V. Bains[1], M. Young[1], W. Moore, II[2], A. Chopra[3], **J. Cies**[2]; [1]St. Christopher's Hosp. for Children, Philadelphia, PA, [2]The Ctr. for Pediatric Pharmacotherapy, Pottstown, PA, [3]NYU Langone Med. Ctr., New York, NY

Abstract Body:

Background: Perinatal asphyxia and subsequent therapeutic controlled hypothermia (CH) are independently known to alter renal function but the combined effect on the pharmacokinetics (PK) of commonly used medications is unknown. We aimed to evaluate the combined effect of moderate to severe hypoxic ischemic encephalopathy (HIE) and CH on gentamicin (G) pharmacokinetics (PK).**Methods:**This was a PK study of hospitalized neonates with moderate to severe HIE undergoing CH receiving IV G 4-5 mg/kg q36 infused over 0.5 hr for suspected meningitis between Sept-Dec 2015. Each patient contributed at least 5 (range 5-9) scavenged blood samples over multiple dosing intervals for G concentration determination. G concentrations were determined by liquid chromatography/tandem mass spectroscopy. G concentrations were modeled using Pmetrics, a nonparametric pharmacometric modeling and simulation package for R. Multiple compartmental and covariate (body weight) models were explored.**Results:**5 neonates contributed 29 G samples. The median gestational age was 39 weeks (range 36-41 weeks). Mean ± SD birth weight was 3.2 ± 0.62 kg. G concentration data best fit a 2-compartment model using weight as a covariate for clearance (CL) and volume of the central compartment (Vc). Population estimates for CL, Vc, and intercompartment transfer constants were 0.69 ± 0.21 mL/min/kg, 0.11 ± 0.01 L/kg, 0.62 ± 1.3 h^{-1} and 0.72 ± 1.48 h^{-1}, respectively. R^2, bias, and precision for the individual predicted versus observed fit were 0.97, -0.82 and 2.32 µg/mL, respectively. The mean ± SD $t_{1/2}$ was 13.8 ± 8 hrs as compared to a reported $t_{1/2}$ range of 3-11 hrs in term newborns. The mean ± SD Vd was 0.24 ± 0.1 L/kg as compared to a reported Vd of 0.45 ± 0.1 L/kg in term newborns.**Conclusions:**These data suggest that CH does have an impact on the PK of G in neonates with HIE undergoing CH, most notably a smaller volume of distribution with a similar CL estimate.

Author Disclosure Block:

T. Habib: None. **O. Menkiti:** None. **V. Bains:** None. **M. Young:** None. **W. Moore:** None. **A. Chopra:** None. **J. Cies:** C. Consultant; Self; Atlantic Diagnostic Laboratories.

Poster Board Number:

MONDAY-499

Publishing Title:

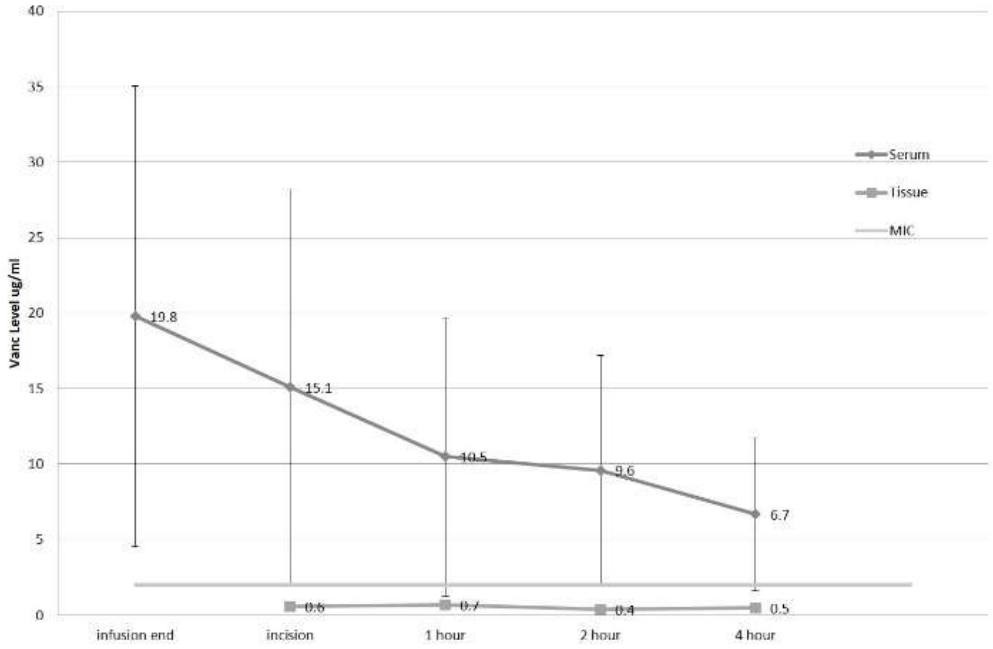
Tissue Concentrations Of Vancomycin During Posterior Spinal Fusions In Neuromuscular Scoliosis

Author Block:

S. M. Brown; Washington Univ. Sch. of Med., St. Louis, MO

Abstract Body:

Due to the rate of surgical site infections (SSI) in posterior spinal fusions (PSF) in neuromuscular (NM) scoliosis and the prevalence of multi-drug resistant organisms, vancomycin is part of standard antibiotic prophylaxis. This prospective study of 17 consecutively enrolled patients who underwent definitive PSF for NM scoliosis received vancomycin infusion pre-operatively evaluated tissue penetrance of vancomycin in the neuromuscular scoliosis population. Serum vancomycin concentrations were obtained immediately after infusion, at surgical incision, and then at 1 hour, 2 hours, and 4 hours post incision. Perispinal muscle tissue samples were obtained at incision, 1 hour, 2 hours, and 4 hours post incision and analyzed by a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) method. The study consisted of 10 males and 7 females with a mean age of 15y1.5m (10-20y). Mean infusion amount was 15.03 mg/kg. Median vancomycin concentrations were 19.8 (µg/ml) after infusion, 15.1 at incision, 10.5 at 1 hour, 9.6 at 2 hours, and 6.7 at 4 hours post infusion. Median muscle concentrations were 0.6 (µg/ml) at incision, 0.7 at 1 hour, 0.4 at 2 hours, and 0.5 at 4 hours post infusion. Hence, median serum concentrations were demonstrated to reach MIC at incision and at all time points during surgery, but median muscle concentrations never reached MIC at any time point. No patients had any cardiac or kidney disease, and all patients had normal kidney function based on laboratory values. Using accepted guidelines for the administration of intravenous vancomycin, serum vancomycin concentrations reached MIC at all time points tested during PSF for NM scoliosis. However, at no time point tested did muscle concentrations reach MIC.



Author Disclosure Block:

S.M. Brown: None.

Poster Board Number:

MONDAY-500

Publishing Title:

Comparison of Ceftazidime-Avibactam (CAZ-AVI) Exposure and PK/PD Target Attainment (TA) Across Patient Subgroups

Author Block:

S. Das[1], J. G. Wright[2], **T. Riccobene**[3], M. Macpherson[2], T. J. Carrothers[3], M. Lovern[4];
[1]AstraZeneca, Macclesfield, United Kingdom, [2]Wright Dose, Altrincham, United Kingdom, [3]Allergan, Jersey City, NJ, [4]Quantitative Solutions, Raleigh, NC

Abstract Body:

Background: Factors such as rate of creatinine clearance (CrCL), obesity, age or severity of inflammation could potentially impact CAZ and AVI pharmacokinetics (PK). Using population PK (popPK) models, individual patient exposures of Phase 3 patients with complicated intra-abdominal or urinary tract infections (cIAI or cUTI) were estimated. The impact of various covariates on CAZ and AVI exposures and PK/PD TA was explored in relevant patient subgroups.**Methods:** Data from ~1350 patients with cIAI or cUTI from Phase 3 studies were used along with Phase 1 and 2 study data to develop full covariate CAZ and AVI popPK models. Most patients received CAZ-AVI 2000-500 mg as a 2 h IV infusion q8h. These models were used to generate individual predictions for Phase 3 patients of CAZ and AVI area under the plasma concentration-time curve at steady state during the dosing interval (AUC_{SS}), maximum plasma concentration at steady state (C_{maxSS}) and TA (targets: CAZ 50% fT>CAZ-AVI MIC of 8 mg/L; AVI 50% fT>C_T of 1.0 mg/L). Exposures and TA were summarized across subgroup categories: CrCL (>150 vs. 80-150 mL/min); BMI (>39.9 vs. <39.9 kg/m^2); and age (>65 vs. <65 y). More severe systemic inflammation at baseline was also assessed by comparing exposure: in the presence or absence of SIRS, bacteremia, and fever; APACHE II score >10 or ≤10; and white blood cell count (WBC) ≤12,000/mm^3 or >12,000/mm^3.**Results:** High estimated CrCL (>150 mL/min), obesity and older age were associated with modest decreases in AUC_{SS} for both CAZ and AVI compared with patients with CrCL 80-150 mL/min normal renal clearance, lower BMI and younger age; however, PK/PD TA remained >95% across all patient categories. Presence of SIRS, bacteremia, fever, APACHE II score >10 or high WBC (>12,000/mm^3) at baseline had no significant impact on CAZ or AVI exposure and TA was >95% in all subgroups.**Conclusions:** CAZ-AVI 2000-500 mg by 2 h infusion q8h provides robust PK/PD TA for MICs ≤8 mg/L in patients with high CrCL (>150 mL/min), obesity, or aged over 65. Systemic inflammation did not impact CAZ or AVI exposure, demonstrating the dose is sufficient in patients with more severe disease.

Author Disclosure Block:

S. Das: D. Employee; Self; AstraZeneca. **J.G. Wright:** C. Consultant; Self; AstraZeneca. D. Employee; Self; Wright Dose. **T. Riccobene:** D. Employee; Self; Allergan PLC. **M. Macpherson:** C. Consultant; Self; AstraZeneca. D. Employee; Self; Wright Dose. **T.J. Carrothers:** D. Employee; Self; Allergan PLC. **M. Lovern:** C. Consultant; Self; AstraZeneca. D. Employee; Self; Quantitative Solutions.

Poster Board Number:

MONDAY-501

Publishing Title:

Clinical Pharmacokinetics of Levornidazole in Elderly Subjects and Dosing Regimen Evaluation

Author Block:

B. Guo, G. He, X. Wu, J. Yu, G. Cao, Y. Li, Y. Fan, Y. Chen, Y. Shi, Y. Zhang, J. Zhang; Inst. of Antibiotics, Huashan Hosp., Fudan Univ., Shanghai, China

Abstract Body:

Background: Levornidazole, the levo isomer of ornidazole, is a third-generation nitroimidazole derivative newly developed after metronidazole, tinidazole and ornidazole. Levornidazole was approved by the China Food and Drug Administration in 2009. The objective of this study was to investigate the pharmacokinetic (PK) profile of levornidazole and metabolites in healthy Chinese elderly subjects, and to evaluate the dose regimens for the elderly patients.**Methods:** An open-label, non-randomized, parallel control design, single-dose study was conducted at Huashan Hospital, Fudan University, Shanghai, China. Levornidazole was intravenous administered at 500 mg in 100 mL of sodium chloride injection to 12 healthy Chinese elderly or young subjects. The PK profiles of levornidazole and its five metabolites (M1, M2, M4, M6 and M16) and drug disposition in the elderly subjects were evaluated and compared with the young group. WinNonlin software was used to simulate the PK profiles of levornidazole in the elderly population following the dosing regimens of 500 mg twice daily and 750 mg once a day for 7 days. The cumulative fraction of response (CFR) of levornidazole against *Bacteroides fragilis* and other *Bacteroides spp* and the probability of target attainment (PTA) for various MIC levels were estimated using Monte Carlo simulation.**Results:** The C_{max}, AUC_{0-24} and $AUC_{0-\infty}$ of levornidazole were 11.98 μg/mL, 131.36 μg·h/mL and 173.61 μg·h/mL in the elderly group. The $T_{1/2}$, CL_t and $MRT_{0-\infty}$ of the elderly subjects were 12.21 h, 2.91 L/h and 16.46 h. The metabolic ratios of M1, M2, M4, M6 and M16 in the elderly groups were 2.14%, 0.08%, 2.91%, 0.03% and17.70%, respectively. The recoveries in urine of levornidazole, M1, M2, M4, M6 and M16 over 96 hours for the elderly subjects were 10.21%, 0.92%, approximate 0%, 2.69%, 0.54% and 41.98%. The PK parameters of levornidazole and urinary recoveries of all compounds did not show statistical difference between two groups ($p > 0.05$). For both dose regimens, the CFR was >90% against *Bacteroides fragilis* and other *Bacteroides* when the PK/PD target of AUC_{0-24}/MIC =157.6 and C_{max}/MIC=14.1 were used, and PTA was greater than 90% when MIC not greater than 1μg/mL.**Conclusion:** Both regimens may achieve satisfactory clinical and microbiological efficacies against *Bacteroides spp* in the elderly.

Author Disclosure Block:

B. Guo: None. **G. He:** None. **X. Wu:** None. **J. Yu:** None. **G. Cao:** None. **Y. Li:** None. **Y. Fan:** None. **Y. Chen:** None. **Y. Shi:** None. **Y. Zhang:** None. **J. Zhang:** None.

Poster Board Number:

MONDAY-502

Publishing Title:

A Pilot Study of Immune Activation and Rifampin Absorption in Hiv-infected Patients

Author Block:

C. Vinnard[1], I. Manley[1], B. Scott[1], M. Bernui[1], S. Varghese[1], J. Adams[2], M. Kutzler[1]; [1]Drexel Univ. Coll. of Med., Philadelphia, PA, [2]Brown Univ., Providence, PA

Abstract Body:

Background: Rifampicin malabsorption is frequently observed in tuberculosis (TB) patients co-infected with HIV, but cannot be predicted by patient factors such as CD4+ T cell count, HIV viral load, or the presence of diarrhea. We sought to describe the relationship between HIV-associated immune activation, measures of gut absorptive capacity and permeability, and rifampin PK parameters, in a pilot study of HIV-infected patients who were naïve to antiretroviral therapy (ART).**Methods:** We conducted an intensive pharmacokinetic (PK) study of single-dose rifampin among 6 HIV-infected patients naïve to antiretroviral therapy (ART). Blood samples were obtained for measurement of HIV-associated immune activation, enterocyte damage, and microbial translocation. We conducted the D-xylose assay to measure overall intestinal absorptive capacity, and the lactulose/mannitol assay to measure intestinal permeability.**Results:** The median rifampin AUC(0-8) was 42.8 mg•hr/L (range 21.2 to 57.6), with a median Cmax of 10.1 mg/L (range 5.3 to 12.5). We observed delayed rifampin absorption, defined as a time to maximum concentration (Tmax) greater than 2 hours, in 2 of 6 participants. Neither of the participants with delayed rifampin absorption demonstrated low intestinal absorptive capacity by D-xylose testing, or increased intestinal permeability by the lactulose/mannitol ratio. There was a trend towards increased serum concentrations of sCD14, a marker of monocyte activation in response to LPS, among participants with delayed rifampin absorption (median 1925 ng/mL) compared to participants with rapid absorption (median 1225 ng/mL, p=0.06).**Conclusions**: Delayed rifampin absorption may be associated with elevated markers of bacterial translocation among ART-naïve HIV-infected individuals. The limited sample size precluded the conduct of compartmental PK analysis using non-linear mixed effects models, and therefore we were unable to evaluate the individual measures of immune activation as covariate effects on the coefficient of absorption. These pilot data will inform the design of future studies to test the hypothesis that measures of HIV-associated immune activation can predict delayed rifampin absorption at the time of initiation of anti-TB therapies.

Author Disclosure Block:

C. Vinnard: E. Grant Investigator; Self; Janssen. **I. Manley:** None. **B. Scott:** None. **M. Bernui:** None. **S. Varghese:** None. **J. Adams:** None. **M. Kutzler:** None.

Poster Board Number:

MONDAY-503

Publishing Title:

Population Pharmacokinetics (Pk) and Pharmacodynamics (Pd) of Teicoplanin (Teic) in Neonates

Author Block:

V. Ramos-Martín[1], M. Neely[2], P. McGowan[3], S. Siner[4], K. Padmore[4], E. Scott[4], M. Peak[4], M. W. Beresford[1], M. A. Turner[1], S. Paulus[4], W. W. Hope[1]; [1]Univ. of Liverpool, Liverpool, United Kingdom, [2]Univ. of Southern California, Los Angeles, CA, [3]Liverpool Women´s Hosp., Liverpool, United Kingdom, [4]Alder Hey Children´s Hosp., Liverpool, United Kingdom

Abstract Body:

TEIC is frequently administered to neonates to treat Gm (+) infections. Little is known about the PK profile of TEIC in neonates and there is uncertainty about the optimal regimens to achieve desired target concentrations. The aim of the study was to determine the population PK of TEIC in neonates, evaluate currently recommended regimens and explore exposure-effect relationships.An open label PK study was conducted. Neonates from 26-44 weeks post-menstrual age were recruited (n=18). Patients received a loading dose (16 mg/kg) followed by 8 mg/kg q24h. Serum was collected at 1, 3, 6 and 24 h, and at steady state. A standard 2-compartment PK model was developed, followed by models that incorporated weight (wt). Wt was allowed to affect clearance using linear and allometric scaling terms. CRP serial concentrations were also collected as a PD input. Monte Carlo simulations were performed using Pmetrics. The AUCs at steady state and the proportion of patients achieving the recommended drug exposures in terms of Cmin (>15 mg/L) were determined.A total of 96 PK samples and 106 CRP serial concentrations were available for analysis. The PK allometric model best accounted for the observed data and was chosen for further analysis. The PK parameters medians were: Clearance=0.435*(wt /70)$^{0.75}$ (L/h), Volume 0.765 (L), Kcp 1.3 (h^{-1}), Kpc 0.629 (h^{-1}). The fit of the PK-PD data was acceptable. The time-course of CRP was well described using the Bayesian posterior median estimates for each patient. All patients except three were able to suppress CRP levels after the 5th dose of therapy. The simulated median AUC $_{96\text{-}120}$ was 297.8 mg*h/L, median Cmin at 120 h was 12.8 mg/L. The percentage of patients with Cmin >15 mg/L was 38.1 %TEIC population PK is highly variable in neonates. Wt (allometric relationship) is the best descriptor of PK variability (clearance) in the population. AUC drug exposures were comparable to exposures in adults (400 mg/day) and effective in terms of suppressing CRP levels in this study. However, only a low percentage of neonates were able to achieve Cmin >15 mg/L. The routine use of TDM and improved knowledge on the PD relationships between target exposures and response is required.

Author Disclosure Block:

V. Ramos-Martín: None. **M. Neely:** None. **P. McGowan:** None. **S. Siner:** None. **K. Padmore:** None. **E. Scott:** None. **M. Peak:** None. **M.W. Beresford:** None. **M.A. Turner:** None. **S. Paulus:** None. **W.W. Hope:** C. Consultant; Self; F2G, Pfizer, Astellas, AiCuris, Basilea, InfectoPharm, Pulmocide. E. Grant Investigator; Self; F2G, Pfizer, Astellas, AiCuris.

Poster Board Number:

MONDAY-504

Publishing Title:

Immune Activation and Isoniazid Metabolism in Hiv-associated Tuberculosis

Author Block:

C. Vinnard[1], S. Ravimohan[2], N. Tamuhla[3], V. Ivaturi[4], J. Pasipanodya[5], S. Kant[5], D. Weissman[2], G. P. Bisson[2]; [1]Drexel Univ. Coll. of Med., Philadelphia, PA, [2]Univ. of Pennsylvania, Philadelphia, PA, [3]Botswana-Upenn Partnership, Gaborone, Botswana, [4]Univ. of Maryland, Baltimore, Baltimore, MD, [5]Baylor Inst. for Immunology Res., Dallas, TX

Abstract Body:

Background: Adverse drug events frequently complicate the course of anti-tuberculosis (TB) therapy in HIV-infected patients. Certain cytokines that are elevated in HIV and TB can regulate expression of enzymes involved in metabolism of isoniazid, raising the possibility that host immune status affects risk of drug toxicity. Furthermore, HIV-infected individuals with rapid acetylator NAT2 genotypes often demonstrate slow acetylation phenotypes. We hypothesized that levels of systemic immune activation in adults with HIV/TB would be associated with variability in the clearance of isoniazid.**Methods:** We conducted a prospective cohort study of isoniazid pharmacokinetics (PK) in HIV/TB patients in Gaborone, Botswana. Because antiretroviral therapy (ART) can alter levels of immune activation in HIV/TB, we performed an intensively sampled PK visit before ART initiation. Immune activation parameters included CD38 and HLA-DR co-expression on CD8+ T cells, IL-6, and CRP. Population PK modeling was performed to determine whether levels of HIV-associated immune activation contributed to between subject variability in isoniazid clearance (CL).**Results:** We enrolled 40 HIV/TB patients into the PK study, after a median of 20 days of anti-TB therapy (range 7-65 days). No patients were receiving ART at the time of the PK visit. The isoniazid concentration data were best fitted with a 2-compartment model with first-order elimination, BSV on the central volume (V) and clearance (CL), and a combined additive/proportional residual error model NAT2 genotype (slow/intermediate/rapid) was a strong predictor of isoniazid CL (reduction of objective function value from 261 to 249). No additional covariate relationships were identified by the stepwise selection process.**Discussion:** Immune activation did not contribute to variability in isoniazid metabolism prior to initiation of ART. Future efforts will determine the covariate effect of immune activation on intra-occasional variability in isoniazid metabolism before and after ART initiation.

Author Disclosure Block:

C. Vinnard: None. **S. Ravimohan:** None. **N. Tamuhla:** None. **V. Ivaturi:** None. **J. Pasipanodya:** None. **S. Kant:** None. **D. Weissman:** None. **G.P. Bisson:** None.

Poster Board Number:

MONDAY-505

Publishing Title:

Safety and Efficacy of High Dose (Hi-D) Polymyxin B (Pb) Regimens Against Extensively Drug Resistant (Xdr) Gram Negative Bacteria (Gnb) in Critically Ill Patients: A Clinical Study

Author Block:

Y. Cai, J. Teo, T-P. Lim, W. Lee, A-L. Kwa; Singapore Gen. Hosp., Sg, Singapore

Abstract Body:

Background: Recent simulation studies suggested that higher doses (30000U/kg/day) of PB may be beneficial, but its clinical efficacy & safety have yet to be described. This study described the safety and efficacy of Hi-D PB regimens in critically-ill patients. **Methods:** An observational cohort study was done from Jan 2013 - Dec 2014 in a tertiary Singapore hospital. All critically ill patients prescribed ≥48h Hi-D PB (≥30000U/kg/day TBW) were included. Demographics, comorbidities, infection-related details, & antibiotic use & doses were collected. Outcomes measured were 30-day all-cause mortality, clinical cure, bacteriological clearance & presence of acute kidney injury (AKI). Daily serum creatinine (SCr) was trended. A multivariable regression model was used to determine predictors of AKI . **Results:** 45 patients were included. Mean (s.d.) APACHEII score was 19 (8) at time of Hi-D PB initiation; majority was ICU patients (69%). Hi-D PB was culture-directed in 36 (80%) patients. The most common indication for Hi-D PB was pneumonia (24%) & soft tissue infection (24%). 22 (49%) patients also had positive blood cultures. Pathogens were *A. baumannii*, *P. aeruginosa* & Enterobacteriaceae in 24 (53%), 11 (24%) & 10 (22%) patients respectively - 38 (84%) were extensively drug-resistant, but all were fully susceptible to PB (MIC range: 0.25 - 2mg/L). 43 (96%) patients received Hi-D PB as part of a 2- or 3-antibiotic combination regimen. Median (range) daily Hi-PB dose was 31310 (28170 - 40704) U/kg, & median cumulative Hi-D PB dose was 20 (4 - 127) MU. Clinical cure & 30-day mortality was observed in 35 (76%) & 11 (24%) patients respectively. In the 22 patients with positive blood culture, bacteriologic clearance was seen in 21 (95%) patients. 19 (42%) patients developed AKI during Hi-D PB; of these, AKI resolved fully in 10 (53%) patients. Peak SCr in AKI patients occurred at a median of 12 (2 - 40) days. In the multivariable regression model, only total cumulative PB was associated with AKI (OR 1.04, 95% C.I. 1.00 - 1.08). **Conclusion:** We observed favorable mortality rates & bacteriologic clearance in Hi-D PB patients. PB-induced AKI appeared to be related to total cumulative PB dose as opposed to high daily doses. Clinicians should balance the risk of AKI against the benefits of adequate PB dose.

Author Disclosure Block:

Y. Cai: None. **J. Teo:** None. **T. Lim:** None. **W. Lee:** None. **A. Kwa:** None.

Poster Board Number:

MONDAY-506

Publishing Title:

Nafithromycin (WCK 4873) Concentrations in Plasma, Epithelial Lining Fluid, and Alveolar Macrophages of Healthy Subjects

Author Block:

R. Chugh[1], A. Bhatia[1], M. Gupta[1], N. Sharma[1], M. Gotfried[2], K. Rodvold[3]; [1]Wockhardt, Mumbai, India, [2]Pulmonary Associates, PA, Phoenix, AZ, [3]Univ. of Illinois at Chicago, Chicago, IL

Abstract Body:

Nafithromycin is a novel oral antibacterial agent belonging to the ketolide class with potent in vitro antimicrobial activity against typical and atypical pathogens commonly associated with community-acquired lower respiratory tract infections. The primary objective of this study was to determine and compare plasma, epithelial lining fluid (ELF), and alveolar macrophages (AM) concentrations of nafithromycin in healthy adult male and female subjects. Nafithromycin concentrations in plasma, ELF, and AM of 37 subjects were measured by LC-MS/MS following repeat oral dosing of nafithromycin (800 mg once daily for 3 days). Noncompartmental pharmacokinetic (PK) parameters were determined from serial total plasma concentrations collected over a 24-hour interval following the first and third oral doses. Each subject underwent one standardized bronchoscopy with bronchoalveolar lavage (BAL) at 3, 6, 9, 12, 24 or 48 hours after the third dose of nafithromycin. Mean (± SD) PK parameters after the first dose included maximum concentration (Cmax) of 1.02 ± 0.31 µg/mL, time to Cmax (tmax) of 3.97 ± 1.30 h, clearance (CL/F) of 67.3 ± 21.3 L/h, and elimination half-life (t½) of 7.7 ± 1.1 h. Plasma PK parameters after the third dose were Cmax of 1.39 ± 0.36 µg/mL, tmax of 3.69 ± 1.28 h, CL/F of 52.4 ± 18.5 L/h, and t½ of 9.1 ± 1.7 h. Nafithromycin concentrations (Table) and AUC0-24 values of nafithromycin based on mean or median plasma concentrations at BAL sampling times were 16.2 µg•h/mL. For ELF, the respective AUC0-24 values were 224.1 and 176.3 µg•h/mL, whereas AUC0-24 values for AM were 8538 and 5894 µg•hr/mL. Penetration ratios based on ELF to plasma AUC0-24 values were 13.8 and 10.9, whereas ratios of AM to plasma were 527 and 364, respectively. The in vitro antibacterial activity and the sustained ELF and AM concentrations for 48 hours after the third dose suggest that nafithromycin has the potential to be a useful agent for the treatment of lower respiratory tract infections.

Nafithromycin concentrations (mean ± SD) at time of bronchoscopy and BAL

Sampling Time	Plasma (µg/mL)	ELF (µg/mL)	AM (µg/mL)
3-hour	1.105 + 0.140	18.48 + 12.31	290.2 + 179.0
6-hour	0.966 + 0.439	15.29 + 14.53	375.4 + 218.2
9-hour	0.922 + 0.286	8.37 + 4.38	401.1 + 420.3

12-hour	0.817 + 0.261	9.67 + 6.20	729.4 + 739.0
24-hour	0.239 + 0.055	4.13 + 1.38	120.9 + 66.2
48-hour	0.060 + 0.029	1.62 + 0.86	22.4 + 10.4

Author Disclosure Block:

R. Chugh: D. Employee; Self; Wockhardt. **A. Bhatia:** D. Employee; Self; Wockhardt. **M. Gupta:** D. Employee; Self; Wockhardt. **N. Sharma:** D. Employee; Self; Wockhardt. **M. Gotfried:** E. Grant Investigator; Self; Wockhardt. **K. Rodvold:** C. Consultant; Self; Wockhardt.

Poster Board Number:

MONDAY-507

Publishing Title:

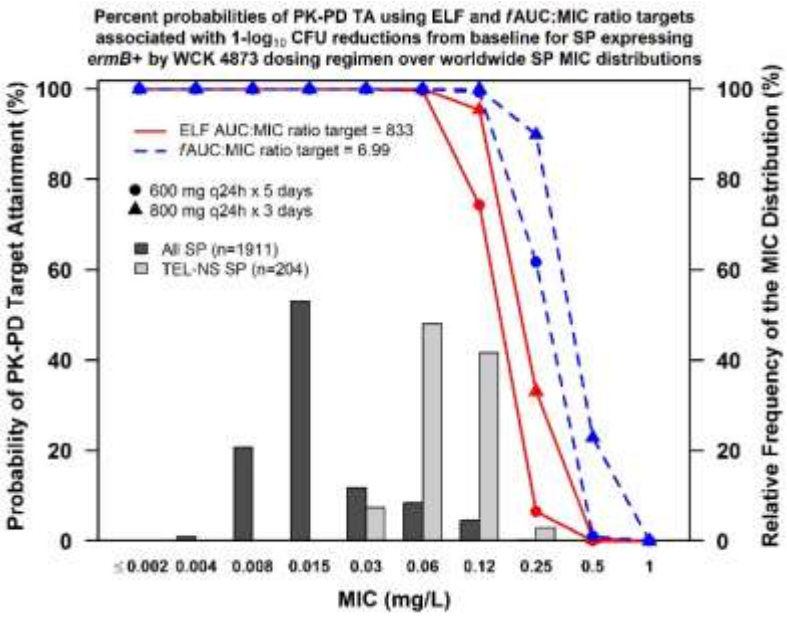
Pharmacokinetic-Pharmacodynamic (PK-PD) Target Attainment (TA) Analyses to Support WCK 4873 Dose Selection for the Treatment of Community-Acquired Bacterial Pneumonia (CABP)

Author Block:

J. C. Bader[1], E. A. Lakota[1], C. M. Rubino[1], M. V. Patel[2], S. S. Bhagwat[2], P. G. Ambrose[1], S. M. Bhavnani[1]; [1]ICPD, Latham, NY, [2]Wockhardt Res. Ctr., Aurangabad, India

Abstract Body:

WCK 4873 is a novel oral (PO) lactone ketolide, active against multi-drug resistant *S. pneumoniae* (SP). PK-PD TA analyses were performed to provide WCK 4873 dose selection support for a Phase 2 CABP clinical trial. Analyses utilized a Phase 1 population PK (PPK) model, non-clinical PK-PD targets from a neutropenic murine-lung infection model, and WCK 4873 MIC data for SP. The PPK model was a 4-compartment model (2 absorptive, 2 distributive) with parallel linear and non-linear clearance. Epithelial lining fluid (ELF) PK was described using a 2-compartment model. Using PK parameter estimates, ELF and free-drug plasma (*f*) concentration-time profiles were simulated for 2000 patients administered WCK 4873 600 mg PO q24h x 5 d or 800 mg PO q24h x 3 d. Average $AUC_{0\text{-}24}$ over Days 1-3 was calculated. The median and second highest ELF AUC:MIC ratio (833 and 1553, respectively) and *f*AUC:MIC ratio (6.99 and 14.1, respectively) targets associated with 1-$\log_{10}$ CFU reduction from baseline for SP *ermB*$^+$ were evaluated. Percent probabilities of PK-PD TA (%PTA) by MIC and over SP MIC distributions were determined. Using median targets, %PTA by MIC for both dosing regimens were $\geq$99.6% at the MIC_{90} of 0.06 mg/L for SP isolates collected worldwide (see Figure). Sensitivity analyses based on the second highest targets showed %PTA of $\geq$81.0% and $\geq$97.1% for the 600 and 800 mg regimens, respectively, at this MIC. Using the worldwide MIC distribution for all SP and all 4 targets, overall %PTA were $\geq$94.0 % for both dosing regimens. Results of these PK-PD TA analyses provide WCK 4873 dose selection support for the treatment of patients with CABP, including patients with SP *ermB*$^+$.



Author Disclosure Block:

J.C. Bader: I. Research Relationship; Self; Wockhardt Research Center. **E.A. Lakota:** I. Research Relationship; Self; Wockhardt Research Center. **C.M. Rubino:** I. Research Relationship; Self; Wockhardt Research Center. **M.V. Patel:** D. Employee; Self; Wockhardt Research Center. K. Shareholder (excluding diversitied mutual funds); Self; Wockhardt Research Center. **S.S. Bhagwat:** D. Employee; Self; Wockhardt Research Center. K. Shareholder (excluding diversitied mutual funds); Self; Wockhardt Research Center. **P.G. Ambrose:** I. Research Relationship; Self; Wockhardt Research Center. **S.M. Bhavnani:** I. Research Relationship; Self; Wockhardt Research Center.

Poster Board Number:

MONDAY-508

Publishing Title:

Population Pharmacokinetic (PPK) Model Describing the Disposition of WCK 4873 in the Plasma and Epithelial Lining Fluid (ELF) of Healthy Subjects

Author Block:

E. A. Lakota[1], J. C. Bader[1], S. M. Bhavnani[1], K. A. Rodvold[2], M. V. Patel[3], S. S. Bhagwat[3], P. G. Ambrose[1], C. M. Rubino[1]; [1]ICPD, Latham, NY, [2]Univ. of Ill, Chicago, IL, [3]Wockhardt Res. Ctr., Aurangabad, India

Abstract Body:

WCK 4873 is a novel oral (PO) lactone ketolide currently in clinical development. WCK 4873 is active against pathogens commonly associated with community-acquired bacterial pneumonia (CABP), including multi-drug resistant *S. pneumoniae*. Data from 3 Phase 1 studies were used to develop a PPK model to describe the time course of WCK 4873. The PPK model was developed in NONMEN 7.1.2 using 2800 plasma and 37 ELF concentrations from 117 and 37 healthy subjects, respectively. Subjects received WCK 4873 as single (100 to 1200 mg) or multiple (600 to 1000 mg) PO doses. Various structural PK models were evaluated. A covariate analysis was conducted to assess the impact of food and various patient demographic features on WCK 4873 PK. A 4-compartment model (2 absorptive, 2 distributive) with parallel linear and non-linear clearance best described the plasma WCK 4873 PK data. Bi-phasic absorption was accomplished using separate processes: one with a lag time and zero-order absorption and one with a time delay, using 4 transit compartments. The mean (%CV) of the linear clearance component was 34.8 L/hr (45.6%). The nonlinear clearance component had a maximum elimination rate of 14.8 mg/hr (4.97%) with a concentration at which clearance is half-maximal of 253 mcg/L. The effect of food was significant; bioavailability was reduced by 12.6% in the fasted state. The ELF data was described using a 2-compartment model for the lung. Bi-directional rate constants (%CV) between ELF central compartment and plasma were 0.0436 (23.7%) and 0.228 hr^{-1}, respectively. The PPK model provided acceptable fits to the plasma and ELF data (r^2=0.941 and 0.350 for observed vs fitted concentrations, respectively). Goodness-of-fit diagnostics indicated an unbiased fit to the data. From Day 1 to 5, the mean (%CV) accumulation was 42.1% (17.7%). Mean (%CV) Day 3 ELF and total- and free-drug plasma $AUC_{0\text{-}24}$ for a 800 mg PO daily regimen were 213 (24.1%), 15, and 2.96 (23.7%) mg·h/L, respectively. Using Day 3 $AUC_{0\text{-}24}$, mean (%CV) ELF:total and ELF:free penetration ratios were 14.4 and 73.0 (16.0%), respectively. A PPK model was successfully developed to describe WCK 4873 PK. This model will be useful to support dose selection for future CABP clinical trials.

Author Disclosure Block:

E.A. Lakota: I. Research Relationship; Self; Wockhardt Research Center. **J.C. Bader:** I. Research Relationship; Self; Wockhardt Research Center. **S.M. Bhavnani:** I. Research Relationship; Self; Wockhardt Research Center. **K.A. Rodvold:** I. Research Relationship; Self; Wockhardt Research Center. **M.V. Patel:** D. Employee; Self; Wockhardt Research Center. K. Shareholder (excluding diversitied mutual funds); Self; Wockhardt Research Center. **S.S. Bhagwat:** D. Employee; Self; Wockhardt Research Center. K. Shareholder (excluding diversitied mutual funds); Self; Wockhardt Research Center. **P.G. Ambrose:** I. Research Relationship; Self; Wockhardt Research Center. **C.M. Rubino:** I. Research Relationship; Self; Wockhardt Research Center.

Poster Board Number:

MONDAY-509

Publishing Title:

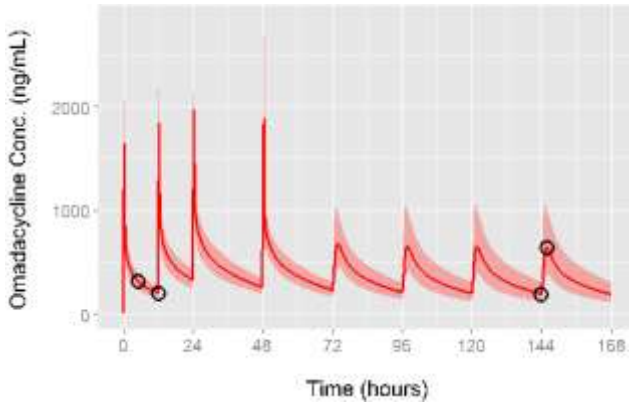
Population Pharmacokinetics (PPK) of Omadacycline (OMC) Following Intravenous (IV) or Oral Administration and Evaluation of Phase 3 Sparse PK Sampling Strategies

Author Block:

S. A. VanWart[1], A. Manley[2], S. M. Bhavnani[1], K. Tanaka[2], E. Loh[2], E. Tzanis[2], P. G. Ambrose[1]; [1]ICPD, Latham, NY, [2]Paratek Pharmaceuticals, Inc., Boston, MA

Abstract Body:

OMC is a first in class aminomethylcycline antibiotic currently being developed to treat acute bacterial skin and skin structure infections and community-acquired bacterial pneumonia. A population PK model for OMC developed using Phase 1 data was used to evaluate potential Phase 3 sparse PK sampling strategies. A 3-compartment model with zero-order IV input, or first-order oral absorption with transit compartments, best described plasma PK data from 281 subjects. Clearance (CL) was a function of creatinine clearance; bioavailability (F) was estimated based upon the timing of a meal relative to dosing. Using this model, PK data were simulated for 2000 patients administered 100 mg IV q12h on Day 1 followed by 100 mg q24h thereafter. Patients were randomly switched to 300 mg oral OMC q24h between Days 4 and 7. Maximum a-posteriori (MAP) Bayesian estimation was performed in NONMEM for various PK datasets with 2 samples each on Days 1 and 7. Absolute prediction error percent (|PE%|) for the Bayesian PK estimates relative to the true values were calculated for CL, steady-state volume of distribution, F, and the maximum concentrations after IV and oral dosing. The median |PE%| for each parameter within each sparse PK sampling scheme was calculated. The simulated patient data, with circles depicting suggested PK sampling times, are shown in Figure 1 using an interactive R-Shiny app. Based on a composite ranking of |PE%| across parameters, PK samples should be collected 3 to 5 h and at 12 h after first IV dose on Day 1, and pre-dose and 1 to 3 h after oral dosing on Day 7. A population PK model for OMC developed using Phase 1 data was used to recommend a Phase 3 sparse PK sampling strategy.



Author Disclosure Block:

S.A. VanWart: I. Research Relationship; Self; Paratek. **A. Manley:** D. Employee; Self; Paratek. **S.M. Bhavnani:** I. Research Relationship; Self; Paratek. **K. Tanaka:** D. Employee; Self; Paratek. **E. Loh:** D. Employee; Self; Paratek. **E. Tzanis:** D. Employee; Self; Paratek. **P.G. Ambrose:** I. Research Relationship; Self; Paratek Pharmaceuticals.

Poster Board Number:

MONDAY-510

Publishing Title:

Evaluation of the *In Vitro* Activity Profile of Omadacycline against *Haemophilus influenzae*

Author Block:

B. D. VanScoy[1], H. Conde[1], K. Tanaka[2], S. M. Bhavnani[1], J. N. Steenbergen[2], P. G. Ambrose[1]; [1]ICPD, Latham, NY, [2]Paratek Pharmaceuticals, Inc., Boston, MA

Abstract Body:

Background: Omadacycline is a novel aminomethylcycline in Phase 3 clinical development for use against multiple bacterial pathogens, including *H. influenzae.* In order to evaluate the *in vitro* activity of omadacycline, a series of foundation studies were undertaken. **Methods:** *In vitro* studies were performed using a panel of 5 *H. influenzae* clinical isolates. The studies included evaluating the MIC, the resistance mutation frequency, static time-kill activity and the post-antibiotic effect of omadacycline. All studies were performed using the Clinical Laboratory Standards Institute and European Committee on Antimicrobial Susceptibility Testing guidelines for broth and agar methodologies using their respective growth mediums (Haemophilus Test Medium and Mueller Hinton broth medium supplemented with 5% defibrinated horse blood, plus 20 mg/L β-nicotinamide adenine dinucleotide MH-F). **Results:** The MIC values for omadacycline ranged from 0.5 to 2 mg/L using broth and agar dilution methodologies. No isolates were recovered from the omadacycline drug-containing agar plates utilized in the mutation frequency studies at concentrations of 3 times the baseline MIC, using an average inoculum of 6.2×10^9 CFU/mL. These results demonstrated omadacycline's resistance mutation frequency to be below detection for the 5 clinical isolates. The results of the static time-kill studies showed that concentrations of 1 to 2 times the omadacycline MIC achieved a ≥ 3 Log_{10} CFU reduction from baseline. The median (min, max) duration of the post-antibiotic effect for omadacycline was 2.95 (1 to 4) hrs when examined against four clinical *H. influenzae* isolates from the original panel. **Conclusion:** Bactericidal *in vitro* activity for *H. influenzae* was observed at omadacycline concentrations either matching or twice the MIC value for each individual isolate. This bactericidal activity along with low resistance mutation frequency demonstrate potent activity of omadacycline against *H. influenzae* and warrant further *H. influenzae* studies.

Author Disclosure Block:

B.D. VanScoy: I. Research Relationship; Self; Paratek. **H. Conde:** I. Research Relationship; Self; Paratek. **K. Tanaka:** D. Employee; Self; Paratek. **S.M. Bhavnani:** I. Research Relationship; Self; Paratek. **J.N. Steenbergen:** D. Employee; Self; Paratek. **P.G. Ambrose:** I. Research Relationship; Self; Paratek.

Poster Board Number:

MONDAY-511

Publishing Title:

Antibacterial Efficacy of Eravacycline (Erv) *In Vivo* Against Gram-positive and Gram-negative Organisms

Author Block:

M. L. Monogue[1], A. K. Thabit[1], Y. Hamada[2], D. P. Nicolau[1]; [1]Ctr. for Anti-Infect. Res. & Dev., Hartford Hosp., Hartford, CT, [2]Aichi Med. Univ. Hosp. Sch. of Med., Aichi, Japan

Abstract Body:

Background: Members of the tetracycline class are frequently classified as bacteriostatic; conversely, recent findings have demonstrated an improved bacterial killing profile, often achieving bactericidal activity, when given for periods of > 24 hours. The objective of this study was to elucidate the bacterial killing effect of ERV, a novel fluorocycline, in the setting of humanized exposures over a 72-hour treatment period. **Methods:** Antibacterial efficacy was studied against two methicillin-resistant *Staphylococcus aureus* (MRSA) (ERV MICs = 0.03 and 0.25 μg/ml) and three Enterobacteriaceae isolates (ERV MICs = 0.125-0.25 μg/ml) in an immunocompetent murine thigh infection model. The humanized doses of ERV (2.5 mg/kg IV q12h), in addition to comparator antibiotics (linezolid, tigecycline, vancomycin, meropenem), were administered starting two hours after infection. Efficacy was measured as the change in $\log_{10}$CFU at 24h, 48h, and 72h compared with 0h controls.**Results:** Progressively enhanced activity of ERV was noted over the 72-hour study period against the tested isolates. The bacterial density was reduced by > 3 $\log_{10}$CFU at 72 hours against the MRSA isolates (Table) and one *E. coli* isolate, and > 1 $\log_{10}$CFU against *C. freundii* and the second *E. coli* isolate. Overall, bacterial killing by ERV was similar to the comparator antibiotics, although more variability in bacterial killing was observed for all antibacterial agents against the Gram-negative isolates.**Conclusions:** ERV has shown strain- and species-specific bactericidality *in vitro*. These studies demonstrate that the drug exhibits a cumulative dose response and may achieve bactericidal activity *in vivo* after 72 hours of therapy against MRSA and Enterobacteriaceae. Therefore, an analysis of the pharmacodynamic profile beyond 24 hours should be considered when assessing the clinical efficacy of this novel agent.

Group	Time of Collection	$\log_{10}$ CFU		Change in $\log_{10}$ CFU	
		Mean	SD	Mean	SD
S. aureus 426	0h	6.98	0.20	-	-
Control	24h	7.75	0.20	0.77	0.20
	48h	7.71	0.18	0.72	0.18
	72h	7.56	0.34	0.58	0.34

ERV	24h	5.05	0.05	-1.94	0.05
	48h	3.96	0.10	-3.03	0.10
	72h	2.88	0.19	-4.11	0.19
Tigecycline	24h	5.58	0.44	-1.4	0.44
	48h	4.74	0.64	-2.24	0.64
	72h	3.24	0.66	-3.74	0.66

Author Disclosure Block:

M.L. Monogue: None. **A.K. Thabit:** None. **Y. Hamada:** None. **D.P. Nicolau:** A. Board Member; Self; Tetraphase Pharmaceuticals, Inc.. E. Grant Investigator; Self; Tetraphase Pharmaceuticals, Inc..

Poster Board Number:

MONDAY-512

Publishing Title:

Post-Antibiotic Effect of Omadacycline against Target Pathogens

Author Block:

R. Hinshaw, L. Stapert, D. Shinabarger, **C. Pillar**; Micromyx, Kalamazoo, MI

Abstract Body:

Background: Omadacycline (OMC) is a new once daily oral and IV aminomethylcycline under phase 3 development for the treatment of serious community-acquired skin and respiratory infections. To better understand the pharmacodynamic properties during dosing, it is important to evaluate the post-antibiotic effect (PAE). In this study, the PAE of OMC, tigecycline (TIG), and linezolid (LZD) was evaluated against target pathogens.**Methods:** MICs of OMC, TIG, and LZD were determined in accordance with CLSI M7 against clinical isolates of *S. aureus* (n=2; 1 MRSA), *E. faecalis* (n=1), *E. faecium* (n=1; VRE), *S. pneumoniae* (n=2; 1 PRSP), and *E. coli* (n=1). PAE was evaluated after initial exposure of log-phase bacteria to 5X the MIC of OMC,TIG, or LZD for 1 hr alongside an unexposed control. Post-exposure, the drug was removed via centrifugation and washing and the bacteria were incubated in fresh drug free media for 10 hr. Viable bacteria were enumerated at 2 h intervals post-exposure. PAE was calculated as the time it took for bacteria to grow 1-log after initial exposure and drug washout relative to unexposed controls.**Results:** The PAE (hr) of OMC, TIG, and LZD after exposure to 5X the MIC is shown in the table below.

Test Agent	***S. aureus* MSSA/MRSA**	***E. faecalis* VSE**	***E. faecium* VRE**	***E. coli***	***S. pneumoniae* PSSP/PRSP**
OMC	2.6/2.2	2.0	2.1	1.4	3.3/2.3
TIG	3.9/2.5	3.8	4.4	1.4	3.3/3.6
LZD	1.3/1.0	1.2	1.7	not tested	2.2/1.5

Similar initial exposure concentrations (µg/mL) were used for OMC (1.25 - 5) and TIG (0.6 - 2.5) across isolates except for *S. pneumoniae* (0.15 - 0.3 for OMC and TIG). Initial exposure for LZD was higher (5 - 20 µg/mL). OMC PAEs varied between 1.4 hr (*E. coli*) and 3.3 hr (*S. pneumoniae*). TIG PAEs were similar to OMC PAEs with the exception of enterococci where the TIG PAE was slightly longer (3.8 - 4.4 hr) compared to OMC (2.0 - 2.1 hr). OMC and TIG PAEs were longer than LZD (PAEs between 1.0 and 2.2 hr) for all organisms tested.**Conclusions:** Overall, the PAE of OMC was similar to that of TIG with the exception of the evaluated enterococci where slightly longer PAEs were observed with TIG relative to OMC. Both OMC and TIG exhibited prolonged PAE relative to LZD. These PAE data demonstrate some prolonged

activity of OMC and TIG which may provide a benefit in the treatment of serious community-acquired bacterial infections.

Author Disclosure Block:

R. Hinshaw: H. Research Contractor; Self; Paratek. **L. Stapert:** H. Research Contractor; Self; Paratek. **D. Shinabarger:** H. Research Contractor; Self; Paratek. **C. Pillar:** H. Research Contractor; Self; Paratek.

Poster Board Number:

MONDAY-513

Publishing Title:

Nafithromycin Phase 1 Multiple Ascending Dose Study in Healthy Subjects

Author Block:

R. Chugh[1], M. Gupta[1], P. Iwanowski[2], A. Bhatia[1]; [1]Wockhardt, Mumbai, India, [2]Wockhardt, Warsaw, Poland

Abstract Body:

Background: Nafithromycin (WCK 4873) is a novel antibacterial agent belonging to the ketolide class with potent *in vitro* antimicrobial activity against typical and atypical pathogens commonly associated with community-acquired lower respiratory tract infections. This study was conducted to determine the safety, tolerability and pharmacokinetics (PK) of multiple ascending doses of nafithromycin in healthy subjects. **Methods:** 30 healthy subjects were enrolled in 3 cohorts (8 subjects on nafithromycin and 2 on placebo in each cohort) in a double-blind manner. Concentrations of nafithromycin in plasma, urine, feces, and polymorphonuclear white blood cells (PMN WBCs) were measured following multiple oral doses of 600 mg, 800 mg, and 1000 mg nafithromycin OD for 7 days.**Results:** C_{max} and AUC_{0-24} values were approximately 1.2 to 1.6 fold and 1.4 to 2 fold higher on Day 7 compared to the respective C_{max} and AUC_{0-24} values on Day 1 (Table 1). After single and multiple dosing with nafithromycin, the mean $t_{1/2}$ was between 9.16 and 14.4 h and tended to increase with increasing dose. On average, steady-state was achieved after Day 3 of nafithromycin administration. The mean recovery of nafithromycin via urine over 24-hours interval was higher (between 16.3 and 22.4%) than the mean recovery of nafithromycin via feces (between 3.2 and 9.4%). High intracellular accumulation of nafithromycin was observed in PMN WBCs with a maximum accumulation ratio ranging between 120.3 and 138.6 on Day 1 and between 142.3 and 366.6 on Day 7 across the dose range tested. All treatment-emergent adverse events were mild except one (diarrhoea) of moderate severity with 1000 mg, and resolved at follow-up. There were no deaths, serious adverse events or clinically significant findings in laboratory, 12-lead ECG, and physical examination.**Conclusions:** Nafithromycin demonstrated non-linear PK with a 2-fold maximum accumulation, and was safe and well tolerated following 7 days of dosing with 600, 800, and 1000 mg.**Table 1. Nafithromycin - AUC_{0-24} and C_{max} on Day 1 and Day 7**

Nafithromycin	**C_{max} (ng/mL)**		**AUC_{0-24} (h.ng/mL)**	
	Day 1	**Day 7**	**Day 1**	**Day 7**
600 mg	1121	1340	9418	13478
800 mg	1207	1888	11986	22198

1000 mg	1968	2987	21262	43464

Author Disclosure Block:

R. Chugh: None. **M. Gupta:** None. **P. Iwanowski:** None. **A. Bhatia:** None.

Poster Board Number:

MONDAY-514

Publishing Title:

Nafithromycin Single Ascending Dose (SAD) and Food Effect (FE) Study in Healthy Subjects

Author Block:

A. Bhatia[1], R. Chugh[1], M. Gupta[1], P. Iwanowski[2]; [1]Wockhardt, Mumbai, India, [2]Wockhardt, Warsaw, Poland

Abstract Body:

Background: Nafithromycin (WCK 4873) is a novel antibacterial agent belonging to the ketolide class with potent *in vitro* antimicrobial activity against typical and atypical pathogens commonly associated with community-acquired lower respiratory tract infections. The primary objective of this study was to determine safety, tolerability, and pharmacokinetics of SAD of nafithromycin, and the effect of food on nafithromycin in healthy adult subjects. **Methods:** Subjects in the SAD part (4 cohorts) were administered a single oral dose of 100, 200, 600 or 1200 mg of nafithromycin (8 subjects in each cohort) or placebo (2 subjects in each cohort) in a double-blind manner. Subjects in the 2 FE cohorts were administered a single oral dose of 400 mg or 800 mg nafithromycin (12 subjects each) or placebo (2 subjects each) in a double-blind cross-over method (fed and fasted state). Analysis of plasma, urine and feces samples were performed for nafithromycin using validated methods. **Results:** Median t_{max} was reached at 1 to 4 h post-dose in the SAD part. Mean maximum concentrations (C_{max}) and exposures based on $AUC_{0\text{-}inf}$ increased with increasing dose and ranged from 99.4 to 1742 ng/mL and 662 to 22,925 h.ng/mL, respectively (Table 1). The half-life ($t_{1/2}$) ranged from 5.15 to 8.95 h. For the food effect cohorts, fed state $AUC_{0\text{-}t}$ and C_{max} were approximately 1.2 to 1.3 higher than in the fasted state (Table 1). All treatment-emergent adverse events (TEAEs) were mild, except for one event of vomiting of moderate intensity, and resolved without sequelae. There were no severe or serious AEs (SAEs) or deaths reported during the study. There were no clinically significant findings in laboratory values, vital signs, 12-lead ECG, and physical examination. **Conclusions:** Plasma exposure to nafithromycin appeared to increase more than doseproportional over the 100 to 1200 mg single dose range and was only mildly affected by food. Overall, single oral treatment with nafithromycin up to a dose level of 1200 mg was safe and well-tolerated by healthy adult subjects.**Table 1. Plasma PK parameters of nafithromycin**

Study Part	**Treatment**	**C_{max}**	**t_{max}**	**$AUC_{0\text{-}inf}$**	**$t_{1/2}$**
		(ng/mL)	**(h)**	**(h.ng/mL)**	**(h)**
SAD	100 mg	99.4	1.26	662	5.15
	200 mg	175	2	1508	7.08

	600 mg	911	4	10655	7.9
	1200 mg	1742	3.5	22925	8.09
FE	400 mg fasted	345	2.5	4106	7.87
	400 mg fed	444	3	4448	7.43
	800 mg fasted	932	4	12487	8.95
	800 mg fed	1203	3.88	14848	8.56

Author Disclosure Block:

A. Bhatia: None. **R. Chugh:** None. **M. Gupta:** None. **P. Iwanowski:** None.

Poster Board Number:

MONDAY-515

Publishing Title:

The Effect of Gepotidacin (Gsk2140944), a Novel Triazaacenaphthylene Bacterial Topoisomerase Inhibitor, on Cardiac Repolarization Parameters

Author Block:

M. Hossain[1], **C. Tiffany**[2], M. McDonald[2], E. Dumont[2]; [1]GlaxoSmithKline, King of Prussia, PA, [2]GlaxoSmithKline, Collegeville, PA

Abstract Body:

Background: Gepotidacin (GEP) is a novel triazaacenaphthylene antibiotic, which inhibits bacterial DNA replication and has *in vitro* activity against susceptible and drug-resistant pathogens associated with a range of conventional and biothreat infections. **Methods:** 4-period, randomized, active- and placebo-controlled, double-blinded, crossover study in 55 healthy male and female subjects. Single doses of gepotidacin 1000 mg and 1800 mg were infused over 2 hours; placebo, and oral moxifloxacin 400 mg were administered using double-dummy methods. PK (blood and urine) and PD (12-lead Holter ECG) sampling, and safety assessments were performed at specified intervals. **Results:** The infusion of gepotidacin caused an increase from baseline in HR (ΔHR). The placebo corrected ΔHR (ΔΔHR) was elevated with a largest effect observed at 2 hours (end of infusion) of 6.5 bpm and 10.4 bpm after 1000 mg and 1800 mg, respectively. QTcF was selected as the primary endpoint based on the ability to remove the HR dependence of the QT interval. The ΔΔQTcF was observed at the end of the infusion with a largest value of 12.1 msec (90% CI: 9.5 to 14.8) after 1000 mg and 22.2 msec (90% CI: 19.6 to 24.9) after 1800 mg. The ΔΔQTcF fell after the end of the infusion. For moxifloxacin, the largest mean ΔΔQTcF was observed at 3 hours (12.7 msec) with the lower bound of the 90% CI above 5 msec at all prespecified time points (2, 3, and 4 hours) confirming assay sensitivity. Exposure response analysis using a linear model with an intercept demonstrated a statistically significant positive relationship between gepotidacin plasma levels and ΔΔQTcF(slope 1.45 msec per µg/mL). There were no severe AEs, SAEs, or deaths reported during this study. **Conclusions:** Infusion of gepotidacin at a dose of 1000 mg and 1800 mg caused a mild increase in HR (6 to10 bpm) and QT prolongation measured as ΔΔQTcF of 12 msec to 22 msec. QT prolongation was quickly reversed over 2 hours after the end of the infusion. Gepotidacin did not have a clinically relevant effect on cardiac conduction (PR and QRS intervals).

Author Disclosure Block:

M. Hossain: None. **C. Tiffany:** None. **M. McDonald:** None. **E. Dumont:** None.

Poster Board Number:

MONDAY-516

Publishing Title:

Activity of S-649266 Siderophore Cephalosporin and Comparators against *Pseudomonas aeruginosa* in Murine Thigh Infection Model

Author Block:

I. Ghazi[1], M. Tsuji[2], D. P. Nicolau[1]; [1]Hartford Hosp., Hartford, CT, [2]Shionogi, Osaka, Japan

Abstract Body:

Background: S-649266 is a siderophore cephalosporin that has demonstrated activity against Gram-negative bacteria as well as stability against serine- and metallo-carbapenemases in animal models. The objectives of this work were to demonstrate the efficacy of S-649266 as compared to two other siderophore based compounds against *P. aeruginosa* and to explore the pharmacodynamic (PD) profile of this novel compound using the neutropenic murine thigh infection model.**Methods: :** Six clinical *P. aeruginosa* isolates (1403, JJ4-36, JJ11-54, JJ8-16, AZ32-13 and JJ5-35) were tested *in vivo*. MICs were determined by broth microdilution in triplicate and modal MIC reported. Groups of 3 mice were inoculated and 2 hours later were treated with ascending doses of S-649266 12.5, 25, 50, 100, 200, 300, 400 and 500 mg/kg/day divided q8h or humanized doses of siderophores MB1 and AZ3167 as determined in previous studies. After 24 hours, the animals were sacrificed for bacterial enumeration and determination of the change in bacterial density ($\log_{10}$ CFU) relative to the starting inoculum (0h). Analysis of PD driver of efficacy was carried out using the inhibitory sigmoid E_{max} model.**Results:** The *P. aeruginosa* MIC ranges for three siderophores S-649266, AZ3167 and MB1 were: 0.063-0.5, 0.063-0.5 and ≤0.031-1 mg/L, respectively. In controls mean ± SD bacterial density at 0h was 5.5 ± 0.4 which increased to 8.5 ± 0.5 $\log_{10}$CFU at 24h. CFU reductions exceeding 1 log were observed for all *P. aeruginosa* after the administration of S-649266 at doses ≥ 100mg/kg/day. As previously reported, the current study confirmed that a humanized exposure of MB1 was effective against 1403 but not against JJ4-36, JJ11-54, JJ8-16, AZ32-13 and JJ5-35. Similar to historical data AZ3176 displayed no efficacy against JJ4-36. The pharmacodynamic analysis revealed that the magnitudes of *f*T>MIC, *f*C_{max}/MIC and *f*AUC/MIC were inversely proportional to observed CFU counts.**Conclusions:** Unlike the previously reported lack of efficacy with siderophores MB1 and AZ3175 against the *P. aeruginosa* studied, S-649266 displayed sustained antibacterial effects for all isolates over the treatment period. Enhanced bacterial kill was observed over the dose range studied and as previously observed % *f*T>MIC was well correlated to the efficacy of S-649266.

Author Disclosure Block:

I. Ghazi: None. **M. Tsuji:** D. Employee; Self; Shionogi & CO LTD. **D.P. Nicolau:** C. Consultant; Self; Shionogi. E. Grant Investigator; Self; Shionogi.

Poster Board Number:

MONDAY-517

Publishing Title:

Application of Pharmacokinetic-Pharmacodynamic (Pk-Pd) Models for Brilacidin (Bri) Dose Selection Support for Patients with Acute Bacterial Skin and Skin Structure Infections (ABSSSI)

Author Block:

S. M. Bhavnani[1], J. P. Hammel[1], A. Forrest[1], S. A. VanWart[1], P. Sager[2], K. J. Tack[3], R. W. Scott[4], D. M. Jorgensen[3], P. G. Ambrose[1]; [1]ICPD, Latham, NY, [2]Stanford Univ., Palo Alto, CA, [3]Cellceutix Corp., Beverly, MA, [4]Fox Chase Chemical Diversity Ctr., Doylestown, PA

Abstract Body:

Introduction: BRI is a synthetic molecule of a novel class of agents that demonstrates potent antimicrobial activity against Gram-positive organisms, including MRSA. BRI is being developed for the treatment of patients with ABSSSI. Using population PK and PK-PD models for efficacy and safety developed using data from two Phase 2 studies of BRI-treated patients with ABSSSI and Monte Carlo simulation (MCS), BRI dosing regimens were evaluated. **Methods:** MCS was used to generate 5000 patients with distributions for covariates of PK based on data included in the previous PK analysis population. MCS was carried out using the BRI population PK model. Individual post-hoc parameter estimates were used to generate plasma BRI concentration-time profiles for simulated patients following 8 BRI dosing regimens, 4 of which were single doses (0.4, 0.6, 0.8 and 1 mg/kg) and 4 of which were 3-day dosing regimens. These included 0.4 mg/kg on Day 1, followed by 0.2 mg/kg on Days 2 and 3; 0.5 mg/kg on Day 1, followed by 0.2 mg/kg on Days 2 and 3; 0.5 mg/kg on Day 1, followed by 0.3 mg/kg on Days 2 and 3; and 0.6 mg/kg on Day 1, followed by 0.3 mg/kg on Days 2 and 3. Daily AUCs were calculated. Using parameter estimates from PK-PD models for clinical response at test of cure (TOC) and systolic blood pressure (SBP) and appropriate distributions or assumptions for independent variables retained in multivariable models, average predicted % probabilities of these endpoints were determined for each BRI dosing regimen. **Results:** Across BRI dosing regimens, % probabilities of clinical success averaged over a BRI MIC distribution for *S. aureus* ranged from 86.4 to 93.1%. During Days 1-7, the % of simulated patients with sustained SBP ≥160 and ≥180 mmHg over 24 h ranged from 1.44 to 4.06% and 0.02 to 0.10%, respectively. A single 0.6 mg/kg dose best balanced safety and efficacy considerations, with % probabilities of 89.2, 1.7, and 0.02% for these endpoints, respectively. **Conclusions:** Application of PK-PD relationships for efficacy and safety to simulated data allowed for benefit/risk discrimination among the BRI dosing regimens assessed for patients with ABSSSI.

Author Disclosure Block:

S.M. Bhavnani: I. Research Relationship; Self; Cellceutix Corp. **J.P. Hammel:** I. Research Relationship; Self; Cellceutix Corp. **A. Forrest:** I. Research Relationship; Self; Cellceutix Corp. **S.A. VanWart:** I. Research Relationship; Self; Cellceutix Corp. **P. Sager:** C. Consultant; Self; Cellceutix Corp. **K.J. Tack:** C. Consultant; Self; Cellceutix Corp. **R.W. Scott:** C. Consultant; Self; Cellceutix Corp. **D.M. Jorgensen:** D. Employee; Self; Cellceutix Corp. K. Shareholder (excluding diversitied mutual funds); Self; Cellceutix Corp. **P.G. Ambrose:** I. Research Relationship; Self; Cellceutix Corp.

Poster Board Number:

MONDAY-518

Publishing Title:

***in Vitro* Protein Binding With Omadacycline, A First In Class Aminomethylcycline Antibiotic**

Author Block:

S. J. Villano, E. Tzanis, S. K. Tanaka; Paratek Pharmaceuticals, King of Prussia, PA

Abstract Body:

Background: Omadacycline is a first in class aminomethylcycline antibiotic that is undergoing clinical development as once daily oral or intravenous monotherapy for acute bacterial skin and skin structure infections and community-acquired bacterial pneumonia. Omadacycline protein binding was investigated during *in vitro* studies in monkey, mouse, rat, and human plasma. **Methods:** *In vitro* protein binding of omadacycline was determined using human plasma and in mouse (CD-1), rat (HanWistar), and monkey (Cynomolgus) using defrosted plasma pools. Binding was determined at nominal omadacycline concentrations of 10, 100, 1000, and 10000 ng/mL. For human binding studies, plasma was obtained from the blood of three healthy male volunteers. Plasma was defrosted from storage at -20°C before use, and pools of human plasma were used for all studies. Plasma from mouse, rat, and monkey was supplied by Harlan Laboratories Ltd. (Netherlands), Milan Analytica AG (Rheinfelden, Switzerland) and Centre de Primatologie ULP (France), respectively. Protein binding was measured using an ultrafiltration method. Plasma bound and unbound concentrations of omadacycline were determined by a LC-MS/MS method with a lower limit of quantification = 5.0 ng/mL.**Results:** Omadacycline was weakly bound to plasma proteins in human plasma as well as in monkey, mouse, and rat plasma. Over the concentration range of 10-10000 ng/mL, no apparent concentration dependent effect of omadacycline on plasma protein binding was observed. The mean ± SD unbound protein fraction in human plasma was 78.7 ± 9.7%. In animals, the mean unbound plasma protein fractions were rat (73.9 ± 12.1%), monkey (78.8 ± 7.3%), and mouse (84.7 ± 5.3%). The low human plasma protein binding of omadacycline (21%) contrasts markedly with other tetracyclines, which exhibit high concentration dependent protein binding (approximately 80%).**Conclusions:** In contrast to other tetracycline-derived antibiotics, omadacycline has markedly low human plasma protein binding (21%), with no concentration-dependent binding. These data may be relevant to the clinical effectiveness of omadacycline since the free, unbound fraction of the drug typically is most closely correlated with antimicrobial activity.

Author Disclosure Block:

S.J. Villano: D. Employee; Self; Paratek Pharmaceuticals. **E. Tzanis:** D. Employee; Self; Paratek Pharmaceuticals. **S.K. Tanaka:** D. Employee; Self; Paratek Pharmaceuticals.

Poster Board Number:

MONDAY-519

Publishing Title:

Effect Of Food On The Bioavailability Of Omadacycline In Healthy Volunteers

Author Block:

E. Tzanis, A. Manley, S. J. Villano, S. K. Tanaka, S. Chitra, E. Loh; Paratek Pharmaceuticals, King of Prussia, PA

Abstract Body:

Background: Omadacycline is a first-in-class aminomethylcycline antibiotic that is being studied in Phase 3 studies as an oral and intravenous monotherapy for skin infections and community-acquired pneumonia. This study evaluated the effect of food consumption on the oral bioavailability of a single 300 mg dose. **Methods:** This was a phase 1, randomized, open-label 4-period, crossover study. Healthy subjects were randomized to 1 of 4 treatment sequences: A) standard 6-hour fast, B) standard, high fat, non-dairy meal 4 hours before dosing, C) standard, high fat, non-dairy meal 2 hours before dosing, and D) standard, high fat meal containing dairy 2 hours before dosing, separated by a 5 day washout period. Patients received a single 300 mg oral dose of omadacycline during each treatment sequence. Blood samples for pharmacokinetic (PK) analysis were collected at frequent intervals for 24 hours after each dose, and safety assessments included adverse events, clinical laboratory tests, vital signs, and electrocardiogram (ECG). A linear mixed-effect model with treatment condition, sequence, and period as fixed effects and subject nested within sequence as a random effect was fitted to the natural log-transformed PK parameters for estimation of effects and 90% confidence intervals (CIs) for the fed states compared with the fasted state.**Results:** Thirty-one subjects were included in the PK analysis. Fasted $AUC_{0\text{-}inf}$, $AUC_{0\text{-}t}$, and $AUC_{0\text{-}24}$ were 10158, 7206, and 7205 ng*h/mL, respectively and C_{max} was 641 ng/mL Compared with a fasted dose, bioavailability was reduced by 15% to 17% for a nondairy meal 4 hours before dosing, 40% to 42% reduction for a nondairy meal 2 hours before dosing, and 59% to 63% reduction for a dairy meal 2 hours before dosing. The effect of food was more pronounced when a high-fat meal was consumed closer to dosing and when dairy was included in the meal. Across all treatment sequences, mean $T_{1/2}$ ranged from 13.5 to 13.8 hours, and median T_{max} ranged from 2.5 to 2.9 hours. No treatment-related adverse events or clinically relevant changes in laboratory values, ECG or vital signs occurred.**Conclusions:** A single oral dose of omadacycline was well tolerated. Administration of a 300 mg dose within 2 to 4 hours of food reduced the bioavailability compared with the fasted state. Once daily oral omadacycline should be administered at least 6 hours following a meal.

Author Disclosure Block:

E. Tzanis: D. Employee; Self; Paratek Pharmaceuticals. **A. Manley:** D. Employee; Self; paratek Pharmaceuticals. **S.J. Villano:** D. Employee; Self; Paratek Pharmaceuticals. **S.K.**

Tanaka: D. Employee; Self; Paratek Pharmaceuticals. **S. Chitra:** D. Employee; Self; Paratek Pharmaceuticals. **E. Loh:** D. Employee; Self; Paratek Pharmaceuticals.

Poster Board Number:

MONDAY-520

Publishing Title:

Pyranopyridine Efflux Pump Inhibitors (Epi) with Improved Adme Properties Maintain Potent Epi Activity

Author Block:

S. M. Kwasny, Z. Aron, J. D. Williams, T. L. Bowlin, **T. J. Opperman**; Microbiotix, Inc., Worcester, MA

Abstract Body:

Background: The RND family of efflux pumps plays an important role in Multi-Drug Resistance (MDR) in Gram-negative pathogens, making efflux pump inhibitors (EPIs) potentially useful weapons in the fight against MDR. We have developed a novel family of pyranopyridine EPIs that exhibit potent EPI activity against AcrB, the archetypal RND efflux pump. Using co-crystal structures of pyranopyridine analogs bound to the hydrophobic trap of AcrB, we are designing analogs with improved drug-like properties that maintain potency. Here, we present the biological activities of the most potent pyranopyridine analogs. **Methods:** Pyranopyridine analogs were designed and synthesized using published methods. Checkerboard MIC assays were used to measure the MPC4 (Minimum Potentiation Concentration that decreases the MIC of an antibiotic 4-fold). Killing curve assays measured potentiation of bactericidality, and the rate of accumulation of H33342 was used to estimate efflux activity. The cationic dye 3,3'-dipropylthiadicarbocyanine Iodide was used to measure the effect of compounds on membrane potential.**Results:** Analogs that carry substitutions on the phenyl and morpholino moieties exhibited improved drug-like properties while maintaining potency. In particular, compounds with dimethyl morpholinyl and urea containing substituents on the phenyl group (e.g. MBX3795, MBX3796, and MBX3797) were most active in killing curve and checkerboard MIC assays, with MPC4 values 0.1-0.4 µM against a strain of *Escherichia coli* that overexpresses AcrAB-TolC. In addition, MBX3795, MBX3796, and MBX3797 exhibited submicromolar MPC4s vs. pathogenic Enterobacteriaceae, but were not active vs. *Pseudomonas aeruginosa*. The antibiotic potentiation activity of these compounds was correlated with inhibition of efflux pumps, but not with membrane activity.**Conclusion:** Pyranopyridine analogs with improved ADME and PK properties maintain potent EPI activity against pathogens of the Enterobacteriaceae, paving the way for *in vivo* proof of concept experiments.

Author Disclosure Block:

S.M. Kwasny: D. Employee; Self; Microbiotix, Inc. **Z. Aron:** D. Employee; Self; Microbiotix, Inc. **J.D. Williams:** D. Employee; Self; Microbiotix, Inc. **T.L. Bowlin:** D. Employee; Self; Microbiotix, Inc. **T.J. Opperman:** D. Employee; Self; Microbiotix, Inc.

Poster Board Number:

MONDAY-521

Publishing Title:

A Phase 1, Open-label Study of the Effect of Delafloxacin (Dlx) on the Pharmacokinetics (Pk) of a Single Oral Dose of Midazolam (Mid)

Author Block:

L. Lawrence[1], M. Quintas[1], R. Hoover[1], R. Wood-Horrall[2], M. Benedict[2], S. Paulson[3], R. Criste[4], S. Cammarata[1]; [1]Melinta Therapeutics, New Haven, CT, [2]PPD, Inc., Austin, TX, [3]Pharma Start, LLC, Northbrook, IL, [4]PPD, Inc., Richmond, VA

Abstract Body:

DLX is an anionic fluoroquinolone with activity against methicillin-resistant *Staphyloccocus aureus* and susceptible gram-negative bacteria currently in Phase 3 development. This Phase 1 study was performed to determine the impact of DLX treatment on the sensitive CYP substrate MID.Twenty-two male and female subjects were enrolled in this single sequence, single group study. A single 5 mg oral dose of MID was given to fasted subjects on Days 1 and 8. Subjects received oral DLX (450 mg) twice daily on Days 3 - 8. Plasma samples were analyzed for DLX, MID, and 1-hydroxymidazolam (1OH-MID) concentrations with validated LCMS/MS methods. Analysis of variance was performed on the ln-transformed AUC_{0-t}, $AUC_{0\infty}$, and C_{max} of MID and 1OH-MID to estimate the ratio of geometric least squares (LS) means between treatments and their 90% confidence interval (CI).Co-administration of DLX with MID did not alter the PK of MID as the 90% CI of geometric mean ratios of C_{max} (ng/mL) and AUC (h•ng/mL) values were within 80 to 125% acceptance criteria. The 1-OH-MID AUC values were also not affected by co-administration, whereas 1-OH-MID C_{max} values were slightly increased. The statistical analyses of plasma noncompartmental PK parameters of MID and 1OH-MID (MID/1-OH + DLX vs. MID alone) are presented:

Parameter	**Substrate**	**Ratio (%) of Geometric LS Means**	**90% CI of the Ratio (%)**
AUC_{0-t}	MID	89.4	(83.2, 96.1)
$AUC_{0-\infty}$		89.4	(83.2, 96.0)
C_{max}		93.6	(83.7, 104.6)
AUC_{0-t}	1OH-MID	106.6	(98.8, 114.9)
$AUC_{0-\infty}$		105.7	(97.7, 114.3)
C_{max}		116.1	(101.7, 132.4)

DLX reached steady state by Day 7 after 4 days of dosing. A similar C_{max} was observed on Days 3 and 7, and terminal elimination half-life was 2.5 and 2.9 hours, respectively. Five subjects

(22.7%) reported at least 1 mild treatment-emergent adverse event (TEAE), and no deaths, serious AEs or TEAEs leading to study discontinuation were reported. All TEAEs resolved by study end.DLX did not affect the C_{max} and $AUC_{0-\infty}$ of MID/1OH-MID, and minimal effect was indicated for 1OH-MID C_{max}. DLX was shown to have no clinically significant interaction with CYP3A substrate MID in a Phase 1 study.

Author Disclosure Block:

L. Lawrence: D. Employee; Self; Melinta Therapeutics. **M. Quintas:** D. Employee; Self; Melinta Therapeutics. **R. Hoover:** C. Consultant; Self; Melinta Therapeutics. **R. Wood-Horrall:** H. Research Contractor; Self; Melinta Therapeutics. **M. Benedict:** H. Research Contractor; Self; Melinta Therapeutics. **S. Paulson:** C. Consultant; Self; Melinta Therapeutics. **R. Criste:** H. Research Contractor; Self; Melinta Therapeutics. **S. Cammarata:** D. Employee; Self; Melinta Therapeutics.

Poster Board Number:

MONDAY-522

Publishing Title:

Optimization of Pyranopyridine Efflux Pump Inhibitor (Epi) Adme Properties

Author Block:

Z. Aron[1], S. M. Kwasny[1], H. Sjuts[2], S. Cardinale[1], J. D. Williams[1], T. L. Bowlin[1], K. M. Pos[2], T. J. Opperman[1]; [1]Microbiotix, Inc., Worcester, MA, [2]Goethe Univ. Frankfurt, Frankfurt, Germany

Abstract Body:

Background: The RND family of efflux pumps plays an important role in Multi-Drug Resistance (MDR) in Gram-negative pathogens. Recently, we reported analogs of MBX 2319 as being potent pyranopyridine efflux pump inhibitors (EPIs) that target the major RND pumps of Enterobacteriaceae. Co-crystal structures of these materials reveal them bound to the hydrophobic trap of the *Escherichia coli* RND pump AcrB. Here, we detail our use of structure-based drug design and SAR analysis to improve the ADME properties of our molecules providing access to EPIs with improved pharmacokinetic profiles.**Methods:** Pyranopyridine analogs were synthesized using published methods. Potency was determined through checkerboard MIC assays that measure the Minimum Potentiation Concentration which decreases the MIC of levofloxacin or piperacillin four fold (MPC4). *In vitro* ADMET assays (cytotoxicity, solubility, human liver microsome stability, and CYP3A4 inhibition) were used to estimate pharmacokinetic properties of analogs. Pharmacokinetic parameters from *In vivo* assays were calculated using WinNonLin. Co-crystal structures of pyranopyridine analogs bound to the periplasmic domain of AcrB were generated in the laboratory of Martin Pos.**Results:** Optimization efforts leveraged existing SAR and structural analyses of pyranopyridines bound to AcrB to improve ADME properties and maintain potency, improving the solubility, stability and ligand efficiencies of lead compounds. This work identified “hot spots” of metabolic activity such as the C4’ amide of MBX3132 that could be modified to improve stability. Additionally, the placement of basic amines and alcohols in water-filled pockets identified in the crystal structure allowed for improved solubility profiles. Further co-crystal structures of new analogs further aided these efforts. Based on this work, Compounds MBX3132, MBX3795, MBX3796 and MBX3797 were profiled for PK in mouse, revealing excellent overall profiles (AUCs ranging from 10,000-40,000, CL<1000 mL/hr/kg with 10 mg/kg IV), paving the way for *in vivo* proof of concept experiments.**Conclusion:** Structural alterations of the pyranopyridine scaffold improved potency, metabolic stability, and solubility allowing the identification of materials with excellent PK profiles.

Author Disclosure Block:

Z. Aron: D. Employee; Self; Microbiotix, Inc. **S.M. Kwasny:** D. Employee; Self; Microbiotix, Inc. **H. Sjuts:** None. **S. Cardinale:** D. Employee; Self; Microbiotix, Inc. **J.D. Williams:** D.

Employee; Self; Microbiotix, Inc. **T.L. Bowlin:** D. Employee; Self; Microbiotix, Inc. **K.M. Pos:** None. **T.J. Opperman:** D. Employee; Self; Microbiotix, Inc..

Poster Board Number:

MONDAY-523

Publishing Title:

The Assessment of SPR741 for Nephrotoxicity in Cynomolgus Monkeys and Sprague-Dawley Rats

Author Block:

S. Coleman[1], M. Bleavins[2], T. Lister[1], M. Vaara[3], T. J. Parr, Jr[1]; [1]Spero Therapeutics, Cambridge, MA, [2]White Crow Innovation, LLC, Dexter, MI, [3]Northern Antibiotics, Espoo, Finland

Abstract Body:

Background: SPR741 is a polymyxin derivative devoid of direct antibacterial activity that is currently being investigated as a partner in combination with antibiotics for the treatment of multi-drug resistant Gram-negative infections. Antibiotic polymyxins have been shown to exhibit kidney toxicity and as such, we investigated the nephrotoxicity of SPR741 in monkeys and rats to determine the impact on nephrotoxicity. **Methods:** SPR741 was administered to female cynomolgus monkeys as a one-hour infusion three times per day for seven days at dose levels of 12, 30, and 60 mg/kg/day. SPR741 was also administered to male Sprague-Dawley rats as a one-hour infusion three times per day for seven days at dose levels of 5, 15, and 30 mg/kg/day. Control animals received the vehicle (sterile saline) only for both studies while Polymyxin B (PMB) was a positive control in each study. Blood samples were taken for toxicokinetic analysis on study days 1 and 7 in monkeys while only taken on study day 7 in rats while in both studies blood was taken for serum chemistry. All animals were euthanized on study day 8 and kidneys were processed to slides stained with hematoxylin and eosin, and read by a board certified veterinary pathologist. **Results:** In monkeys, SPR741 was not associated with nephrotoxicity at any dose level as determined by histopathology while PMB demonstrated the anticipated nephrotoxicity. The exposure of SPR741 in monkeys at the highest dose tested of 60 mg/kg/day was an $AUC_{0\text{-}24}$ of 163 ug*hr/mL and a C_{max} of 66 ug/mL. In rats, SPR741 was associated with minimal nephrotoxicity at 30 mg/kg/day demonstrated by pathology of a low degree of nephrotoxicity accompanied with slight increases (1.7X) in serum creatinine. PMB did not produce nephrotoxicity in rats. In rats, the exposure of SPR741 at the NOAEL of 15 mg/kg/day was an $AUC_{0\text{-}24}$ of 54 ug*hr/mL and a C_{max} of 7 ug/mL. **Conclusions:** SPR741 demonstrated a no observed adverse effect level (NOAEL) of greater than 60 mg/kg/day (the highest dose tested) following seven days of repeated three times daily one hour infusions in cynomolgus monkeys whereas in rats the NOAEL was 15 mg/kg/day following seven days of repeated three times daily one hour infusions.

Author Disclosure Block:

S. Coleman: D. Employee; Self; Spero Therapeutics. **M. Bleavins:** C. Consultant; Self; Spero Therapeutics. **T. Lister:** D. Employee; Self; Spero Therapeutics. **M. Vaara:** D. Employee; Self; Northern Antibiotics. **T.J. Parr:** D. Employee; Self; spero Therapeutics.

Poster Board Number:

MONDAY-524

Publishing Title:

A Single-centre, Double-blind, Placebo-controlled Study in Healthy Men to Assess the Safety and Tolerability of Single and Repeated Ascending Doses of mgb-Bp-3, a New Class of Antibacterial Agent

Author Block:

M. Ravic, MD, PhD[1], D. Firmin, PhD[1], I. Hunter, PhD[2], C. Suckling, PhD[2], F. van den Berg[3], O. Sahgal[3]; [1]MGB Biopharma Ltd, Glasgow, United Kingdom, [2]Univ. of Strathclyde, Glasgow, United Kingdom, [3]Hammersmith Med.s Res. Ltd, London, United Kingdom

Abstract Body:

Background: MGB-BP-3 (MGB) binds selectively to the bacterial DNA minor groove and has strong bactericidal *in vitro* and *in vivo* activity against *Clostridium difficile*. Completion of a full non-clinical package, including formal GLP toxicology and safety pharmacology, allowed progression to a Phase I human study, to assess the safety, tolerability and PK profile of single (Part A) and multiple (Part B) ascending oral doses of MGB.**Methods:** Part A was a randomised, double-blind, placebo-controlled, cross-over, single ascending-dose trial in 16 healthy men. Subjects were enrolled in 2 groups of 8 (Groups 1-2). Each subject received a single oral dose of MGB in each of 3 study sessions; Group 1 - 250mg, 500mg, 750mg; Group 2 - 1000mg, 1500mg, 2000mg; 6 volunteers received matching placebo in 1 study session. Part B was a randomised, double-blind, placebo-controlled, sequential-group, repeated ascending-dose trial in 24 healthy men. Subjects were enrolled in 3 groups of 8 (Groups 3-5). Each subject had 1 study session, receiving twice-daily oral doses of MGB, or matching placebo, for 9 days (Days 1-9) and a single dose on the morning of Day 10. Doses were 2X250mg, 2X500mg and 2X1000mg. Primary safety and tolerability variables, and secondary variables were assessed throughout.**Results:** No serious adverse events were reported during Parts A or B at any dose level tested. Adverse events were mild to moderate and primary safety variables (including vital signs, cardiac monitoring, lab safety tests, medical examinations and FOB) were all within acceptable limits. Plasma PK assessment showed no absorption after oral administration. Details of intestinal permeability, urine and faecal PK and faecal flora analysis will be presented.**Conclusion:** Orally-administered MGB was tolerated well in healthy volunteers at all dose levels tested in this Phase I clinical trial, with no dose limiting toxicity or serious adverse events. The safe dose was 2000mg MGB, administered orally at 1000mg twice a day over 10 days. MGB is suitable for progression into Phase II clinical trials to assess efficacy in patients with *C. difficile* infection.

Author Disclosure Block:

M. Ravic: D. Employee; Self; MGB Biopharma LTD. **D. Firmin:** D. Employee; Self; MGB Biopharma Ltd. **I. Hunter:** None. **C. Suckling:** None. **F. van den Berg:** F. Investigator; Self; HMR. **O. Sahgal:** F. Investigator; Self; HMR.

Poster Board Number:

MONDAY-525

Publishing Title:

Aminoglycoside (Ag) Activity Against *Enterobacter* spp., and Correlation with the Presence of Aminoglycoside Modifying Enzymes (Ames), *Klebsiella pneumoniae* Carbapenemases (Kpcs) and Extended-spectrum-Beta-Lactamases (Esbls)

Author Block:

G. Haidar, S. Cheng, T. M. Churilla, B. M. Churilla, A. Alkroud, R. K. Shields, Y. Doi, C. J. Clancy, M. H. Nguyen; Univ. of Pittsburgh, Pittsburgh, PA

Abstract Body:

Background: KPC-producing *Enterobacter* spp. are emerging. Gentamicin (GEN) is active against the majority of carbapenem-resistant *K. pneumoniae*. The activity of GEN and other AGs against KPC-producing *Enterobacter* is not known. **Methods:** We compared the *in vitro* susceptibility of KPC-producing (KPC+) and non-producing (KPC-) *Enterobacter* isolates to aminoglycosides (GEN, tobramycin TOB, amikacin AMK). We determined the presence of KPC, ESBL and AME genes by PCR. **Results:**21 KPC+ (12 *E. aerogenes* (EA) and 9 *E. cloacae* (EC)) and 44 KPC- isolates (12 EA and 32 EC) were compared. 71% (15) and 7% (3) of KPC+ and KPC- isolates were resistant to GEN, respectively ($p<0.0001$). Corresponding rates were 67% (14) and 9% (4) for TOB ($p<0.0001$), and 9% (2) and 0% for AMK ($p=0.10$). AMEs were more common among KPC+ (95%) than KPC- isolates (20%, $p<0.0001$), as were ESBLs (41% vs. 30%, $p<.0001$). Likewise, mean and median numbers of AMEs were higher for KPC+ (4 and 5) than KPC- isolates (0.4 and 0). The most common AME genes among KPC+ isolates were: *aac(6')-Ib* (62%), *aadA2* (62%), *aac(6')-II* (57%) and *aadA1* (57%). The presence of either *aac(6')-Ib* or *aac(6')-II* was associated with resistance to all 3 AGs. The presence of any one of *aac(3)-II* and *-IV*, *aadA1* and *aadA2* was associated with resistance to GEN and TOB but not AMK. The presence of *aph(3')-Ia* was associated with GEN resistance. GEN, TOB and AMK MICs were significantly increased as the number of β lactamases (either KPC or ESBL) increased (<0.001). There was a direct correlation between presence of KPC and presence of *aac(6')-Ib* and *aph(3')-Ia*, and between presence of ESBL and *aac(6')-II* and *aph(3')-Ib* (all $p<0.01$). **Conclusion:**A significant majority of KPC+ *Enterobacter* spp. were resistant to GEN and TOB. Associations between presence of AME, KPC and ESBL genes suggest that they may be acquired on the same plasmids. The high prevalence of KPC+ isolates with *aac(6')-Ib*, known to confer resistance to GEN, TOB and AMK, suggests that these AG are unlikely to have a significant role in the treatment of KPC+ *Enterobacter* infections.

Author Disclosure Block:

G. Haidar: None. **S. Cheng:** None. **T.M. Churilla:** None. **B.M. Churilla:** None. **A. Alkroud:** None. **R.K. Shields:** None. **Y. Doi:** E. Grant Investigator; Self; The Medicines Company Advisory Board: Meiji, Tetraphase. **C.J. Clancy:** None. **M.H. Nguyen:** None.

Poster Board Number:

MONDAY-526

Publishing Title:

A Conjugative A*cinetobacter baumannii* Plasmid Carrying the *sul2* Sulfonamide Resistance Gene

Author Block:

M. Hamidian, S. J. Ambrose, R. M. Hall; Univ. of Sydney, Sydney, Australia

Abstract Body:

Background: Two globally disseminated clones of *Acinetobacter baumannii*, GC1 and GC2, are responsible for the majority of resistance to multiple antibiotics. We previously showed that the GC1 reference strain, A297 (RUH875) isolated in the Netherlands in 1984, is resistant to multiple antibiotics including tobramycin, gentamicin, kanamycin, neomycin, tetracycline, sulfonamides, trimethoprim and streptomycin. Resistance genes *aadB* in the 6-kb plasmid pRAY*, and *aphA1b*, *tetA*(A), *bla*$_{TEM}$, *catA1*, *sul1* and *dfrA5* in the AbaR21 resistance island in the chromosome accounted for most of the phenotype. However, *strAB* and *sul2* genes, which are rarely seen in GC1 isolates, were also found and shown to be linked. The objective of this study was to determine the context of the region containing *sul2* and *strAB*.**Methods:** A297 was sequenced using Illumina HiSeq and reads were used to generate a de novo assembly. Standalone BLAST was used to identify contigs that carry resistance genes. PCR followed by sequencing was used to join the contigs. Conjugation experiments were also conducted.**Results:** The *sul2* and *strAB* genes were found in pA297-3, a 200 kb plasmid that included a set of genes encoding conjugative transfer functions. However, no *rep* gene was in pA297-3. pA297-3 is conjugative and transferred streptomycin and sulfonamide resistance to a sensitive recipient. The *sul2* gene was part of ISAba1-*sul2*-CR2-*strAB* within a transposon structure named Tn*6172*. Tn*6172* was found to be identical to the right hand side of ABGRI1-2 resistance island found in the chromosome of GC2s. Exploring GenBank also revealed that pAB3, a 148 kb plasmid in the ATCC17978 strain, contains a transposon similar to Tn*6172*, named Tn*6174*. Tn*6174* was flanked by an orf region followed by a copy of Tn*6021*. Detailed analyses revealed that the structure found in pAB3, including orf region and Tn*6174*, forms an ancestral structure for the ABGRI1 group of islands.**Conclusions:** pA297-3, which caries Tn*6172* explains the presence of *sul2* in the A297 genome. Tn*6172* appears to be derived from of Tn*6174*. Tn*6174* together with its adjacent region is an ancestral form of the ABGRI1-2, the main resistance island found in the most globally widespread clone of *A. baumannii*, GC2. This novel finding highlights the role of plasmids in evolution and crafting complex genomic resistance islands in *A. baumannii*.

Author Disclosure Block:

M. Hamidian: None. **S.J. Ambrose:** None. **R.M. Hall:** None.

Poster Board Number:

MONDAY-527

Publishing Title:

Detection of Extended-Spectrum β-Lactamase from Carbapenem-resistant *Acinetobacter baumannii* from Philadelphia Tertiary-Care Hospital

Author Block:

K. M. Raible[1], B. Sen[1], C. Emery[2], K. Krevolin[3], T. Bias[3], N. Law[1], G. Ehrlich[1], S. Joshi[1]; [1]Drexel Univ., Philadelphia, PA, [2]Drexel Coll. of Med., Philadelphia, PA, [3]Hahnemann Univ. Hosp., Philadelphia, PA

Abstract Body:

Background: *Acinetobacter baumannii* is the third most common Gram-negative bacillus isolated from patients in intensive care units associated with ventilator-associated pneumonia and is considered major nosocomial pathogen. Currently, treating these infections is difficult due to pathogen's innate resistances, and its high propensity to acquire exogenous resistance genes through horizontal gene transfer. Carbapenem resistance genes in *A. baumannii* require special attention in this regard. In the present study, we tested for the four major β-lactamase classes, as well as mobile gene elements in clinical isolates of carbapenem-resistant *Acinetobacter baumannii* (CRAB) from Hahnemann University Hospital in Philadelphia, PA.**Methods:** Carbapenem minimum inhibitory concentration results were obtained for each CRAB isolate (n=28) using VITEK$^{@}$2 and the results were confirmed by broth microdilution. Detection of β-lactamase genes and mobile elements were performed using PCR-based assays. The PCR assays were validated using Clinical Laboratory Standards Institute (CLSI)- recommended quality control (QC) strains, and the results were verified by Sanger sequencing.**Results: :** PCR-based detections established the presence of both innate and exogenous β -lactamase genes (particularly Classes A, C, and D) in all CRAB isolates. In addition, the presence of mobile elements (integron-cassettes, integrase genes, and insertion sequence (IS) elements) were also present in all isolates.**Conclusions:** IS-elements upstream of innate A. baumannii β -lactamase genes were found in all 28 CRAB isolates leading to increased expression of these genes. Insertion type correlated with resistance phenotype. The presence of plasmid-borne β -lactamase genes (OXA-23 and OXA-40) as well as integron cassettes in the CRAB isolates suggests the acquisition of these genes through multiple recombinatory events. Further sequencing studies are currently being performed to gain a better understanding of the local distribution and phenotypic impact of these resistance genes, and possibly provide a mechanism of how these resistance genes spread.

Author Disclosure Block:

K.M. Raible: None. **B. Sen:** None. **C. Emery:** None. **K. Krevolin:** None. **T. Bias:** None. **N. Law:** None. **G. Ehrlich:** None. **S. Joshi:** None.

Poster Board Number:

MONDAY-528

Publishing Title:

Selenium Increases the Sensitivity of Multidrug-Resistant *Acinetobacter baumannii* to Antibiotics through Synergistic Interactions

Author Block:

M. Surendran Nair, Y. Liu, K. Venkitanarayanan; Univ. of Connecticut, Storrs, CT

Abstract Body:

Background: Multidrug resistant (MDR) *Acinetobacter baumannii* is a nosocomial pathogen causing a wide spectrum of clinical conditions in humans. *A. baumannii* is equipped with a multitude of antibiotic resistance mechanisms, rendering them resistant to most of the antibiotics. Thus, it is critical to devise novel approaches for controlling *A. baumannii* infections. This study investigated the efficacy of Selenium (Se), a dietary essential mineral, in increasing *A. baumannii*'s sensitivity to three classes of antibiotics, namely beta-lactams (Ampicillin), tetracyclines (Tetracycline) and quinolones (Ciprofloxacin). Additionally, the synergistic interactions of Se with the antibiotics were determined using checkerboard assay.**Methods:** Two MDR *A.baumannii* isolates (~5 log CFU/ml) were separately inoculated into Muller Hinton broth supplemented with or without the sub-inhibitory concentration (SIC, highest concentration not inhibiting growth) of Se (5.6 mM) along with or without ampicillin, tetracycline or ciprofloxacin at their respective break point concentration. Bacterial growth was determined at 600 nm following incubation at 37°C for 24 h. The interactions of Se with the three antibiotics was investigated using microdilution checkerboard assay. Moreover, E-test was used to measure the reduction in the MIC of antibiotics when *A. baumannii* was exposed to the antibiotics with Se. The entire study was repeated twice with duplicate samples.**Results and Conclusion:** Se increased *A.baumannii*'s sensitivity to ampicillin, tetracycline and ciprofloxacin ($P \leq 0.05$) as evident from the decreased bacterial population in samples containing Se and antibiotic compared to those with antibiotics alone. Furthermore, the combination of Se with ampicillin produced a synergistic interaction ($FIC_{index} = \leq 0.5$), whereas with tetracycline and ciprofloxacin produced a synergistic ($FIC_{index} = \leq 0.5$) or additive ($0.5 \leq FIC_{index} \leq 1.10$) interaction was observed. The E test results showed a two-fold reduction in the MIC of the antibiotics when combined with Se. The results suggest that Se could potentially be used to enhance *A. baumannii*'s antibiotic sensitivity, thereby making the pathogen susceptible to the drugs.

Author Disclosure Block:

M. Surendran Nair: None. **Y. Liu:** None. **K. Venkitanarayanan:** None.

Poster Board Number:

MONDAY-529

Publishing Title:

Antibiotic Resistance Determinants in the Cystic Fibrosis Pathogen *Achromobacter xylosoxidans*

Author Block:

A. K. Lee; Univ. of Minnesota, Minneapolis, MN

Abstract Body:

Cystic fibrosis (CF) is an autosomal, recessive disease caused by a mutation in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) causing an increase in mucus viscosity. This creates an ideal environment for microbial colonization resulting in the establishment of a diverse microbial community. Recently, *Achromobacter xylosoxidans*, an opportunistic pathogen often associated with nosocomial infections, has been implicated in later disease states in CF patients. It has proved resistant to a broad range of antibiotics, transmissible between patients, and shows robust biofilm formation as well as other virulence factors, increasing its importance as a pathogen and creating difficulties in identifying effective therapeutic strategies. Despite its relevance in disease, little is known regarding mechanisms involved in pathogenicity, including antibiotic resistance. In this study, we used transposon mutagenesis to identify genes contributing to antibiotic resistance in a transmissible, multidrug-resistant, clinical isolate of *A. xylosoxidans*. In the presence of the minimum inhibitory concentration of four antibiotics (ampicillin, gentamicin, chloramphenicol, and ciprofloxacin), we screened approximately 10,000 mutants, representing 90 percent of the *A. xylosoxidans* genome, for drug resistance determinants. Genes we identified included efflux pumps, transcriptional regulators, and hypothetical proteins. One particular gene of interest was a HTH-type transcriptional regulator, *dmlR*. This gene confers resistance to ciprofloxacin, gentamicin and chloramphenicol. DmlR, in addition to other determinants were further screened for their involvement in resistance to 10 antibiotics commonly used in CF treatment, revealing several other genes involved in multidrug resistance. With this information, we can tailor future studies toward characterizing the role of these genetic determinants in antibiotic resistance, which will potentially inform the design of novel therapeutic strategies.

Author Disclosure Block:

A.K. Lee: None.

Poster Board Number:

MONDAY-530

Publishing Title:

Investigations into the *hipa7* High Persister Mutation in Clinical *Burkholderia cenocepacia* Isolates

Author Block:

S. E. Nicolau, Y. Shan, K. Lewis; Northeastern Univ., Boston, MA

Abstract Body:

Background: *Burkholderia cenocepacia* is a difficult pathogen to treat because it possesses an abundance of virulence factors, multiple resistance mechanisms and often infects compromised hosts. Moreover, this pathogen often presents as a reoccurring infection and is associated with increased mortality in cystic fibrosis (CF) patients. Historically, beta-proteobacteria, like *Burkholderia* species, do not possess the *hipA* gene, a previously identified persister gene. Persisters are phenotypic variants of a population that go dormant, allowing them to survive antibiotic treatment. However, for some reason that remains unclear at the present time, human pathogenic isolates of *Burkholderia* appear to contain this gene. The purpose of this experiment was to evaluate the presence of the *hipA7* allele, a high persister mutation in *E. coli* that has been linked to reoccurring clinical urinary tract infections, in clinical isolates of *Burkholderia cenocepacia*. **Methods:** In this study, colony polymerase chain reaction (PCR) was done on 30 clinical isolates of *B. cenocepacia* using predesigned primers upstream and downstream of the *hipA* gene. *E. coli* MG1655 was run simultaneously as a positive control. Success of the PCR was confirmed by gel electrophoresis. PCR products were then purified and samples were sequenced by GENEWIZ (South Plainfield, NJ).**Results:** The *E. coli* MG1655 control presented on the gel with a band size representative of the *hipA* gene, confirming the viability and specificity of the PCR protocol utilized. Of the 30 *B. cenocepacia* clinical isolates screened, none contained the *hipA7* allele. In the absence of this specific high persister mutation, P77S, I91T, A107S, P155L, L168P, N394D and A403S were found to be common mutations among many of the clinical isolates.**Conclusions:** In this study we were unable to locate the presence of the *hipA7* high persister mutation in the 30 clinical isolates of *B. cenocepacia* screened. While these data suggest that the *hipA7* allele may not be present in this organism, many of these isolates contained mutations in their amino acid sequence, which could translate into currently unidentified high persister alleles.

Author Disclosure Block:

S.E. Nicolau: None. **Y. Shan:** None. **K. Lewis:** None.

Poster Board Number:

MONDAY-531

Publishing Title:

Complex Regulation of the *Burkholderia pseudomallei bpeef-oprc* Multidrug-Resistance Operon

Author Block:

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Abstract Body:

Background: *Burkholderia pseudomallei* is an emerging pathogen and the etiologic agent of melioidosis. *B. pseudomallei* infections are difficult to treat because of the bacterium's intrinsic and acquired antibiotic resistance. Efflux mediated by members of the resistance nodulation cell division (RND) family of efflux pumps is the dominant resistance mechanism. Of the three RND pumps characterized in some detail, the BpeEF-OprC pump encoded by the *llpE-bpeE-bpeF-oprC* efflux operon located on chromosome 2 represents the clinically most relevant efflux pump. Genetic evidence indicates that efflux operon expression is governed by the product of the *bpeT* gene, located upstream of and transcribed divergently from the efflux operon. BpeT is a LysR-type transcriptional regulator and mutations to the carboxy-terminal domain cause constitutive operon expression. Pump expression results in multidrug resistance, including clinically used trimethoprim and in some instances trimethoprim-sulfamethoxazole [SXT]). **Methods:** Genetic, biochemical and molecular methods were used to study molecular mechanisms governing BpeEF-OprC efflux pump expression. To avoid dual use of research concerns, resistance and recombinant experiments were performed using a select agent excluded *B. pseudomallei* strain. **Results:** BpeT overexpression activates transcription of the efflux operon. Purified BpeT binds to the 187-nucleotide *bpeT-llpE* intergenic region. Genome sequence analysis of a SXT resistant mutant identified *bpeS*, on chromosome 1 whose product shares >60% amino acid homology to BpeT. Mutations affecting the BpeS carboxy-terminal domain and its presumptive DNA binding domain, but not BpeS overexpression, cause high-level *llpE-bpeE-bpeF-oprC* operon expression. Purified BpeS also binds to the *bpeT-llpE* intergenic region. While BpeT and BpeS are both involved in regulation of BpeEF-OprC expression, their impact on drug resistance is curiously not the same. **Conclusions:** Our data show that expression of BpeEF-OprC is complex, and further studies are needed to understand the interplay of regulatory factors causing increased pump expression and subsequent decreased antimicrobial susceptibility in *B. pseudomallei*.

Author Disclosure Block:

K.A. Rhodes: None. **N.L. Podnecky:** None. **H.P. Schweizer:** None.

Poster Board Number:

MONDAY-532

Publishing Title:

Fluoroquinolone Resistance (FQR) Mechanisms in Extended-Spectrum β-Lactamase Producing *Enterobacteriaceae* (ENT) Isolates from Children

Author Block:

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Abstract Body:

Background: Fluoroquinolones are not commonly prescribed in children, yet the increasing incidence of MDR GNR infections in this population is escalating to a threatening level. Our long term goal is to define the molecular epidemiology of MDR GNRs in children, in order to devise more effective treatment and prevention strategies. Here we report our findings regarding FQR *Ent* in children. **Methods:** A retrospective cohort study of clinical GNR isolates obtained from children ages 0-21 years hospitalized from 2011-14 at three Chicago hospitals was performed. Plasmid-mediated FQR (PMFQR) and mutations in the quinolone resistance-determining regions (QRDR) of *gyrA* and *parC* in isolates were investigated. Rep-PCR determined genetic relatedness. DNA microarray (Check-Points CT101/103XL) was used to detect β-lactamase genes (*bla*). Multilocus sequence typing (MLST), PCR and DNA sequencing were also performed. **Results:** Of 169 ESBL-producing *Ent* isolates, 85 (50%) were FQR of which 82 were analyzed (96.4%). The median age of patients was 4.8 years. All patients were deemed to have infection by these isolates. The predominant FQR organism was *E. coli* 65/82 (79%), and the predominant *bla* genotype associated with FQR in *Ent* was $bla_{\text{CTX-M-1}}$ group in 51/82 cases (62%). Within ESBL *E. coli*, FQR was commonly associated with phylogenetic group B2 and ST43/ST131 containing $bla_{\text{CTX-M-1}}$ group in 47/63 cases (75%). PMFQR was found in 56/82 (62%) isolates, of which *aac 6'1b-cr* (Y102R and/or D179Y), *oqx A/B*, *qnr A/B/D/S*, and *qepA* gene mutations were found in 51%, 10%, 10%, and 9%, respectively. PMFQR was found with *gyrA* and/or *parC* mutations in 84% of cases. Several isolates contained >1 *bla*/PMFQR gene. **Conclusions:** This report defines the emerging molecular epidemiology of FQR in ESBL-producing *Ent* in this population of hospitalized children in Chicago. PMFQR and QRDR mutations are the primary mechanisms. Knowledge of these genotypes among children without direct clinical exposure to FQs raises the concern that in this population, transmission is readily occurring by multiple means.

Author Disclosure Block:

L.K. Logan: None. **S.H. Marshall:** None. **A.M. Hujer:** None. **T.N. Domitrovic:** None. **S.D. Rudin:** None. **F.A. Scaggs:** None. **X. Xheng:** None. **N.K. Qureshi:** D. Employee; Spouse/Life Partner; Baxter. **A. Karadkhele:** None. **M.K. Hayden:** None. **R.A. Bonomo:** E. Grant Investigator; Self; AstraZeneca, Merck, Melinta.

Poster Board Number:

MONDAY-533

Publishing Title:

Compassionate Use Ceftazidime/Avibactam (Cazavi) for Carbapenem-Resistant (Cr) Gram-Negative Rod (Gnr) Infections

Author Block:

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Abstract Body:

Background: CAZAVI is a novel agent with activity against MDR GNRs, including carbapenem-resistant Enterobacteriaceae (CRE). We collected the experience of US clinicians who used CAZAVI during a compassionate use program available before its FDA approval for CR-GNR infections.**Methods:** Clinicians of record who requested and were granted approval for compassionate use CAZAVI were contacted and asked to contribute blinded data on their patient cases into a central database. Patients were included if they had received CAZAVI and had sufficient data to determine outcomes. CRE was defined as isolates resistant to all carbapenems by CLSI standards. Outcomes included in-hospital mortality and microbiological and clinical success. Microbiological success required a negative culture at the end of therapy. Clinical success was judged by improved symptoms and defervesence.**Results:**

Characteristic	**Results (N=15)**
Male gender (n,%))	7 (47%)
Age, median (range)	61 (31-92)
Length of stay, median (range)	72 (26-585)
Charlson Comorbidity Index, median (range)	3 (1-12)
Pitt Bacteremia Score, median (range)	3 (1-8)
ICU residence, n (%)	12 (80%)
Organ transplant, n (%)	5 (33%)
Acute renal failure, n (%)	7 (47%)
Ventilator requirement, n (%)	9 (60%)
Septic shock, n (%)	8 (53%)
Hepatic disease, n (%)	11 (73%)
Organism, n *K. pneumoniae*	12 1

E. coli *E. aerogenes* *P. aeruginosa*	1 1
Infection(s), n Bacteremia Intra-abdominal Pneumonia Urinary tract	10 7 2 1
Isolates susceptible to CAZAVI, n/N (%)	8/10 (80%)
Days of CAZAVI use, median (range)	24 (3-50)
In-hospital mortality, n (%)	10 (67%)
Microbiologic success, n (%)	13 (87%)
Clinical success, n/N (%)	5/11 (45%) 4 indeterminate outcomes

All patients received therapy with alternate antibiotics prior to CAZAVI. While receiving CAZAVI, one patient had a hypersensitivity reaction and 2 had transient LFT elevations.**Conclusion:** In this seriously ill population with CR-GNR infections and delayed receipt of CAZAVI, high rates of microbiological success coupled with moderate clinical success were seen.

Author Disclosure Block:

J.C. Gallagher: C. Consultant; Self; Allergan. J. Scientific Advisor (Review Panel or Advisory Committee); Self; Allergan. L. Speaker's Bureau; Self; Allergan. **J. McKinnell:** C. Consultant; Self; Allergan. I. Research Relationship; Self; Allergan. L. Speaker's Bureau; Self; Allergan.

Poster Board Number:

MONDAY-534

Publishing Title:

Susceptibility and Mechanisms of Resistance to Carbapenems Among Us Gram-negative Bacilli (Gnb)

Author Block:

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Abstract Body:

Introduction: Strains of GNB (n = 3843) obtained from a systematic surveillance at 50 clinical laboratories in the US and Puerto Rico were evaluated for susceptibility to imipenem (IMP), meropenem (MER), doripenem (DOR) based on CLSI and FDA breakpoints; a subset of DOR non-susceptible isolates (41 *Enterobacteriaceae*, 20 *P. aeruginosa*, 20 *Acinetobacter* spp.) were analyzed for their underlying resistance mechanisms.**Methods***: In vitro* activity of the carbapenems was determined centrally (Eurofins, Chantilly, Va) according to CLSI guidelines for broth microdilution. Real-time PCR was used to detect mobile β-lactamase targets, including genes for carbapenemases (KPC, NDM, IMP,VIM, OXA-48, OXA-23, OXA-40, OXA-51, and OXA-58), ESBLs (CTX-M-1, CTX-M-2, CTX-M-9, and CTX-M-8/25) and AmpC (CMY1/MOX, ACT/MIR, CMY2/LAT/CEF,DHA, FOX, and ACC). Chromosomally encoded *ampC* gene, SHV and TEM genes and *K. pneumoniae* sequence type were determined. Porin genes for *K. pneumoniae* (*ompK35, ompK36*), *E. coli* (*ompF, ompC, ompR*), *A. baumannii* (*oprD, carO*) and *P. aeruginosa oprD* were determined. Gene expression of efflux pumps in *P. aeruginosa* was compared to the reference strain PAO1.**Results**: DOR was the most active carbapenem *vs P. aeruginosa* (N=1175). 8.1% isolates were multi-drug resistant (MDR). 50-53% of *Acinetobacte*r spp (N=335) were susceptible to DOR, MER or IMP. 41 (1.75%) Enterobacteriaceae (N=2333) were non-susceptible to DOR and all contained KPC. 89% KPC *K. pneumoniae* were ST258. Among 20 DOR-non-susceptible *P. aeruginosa*, only 7 contained KPC while 13 different *oprD* porin mutants were identified. OXA-51 was present in 95% *Acinetobacter* isolates and strains harboring only OXA-51 showed DOR MIC of 2 μg/ml. All 10 *Acinetobacter* strains with doripenem MIC >32 μg/ml contained either OXA-23 or OXA-40 in combination with OXA-51.**Conclusion**: This study contains a robust collection of phenotypic and genetic profiles of key Gram-negative pathogens. While KPCs among *K. pneumoniae* are directly correlated with higher doripenem MICs, the overall picture is that multidrug resistance is the result of several complex factors (i.e. enzymes, porins, and efflux) that combine to create carbapenem resistance phenotypes, especially among *P. aeruginosa* and *Acinetobacter* spp.

Author Disclosure Block:

R.M. Echols: C. Consultant; Self; consultant. **B.N. Kreiswirth:** None. **D. Sahm:** None. **M. Ariyasu:** None.

Poster Board Number:

MONDAY-535

Publishing Title:

A Bottom-Up Approach for Rationalising Molecule Permeation in Gram-Negative Bacteria

Author Block:

M. Ceccarelli[1], S. Acosta Gutierrez[1], I. Bodrenko[1], G. Malloci[1], M. A. Scorciapino[2], J. Wang[3], H. Bajaj[4], M. Winterhalter[4]; [1]Dept. of Physics, Univ. of Cagliari, Monserrato, Italy, [2]Dept. of BioMed. Sci., Univ. of Cagliari, Monserrato, Italy, [3]Nanion, Munich, Germany, [4]Jacobs Univ., Bremen, Germany

Abstract Body:

Background: The demand of new drugs for combating multidrug-resistant bacteria appears more urgent for Gram-negative bacteria: the presence of the outer membrane, which hinders the access of molecules to internal targets, renders the development of anti-infectives more challenging. Today neither a robust screening method for permeation nor defined physical/chemical rules governing permeation through the outer membrane are available.As part of the IMI-ND4BB platform, a Public-Private Partnership funded by the EU and EFPIA, we are applying a bottom-up approach to investigate the problem of molecule permeation through the outer membrane. We started from high-resolution crystal structures of simple porins, followed by monitoring their expression in the bacteria in different growth conditions. With this information we defined a priority list of proteins and selected antibiotics and inhibitors from different families to rationally explore the chemical space. We then combined molecular modeling with molecular dynamics enhanced sampling techniques to characterize electrostatic of porins and simulate the permeation of antibiotics through porins, identifying the key molecular parameters.We focused initially on Enterobacteriaceae, where OmpF/OmpC orthologs constitute the main influx routes for polar antibiotics. The results were analyzed with a simple Hamiltonian model, showing how (i) the minimal projection area and (ii) the molecular dipole moment represent key parameters to modulate permeation through simple porins. Other parameters such as size/shape fluctuations, which correlate with rotatable bonds, were also considered. Cell-free electrophysiology at single molecule level on reconstituted porins was used to confirm our data whenever possible.Without a strong and accurate experimental method to assess the flux of antibiotics through porins, our Hamiltonian model, based on steric hindrance and electrostatic of pore/antibiotic complex, represents a useful tool to explore the chemical space for searching new scaffolds and/or optimize at the molecular level existing molecule for an enhanced permeation through bacterial porins.

Author Disclosure Block:

M. Ceccarelli: None. **S. Acosta Gutierrez:** None. **I. Bodrenko:** None. **G. Malloci:** None. **M.A. Scorciapino:** None. **J. Wang:** None. **H. Bajaj:** None. **M. Winterhalter:** None.

Poster Board Number:

MONDAY-536

Publishing Title:

Understanding Epistasic Interactions in Evolving Antibiotic Resistant Populations

Author Block:

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Abstract Body:

Background: Bacterial evolution of antibiotic resistance is significant public health problem contributing to treatment failures for an increasing number of infections. We have previously used amplicon sequencing to screen mutagenic genomic hot-spots at various time points from five drug adaption 14-day *E. coli* evolution experiments done in triplicate. The drugs used were amikacin, chloramphenicol, ciprofloxacin, amikacin + chloramphenicol and chloramphenicol + ciprofloxacin, we were interested in these drugs as they are all clinically relevant, and the last two combinations the first exhibits collateral sensitivity (amikacin + chloramphenicol), whilst the second exhibits collateral resistance (chloramphenicol + ciprofloxacin). We identified mutations that where closely correlated (positively or negatively) with other mutations. In this study we explore these potential epistasic interactions and use recombineering to introduce representative sets of resistance conferring mutations and explore their fitness effects and population dynamics.**Method:** To recreate all the mutants we used PMA7 based recombineering in *E. coli*. Once all mutants were created doubling times were measured to assess fitness and antibiotic IC90s to assess selective advantage compare to their ancestral wild type. Then competition experiments were performed with strains transformed with plasmids inducing fluorescent proteins to simulate population dynamics previously seen.**Results:** Our results indicate that antibiotic resistance development is more complex than previously reported in the literature. We found that double mutants that were positively correlated had a fitness cost but a significant selective advantage, whilst this selective advantage did not occur with the negatively correlated mutations.**Conclusion:** These results demonstrate that epistasic interactions exist amongst resistance mutations and likely play a role in shaping the evolutionary trajectory leading to resistance.

Author Disclosure Block:

R.A. Hickman: None. **M.O.A. Sommer:** None.

Poster Board Number:

MONDAY-537

Publishing Title:

Identification of a Novel Meropenem-intermediate Resistance Factor Using a Genomic Dna Library of *Bacteroides fragilis* gai92214 Strain

Author Block:

T. Goto[1], Y. Morita[2], M. Hayashi[1], K. Tanaka[1]; [1]Gifu Univ., Gifu city, Gifu, Japan, [2]Aichi Gakuin Univ., Nagoya city, Aichi, Japan

Abstract Body:

Background: *Bacteroides fragilis*, an anaerobic opportunistic pathogen, is resistant to a broader range of antimicrobials. Approximately 2% of *B. fragilis* clinical isolates are resistant to carbapenems. We previously reported a highly carbapenem-resistant isolate, GAI92082 strain, in which carbapenemase (CfiA) gene was encoded by a plasmid (pBFUK1). As carbamenem-intermediate resistant *B. fragilis* isolates are also problems in chemotherapy recently, we aimed to identify a novel resistance factor using *B. fragilis* GAI92214 strain (*cfiA* gene negative) conferred meropenem (MP)-intermediate resistance (MP MIC, 16 µg/ml). **Methods:** We constructed a genomic DNA library of the GAI92214 strain. Briefly, we purified 3-5 kb DNA fraction from sheared GAI92214 genomic DNA and ligated it into a high copy vector, pHSG398. Resulting recombinant plasmids were electroporated into *Escherichia coli* KAM3 Δ*tolC* strain, hypersusceptible to antimicrobials. We performed screening a novel MP resistance factor from forty thousand clones, using LB agar plates contained 0.04 µg/ml MP. **Results:** One of forty thousand clones showed slightly higher resistance (MP MIC, 0.06 µg/ml) than the host strain, KAM3 Δ*tolC*. The partial sequence of 'major facilitator superfamily (MFS) transporter' was revealed by sequencing of insert DNA in recombinant plasmid purified from this resistant clone. We sequenced the complete sequence of this transporter, its promoter and terminator regions (named as MFS whole region). This complete MFS transporter contained 12 transmembrane regions. The MFS whole region was ligated into the pHSG398 and transformed into the KAM3 Δ*tolC*. Resulting recombinant clone showed MP resistance (MIC, 0.06 µg/ml) slightly higher than the KAM3 Δ*tolC*. **Conclusions:** Identified MFS transporter may contribute slightly to MP resistance in the GAI92214 strain. Not only gene expression analyses of this MFS transporter in the GAI92214 strain is necessary, but also other genomic approaches will be needed to identify other factors (e.g., other pumps, mutation of porins) contributed greatly to MP intermediate resistance in the GAI92214 strain.

Author Disclosure Block:

T. Goto: None. **Y. Morita:** None. **M. Hayashi:** None. **K. Tanaka:** None.

Poster Board Number:

MONDAY-538

Publishing Title:

A Novel Ndm-1 Gene Carrying *Acinetobacter baumannii* from University Hospital in a Resource Starved Country, Nepal

Author Block:

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Abstract Body:

Background: In recent years, the emergence of multidrug-resistant *Acinetobacter baumannii* associated with hospital-acquired infections has been increasingly reported worldwide and in particular emergence of $bla_{\text{NDM-1}}$ has created the most concern. The aim of this study was to investigate the prevalence of NDM-1 carrying *Acinetobacter baumannii* species from nosocomial infection in a university hospital in Nepal. **Methods:** A total of 246 *Acinetobacter* species isolates obtained from different patients were screened for MDR *A. baumannii* during December 2013 to December 2014. Identification of the MDR *A. baumannii* strains at the species level was confirmed by 16S rRNA sequencing. Minimum inhibitory concentrations (MICs) were determined using the guidelines of the Clinical and Laboratory Standards Institute (CLSI). Carbapenem-resistant isolates were further screened for metallo-β-lactamase (MBL) production by a combination disc method using imipenem and ethylene-diamine -tetraacetic acid (EDTA). Among 122 MDR *A. baumannii*, 52 isolates were tested for the presence of $bla_{\text{NDM-1}}$ by PCR and confirmed by sequencing. **Results:** Among 246 *Acinetobacter* species collected, 122 were MDR *A. baumannii* with the majority resistant to aminoglycosides, carbapenems, and fluoroquinolones but not to colistin and tigecycline. We found a high prevalence of $bla_{\text{NDM-1}}$ among *A. baumannii* clinical isolates with 18/52 (34.6.%) isolates being $bla_{\text{NDM-1}}$ positive. **Conclusion:**This is the first report describing multidrug-resistant *A. baumannii* strains harboring $bla_{\text{NDM-1}}$ isolated from hospital-acquired infection in Nepal. Further investigations to find the origin of this gene and its mechanism of spread is urgently needed.

Author Disclosure Block:

S. Shrestha: None. **B. Pokhrel:** None. **B. Rijal:** None. **J. Sherchand:** None. **T. Tada:** None. **T. Kirikae:** None.

Poster Board Number:

MONDAY-539

Publishing Title:

Macrolide Efflux Genetic Assembly (Mega) Element in Haemophilus Parainfluenzae

Author Block:

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Abstract Body:

Background: Two identical extensively drug-resistant (XDR) *Haemophilus parainfluenzae* isolates non-susceptible to β-lactams, macrolides, quinolones, tetracycline, and chloramphenicol were recently described.**Methods:** In the efforts to analyze how the resistance genes of these pathogens were organized, one isolate (AE-2096513) underwent Illumina HiSeq 200 whole genome sequencing (WGS). Sanger sequencing was additionally performed to bridge the contigs.**Results:** We describe for the first time the tet(M)-Macrolide Efflux Genetic Assembly (MEGA) element in a *H. parainfluenzae* isolate. Furthermore, it was revealed that the tet(M)-MEGA element was inserted 2bp downstream of RF-3 (ORF19190) at position 1'989'391 compared to *H. parainfluenzae* T3T1. Inspecting the ends of the element revealed that there were no inverted repeats. In AE-2096513, tet(M) together with MEGA were in a "non-Tn916" context because Tn916 typically contains the ORF8 to 23 that were absent in our element.**Conclusions:** *H. parainfluenzae* is normally isolated from the pharynx, where there are also streptococci and pneumococci. A genetic exchange between these two bacterial species was therefore likely. Our finding may serve as a paradigmatic example of microbial acquisition of mobile genetic elements carrying resistant genes against clinically implemented antibiotics.

Author Disclosure Block:

A. Endimiani: None. **A. Lupo:** None. **D. Wüthrich:** None. **A. Allemann:** None. **M. Hilty:** None.

Poster Board Number:

MONDAY-540

Publishing Title:

Structure-Guided Drug Design for the Antibiotic Resistance Enzyme OXA-48

Author Block:

B. A. Lund, S. Ahkter, Y. Guttormsen, T. Christopeit, A. Bayer, H-K. S. Leiros; UiT - The Arcitc Univ. of Norway, Tromsø, Norway

Abstract Body:

Background: Antibiotic resistance shakes the foundation of modern healthcare. While there are several mechanisms, some of the worst problems are caused by antibiotic resistance enzymes such as the class D β-lactamase OXA-48. These enzymes catalyze the hydrolysis of antibiotics, making the bacteria resistant to penicillins and carbapenems. Avibactam is currently the only approved drug on the market that is effective in inhibiting the class D β-lactamases, and there is a need for more compounds in the pipeline to have a backup when resistance arises. **Methods:** The project focuses on designing inhibitors towards these enzymes based on the structural information we have from X-ray crystallographic structures, in order to prolong the lifetime of the current antibiotics with combination therapies. We have performed a surface plasmon resonance (SPR) screen with 490 fragments to find inhibitor scaffolds for OXA-48, and validated the hits using a biochemical assay. **Results:** Several interesting starting points for further optimization have been identified in this manner. By systematically varying the substitutions on the scaffolds we have identified the key interactions of these compounds, and the binding mode for several compounds by X-ray crystallography. The compounds bind non-covalently and reversibly, and with good ligand efficiency (Above 0.3 kcal/mol per heavy atom). We were also able to use two alternate conformations from a structure to design and synthesize a merged compound with improved inhibitory potency.

Conclusions: Our most potent compound has a K_D of 50 µM and an IC_{50} of 18 µM, and the compounds represent a novel inhibitory scaffold for an important drug target involved in antibiotic resistance.

Author Disclosure Block:

B.A. Lund: None. **S. Ahkter:** None. **Y. Guttormsen:** None. **T. Christopeit:** None. **A. Bayer:** None. **H.S. Leiros:** None.

Poster Board Number:

MONDAY-541

Publishing Title:

Discovery of Novel Antibacterials Targeting the MEP Pathway Using the Crystal Structure of *F. tularensis* lspD

Author Block:

S. M. Noble[1], A. Tsang[1], N. Pattabiraman[2], C. Johny[3], A. Haymond[3], R. Couch[3]; [1]Walter Reed Army Inst. of Res., Silver Spring, MD, [2]MolBox, Silver Spring, MD, [3]George Mason Univ., Fairfax, VA

Abstract Body:

Francisella tularensis is classified as a category A agent by the US Centers for Disease Control and Prevention due to its ease of transmission, low infectious dose and significant potential to cause severe illness and death. Due to the prevalence of antimicrobial resistance and the feasibility by which resistance can be engineered into bacteria such as *F. tularensis* and *Y. pestis*, continued development of novel antibiotics is needed. The methylerythritol phosphate (MEP) pathway presents a series of enzymes that may be exploited for antibacterial development. The MEP pathway is utilized by bacteria, apicomplexan protozoa, and plants for biosynthesis of isoprenoids, which are vital for cellular processes such as electron transport, cell wall biosynthesis, and signal transduction. All isoprenoids are derived from either the MEP pathway or the mevalonic acid (MVA) pathway. Since humans acquire isoprenoids through the non-homologous MVA pathway, enzymes within the MEP pathway provide attractive targets for antimicrobial development. We hypothesize that inhibitors of the MEP pathway in *F. tularensis* will serve as effective antibiotics by blocking isoprene biosynthesis. Our objective is to determine crystal structures of MEP pathway enzymes to facilitate development of inhibitors effective against *F. tularensis* and other pathogenic bacteria. We have expressed, purified, crystallized and determined the 2.4 Å crystal structure of *F. tularensis* IspD (*Ft*IspD). The *Ft*IspD structure was then used for *in silico* screening of the PubChem compound database. Of 100 compounds identified from *in silico* screening, 36 were evaluated for enzyme inhibition. Two compounds exhibited IC_{50}'s of 2.47 μM and 1.58 μM and inhibited growth of *F. tularensis* in liquid culture. One of these compounds also inhibited growth of *Y. pestis*. In conclusion, using structure-based drug design methods, an inhibitor was identified that demonstrated antimicrobial activity against *F. tularensis* and *Y. pestis*. We are in the process of determining structures of *Ft*IspD bound to the two compounds with the lowest IC_{50}s for rational design and further improvement of antimicrobial activity.

Author Disclosure Block:

S.M. Noble: None. **A. Tsang:** None. **N. Pattabiraman:** None. **C. Johny:** None. **A. Haymond:** None. **R. Couch:** None.

Poster Board Number:

MONDAY-542

Publishing Title:

Structure-Function Analysis of the *P. aeruginosa* Type III Secretion Needle

Author Block:

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Abstract Body:

Background: The type III secretion system (T3SS) is a clinically important virulence mechanism in *Pseudomonas aeruginosa* (*Paer*) that secretes and translocates protein effector toxins into human cells. We previously identified a highly stereoselective phenoxyacetamide (PhA) inhibitor of *Paer* T3SS and demonstrated that mutations selected for resistance to PhA mapped to the needle protein gene, *pscF* (Bowlin et al., 2014). To better understanding the T3SS needle protein and its interaction with PhA, we initiated a structure-function analysis of *Paer* PscF. **Methods:** Random *pscF* mutants were generated by error-prone PCR, fused to the chaperone genes *pscE* and *pscG*, expressed from the *lac* promoter on pUCP24, introduced into Δ*pscF* or wild-type (WT) *Paer* cells, and assayed for T3SS secretion +/-PhA, +/-IPTG, +/-EGTA. Mutant and wild-type *pscF* alleles were combined in the same strain for dominance studies by expressing the second allele from the *ara* promoter and integrating it into the chromosome of Δ*pscF* cells with miniTn7. **Results:** Alleles of *pscF* with 80 non chain-terminating mutations in 54 different residues of the 84 residue PscF protein were identified. About 50% of the mutations eliminated secretion, 34% had no detectable effect on secretion, and nearly 19% resulted in secretion that was weakly or strongly PhA-resistant. Mutations conferring strong PhA resistance mapped to 8 distinct residues in the C-terminal 25% of PscF, and the side chains face the needle lumen, according to a *Paer* needle model based on the NMR/EM structure of the assembled Salmonella needle protein PrgI. None of the mutants was resistant to 3 other published T3SS inhibitors. Three mutant *pscF* alleles (D14V, D76N, and Q83H) caused constitutive T3SS expression. PhA resistance appeared to be co-dominant with the WT allele, revealing a continuous increase in PhA IC_{50} as the proportion of mutant allele expression was increased. In contrast, the constitutive expression phenotype was recessive to the WT allele. **Conclusions:** *Paer* T3SS resistance to PhA arises predominately from mutations in the C-terminal quarter of PscF. PscF subunits appear to play a role in T3SS regulation based on the constitutive expression phenotype of three *pscF* mutants.

Author Disclosure Block:

N.O. Bowlin: D. Employee; Self; Microbiotix, Inc.. **B.J. Berube:** None. **J.D. Williams:** D. Employee; Self; Microbiotix, Inc.. **J. Mecsas:** None. **A.R. Hauser:** None. **T.L. Bowlin:** D. Employee; Self; Microbiotix, Inc. **D.T. Moir:** D. Employee; Self; Microbiotix, Inc..

Poster Board Number:

MONDAY-543

Publishing Title:

Identification of Small Molecule Inhibitors of the Type II Secretion System in *Acinetobacter baumannii*

Author Block:

U. B. Waack, L. Simmons, H. L. T. Mobley, M. Sandkvist; Univ. of Michigan, Ann Arbor, MI

Abstract Body:

Multi-drug resistant (MDR) bacterial infections pose a major health threat. The increasing frequency of antibiotic resistance in many bacteria, including the nosocomial pathogen *Acinetobacter baumannii*, highlights the need for new therapeutic targets and treatment approaches. The Type II Secretion (T2S) system and its secreted substrates may be one such target as inactivation of the T2S system in *A. baumannii* results in reduced fitness in a murine model of bacteremia. Similarly, deletion of the genes coding for the T2S substrates, lipase (LipA) and metalloprotease (CpaA), results in reduction of *in vivo* colonization. In this study, we have designed and employed a high throughput (HT) screen for the identification of inhibitors of the *A. baumannii* T2S system. A HT lipase assay was developed to detect the functionality of the T2S system using the activity of LipA as a reporter. To eliminate false positives, counter screens were also developed. These counter screens were designed to remove compounds that interfere with bacterial growth, LipA expression or LipA activity. The lipase secretion assay and counter screens were evaluated in a pilot screen in which we tested 6400 compounds of known biological function. Following validation in the proof-of-concept pilot study, we screened 140,000 compounds for inhibition of the *A. baumannii* T2S system. One hundred thirty-three compounds that tested positive for the lipase secretion assay, but negative for the counter screens, were retested over a range of concentrations. Seventy-three of these compounds had an $IC_{50} \leq 30$ μM and inhibited lipase secretion by ≥ 35% at the highest dose tested. Fifty-four compounds were commercially available for reorder and will be retested. Following confirmation of activity, positive compounds will be analyzed for their ability to inhibit secretion of CpaA, another T2S substrate from *A. baumannii*. After verifying that the remaining T2S inhibitors have no adverse effect on the integrity of the cell envelope, they will be subjected to structure activity relationship and pharmacokinetic analysis. The ultimate goal is to identify compounds that may be used as chemical probes for studies of T2S or as therapeutics that, either alone or in tandem with other therapies, can treat MDR *A. baumannii* infections.

Author Disclosure Block:

U.B. Waack: None. **L. Simmons:** None. **H.L.T. Mobley:** None. **M. Sandkvist:** None.

Poster Board Number:

MONDAY-544

Publishing Title:

Validation of a High-Thoughput Screening Assay for Identification of Adjunctive and Directly Acting Antimicrobials Targeting Carbapenem-Resistant *Enterobacteriaceae*

Author Block:

K. Smith, J. E. Kirby; Beth Israel Deaconcess Med. Ctr., Boston, MA

Abstract Body:

Carbapenem resistant Enterobacteriaceae (CRE) are classified as an urgent threat by the CDC and new strategies for treatment are needed. We hypothesized that some known bioactive compounds will restore efficacy of carbapenems (termed antimicrobial adjunctives) or directly inhibit CRE. In this study, we developed, validated, and performed a high-throughput screen to simultaneously identify both of these antimicrobial types.Briefly, a representative CRE strain, *Klebsiella pneumoniae* BIDMC12A, was inoculated into 384-well plates containing test compounds in the presence (screen) or absence (counterscreen) of meropenem. Inocula and media corresponded to standards for in vitro susceptibility testing. Output was optical density with hits selected by z-score. Compounds inhibitory solely in the screen were considered potential adjunctives while those inhibitory in the screen and counterscreen were considered direct antimicrobials. Throughout screening, Z' scores routinely exceeded 0.6.Primary screening of 11,698 known bioactive compounds at the Institute of Chemistry and Cell Biology (Harvard Medical School) revealed 14 adjunctives and 79 direct antimicrobials, 74 of which were subject to abbreviated synergy testing in secondary analysis. Compounds showing positive results in secondary analysis were subject to dose-response and high-resolution synergy assays. Triclosan, a fatty acid synthesis inhibitor, was synergistic with meropenem (Fractional inhibitory concentration = 0.48). NH125 (MIC = 4 µg/ml), diphenyleneiondonium chloride (MIC = 8 µg/ml), and azidothymidine (AZT, MIC = 4 µg/ml) acted as direct antimicrobials. AZT, a nucleoside analog antiviral, was selected for a spectrum of activity assay due to its known low toxicity in humans. Using a panel of 103 clinical Enterobacteriaceae isolates, half of which were CRE, AZT showed an MIC_{50} of 2 µg/ml suggesting potential for clinical efficacy based on known human pharmacokinetic-pharmacodynamic parameters.Taken together, our data demonstrate proof of concept for our high-throughput screen and secondary analyses to identify compounds with direct antimicrobial activity or meropenem synergy. We are currently continuing our screen to identify novel and as yet uncharacterized inhibitors of CRE.

Author Disclosure Block:

K. Smith: None. **J.E. Kirby:** None.

Poster Board Number:

MONDAY-545

Publishing Title:

Development and Application of Bioluminescent Reporter Screens for the Discovery of Isoprenoid Biosynthesis Inhibitors in *P. aeruginosa*

Author Block:

D. M. Mills[1], J. D. Williams[1], D. C. Crick[2], T. L. Bowlin[1], D. T. Moir[1]; [1]Microbiotix, Inc., Worcester, MA, [2]Colorado State Univ., Ft. Collins, CO

Abstract Body:

Background: Isoprenoids are essential for cell viability due to their role in electron transport and cell wall biosynthesis. Many Gram-negative nonfermenters, including *Pseudomonas aeruginosa (Paer)*, utilize an alternate isoprenoid synthesis pathway, the 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway, which is quite distinct from that found in humans, the mevalonate (MVA) pathway. To identify new inhibitors of this pathway as potential antibacterials, we developed and applied cellular reporter screens. **Methods:** A Δ*ispC* (aka *dxr*) *Paer* efflux-deficient strain with a deletion of the gene encoding the first step in the MEP pathway was constructed by complementation with a *lac*-regulated *ispC* copy on a plasmid. Growth of the regulated Δ*ispC* strain is directly proportional to the amount of added IPTG. To identify promoters that respond to reduction in *ispC* levels, the Δ*ispC* strain was grown in limiting IPTG, and RNA was isolated and subjected to RNASeq transcriptional profiling. **Results:** Profiles revealed that transcription of MEP genes does not respond to depletion of the first enzymatic step. Therefore, we selected responsive promoters apparently unrelated to the MEP pathway. To reduce identification of compounds with non-specific responses, we selected two promoters -- one (PA1317) that responded by up-regulation and one (PA2126) by down-regulation to *ispC* depletion. Promoter regions from the respective genes were inserted into a miniTn7 vector upstream of *luxCDABE* and integrated into efflux-deficient *Paer* cells. Application of both reporter strain assays to >150,000 compounds followed by secondary validation assays resulted in the discovery of 10 apparent MEP pathway inhibitors. All 10 have drug-like scaffolds and failed to stimulate reporter strains designed to detect inhibitors of other targets. Furthermore, they inhibit the growth of the *Paer* efflux-deficient Δ*ispC*/pUCP24-P*lac*-*ispC* strain inversely proportional to the concentration of IPTG provided. Inhibitors exhibited low or undetectable cytotoxicity (HeLa CC_{50} >100 μM), and did not inhibit the growth of *S. aureus* cells, which utilize the MVA pathway. **Conclusions:** This research resulted in the discovery of several novel scaffolds with characteristics of selective MEP pathway inhibitors.

Author Disclosure Block:

D.M. Mills: D. Employee; Self; Microbiotix, Inc. **J.D. Williams:** D. Employee; Self; Microbiotix, Inc.. **D.C. Crick:** None. **T.L. Bowlin:** D. Employee; Self; Microbiotix, Inc. **D.T. Moir:** D. Employee; Self; Microbiotix, Inc..

Poster Board Number:

MONDAY-546

Publishing Title:

Combating Antibiotic Resistant Bacteria (Carb) Program Leads to Novel Screening and Drug Development Efforts

Author Block:

D. V. Zurawski, R. J. Sciotti, Y. Si, M. H. Khraiwesh, R. K. Kim, C. E. Bane, R. A. Heitkamp, P. E. Waterman, L. T. Read, S. D. Tyner; Walter Reed Army Inst. of Res., Silver Spring, MD

Abstract Body:

In 2014 the President initiated a National Strategy for Combating Antibiotic Resistant Bacteria (CARB). This initiative tasked the Walter Reed Army Institute of Research (WRAIR) with the delivery of a novel Gram negative antibiotic drug candidate. Two research divisions at the WRAIR collaborated to construct a drug development pipeline that encompassed: initial compound screening with medicinal chemistry input, followed by typical ADME analysis and subsequent safety and efficacy testing in animal models of infection. The primary in vitro screen employed a novel approach using clinically relevant multidrug-resistant (MDR) Gram negative isolates against a proprietary compound library of about 10,000 compounds from WRAIR's 500,000 compound library. The isolates tested were previously characterized imipenem-resistant *Acinetobacter baumannii* (AB5711) and *Klebsiella pneumoniae* (KP4640). Both strains are clinical isolates from wound infections, harbor multiple carbapenemases and develop robust biofilms in vitro. Compounds were screened in CAMHB, and each isolate was grown in the presence or absence of imipenem (IMP). From the primary screen, 208 (about 2%) were found to inhibit growth with: 133 positives w/IMP against AB5711; 40 positives w/IMP against KP4640; 51 positives w/o IMP against AB5711; and 18 positives w/o IMP against KP4640. We then employed a novel secondary screen against these hits utilizing a more physiologically relevant minimal medium against positives from the primary screen. This secondary screen yielded further potential drug candidates, representing an additional down selection from our original library. Now, we will determine the MIC for these candidate compounds against a broad range of Gram positive and Gram negative bacteria. In addition, we will perform a diversity set screen against a representative panel of currently circulating MDR clinical isolates if that species appears susceptible. This screening approach, utilizing clinically relevant isolates in physiologically relevant media, with and without the current antibiotic standard of care, can identify novel small molecules that are potential antibiotics as well as antibiotic adjuvants.

Author Disclosure Block:

D.V. Zurawski: None. **R.J. Sciotti:** None. **Y. Si:** None. **M.H. Khraiwesh:** None. **R.K. Kim:** None. **C.E. Bane:** None. **R.A. Heitkamp:** None. **P.E. Waterman:** None. **L.T. Read:** None. **S.D. Tyner:** None.

Poster Board Number:

MONDAY-547

Publishing Title:

Discovery of Novel Inhibitors of Multi-drug Resistant *Acinetobacter baumannii*

Author Block:

I. Soojhawon[1], N. Pattabiraman[2], A. Tsang[1], A. Roth[1], E. Kang[1], S. Noble[1]; [1]Walter Reed Army Inst. of Res., Silver Spring, MD, [2]MolBox LLC, Silver Spring, MD

Abstract Body:

Background: The rapid emergence of multidrug resistant *Acinetobacter baumannii* strains significantly reduces the efficacy of currently available antibiotics in the treatment of nosocomial infections. To combat antimicrobial resistance, novel drugs with new mechanisms of actions are urgently needed. Promising drug targets in Gram-negative bacteria include the outer membrane proteins (OMPs), especially the outer membrane protein W (OmpW). OmpW is a key transport channel and functions in the maintenance of homeostasis during cellular stress. Previous research studies have shown that *E. coli* colicin S4, a naturally occurring antimicrobial protein, exploits OmpW for its entry into the cell, and deletion of the OmpW gene from *E. coli* decreased the minimum inhibitory concentrations (MICs) for a range of antibiotics. Our overall objective is to use a structure-based approach to develop novel inhibitors of *A. baumannii* OmpW2.**Methods:** Computational modeling was utilized to generate a homology model of one of the isoforms of *A. baumannii* OmpW (OmpW2) based on the x-ray crystal structure of *E. coli* OmpW. Molecular docking studies were performed using the homology model to determine the binding pockets in the protein. Using a 3D stereo-electronic pharmacophore, *in silico* screening of the PubChem chemical library was performed for potential *A. baumannii* inhibitors. The antimicrobial activities of the identified compounds were then evaluated on the growth of a panel of *A. baumannii* strains by determining the MICs according to CLSI guidelines.**Results:** Fifty compounds were identified from the *in silico* screen and evaluated for antimicrobial activities. Three lead inhibitors designated as WRAIR_D5, WRAIR_D6 and WRAIR_D12 inhibited the growth of the *A. baumannii* strains with MICs ranging between 1 to 32 μg/ml.**Conclusions:** These results provide the preliminary information to refine the pharmacophore, perform further structure based drug screening and synthesize optimized derivatives of the leads. We conclude that this structure-based drug screening strategy offers promising inhibitors of *A. baumannii*, and that further studies are warranted to validate OmpW2 as a novel drug target.

Author Disclosure Block:

I. Soojhawon: None. **N. Pattabiraman:** None. **A. Tsang:** None. **A. Roth:** None. **E. Kang:** None. **S. Noble:** None.

Poster Board Number:

MONDAY-548

Publishing Title:

Developing an Antimicrobial Screening Approach Using a T7 Phage Library

Author Block:

W. R. Rodriguez Polanco, C. Rios-Velazquez; Univ. of Puerto Rico at Mayaguez, Mayaguez, PR

Abstract Body:

The use of antibiotics is essential to treat microbial infections, patients that need surgery, chemotherapy or are immunocompromised need to be treated with antibiotics, but their misuse is causing the development of antibiotic resistant strains (ARS). Since the ARS spreading surpass the generation of new antibiotics, it is imperative to find novel antimicrobial agents, capable of inhibiting the antibiotic resistant pathogens. Also, due to the difficulty to find new antimicrobial agents by conventional screening, novel techniques and technologies needs to be developed. The main focus of this research is to test a novel antimicrobial screening strategy to detect antimicrobial agents using combinatorial chemistry. A bacterial bioprospect from our laboratory collection, previously isolated from Guánica Dry Forest in Puerto Rico and capable of inhibits *Staphylococcus aureus*, was used as a candidate to generate a combinatorial chemistry Phage Display Library (PDL). After extracting the bioprospect genomic DNA by a direct method, a genomic library was generated using T7 Select 10-3 cloning kit (Novagen). Three different screening types were done to the generated library. The first one was a plaque assay for inhibition, where a *Escherichia coli* lawn with individual T7-Bioprospect PDL plaques were exposed to the target, by pouring top agar inoculated with *Staphylococcus aureus* over the plaques. The second screening combined the T7-Bioprospect PDL with *E. coli* and *S. aureus* in the same top agar solution. A third screening was done by selecting and amplifying individual phage plaques in a 96 well plate, follow by transferring two micro-litters aliquot from each amplification on a Petri plate with *Staphylococcus aureus* lawn. In all the screening methods, the presence of a clear zone on the target was scored as a positive result. Preliminary tests have been done and no candidate has been found yet. A bioprospect T7 Phage display library was generated with approximately 9×10^9 plaque forming units. After screening approximately 10,000 clones (0.00001%) of the combinatorial library no visible inhibition zones have been detected. There is a possibility that the size and expression of the displayed peptides is affecting the antimicrobial activity. While more library screening and optimization processes are is in progress, this is a promising strategy to find molecules with antibiosis activity.

Author Disclosure Block:

W.R. Rodriguez Polanco: None. **C. Rios-Velazquez:** None.

Poster Board Number:

MONDAY-549

Publishing Title:

Phage Display Selected Peptides as Potential Anti-Adhesives Molecules in Paracoccidioidomycosis Therapy

Author Block:

H. C. de Oliveira[1], J. Michaloski[2], J. F. Silva[1], L. Scorzoni[1], A. C. A. de Paula e Silva[1], C. M. Marcos[1], P. A. Assato[1], A. M. Fusco-Almeida[1], R. J. Giordano[2], M. J. S. Mendes-Giannini, 14801902[1]; [1]Faculdade de Ciências Farmacêutica de Araraquara - FCFAr UNESP, Araraquara, Brazil, [2]Univ.e de São Paulo, USP, São Paulo, Brazil

Abstract Body:

Paracoccidioides brasiliensis and *P. lutzii* are dimorphic fungi, etiologic agents of paracoccidioidomycosis (PCM). Fungi of *Paracoccidioides* genus have mechanisms that enable them to adhere and invade host tissues and adhesion is one of the most important steps in the *Paracoccidioides* infection, being responsible for differences in virulence of isolates of these fungi. Regarding the importance of the adhesion to the establishment of the infection, this study focused in a preliminary development of a new therapeutic strategy to avoid the adhesion of *Paracoccidioides*, inhibiting infection and preventing the development of this systemic mycosis. This disease is an important public health issue in Latin America, mainly in Brazil, country with most endemic areas for this disease. We used two phage display libraries with the aim to select peptides that strongly bind to *Paracoccidiodes* cell wall and avoid the adhesion to host cells and extracellular matrix (ECM) components: laminin, fibronectin and types I and IV collagen. With this approach we identify four peptides that inhibits *in vitro* up to 64% of the adhesion to pneumocytes and up to 57% the adhesion to the different ECM components. With these results, we decided to evaluate, in a preliminary way, if these selected peptides can protect the alternative animal model *Galleria mellonella* of *Paracoccidioides* infection. For this, we treated *G. mellonella* larvae with the different peptides for three hours prior to infection with *P. brasiliensis* and *P. lutzii* and them we observed the survivor of these larvae. The results shows us that all tested peptides increasing up to 64% the survivor of larvae infected with *P. brasiliensis* and up to 60% when infected with *P. lutzii*. These data, besides preliminary, open us new horizons in therapeutic strategies to face PCM. The important role of the adhesion in *Paracoccidioides* infection show us that an anti-adhesive therapy could be an important strategy and the selected peptides have potential as prototypes to be explored in the PCM therapy.

Author Disclosure Block:

H.C. de Oliveira: None. **J. Michaloski:** None. **J.F. Silva:** None. **L. Scorzoni:** None. **A.C.A. de Paula e Silva:** None. **C.M. Marcos:** None. **P.A. Assato:** None. **A.M. Fusco-Almeida:** None. **R.J. Giordano:** None. **M.J.S. Mendes-Giannini:** None.

Poster Board Number:

MONDAY-550

Publishing Title:

Eravacycline *In Vitro* Antibacterial Activity against 110 Carbapenemase-Producing *Enterobacteriaceae* Clinical Isolates

Author Block:

Y. Zhang, X. Lin, K. Bush; Indiana Univ., Bloomington, IN

Abstract Body:

Background: Carbapenem-resistant *Enterobacteriaceae* (CRE) are among the most urgent threats in the antibiotic resistance spectrum and have been increasing in central Indiana Health Care Centers since 2009. Our laboratory has characterized Indiana CRE isolates that produce both serine and metallo-carbapenemases resulting in resistance to most beta-lactam antibiotics. Eravacycline (ERV) is a novel synthetic fluorocycline antibiotic of the tetracycline class with broad-spectrum activity, being developed for the treatment of serious infections, including those caused by multidrug-resistant pathogens. In this study we compare its activity to other agents used to treat enteric infections.**Materials:** CRE isolates from 2010 - 2013 (n=110) were characterized for carbapenemase genes by PCR and sequencing. MICs for ERV, tetracycline, tigecycline, amikacin, imipenem, ceftazidime, cefotaxime and levofloxacin were determined in broth microdilution assays according to CLSI standards.**Results:** Among the CRE isolates were 96 *Klebsiella pneumoniae* with the serine carbapenemases KPC-2 (n=15) or KPC-3 (n=81); 6 *Escherichia coli* isolates with KPC-2 (n=1) or KPC-3 (n=5); 6 *Serratia marcescens* isolates with SME-1 (n=3) or KPC-3 (n=3); and 2 *Enterobacter cloacae* isolates with both KPC-3 and a VIM-1-type metallo-beta-lactamase (MBL). Other MBL-producing isolates included 5 KPC-3-producing *K. pneumoniae* isolates with VIM-1 (n=4) or NDM-1 (n=1). The isolates were highly resistant to all beta-lactams tested and to levofloxacin and were intermediate to tetracycline and amikacin, based on MIC_{50}/MIC_{90} values. MIC_{50}/MIC_{90} values for ERV were 1 / 2 μg/ml compared to 2 / 2 μg/ml for tigecycline. ERV MICs were often 2-fold lower than for tigecycline, with only 4% of tigecycline MICs <2 μg/ml compared to 64% of the ERV MICs.**Conclusions:** Eravacycline demonstrated the lowest cumulative MICs against this panel of recent carbapenem-resistant *Enterobacteriaceae* with MIC_{50}/MIC_{90} values of 1 and 2 μg/ml. Thus, eravacycline may have the potential to treat infections caused by CRE.

Agent	MIC range μg/ml	MIC_{50} in μg/ml	MIC_{90} in μg/ml
Eravacycline	0.5-4	1	2
Tetracycline	4 - >64	8 (I)	8 (I)
Tigecycline	1 - 4	2 (S)	2 (S)

Amikacin	2 - >64	32 (I)	32 (I)
Imipenem	4 - >64	8 (R)	32 (R)
Ceftazidime	1 - >32	>32 (R)	>32 (R)
Cefotaxime	1 - >8	>8 (R)	>8 (R)
Levofloxacin	<0.25 - >16	>16 (R)	>16 (R)

Author Disclosure Block:

Y. Zhang: None. **X. Lin:** None. **K. Bush:** C. Consultant; Self; Achaogen, Naeja. H. Research Contractor; Self; Achaogen, Actavis, Tetraphase. J. Scientific Advisor (Review Panel or Advisory Committee); Self; Achaogen, Allecra, Entasis, Gladius, Melinta, Roche, Tetraphase, WarpDrive. K. Shareholder (excluding diversitied mutual funds); Self; Fedora, Johnson & Johnson. N. Other; Self; Bristol-Myers Squibb, Johnson & Johnson, Pfizer.

Poster Board Number:

MONDAY-551

Publishing Title:

In Vitro* Intracellular Activity of Omadacycline against *Legionella pneumophila

Author Block:

J. Dubois[1], M. Dubois[1], J-F. Martel[1], S. K. Tanaka[2]; [1]M360, Sherbrooke, QC, Canada, [2]Paratek Pharmaceuticals, Boston, MA

Abstract Body:

Background: Omadacycline (OMC) is the first once-daily, oral and IV aminomethylcycline in late stage clinical development for serious community-acquired bacterial pneumonia (CABP) and acute bacterial skin and skin structure infection (ABSSI). *In vitro* bacterial activity and intracellular activities using human monocytes against a variety of *L. pneumophila* serogroup one (1) were investigated.**Methods:** The intracellular activity of **OMC** was compared with that of doxycycline (DO), moxifloxacin (MO) and azithromycin (AZ) against a total of three (3) erythromycin-resistant and two (2) erythromycin-susceptible strains of *L. pneumophila* serogroup 1. The minimal extracellular concentration inhibiting the intracellular multiplication of *L. pneumophila* (MIEC) was determined by exposing human monocytes, U937 cell line, with intracellular *Legionella* to antibiotic at the 1X, 1/2X, 1/4X, 1/8X or 1/16X the extracellular MIC of each strain during 4 days exposure. Counts of CFU/mL were performed daily in triplicate using the Buffer Yeast Extract agar with charcoal.**Results:** All tested strains of *L. pneumophila* (5/5 strains) had a MIC of 0.25 mg/L to **OMC.** A mean reduction of ≥50% of *L. pneumophila* grown in human monocytes at Day 3 or Day 5 of **OMC** exposure, was achieved at a MIEC of 0.06 mg/L or at MIEC/MIC ratio of 0.24 (1/4XMIC). An intracellular activity at Day 3 or at Day 5 of **OMC** exposure with a reduction (≥50%) of *L. pneumophila* grown in macrophages and with a MIEC/MIC ratio of 0.12 (1/8XMIC) was observed with only 1 erythromycin-resistant strains of *L. pneumophila*. In this study, the MIEC/MIC ratio of **OMC** (0.24 or 1/4XMIC) was lower than the MIEC/MIC ratio of doxycycline (0.5 or 1/2XMIC), moxifloxacin (0.5 or 1/2XMIC), and azithromycin (1 or 1XMIC).**Conclusions:** These data, demonstrating robust bacterial activity and human monocyte penetration, highlight the potential utility of **OMC** as an effective oral and IV antibiotic for the treatment of CABP caused by *L. pneumophila*.

Author Disclosure Block:

J. Dubois: H. Research Contractor; Self; Paratek Pharmaceuticals. **M. Dubois:** None. **J. Martel:** None. **S.K. Tanaka:** D. Employee; Self; Paratek Pharmaceuticals.

Poster Board Number:

MONDAY-552

Publishing Title:

***In Vitro* Activity of Omadacycline against *E. coli* Biofilms**

Author Block:

D. Diehl[1], **N. Bionda**[1], N. C. Cady[1], A. D. Strickland[1], S. K. Tanaka[2]; [1]iFyber, Ithaca, NY, [2]Paratek Pharmaceuticals, King of Prussia, PA

Abstract Body:

The goal of the current study was to investigate the anti-biofilm properties of omadacycline (OMC), a first-in-class compound of a new class of aminomethylcycline antibiotics. OMC exhibits potent bacteriostatic activity against both Gram-negative and Gram-positive bacteria and is currently in Phase III clinical trials for multiple indications. Microbial biofilms are defined by a dense extracellular polymeric substance that can act as a physical barrier to the external environment, and results in a subpopulation of metabolically quiescent microbes, which can lead to a remarkable tolerance to antibiotics that normally kill planktonic bacteria. Given that OMC exhibits activity against a broad spectrum of organisms and has potential utility against organisms that produce biofilms, the current study aimed to establish the baseline anti-biofilm properties of OMC. Focusing on *Escherichia coli* (ATCC 25922), which is a common cause of community-acquired infections, doses of OMC above and below the minimum inhibitory concentration (MIC) for planktonic cells were assessed for biofilm prevention and/or induction, and against established biofilms using the MBEC™ 96 well plate high throughput screening device. In experiments using sub-MIC and supra-MIC doses of OMC applied to early-phase and established biofilms in the presence of planktonic bacteria, sub-MIC doses (e.g., 0.74 µg/mL) do not appear to induce further biofilm propagation above the levels observed in the absence of OMC. Further, biofilm prevention/induction assays indicate that OMC strongly inhibits biofilm formation at all doses above the MIC. At sub-MIC doses (e.g., 0.74 µg/mL), mean biofilm formation is reduced by approximately 3 to 4 log units compared to controls, in high and low inoculum conditions, respectively. In contrast to other translation inhibitors, sub-MIC doses of OMC not only failed to induce biofilm formation above the level of controls, several doses below the MIC appear to have an inhibitory effect on biofilm formation.

Author Disclosure Block:

D. Diehl: None. **N. Bionda:** None. **N.C. Cady:** None. **A.D. Strickland:** None. **S.K. Tanaka:** None.

Poster Board Number:

MONDAY-553

Publishing Title:

Antibacterial Activity of Kbp-7072 Against Clinical Isolates of Drug-resistant Bacteria

Author Block:

Y. Wang, Q. Liu, **B. Zhang**; KBP BioSci.s, Princeton, NJ

Abstract Body:

The purpose of this study was to investigate the activity of KBP-7072, a novel broad spectrum aminomethylcycline, against Gram positive (GP) and Gram negative (GN) multidrug resistant isolates. The *in vitro* activity of KBP-7072 against multidrug resistant GP and GN strains collected from Chinese hospitals was compared with that of tigecycline. GP strains included at least 12 isolates each of Methicillin-resistant *S. aureus*, MRSE, *E. faecium*, *E. faecalis*, and *S. pneumoniae*. GN strains included at least 12 isolates of ESBLs *E. coli*, ESBLs *K. pneumoniae*, *Acinetobacter,* and *P. aeruginosa*. Susceptibility testing was performed according to the M7-A9 and M7-A11 CLSI-recommended agar dilution methods. The bacteria freshly grown in MHA and BHIA plates containing sterile fiber sheep blood were suspended in 0.9% sodium chloride for injection to 0.5 McFarland standard absorbance and were further diluted 10x. The testing bacterial suspension was then inoculated onto MHA and BHIA plates with sterile fiber sheep blood and test articles by multipoint microplanter at 10^4 CFU per point. Test plates were incubated at 35°C for 16 -20 hrs or with 5% CO_2 for 20 to 24 hrs as specified by CLSI M07-A9 and M07-A11. The range of MIC in GP bacteria was 0.015-2 μg/mL for KBP-7072 vs 0.015 to 2 μg/mL for tigecycline. The MIC_{90} values of KBP-7072 and tigecycline were 0.5 vs 2 μg/mL (MRSA), 1 vs 2 μg/mL (MRSE), 0.125 vs 0.25 μg/mL (*E. faecium)*, 0.25 vs 0.5 μg/mL (*E. faecalis)*, and 0. 5 vs 1 μg/mL (PRSP), respectively. The range of MIC in GN bacteria (excluding *P. aeruginosa*) was 0.06 to 16 μg/mL for KBP-7072 vs 0.125 to 16 μg/mL for tigecycline. The MIC_{90} values of KBP-7072 and tigecycline were 2 vs 2 μg/mL (ESBLs *E. coli*), 4 vs 2 μg/mL (ESBLs *K. pneumoniae*), 1 vs 4 μg/mL (*A. baumannii*), and 1 vs 2 μg/mL (*A. lwoffi*). After a single step culture, KBP-7072 showed better or equal resistance frequency compared to tigecycline ranging from 2.40×10^{-8} to 1.05×10^{-6} in 13 clinical isolates. At 2-fold and 4-fold MICs, the resistance frequency of KBP-7072 was comparable to tigecycline. KBP-7072 demonstrates excellent *in vitro* activity against clinical bacterial isolates and strains including multi-drug resistant GP and GN bacteria. KBP-7072 showed better or equal resistance frequency compared to tigecycline in 13 clinical isolates after a single step culture, as well as at 2-fold and 4-fold MICs.

Author Disclosure Block:

Y. Wang: None. **Q. Liu:** None. **B. Zhang:** None.

Poster Board Number:

MONDAY-554

Publishing Title:

***In Vitro* Activity of Plazomicin aAgainst 110 Carbapenemase-Producing *Enterobacteriaceae* Clinical Isolates**

Author Block:

Y. Zhang, **A. Kashikar**, X. Lin, K. Bush; Indiana Univ., Bloomington, IN

Abstract Body:

Background: Carbapenem-resistant *Enterobacteriaceae* (CRE) represent one of the most stringent challenges to our current antibacterial armamentarium. Our laboratory has been monitoring the presence of CRE in central Indiana Health Care Centers since 2009. These multidrug-resistant isolates with serine and metallo-carbapenemases are not susceptible to most beta-lactam antibiotics. In this study, we tested plazomicin, a new aminoglycoside, for its antibacterial activity against Indiana CRE isolates in comparison to meropenem or piperacillin-tazobactam.**Materials: I**nitial carbapenem MICs for 110 CRE isolates were determined using a Vitek 2. Carbapenemases were characterized by PCR and sequencing. MICs for imipenem, amikacin and plazomicin were determined in broth microdilution assays according to CLSI.**Results:** The CRE isolates included 96 *Klebsiella pneumoniae* isolates producing KPC-2 (n=15) or KPC-3 (n=81) serine carbapenemases, 6 *Escherichia coli* isolates with KPC-2 (n=1) or KPC-3 (n=5), 2 *Enterobacter cloacae* isolates producing both KPC-3 and a VIM-1-type metallo-beta-lactamase, and 6 *Serratia marcescens* isolates producing SME-1 (n=3) or KPC-3 (n=3). VIM-1 was also in 4 *K. pneumoniae* isolates with KPC-3; NDM-1 was in one *K. pneumoniae* with a KPC-3 enzyme. The isolates were highly resistant to carbapenems with MIC_{90} values >4 μg/ml. Amikacin MIC_{50} / MIC_{90} values were in the intermediate range. Plazomicin had the lowest MICs of all the agents, with MIC_{50}/MIC_{90} values 0f 0.5 / 1 μg/ml. The only isolates with plazomicin MICs >4 μg/ml were 1) a *S. marcescens* with SME-1 (MIC 8 μg/ml); and 2) a *K. pneumoniae* isolate with NDM-1 (plazomicin MIC 256 μg/ml, amikacin MIC >64 μg/ml), presumably due to production of a ribosomal methylase.**Conclusions:** Plazomicin was the most potent agent tested against this panel of recent carbapenem-resistant *Enterobacteriaceae* with an MIC_{90} of 1 μg/ml. These data suggest that plazomicin may provide useful activity against CRE that produce serine carbapenemases.

Agent	MIC in μg/ml		
	MIC range	MIC_{50}	MIC_{90}
Imipenem	0.5 - $\geq$ 8	$\geq$ 8	$\geq$ 8
Meropenem	0.5 - $\geq$ 8	$\geq$ 8	$\geq$ 8

Amikacin	2 - >64	32	32
Plazomicin	0.25 - 256	0.5	1

Author Disclosure Block:

Y. Zhang: None. **A. Kashikar:** None. **X. Lin:** None. **K. Bush:** C. Consultant; Self; Naeja, Achaogen. H. Research Contractor; Self; Achaogen, Tetraphase, Actavis. J. Scientific Advisor (Review Panel or Advisory Committee); Self; Achaogen, Allecra, Entasis, Gladius, Melinta, Roche, Tetraphase, WarpDrive. K. Shareholder (excluding diversitied mutual funds); Self; Fedora, Johnson & Johnson. N. Other; Self; Bristol-Myers Squibb, Johnson & Johnson, Pfizer.

Poster Board Number:

MONDAY-555

Publishing Title:

Aminospectinomycins Display Efficacy in Murine Gonorrheal Infections

Author Block:

M. M. Butler[1], S. L. Waidyarachchi[2], R. E. Lee[2], A. E. Jerse[3], K. L. Connolly[3], T. L. Bowlin[1]; [1]Microbiotix, Inc., Worcester, MA, [2]St. Jude Children's Res. Hosp., Memphis, TN, [3]Uniformed Services Univ., Bethesda, MD

Abstract Body:

Background: Bacterial sexually transmitted infections are widespread and common, with an estimated 1.4 million reported cases annually in the US. *Neisseria gonorrhoeae* (*Ng)* causes gonorrhea, a very common, but often asymptomatic, STD that can be urogenital, anorectal or pharyngeal in nature. If left untreated, gonorrhea can cause pelvic inflammatory disease in women, leading to infertility or may disseminate, causing joint and skin symptoms. The recommended treatment for gonorrhea is ceftriaxone (CTX) plus azithromycin, both as a single dose. Antibiotic resistance to all existing cures has developed in gonorrheal infections. The need for new antibiotics is great and the pipeline for new drugs is alarmingly small. The aminospectinomycins (AmSPCs), a new class of semisynthetic analogs of the antibiotic, spectinomycin (SPC), have increased potency against *Ng*. We recently reported *in vitro* potency against resistant isolates.**Methods:** The *in vivo* efficacy of lead AmSPC compounds was evaluated by pretreating estradiol-treated mice with streptomycin (STR) and trimethoprim to suppress overgrowth of commensal flora that occurs under estradiol treatment. On day 0, mice were inoculated with approximately 10^4 CFU of *Ng* strain H041 (STR^R-CTX^R); treatment with 1950, 2324 and SPC by the SC route or positive control, gentamicin (GEN), by the IP route was initiated on day 2 and continued once daily for 5 days. Daily vaginal swabs were tested for the presence of *Ng* during the treatment regimen and for an additional 3 days.**Results:** The percentage of mice in each group that was colonized with *Ng* prior to and after treatment was calculated. 100% of mice given Lee 1950, SPC, or GEN and 90% of mice given 2324 cleared infection by the study endpoint. The effect of test and control compounds on *Ng* colonization was measured by counting CFU recovered in vaginal swabs. The colonization load of *Ng* was significantly reduced after treatment with 1950, 2324, SPC and GEN compared to the vehicle control; colony counts dropped to below the limit of detection.**Conclusions:** In summary, data produced by this study support AmSPCs as a promising series for treating gonorrhea, including infections caused by CTX^R *Ng*. These compounds as well as SPC were comparable to GEN in this model.

Author Disclosure Block:

M.M. Butler: None. **S.L. Waidyarachchi:** None. **R.E. Lee:** C. Consultant; Self; Avisa Pharma, Astra Zenceca, Achaogen. N. Other; Self; Microbiotix, Arietis Pharma, Sequella, Inc.. **A.E. Jerse:** None. **K.L. Connolly:** None. **T.L. Bowlin:** None.

Poster Board Number:

MONDAY-556

Publishing Title:

Comparative Antimicrobial Activity of Mul-1867, a Novel Antimicrobial Compound and Amikacin, against New Clinically Important Airway Pathogens in Cystic Fibrosis *S.maltophilia, A.xylosoxidans, M.abscessus*

Author Block:

G. Tetz[1], M. Cynamon[2], V. Tetz[1]; [1]TGV-Therapeutics, New York, NY, [2]VA Med. Ctr., Syracuse, NY

Abstract Body:

Background: As a result of treatment of "classic" bacterial infections in cystic fibrosis (CF), there are now increasing problems with innately resistant new airway pathogens such as *S.maltophilia, A.xylosoxidans, M.abscessus*. These bacteria are are associated with higher morbidity among CF patients and display high resistance to antimicrobials. The aim of the present study was to compare selected antimicrobials against these planktonic and sessile pathogens. **Methods:** We used clinical isolates of *S.maltophilia* and *A.xylosoxidans* from cystic fibrosis patients and a clinical isolate of *M.abscessus.* Two antimicrobial agents were tested: novel broad spectrum antimicrobial Mul-1867 ($C_{426}H_{852}N_{204}$ * 60 HCl) and amikacin as representative aminoglycosides that are commonly used to treat CF patients. The anti-biofilm activity against 48-h-old S.*maltophilia* and *A.xylosoxidans* biofilms was evaluated as minimum biofilm eliminating concentration that completely eradicated mature biofilms (MBEC100). Biofilms were exposed for 24 h containing antibacterial at 1 - 128× the MIC. All assays were repeated in triplicate. **Results:** Mul-1867 exhibited a high level of antimicrobial activity against all strains with the MIC values 0.125 mg/L against *S.maltophilia*, 0.25 mg/L against *A.xylosoxidans* and 2.0 mg/L against *M.abscessus*. Amikacin was less active than Mul-1867; the MIC for *S.maltophilia* was 128 mg/L, for *A.xylosoxidans* was 25 mg/L, and for *M.*abscessus 8.0 mg/L. Mul-1867 showed MBEC100 values of 2× and 4× the MIC, respectively, against *S.maltophilia* and *A.xylosoxidans*. Amikacin displayed lower antibiofilm activity against *S.maltophilia* and *A.xylosoxidans* with $MBEC_{100}$ values of 128× and 64× the MIC, respectively. **Conclusions:** In the current study, we found that a novel drug candidate, Mul-1867, exhibits a high level of antimicrobial activity against *S.maltophilia, A.xylosoxidans* and *M.abscessus*. We revealed that Mul-1867 was more active than amikacin against selected clinical isolates, displayed lower MICs and possessed low MBEC100/MIC. The efficacy of Mul-1867 raises the possibility that it may serve as a locally acting antimicobial compound in CF patients.

Author Disclosure Block:

G. Tetz: None. **M. Cynamon:** None. **V. Tetz:** None.

Poster Board Number:

MONDAY-557

Publishing Title:

Me1100, a New Formulation of Arbekacin for Inhalation: The Antimicrobial Activity of Arbekacin in Comparison with Other Aminoglycosides Against Aminoglycoside Modifying Enzymes Producing Strains

Author Block:

N. Baba, T. Takata, T. Sugano, Y. Takayama, N. Senju, E. Murase, Y. Tsutsumi, T. Ida, K. Kondo; Meiji Seika Pharma Co., Ltd., Tokyo, Japan

Abstract Body:

Background: ME1100, arbekacin (ABK), is a semi-synthetic aminoglycoside derived from Kanamycin B, designed to be stable to various aminoglycoside modifying enzymes (AMEs). ABK shows strong antibacterial activity against both Gram-positive and Gram-negative bacteria and possesses a possibility to be a drug for HABP/VABP treatment. We determined MICs and MBCs of ABK against AMEs producing MRSA and *P. aeruginasa* (Pa) and compared the values with those of other aminoglycosides.**Methods:** The MICs and MBCs for aminoglycosides were determined by using standard techniques in accordance with CLSI guidelines.**Results:** For MRSA, the geometric mean (GM) MICs and GM-MBCs of ABK against 3 strains without AMEs genes were 0.6 μg/mL and 2.5 μg/mL, respectively, and they were lower than those of amikacin (AMK). The GM-MICs and GM-MBCs of ABK against 9 strains with *aac(6')/aph(2")* gene were 1.5 μg/mL and 4.3 μg/mL, respectively, and they were considerably lower than those of other aminoglycosides. The GM-MICs and GM-MBCs of ABK against 9 strains with *ant (4')-Ia* gene were 0.5 μg/mL and 2.2 μg/mL, respectively, and they were considerably lower than those of AMK and TOB. Furthermore, the GM-MICs and GM-MBCs of arbekacin against 8 strains with both *aac(6')/aph(2")* and *ant (4')-Ia* genes were 1.8 μg/mL and 7.3 μg/mL, respectively, and they were considerably lower than those of other aminoglycosides. For Pa, the GM-MICs and GM-MBCs of ABK against 13 strains without AMEs genes were 2.1 μg/mL and 7.6 μg/mL, respectively, and they were almost similar to those of other aminoglycosides. On the other hand, the GM-MICs and GM-MBCs of ABK against 9 strains with *aac(6')-Ib* gene were 34.6 μg/mL and 101.6 μg/mL, respectively and those of ABK against 12 strains with *aac(6')-Iae* gene were 20.2 μg/mL and 42.7 μg/mL, respectively. These values were not so low, but were considered superior activity among aminoglycosides tested.**Conclusions:** Since ABK is stable to AMEs such as AAC(6'), APH(2") and ANT(4'), ABK showed excellent antibacterial and antibactericidal activities against AME-producing MRSA and Pa among aminoglycosides tested.

Author Disclosure Block:

N. Baba: D. Employee; Self; Meiji Seika Pharma Co., Ltd. **T. Takata:** D. Employee; Self; Meiji Seika Pharma Co., Ltd. **T. Sugano:** D. Employee; Self; Meiji Seika Pharma Co., Ltd. **Y.**

Takayama: D. Employee; Self; Meiji Seika Pharma Co., Ltd. **N. Senju:** D. Employee; Self; Meiji Seika Pharma Co., Ltd. **E. Murase:** D. Employee; Self; Meiji Seika Pharma Co., Ltd. **Y. Tsutsumi:** D. Employee; Self; Meiji Seika Pharma Co., Ltd. **T. Ida:** D. Employee; Self; Meiji Seika Pharma Co., Ltd. **K. Kondo:** D. Employee; Self; Meiji Seika Pharma Co., Ltd..

Poster Board Number:

MONDAY-558

Publishing Title:

In Vitro* Dynamic Pharmacokinetic-Pharmacodynamic (Pk-Pd) Modeling of Arbekacin Inhalation Solution (Me1100) Against *K. pneumoniae

Author Block:

N. Sato, K. Matsumoto, S. Ouchi, N. Baba, Y. Nagira, Y. Tsutsumi, K. Kondo; Meiji Seika Pharma Co., Ltd., Tokyo, Japan

Abstract Body:

Background: Arbekacin (ABK) is an aminoglycoside antibiotic that has been approved for an injectable formulation in Japan. Currently, arbekacin inhalational solution (ME1100) is under development for treatment of ventilator-associated bacterial pneumonia (VABP). We investigated the PK-PD properties of ME1100 against *K. pneumonia* strains using dynamic PK-PD model and evaluated the predictability of the fluctuation of the number of bacteria in the *in vitro* chemostat model by the *in vitro* dynamic PK-PD model.**Methods:** The time-kill study and the *in vitro* chemostat model study were conducted against three *K. pneumonia* strains for 24 hours. The dynamic PK-PD model was established to investigate the PK-PD relationship of ME1100 against the *K. pneumonia* strains. This model hypothesizes two populations (the sensitive and the low sensitive populations) for the bacteria, which differ in growing ability and sensitivity to ABK. The PD parameters, which represent the properties of the two populations of bacteria, were obtained by analysing the time-kill study data with the dynamic PK-PD model. The computer simulation of the fluctuations in $\log_{10}$ CFU was conducted using the PD parameters from the time-kill study and the mimicked ABK concentrations in human ELF. And then, the correlation of the time-dependent fluctuations in $\log_{10}$ CFU between the observed data from the *in vitro* chemostat model and the predicted values based on the dynamic PK-PD model were statistically evaluated.**Results:** The time-kill study data were analyzed with the dynamic PK-PD model and the 12 PD parameters were obtained. The fittings of the observed Log_{10} CFU and the predicted Log_{10} CFU were good. The fluctuations in $\log_{10}$ CFU, which was predicted using the PD parameters and the mimicked ABK concentrations in human ELF, were compared with the *in vitro* chemostat model study data. The 90 percentile confidence intervals of the residues between the predicted and the observed $\log_{10}$ CFU were about -2~3.**Conclusions:** These analyses suggest that our dynamic PK-PD model can predict *in vitro* chemostat model results based on the *in vitro* time-kill data and the mimicked PK profile.

Author Disclosure Block:

N. Sato: D. Employee; Self; Meiji Seika Pharma Co., Ltd. **K. Matsumoto:** D. Employee; Self; Meiji Seika Pharma Co., Ltd. **S. Ouchi:** D. Employee; Self; Meiji Seika Pharma Co., Ltd. **N. Baba:** D. Employee; Self; Meiji Seika Pharma Co., Ltd. **Y. Nagira:** D. Employee; Self; Meiji

Seika Pharma Co., Ltd. **Y. Tsutsumi:** D. Employee; Self; Meiji Seika Pharma Co., Ltd. **K. Kondo:** D. Employee; Self; Meiji Seika Pharma Co., Ltd..

Poster Board Number:

MONDAY-559

Publishing Title:

Aminospectinomycins: Novel Therapeutics for Biothreat Pathogens

Author Block:

S. L. Waidyarachchi[1], M. M. Butler[1], R. Panchal[2], D. Lane[2], T. Bowlin[1], R. E. Lee[3]; [1]Microbiotix, Inc, Worcester, MA, [2]US Army Med. Res. Inst. of Infectious Diseases, Frederick, MD, [3]St.Jude Children's Res. Hosp., Memphis, TN

Abstract Body:

Background: The dramatic rise in the prevalence of antibiotic resistance requires the discovery and development of new antibiotics. There is also a clear need to identify novel therapeutic agents for biodefense purposes that can prevent and treat multiple types of respiratory tract infections. In this regard, we have re-visited the synthetic modification of the low molecular weight aminocyclitol **antibiotic** spectinomycin (SPC) producing a series of aminospectinomycins (AmSPCs). The AmSPCs show excellent efficacy for treatment of otherwise lethal *S. pneumoniae* pneumonia in mice. Herein, we report the exploration of AmSPCs as agents to treat pulmonary infections from biothreat bacterial pathogens. **Methods:** Guided by computational design, compounds were synthesized in 5 steps from SPC and potency evaluated against a broad panel of Gram positive and negative bacterial including the most prominent bacterial biothreat pathogens. *In vivo* efficacy trials were performed with a mouse models of *Burkholderia .mallei and Franciscella tularensis* infection. Target inhibition of protein synthesis was studied at the ribosomal level in a cell free *M. smegmatis* mycobacterium translation assay. Mammalian cytotoxicity was assessed *in vitro* against HepG2 cell line.**Results:** From the AmSPCs synthesized, a clear structure activity relationship was established. Analogs with substituted benzyl moieties displayed the best activities. The most active compound, **1950**, showed substantially improved potency against *B. mallei* (MIC_{90} 12.5 ug/ml), *B. suis* (MIC_{90} 3.1 ug/ml), *F. tularensis* (MIC_{90} 100 ug/ml) and showed limited activity against *Y. pestis* (MIC_{50} 50 ug/ml) and *B. anthracis Ames* (MIC_{50} 50ug/ml). Compounds appeared to be mostly ineffective against *Burkholderia pseudomallei* (MIC_{50} >200 ug/ml). Lead **1950** was active against a panel of drug resistant *B. mallei, B. suis and F. tularensis* clinical isolates *in vitro,* and cleared mice of *B. mallei* infection at a dose of 50 mg/kg BID.**Conclusion:** In summary, amSPCs represent a new chemotype with many suitable properties for further development to treat infections caused by biothreat bacterial pathogens.

Author Disclosure Block:

S.L. Waidyarachchi: None. **M.M. Butler:** None. **R. Panchal:** None. **D. Lane:** None. **T. Bowlin:** None. **R.E. Lee:** None.

Poster Board Number:

MONDAY-560

Publishing Title:

Aminospectinomycins Are Potent Inhibitors of Mdr Pathogens That Cause Pneumonia and Otitis Media

Author Block:

M. M. Butler[1], S. L. Waidyarachchi[2], R. E. Lee[2], J. W. Rosch[2], T. L. Bowlin[1]; [1]Microbiotix, Inc., Worcester, MA, [2]St. Jude Children's Res. Hosp., Memphis, TN

Abstract Body:

Background: *Streptococcus pneumoniae* (*Sp*) is the leading cause of bacterial pneumonia and meningitis in the United States. It also is a major cause of bloodstream infections, otitis media (OM) and sinus infections. *Sp* has developed resistance to drugs in the penicillin and erythromycin groups, as well as to other, less commonly used antibiotics. As described in CDC's *2013 Antibiotic Resistance Threats in the United States*, multi-drug-resistant (MDR) *Sp* is classified as a "serious" threat, with 1.2 million resistant infections per year, along with 22,000 deaths from *Sp* disease overall. Other bacterial causes of pneumonia and OM include MDR *Haemophilus influenzae* (*Hi*) and *Moraxella catarrhalis (Mc)*. Adding to the growing resistance problem, the number of new antibiotics approved has steadily decreased in the past three decades, leaving fewer options to treat MDR bacteria. The aminospectinomycins (AmSPCs), a new class of semisynthetic analogs of the antibiotic, spectinomycin (SPC), have demonstrated improved potency against *Sp, Hi and Mc*. Herein, we report the exploration of AmSPCs as agents to treat pulmonary and ear infections from these MDR bacterial pathogens.**Methods:** The *in vitro* potency of the lead AmSPC compound 1950 was determined against groups of 20-30 isolates of each species using the broth dilution method. Most of the isolates were resistant to at least two antibiotic families. Additionally, the efficacy of 1950 was tested in murine models of *Sp*-associated pneumonia, sepsis, meningitis and OM using luminescent strain BHN97x; assessments included survival rates, colony counts and luminescence.**Results:** Compound 1950 demonstrated good potency, with Minimal Inhibitory Concentrations for *Sp*, *Hi* and *Mc* ranging from 1-16 µg/mL across 90 MDR isolates. Additionally, 1950 was fully protective in mice against lethal *Sp* pneumonia, sepsis, meningitis and OM when dosed at levels of 5-25 mg/kg, dropping bacterial counts to below detectable limits. 1950 had comparable or better potency than the comparator ampicillin and was superior to SPC. Furthermore, 1950 displayed good efficacy in a murine co-infection model with *Hi* and with the influenza virus.**Conclusions:** In summary, these studies support the AmSPCs as a promising new series for treating MDR bacterial pneumonia and OM.

Author Disclosure Block:

M.M. Butler: None. **S.L. Waidyarachchi:** None. **R.E. Lee:** C. Consultant; Self; Avisa Pharma, Astra Zeneca, Achaogen. N. Other; Self; Microbiotix, Arietis Pharma, Sequella, Inc.. **J.W. Rosch:** None. **T.L. Bowlin:** None.

Poster Board Number:

MONDAY-561

Publishing Title:

***In Vivo* Efficacy of Combinations of Novel Antimicrobial Peptide Spr741 and Rifampicin in Short-Duration Murine Thigh Infection Models of Gram-Negative Bacterial Infection**

Author Block:

P. Warn[1], P. Thommes[1], S. Vaddi[1], D. Corbett[1], D. Coles[1], L. Vaccaro[1], S. Standring[1], T. Lister[2], T. R. Parr, Jr[2]; [1]Evotec (UK) Ltd, Manchester, United Kingdom, [2]Spero Therapeutics, Cambridge, MA

Abstract Body:

Background: There is an acute shortage of effective antimicrobial agents to treat multi-drug-resistant Gram-negative infection. An attractive approach to addressing the dearth of treatment options is potentiation of antimicrobial agents to either increase the spectrum of activity or enhance activity. In these studies we assessed the efficacy of combinations of a novel antimicrobial cationic peptide (SPR741) with rifampicin (Rif) in murine models of thigh muscle infection.**Methods:** Male ICR mice were rendered neutropenic using 2 doses of cyclophosphamide on days -4 & -1. Mice were infected by IM injection into the lateral thigh muscle on day 0 with either *E. coli* (ATCC 25922 and IR60 [Bla_{NDM-1}), *Klebsiella pneumoniae* (Kp114 [Bla_{KPC}] or ATCC BAA 2146 [Bla_{NDM-1}]) or *Acinetobacter baumannii* (ATCC BAA 747). Treatment was initiated 1h post infection with SPR741 administered at 1, 3.5 and 7h post infection and Rif administered at 1 and 5h post infection). SPR741 was administered at 10, 20 and 40mg/kg/dose, the doses of Rif were based on preliminary dose response experiments (range 0.376-64mg/kg/dose). Mice were euthanized 9h post infection and the thigh muscle quantitatively cultured.**Results:** SPR741 and Rif were well tolerated and all animals continued to the study end. All isolates demonstrated robust *in vivo* growth of 1.55-3.4Log_{10}cfu/g thigh tissue between pre-treatment and harvest samples. Monotherapy with SPR741 at 40mg/kg/dose or Rif (at the doses used) had little effect on the burdens and did not achieve stasis against any isolate. In contrast in all models combinations with ≤20mg/kg/dose SPR741 with Rif led to highly significant reductions in burden below stasis (2.2, 3.7, 4.7, 1.6 and 2.9Log_{10}cfu/g below stasis for ATCC 25922, IR60, Kp114, ATCC BAA 2146 and ATCC BAA 747 respectively).**Conclusions:** The combination of SPR741 with Rif was highly effective at reducing the thigh burden of mice infected with *E. coli, K. pneumonia* and *A. baumannii* including strains expressing Bla_{KPC} and Bla_{NDM-1}. These studies support continued development of novel antimicrobial cationic peptide for the treatment of multi-drug-resistant Gram-negative infections.

Author Disclosure Block:

P. Warn: H. Research Contractor; Self; Spero. **P. Thommes:** H. Research Contractor; Self; Spero. **S. Vaddi:** H. Research Contractor; Self; Spero. **D. Corbett:** H. Research Contractor; Self;

Spero Therapeutics. **D. Coles:** H. Research Contractor; Self; Spero Therapeutics. **L. Vaccaro:** H. Research Contractor; Self; Spero Therapeutics. **S. Standring:** H. Research Contractor; Self; Spero Therapeutics. **T. Lister:** D. Employee; Self; Spero Therapeutics. **T.R. Parr:** A. Board Member; Self; Spero Therapeutics. D. Employee; Self; Spero Therapeutics.

Poster Board Number:

MONDAY-562

Publishing Title:

The Efficacy of Arbekacin Inhalation (Me1100) as Adjunctive Therapy to Iv Antibiotics in Murine Pneumonia Models

Author Block:

N. Baba, E. Murase, S. Sakakibara, Y. Takayama, T. Takata, S. Ouchi, T. Ida, K. Kondo; Meiji Seika Pharma Co., Ltd., Tokyo, Japan

Abstract Body:

Background: ME1100, arbekacin inhalation solution, has been designated as a Qualified Infectious Drug Product (QIDP) for the adjunctive treatment of mechanically ventilated patients with bacterial pneumonia. *S. aureus* including MRSA and *P. aeruginosa* (Pa) are the most prevalent bacteria in VABP, and the two species account for about 50% in total. We compared the efficacy of ME1100 and vancomycin (VCM) or meropenem (MEM) combination therapy with that of each antibiotic in a murine pneumonia model of MRSA or aminoglycosides resistant Pa.**Methods:** Immunosuppressed mice were nasally inoculated with MRSA or Pa. At 2 hours post-infection, ME1100 was administered by a nebulizer (eFlow®) in conjunction with/without subcutaneous administration of VCM or MEM to mice. Viable cell counts in lungs were measured at 18 hours after infection.**Results:** ME1100 alone or in combination with VCM or MEM demonstrated potent *in vivo* activity. In MRSA, the viable cell counts (Log CFU/lung ± S. D.) of control group was 6.42 ± 0.89, and those of treatment groups with ME1100 (30 mg/mL for 5 min) alone, VCM (10 mg/kg) alone and their combination were 3.49 ± 1.04, 3.95 ± 0.90 and 2.51 ± 0.35 (n=6 or 7), respectively. In Pa, the viable cell counts of control group was 6.92 ± 0.51, and those of treatment groups with ME1100 (30 mg/mL for 5 min) alone, MEM (10 mg/kg) alone and their combination were 5.31 ± 0.65, 4.51 ± 1.86 and 2.08 ± 0.26 (n=3 or 4), respectively.**Conclusions:** The therapeutic effects of inhaled ME1100 in combination with VCM or MEM were superior to those of VCM alone or MEM alone. These data support the potential utility of ME1100 as adjunctive therapy to best available therapy for severe pneumonia due to drug-resistant bacteria.

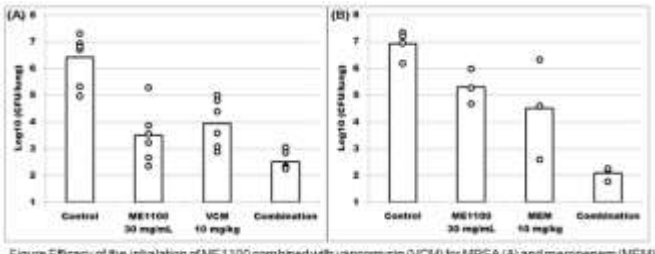


Figure Efficacy of the inhalation of ME1100 combined with vancomycin (VCM) for MRSA (A) and meropenem (MEM) for *P. aeruginosa* (B) in murine pneumonia models.

Author Disclosure Block:

N. Baba: D. Employee; Self; Meiji Seika Pharma Co., Ltd. **E. Murase:** D. Employee; Self; Meiji Seika Pharma Co., Ltd. **S. Sakakibara:** D. Employee; Self; Meiji Seika Pharma Co., Ltd. **Y. Takayama:** D. Employee; Self; Meiji Seika Pharma Co., Ltd. **T. Takata:** D. Employee; Self; Meiji Seika Pharma Co., Ltd. **S. Ouchi:** D. Employee; Self; Meiji Seika Pharma Co., Ltd. **T. Ida:** D. Employee; Self; Meiji Seika Pharma Co., Ltd. **K. Kondo:** D. Employee; Self; Meiji Seika Pharma Co., Ltd..

Poster Board Number:

MONDAY-563

Publishing Title:

Tp-271 is Efficacious in a *Francisella tularensis* Cynomolgus Monkey Treatment Model

Author Block:

T. H. Grossman[1], L. N. Henning[2], M. Gooldy[3], A. K. Radcliff[3], V. H. Tam[4], J. A. Sutcliffe[1]; [1]Tetraphase Pharmaceuticals, Watertown, MA, [2]Battelle, Columbus, OH, [3]CUBRC, Inc., Buffalo, NY, [4]Univ. of Houston Coll. of Pharmacy, Houston, TX

Abstract Body:

Background: *Francisella tularensis* (*Ft*) is a biothreat that can be weaponized for use in an aerosolized mass attack. This study evaluated the therapeutic efficacy of TP-271, a fully synthetic tetracycline with potent broad spectrum antibacterial activity, against inhalation tularemia in cynomolgus monkeys. **Methods:** Cynomolgus monkeys received an average aerosolized dose of 1144 colony forming units of *Ft* (SCHU S4; NR-10492) using a head-only inhalation exposure chamber. Treatment was initiated within 6 hours of a confirmed fever (fever persisting for at least 2 hours). Animals were administered 3 mg/kg/day TP-271, 1 mg/kg/day TP-271, or saline (vehicle control) by a 90 min IV infusion for 21 days (QD), followed by 14 days of post-treatment monitoring. Animals were monitored for changes in body temperature and activity (by telemetry), body weight, clinical pathology, and blood culture, quantitative PCR (qPCR), and bacterial tissue assessment. Gross necropsies were conducted on all animals and target tissues were evaluated microscopically from all animals found dead or euthanized *in extremis* to confirm *Ft* infection. **Results:** All infected animals treated with TP-271 survived to the end of the study, whereas none of the vehicle control animals (0/8) survived inhalational exposure to *Ft*. All of the vehicle control animals were bacteremic before death, had quantifiable levels of *Ft* in all tissues assessed (lung, liver, spleen, mediastinal lymph node), and microscopic findings consistent with tularemia. In contrast, TP-271-treated animals did not have a positive blood culture or quantifiable qPCR result at any time point during the study and *Ft* was detected only in the mediastinal lymph node for 19% (3/16) of the TP-271-treated animals. The TP-271 mean $AUC_{0\text{-}24}$ on Day 1/Day 21 of dosing was 10.41/27.48 µg·hr/mL and 1.95/7.75 µg·hr/mL for the 3 mg/kg and 1 mg/kg doses, respectively, for males and females combined. **Conclusions:** TP-271 was efficacious when administered therapeutically to cynomolgus monkeys after aerosol exposure to *Ft*. Further studies with TP-271 are warranted to confirm efficacy and evaluate the utility of TP-271 for treatment of respiratory infections caused by other biothreat pathogens.

Author Disclosure Block:

T.H. Grossman: D. Employee; Self; T. Grossman. **L.N. Henning:** H. Research Contractor; Self; L. Henning. **M. Gooldy:** H. Research Contractor; Self; M. Gooldy. **A.K. Radcliff:** H.

Research Contractor; Self; A. Radcliff. **V.H. Tam:** J. Scientific Advisor (Review Panel or Advisory Committee); Self; V. Tam. **J.A. Sutcliffe:** D. Employee; Self; J. Sutcliffe.

Poster Board Number:

MONDAY-564

Publishing Title:

Effect of Kbp-7072 on Bacterial Activity and Survival in a *S. pneumoniae*-induced Murine Pneumonia Model

Author Block:

Q. Liu, Y. Wang, **B. Zhang**; KBP BioSci.s, Princeton, NJ

Abstract Body:

KBP-7072 is a novel aminomethylcycline with broad-spectrum activity against Gram+ and Gram- multidrug resistant bacterial isolates and strains. *In vivo* antibacterial activity was assessed in a *S. pneumoniae*-induced murine pneumonia model. 6-8 week old female CD-1 mice, 23 - 27 g, were randomized to treatment groups based on body weight. KBP-7072 was dosed at 5, 15, or 45 mg/kg, po, qd; 2.5 or 7.5 mg/kg, po, bid; linezolid 7.5 mg/kg, po, bid; and vehicle 0.2 mL, po, qd. 15 mice were assigned to each dose group. After randomization, animals were rendered neutropenic with 150 mg/kg of cyclophosphamide ip at Day 4 and Day 1 prior to infection, then anesthetized with 6.5% pentobarbital sodium at a dose of 65 mg/kg ip. Each animal was inoculated with 50 µL of a log phase culture of *S. pneumoniae* 6962spn-310 placed on the tip of the nares. KBP-7072 and antibiotics were started 18 hours post inoculation and continued for 3 days. Animals were euthanized at 0 (control group), 24, 48, and 72 hours post final dose. The lungs were harvested, homogenized in saline, and serial dilutions of the homogenates were cultured overnight on an MH agar plate. Bacterial CFUs were counted and presented as $\log_{10}$CFU/lung. Respiratory infection occurred in all inoculated mice. Treatment with KBP-7072 resulted in a significant dose-dependent decrease in CFUs at all doses and time points tested. At 72 hours post dose, KBP-7072 45 mg/kg qd, or 2.5 mg/kg and 7.5 mg/kg bid, resulted in no detectable bacteria in the lung. Linezolid 7.5 mg/kg bid showed no inhibition of bacteria growth, and linezolid 15 mg/kg bid resulted in 33.9% inhibition of number of bacteria in lung vs vehicle ($p<0.01$). KBP-7072, 5 and 15 mg/kg, resulted in a 133% and 166% dose-dependent increase respectively ($p<0.0001$), and linezolid 7.5 mg/kg resulted in a 66% increase ($p<0.0001$) compared to vehicle in median survival at Day 3. At Day 10, the survival rate in the KBP-7072 45 mg/kg group was 93.3% and median survival was not reached. The survival rate and median survival of all KBP-7072-treated groups were significantly higher than observed in the linezolid group ($p<0.0001$). These results demonstrate that KBP-7072 has greater antibacterial activity compared to linezolid and significantly increased the survival rate and prolonged median survival of treated animals compared to linezolid in a *S. pneumoniae*-induced murine pneumonia model in a dose-dependent manner.

Author Disclosure Block:

Q. Liu: None. **Y. Wang:** None. **B. Zhang:** None.

Poster Board Number:

MONDAY-565

Publishing Title:

Effect of Kbp-7072 on Bacterial Activity and Survival in a *K. pneumoniae*-induced Murine Pneumonia Model

Author Block:

Q. Liu, Y. Wang, **B. Zhang**; KBP BioSci.s, Princeton, NJ

Abstract Body:

KBP-7072 is a novel aminomethylcycline with broad-spectrum activity against Gram+ and Gram- multidrug resistant bacterial isolates and strains. *In vivo* antibacterial activity was assessed in a *K. pneumoniae*-induced murine pneumonia model. 6-8 week old female CD-1 mice, 23 - 27 g, were randomized to treatment groups (15 per group) based on body weight. KBP-7072 was dosed at 150, 300, and 600 mg/kg, po, qd; minocycline 300 mg/kg, po, qd; and vehicle 0.2 mL, po, qd. After randomization, animals were rendered neutropenic with 150 mg/kg of cyclophosphamide ip at Day -4 and Day -1, then anesthetized with 6.5% pentobarbital sodium at a dose of 65 mg/kg ip. Each animal was inoculated with 50 µL of a log phase culture of *K. pneumoniae* 5615kpn-493 placed on the tip of the nares. KBP-7072 and antibiotics were started 3 hours post inoculation and continued for 3 days. Animals were euthanized at 0 (control group), 24, 48, and 72 hours post final dose. The lungs were harvested, homogenized in saline, and serial dilutions of the homogenates were cultured overnight on an MH agar plate. Bacterial CFUs are presented as $\log_{10}$CFU/lung. Respiratory infection occurred in all inoculated mice. Treatment with KBP-7072 resulted in a significant dose-dependent decrease in CFUs at all doses and most time points tested. At 72 hours post dose, KBP-7072 150, 300, or 600 mg/kg qd, or 75 mg/kg and 150 mg/kg bid, resulted in 17.5%, 21.6%, 47.4%, 23.7% and 47.4% inhibition, respectively, vs vehicle ($p<0.05$ to 0.001). Minocycline resulted in no significant inhibition of bacteria growth. Treatment with KBP-7072 resulted in a dose-dependent increase in survival rate. At doses of 150 and 300 mg/kg, KBP-7072 resulted in a median survival of 3 days, a 50% increase compared to median Day 2 survival in vehicle-treated animals ($p=0.0001$). At day 10, the survival rate in the KBP-7072 600 mg/kg group was 73.3% and the median survival was not reached, vs vehicle ($p<0.0001$). Minocycline 300 mg/kg resulted in no increase in median survival. The survival rate and median survival in all KBP-7072-treated groups were significantly higher than for minocycline ($p=0.0001$). These results demonstrate that KBP-7072 has greater antibacterial activity compared to minocycline and significantly increased the survival rate and prolonged median survival of treated animals in a *K. pneumoniae*-induced murine pneumonia model in a dose-dependent manner.

Author Disclosure Block:

Q. Liu: None. **Y. Wang:** None. **B. Zhang:** None.

Poster Board Number:

MONDAY-566

Publishing Title:

Eravacycline is Active against MDR, Cephalosporin- and Carbapenem-Resistant *Enterobacteriaceae* and *Acinetobacter baumannii*

Author Block:

T. H. Grossman[1], I. Morrissey[2], **J. A. Sutcliffe**[1]; [1]Tetraphase Pharmaceuticals, Watertown, MA, [2]HMA Europe Sàrl, Epalinges, Switzerland

Abstract Body:

Background: Eravacycline (ERV) is a novel, fully synthetic fluorocycline antibiotic of the tetracycline class with broad-spectrum activity being developed for the treatment of serious infections, including those caused by MDR pathogens. ERV was tested *in vitro* against Gram-negative pathogens of concern, including 3rd/4th generation cephalosporin-resistant (CEPH-R) and carbapenem-resistant (CP-R) *Enterobacteriaceae*, and CP-R and MDR *Acinetobacter baumannii*. **Methods:** MIC assays were performed as per CLSI methodology and data for ERV was analyzed across multiple non-clinical and recent surveillance studies using a composite database housed at International Health Management Associates (IHMA). The IHMA Surveillance Data Link Network (SDLN) webtool was used to analyze isolates by resistance phenotypes, including MDR (resistant to ≥3 antibiotic classes), as per 2015 CLSI criteria. A subset of cephalosporin and/or carbapenem resistant isolates were screened using published PCR conditions for narrow- and extended-spectrum β-lactamases and carbapenemases.**Results:** For most pathogens, ERV $MIC_{50/90}$ values increased ≤2-fold across resistant subclasses, indicating that ERV had *in vitro* potency against enterobacterial pathogens which are resistant to other antibacterials, including 3rd/4th generation cephalosporins and carbapenems, and MDR organisms (Table). ERV had an overall $MIC_{50/90}$ of 0.5/2 µg/mL for a subset of 416 molecularly characterized CP-R *Enterobacteriaceae* which included 255 isolates positive for bla_{KPC} and 106 isolates positive for bla_{IMP}, bla_{VIM}, or bla_{NDM} metallo-β-lactamase genes. The $MIC_{50/90}$ was 1/2 µg/mL for a subset of 220 CEPH-R, CP-R *Enterobacteriaceae*. The ERV $MIC_{50/90}$ for CP-R *A. baumannii* containing bla_{OXA} (n=94) was 0.5/1 µg/mL, and MIC values for strains with bla_{NDM} (n=5) and bla_{KPC} (n=1) were 0.12-0.25 µg/mL and 0.5 µg/mL, respectively.**Conclusions:** ERV is active against difficult-to-treat CEPH-R, CP-R and MDR Gram-negative pathogens.

Organism	**Phenotype[a] (# isolates)**	**ERV MIC_{50}/MIC_{90}/ Range[b]**
Enterobacteriaceae	**All (12,292)**	**0.25/1/ ≤0.015-16**
	CP-R (1,276)	1/2/ 0.03-16
	CEPH-R (2,480)	0.5/2/ 0.03-16

	MDR (2,580)	0.5/2/ 0.03-16
Acinetobacter baumannii	**All (851)**	**0.5/1/ ≤0.03-8**
	CP-R (511)	0.5/2/ 0.06-8
	CEPH-R (514)	0.5/1/ 0.06-8
	MDR (574)	0.5/2/ 0.06-8
[a]All=all studies combined; CP-R=resistant to meropenem, imipenem or ertapenem; CEPH-R=resistant to cefotaxime, ceftazidime, ceftriaxone or cefepime; MDR= resistant to ≥3 antibiotic classes. [b]values in µg/mL.		

Author Disclosure Block:

T.H. Grossman: D. Employee; Self; T. Grossman. **I. Morrissey:** H. Research Contractor; Self; I. Morrissey. **J.A. Sutcliffe:** D. Employee; Self; J. Sutcliffe.

Poster Board Number:

MONDAY-567

Publishing Title:

Omadacycline (PTK0796) Spectrum of Activity from 2003-2015

Author Block:

M. M. Traczewski; Clinical Microbiol. Inst., Wilsonville, OR

Abstract Body:

Background: Omadacycline (OMC) is the first aminomethylcycline antibiotic under development for use as a once daily oral and IV treatment in skin and soft tissue and respiratory infections. Omadacycline is currently in Phase 3 clinical development for acute bacterial skin and skin structure infections. This study compares spectrum of activity studies of OMC performed in 2003, 2007 and 2015. **Materials and Methods:** All three studies used the Clinical and Laboratory Standards Institute (CLSI) frozen broth microdilution (BMD) methods described in the M7 and M45 documents current at the time of testing. MIC panels were prepared using fresh broth (<12 hrs old) and frozen at -70^{o} C until use. Clinical isolates used for each of these studies were < 3 years old at the time of testing for >80% of the total isolates tested in each study. **Results:** $MIC_{50/90s}$ and range of MICs are listed in Table 1.**Conclusions:** The activity of OMC has remained stable from 2003 through 2015. OMC had good activity against *Enterococcus faecium*, *Enterococcus faecalis*, *Staphlyococcus aureus*, β-hemolyctic streptococci, *Streptococcus pneumoniae* and *Moraxella catarahhalis* with MIC_{50s} ranging from 0.06 to 0.25 μg/ml and MIC_{90s} from 0.06 to 0.25. Haemophilus influenzae had slightly higher MIC_{50s} which ranged from 0.5 to 1 μg/ml and MIC_{90s} from 1 to 2. OMC MIC_{50s} vs. *Escherichia coli* ranged from 1 to 2 μg/ml and MIC_{90s} from 2-4 μg/ml. *Klebsiella pneumonae* MIC_{50s} were 2 μg/ml and MIC_{90s} were 8 μg/ml.

Table 1. Spectrum of Activity of Omadacycline from 2003 to 2015

ORGANISM GROUP	2003 SURVEY		2007 SURVEY		2015 SURVEY	
	$MIC_{50/90}$	MIC Range	$MIC_{50/90}$	MIC Range	$MIC_{50/90}$	MIC Range
All Enterococci	0.12/0.25	0.03-0.5	0.12/0.25	0.06-0.5	0.12/0.25	0.03-1
E. faecalis	0.12/0.25	0.06-0.5	0.25/0.25	0.06-0.5	0.12/0.12	0.03-0.5
E. faecium	0.12/0.12	0.03-0.25	0.12/0.12	0.06-0.5	0.12/0.12	0.03-1
All *Staphylococcus aureus*	0.25/0.25	0.06-4	0.25/0.25	0.12-0.5	0.25/0.25	0.06-2
S. aureus oxacillin susceptible	0.25/0.25	0.06-0.25	0.25/0.25	0.06-0.5	0.12/0.25	0.06-1
S. aureus oxacillin resistant	0.25/0.25	0.06-4	0.25/0.25	0.12-0.5	0.25/0.25	0.06-2
Moraxella catarrhalis	0.25/0.25	0.25	0.25/0.25	0.25-0.5	0.12/0.25	0.12-0.5
All *Escherichia. coli*	1/2	0.5-4	2/4	0.12-8	1/4	0.25-8
Klebsiella pneumoniae	2/8	2-8	2/8	0.5-32	2/8	0.5->16
β-hemolytic streptococci	0.12/0.12	0.06-0.5	0.12/0.12	0.12-0.25	0.06/0.12	0.03-0.25
Streptococcus pneumoniae	0.06/0.12	0.015-0.25	0.12/0.12	0.06-0.25	0.06/0.06	0.015-0.12
Haemophilus influenzae	0.5/2	0.25-4	0.5/1	0.25-1	1/1	0.5-1
	All MIC values listed are in μg/mL.					

Author Disclosure Block:

M.M. Traczewski: None.

Poster Board Number:

MONDAY-568

Publishing Title:

Activity of Eravacycline and Comparators against 6,338 Pathogens Isolated from Canadian Hospitals: Canward 2014 and 2015

Author Block:

G. G. ZHANEL[1], H. Adam[2], M. Baxter[1], B. Weshnoweski[2], R. Vashisht[1], S. Biju[1], A. Golden[1], A. Denisuik[1], A. Walkty[2], P. Lagace-Wiens[2], J. A. Karlowsky[2], D. J. Hoban[2]; [1]Univ. of Manitoba, Winnipeg, MB, Canada, [2]Diagnostic Services Manitoba, Winnipeg, MB, Canada

Abstract Body:

Background: Eravacycline (ERV) is a novel, fully synthetic fluorocycline antibiotic of the tetracycline class with broad-spectrum activity being developed for the treatment of serious infections, including those caused by multidrug-resistant (MDR) pathogens. The activity of this synthetic fluorocycline was compared to a variety of comparators including meropenem (MER) and piperacillin-tazobactam (PTZ) against Gram-negative and Gram-positive pathogens causing infections in Canadian hospitals.**Methods:** From January 2014-December 2015, inclusive, 13 sentinel hospitals submitted pathogens from patients attending hospital clinics, emergency rooms, medical and surgical wards, and intensive care units as part of an ongoing national surveillance program in Canadian hospitals. 6,338 total isolates were collected for 2014 and 2015. Susceptibility testing was performed using CLSI broth microdilution methods.**Results:**The activity (µg/ml) of ERV, MER and PTZ against select pathogens is described below:

Organism (# isolates)	ERV MIC_{50}/MIC_{90}	MER MIC_{50}/MIC_{90}	PTZ MIC_{50}/MIC_{90}
S. agalactiae (115)	0.03/0.06	≤0.06/≤0.06	≤1/≤1
S.pneumoniae (282)	0.015/0.015	≤0.06/0.12	≤1/≤1
S. pyogenes (86)	0.03/0.03	≤0.06/≤0.06	≤1/≤1
SPN - PenR (13)	0.008/0.015	1/1	4/4
MSSA (1216)	0.06/0.12	0.12/0.25	≤1/≤1
MRSA[a] (302)	0.06/0.25	4/32	32/128
CA-MRSA (114)	0.06/0.12	2/4	16/32
HA-MRSA (157)	0.12/0.5	16/32	64/128
S. epidermidis (108)	0.06/0.25	1/32	≤1/32
E. faecalis (202)	0.06/0.12	4/8	4/4

E. faecium (85)	0.03/0.12	>32/>32	>512/>512
VRE (17)	0.06/0.12	>32/>32	>512/>512
C. freundii (19)	0.25/2	≤0.03/≤0.03	4/64
E. aerogenes (32)	0.5/0.5	≤0.03/0.06	4/64
E. cloacae (175)	0.5/1	≤0.03/0.12	2/64
E. coli (1168)	0.25/0.5	≤0.03/≤0.03	≤1/4
E. coli-ESBL (124)	0.25/0.5	≤0.03/≤0.03	2/16
K. oxytoca (88)	0.25/0.5	≤0.03/≤0.03	≤1/128
K. pneumoniae (383)	0.5/1	≤0.03/≤0.03	2/8
M. morgannii (20)	1/2	0.06/0.12	≤1/≤1
P. mirabilis (91)	1/2	0.06/0.12	≤1/≤1
S. marcescens (83)	1/2/ 0.5-8	0.06/0.06	≤1/4
A. baumannii (28)	0.06/ 0.5	0.5/0.5	≤1/64
P. aeruginosa (710)	8/16	1/16	4/64
S. maltophilia (117)	1/4	>32/>32	256/>512
MSSA-methicillin-susceptible *Staphylococcus aureus*, MR-methicillin resistant, VRE-vancomycin-resistant enterococci, SPN-*Streptococcus pneumoniae*, ESBL-extended spectrum beta lactamase producing.			
[a]Based upon oxacillin susceptibility.			

Conclusions: Eravacycline displayed broad-spectrum activity against recent pathogens from Canadian hospitals including MRSA, VRE, ESBL-producing Enterobacteriaceae and *A. baumannii*.

Author Disclosure Block:

G.G. Zhanel: I. Research Relationship; Self; Abbott, Astellas, Cubist, Merck, Galderma, Pharmascience, Sunovion, The Medicines Co, Tetraphase, Zoetis. **H. Adam:** None. **M. Baxter:** None. **B. Weshnoweski:** None. **R. Vashisht:** None. **S. Biju:** None. **A. Golden:** None. **A. Denisuik:** None. **A. Walkty:** None. **P. Lagace-Wiens:** None. **J.A. Karlowsky:** None. **D.J. Hoban:** I. Research Relationship; Self; Abbott, Astellas, Cubist, Galderma, Merck, Pharmascience, Sunovion, The Medicines Co, Tetraphase, Zoetis.

Poster Board Number:

MONDAY-569

Publishing Title:

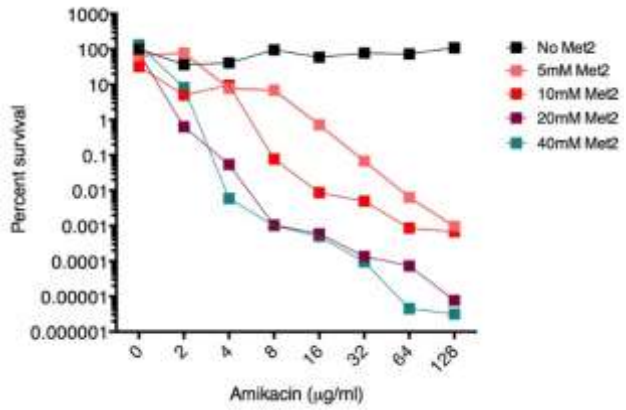
Aminoglycoside Potentiation for the Treatment of Catheter-Associated Urinary Tract Infections

Author Block:

M. Koeva[1], D. Sweeney[2], D. L. Shinabarger[2], **D. Joseph-McCarthy**[1]; [1]EnBiotix, Inc., Cambridge, MA, [2]Micromyx, LLC, Kalamazoo, MI

Abstract Body:

Background: Bacterial persisters are a quasi-dormant sub-population of cells that are tolerant to antibiotic treatment. EBX-002 is a combination of the aminoglycoside amikacin with a bacterial metabolite as a potentiator. This product is being developed with the goal of reducing recurrent infections in the treatment of catheter-associated urinary tract infections (CAUTIs).**Methods:** MICs were determined by standard broth microdilution methods (CLSI M7). *E. coli* cultures in planktonic stationary phase were used to select for bacterial persisters. Experiments were conducted with and without a pre-selection step utilizing the fluoroquinolone ofloxacin. Using the time-kill method (CLSI M26), a panel of 10 *E. coli* UTI amikacin-sensitive clinical isolates were evaluated, varying the concentration of amikacin and metabolite. Cells were enumerated after 4 hr of exposure to the amikacin/metabolite combinations. Finally, the potentiator effect was similarly examined in other UTI relevant species.**Results:** The amikacin MIC range for the 10 *E. coli* isolates was 1-4 μg/mL. Enhanced killing of up to 7 orders of magnitude of *E. coli* persisters was seen (see example in the figure below). Essentially no killing of persister cells occurred upon exposure to as high as 128 μg/mL of amikacin alone. The highest concentration of metabolite tested (40 mM) also did not kill cells when tested alone. In addition, a potentiation effect was observed, as examples, in *P. aeruginosa* and *K. pneumoniae.* **Conclusions:** The combination of amikacin with a metabolite potentiator has the capacity to revitalize existing and novel aminoglycosides through eradication of bacterial persisters, with broad spectrum potential for the treatment of CAUTIs.



Author Disclosure Block:

M. Koeva: D. Employee; Self; EnBiotix, Inc.. K. Shareholder (excluding diversitied mutual funds); Self; EnBiotix, Inc. **D. Sweeney:** H. Research Contractor; Self; EnBiotix. **D.L. Shinabarger:** H. Research Contractor; Self; EnBiotix. **D. Joseph-McCarthy:** D. Employee; Self; EnBiotix, Inc.. K. Shareholder (excluding diversitied mutual funds); Self; EnBiotix, Inc..

Poster Board Number:

MONDAY-570

Publishing Title:

Activity of Plazomicin in Combination with Other Antibiotics Against Multidrug Resistant Enterobacteriaceae

Author Block:

M. Thwaites[1], D. Hall[1], D. Shinabarger[1], A. Serio[2], K. Krause[2], **C. Pillar**[1]; [1]Micromyx, Kalamazoo, MI, [2]Achaogen, San Francisco, CA

Abstract Body:

Background: Plazomicin (PLZ), a novel aminoglycoside, is currently undergoing development for the treatment of severe Gram-negative (GN) infections. Combination therapy is often used for the treatment of multi-drug resistant (MDR) GN infections. To confirm PLZ activity against MDR GN bacilli and whether there was synergy with PLZ, PLZ combined with other GN agents was evaluated in checkerboard assays against MDR Enterobacteriaceae (EB), including isolates with characterized resistance to currently marketed aminoglycosides and beta-lactams. **Methods:** Fractional inhibitory concentrations (FIC) were determined by broth microdilution (CLSI M7) against 30 GN EB clinical isolates (10 *E. coli*, 6 *K. pneumoniae*, 2 *K. oxytoca*, 6 *E. cloacae*, 4 *E. aerogenes*, and 2 *C. freundii*) using test panels containing a dynamic concentration range of plazomicin and other agents alone and in combination. GN agents included ceftazidime (CAZ), piperacillin/tazobactam (P/T), meropenem (MEM), levofloxacin (LVX), tigecycline (TIG), and colistin (COL). FIC indices (FICI) were calculated by row as the MIC of PLZ in combination/MIC of PLZ alone + MIC of the GN agent in combination/MIC of GN agent alone. The FICI was interpreted as follows: $\leq$0.50, synergy; >0.50 - 4, indifference; >4, antagonism. **Results:** Overall, PLZ had an $MIC_{50/90}$ (µg/mL) of 1/2, which was comparable to COL (0.25/0.5) and TIG (1/4) and more potent than CAZ (128/>512), P/T (256/>512), MEM (0.12/64), and LVX (16/64). FICI values indicative of synergy were observed for PLZ in combination with P/T for 17/30 isolates. In general, the majority of combinations evaluated were characterized as indifferent, though there was some synergy observed for PLZ with MEM (6/30 isolates), CAZ and LVX (5/30 isolates), and TIG and COL (3/30 isolates). **Conclusions:** PLZ maintained potent activity against MDR EB including those with characterized resistance to other aminoglycosides. Synergy between PLZ and P/T was commonly observed and synergy with the other evaluated agents, though infrequent, was observed for select concentrations. PLZ has potential as a monotherapy and in combination for the treatment of serious GN infections caused by MDR EB.

Author Disclosure Block:

M. Thwaites: H. Research Contractor; Self; Achaogen. **D. Hall:** H. Research Contractor; Self; Achaogen. **D. Shinabarger:** H. Research Contractor; Self; Achaogen. **A. Serio:** D. Employee;

Self; Achaogen. **K. Krause:** D. Employee; Self; Achaogen. **C. Pillar:** H. Research Contractor; Self; Achaogen.

Poster Board Number:

MONDAY-571

Publishing Title:

An Evaluation of the Bactericidal Activity of Plazomicin and Comparators Against Multidrug Resistant Enterobacteriaceae

Author Block:

M. Thwaites[1], D. Hall[1], D. Shinabarger[1], A. Serio[2], K. Krause[2], **C. Pillar**[1]; [1]Micromyx, Kalamazoo, MI, [2]Achaogen, San Francisco, CA

Abstract Body:

Background: Plazomicin (PLZ) is a novel aminoglycoside (AG) under development for the treatment of serious Gram-negative (GN) infections. Few available agents have potent activity against multidrug resistant (MDR) GN bacilli. In this study, the bactericidal activity of PLZ, comparator AG, and other GN agents was evaluated by determining the minimum bactericidal concentration (MBC) and evaluating the time-kill (TK) kinetic of these agents against MDR Enterobacteriaceae (EB), including isolates with characterized AG and beta-lactam resistance.**Methods:** MICs/MBCs were determined by broth microdilution (CLSI M7/M26) for 30 strains of EB (MDR and characterized AG and beta-lactam resistant isolates; 10 *E. coli*, 6 *K. pneumoniae*, 2 *K. oxytoca*, 6 *E. cloacae*, 4 *E. aerogenes*, and 2 *C. freundii*). PLZ, amikacin (AMK), gentamicin (GM), ceftazidime (CAZ), meropenem (MEM), levofloxacin (LVX), tigecycline (TIG), and colistin (COL) were evaluated. The MBC was reported as the concentration where a 99.9% kill was observed. MBC:MIC ratios ≤4 were considered as bactericidal. TK for PLZ was determined (CLSI M26) for 10 isolates at 2X, 4X, 8X, and 16X the MIC at 1, 2, 4, 6 and 24 hr; comparators (AMK, GM, MEM, COL) were tested at 8X the MIC or 64 µg/mL for resistant isolates.**Results:** MIC/MBC results are summarized for 30 MDR EB in the table below. PLZ and COL were the most potent bactericidal agents.

Agent	**MIC_{50}/MIC_{90} (µg/mL)**	**MBC_{50}/MBC_{90} (µg/mL)**	**MBC:MIC ratio ≤ 4 n/N[a] (%)**
PLZ	0.5/2	0.5/4	29/30 (96.7)
AMK	32/128	64/256	29/30 (96.7)
GM	32/128	64/>512	25/27 (92.6)
CAZ	64/>512	128/>512	21/21 (100)
MEM	0.06/64	0.12/64	30/30 (100)
LVX	16/64	16/64	29/30 (96.7)
TIG	0.5/4	>4/>16	7/29 (24.1)
COL	0.12/0.25	0.12/0.5	28/28 (100)

[a] isolates with MIC/MBC values that were undefined/off-scale were not included for analysis of MBC:MIC ratio

By TK, a sustained 3-log kill through 24 hr was apparent as early as 1 hr with PLZ for *E. coli* (16X MIC; n=3), *K. pneumoniae* (4X/8X/16X MIC; n=3), and *Enterobacter* spp. (4X/8X/16X MIC; n=3). For *K. oxytoca* (n=1) a 2.87-3.05 log kill was observed at 4-24 hr for PLZ at 16X MIC. Though 3-log kills were observed early for the comparators at the evaluated concentrations (8X MIC or 64 µg/mL) there was typically regrowth at 24 hr excluding MEM (3-log kill at 24 hr for 5 of 10 isolates).**Conclusions:** PLZ and COL were the most potent agents by MIC and MBC against MDR EB and PLZ and by TK where rapid and sustained 3-log kills were apparent with PLZ against MDR EB isolates.

Author Disclosure Block:

M. Thwaites: H. Research Contractor; Self; Achaogen. **D. Hall:** H. Research Contractor; Self; Achaogen. **D. Shinabarger:** H. Research Contractor; Self; Achaogen. **A. Serio:** D. Employee; Self; Achaogen. **K. Krause:** D. Employee; Self; Achaogen. **C. Pillar:** H. Research Contractor; Self; Achaogen.

Poster Board Number:

MONDAY-572

Publishing Title:

A Comprehensive Study of Plazomicin Activity against a Panel of Aminoglycoside Resistance Enzymes

Author Block:

G. Cox[1], L. Ejim[1], A. Sieron[1], A. W. Serio[2], K. M. Krause[2], G. D. Wright[1]; [1]McMaster Univ., Hamilton, ON, Canada, [2]Achaogen, San Francisco, CA

Abstract Body:

Background: Aminoglycoside (AG) modifying enzymes (AMEs) chemically alter AGs via *N*-acetylation (AACs), *O*-adenylylation (ANT) or *O*-phosphorylation (APHs). Methyltransferases (AMTs) have also emerged, which modify the ribosome and reduce AG binding. Plazomicin (PLZ) is a derivative of sisomicin under development for the treatment of infections due to multi-drug resistant (MDR) Enterobacteriaceae. The addition of a hydroxyl-aminobutyric acid moiety and a hydroxyethyl substituent are thought to protect the drug against AMEs. Despite many studies revealing PLZ activity against a variety of MDR clinical isolates, a study of PLZ activity in the presence of individual AMEs has not been performed. **Methods:** The MIC of PLZ was measured in *Escherichia coli* BW25113 and BW25113 Δ*bamB*Δ*tolC* (hyper permeable and efflux deficient strain) containing 17 individually cloned AMEs and 2 AMTs. PLZ modification was next assessed utilizing kinetic enzyme assays. To detect *N*-acetylation, 5,5'-Dithio-bis (2-Nitrobenzoic Acid) was used as an indicator. Phosphorylation was detected using a pyruvate kinase/lactate dehydrogenase assay and adenylylation detected using a pyrophosphatase assay. Assays were performed with purified AAC(2')-Ia, AAC(3')-Ia, AAC(3)-II, AAC(3)-IV, AAC(6')-Ii, APH(2")-Ib, APH(2")-Id, APH(3")-Ia, APH(3')-IIIa, APH(4)-Ia, APH(6)-Ia, APH(9)-Ia and ANT(2")-Ia. **Results:** Expression of only two of the AMEs tested led to elevated PLZ MICs (compared to vector alone); *aac(2')-Ia* (>8-fold MIC increase) and *aph(2")-IVa* (*Id*) (>4-fold MIC increase). Expression of either *armA* or *rmtB* AMTs led to high-level MICs (>64 µg/ml and >512 µg/ml, respectively). *In vitro* enzymatic assessment confirmed that only AAC(2')-Ia and APH(2'')-Id were able to utilize PLZ as a substrate with a K_m of 280 µM and 42 µM, respectively. **Conclusion:** Overall, the majority of AMEs tested in this study did not impact PLZ MICs and were not able to utilize PLZ as a substrate, whereas AMT expression led to high-level PLZ MICs. The two AMEs capable of modifying PLZ in this study, AAC(2')-Ia and APH(2")-Id, are limited in their distribution to *Providenica stuartii* and Enterococcus, respectively. Taken together, these data suggest that PLZ will exhibit increased clinical usefulness in comparison to older-generation AGs.

Author Disclosure Block:

G. Cox: None. **L. Ejim:** None. **A. Sieron:** None. **A.W. Serio:** D. Employee; Self; Achaogen. **K.M. Krause:** D. Employee; Self; Achaogen. **G.D. Wright:** B. Collaborator; Self; Achaogen.

Poster Board Number:

MONDAY-573

Publishing Title:

A Cross Sectional Survey: Attitude Towards Adult Vaccination in Karachi-Pakistan

Author Block:

N. Z. Siddiqui; Univ. of Karachi, Karachi, Pakistan

Abstract Body:

As per the estimates of *National Interview Health Survey* (*NHIS*) -2012, adult vaccination is extensively ignored and a very less number of adults could receive recommended vaccine doses. Thus, a cross sectional study was conducted to evaluate the knowledge and attitude towards adult vaccination programs among the adult citizens of Karachi, Pakistan. The survey covered about 54.28% of the total areas of Karachi, Pakistan. A questionnaire comprising of 11 questions was designed and a total of 500 individuals ranging from 11-61 years of age participated in the study. Responses to each question were statistically analyzed and the associations between different variables were established using SPSS 16. According to the results, majority (93.4%) of the individuals considered vaccines safe for health and more than 80% agreed that adult immunization is as necessary as child immunization however, a significant difference ($p<0.05$) was noticed between the vaccination schedule follow up rates during childhood and adulthood and only a few individuals could receive the recommended adult vaccines. A significant association ($p<0.05$) was also found between the frequency of infections and the factors that may affect the efficiency of immune system such as sleeping disorders, smoking, persistent depression/anxiety, alcohol intake and the use of anti-depressive drugs/steroids/cortisones. Results showed that a number of reasons may increase the reluctance of people towards any vaccination program such as the cost, risk of quality assurance and requirement of booster doses. Thus, the study emphasizes on the need of awareness programs for adult vaccination as the lack of awareness and ignorance in this sector may render masses susceptible to serious & life threatening infections.

Author Disclosure Block:

N.Z. Siddiqui: None.

Poster Board Number:

MONDAY-574

Publishing Title:

Efficacy of Measles Virus Vaccine in Different Age Groups of Children in Lahore, Pakistan

Author Block:

T. Ijaz[1], **S. Ijaz**[2], S. Ahmad[3]; [1]Mayo Hosp.,King Edward Med. Univ., Lahore, Pakistan, [2]Shalamar Med. and Dental Coll. Lahore Pakistan, Lahore, Pakistan, [3]CEMB, Lahore Pakistan, Lahore, Pakistan

Abstract Body:

Background: Measles continues to be a serious public health problem in developing as well as developed countries, despite the availability of effective vaccine, the disease causes an estimated 1.4 million deaths annually,even in presence of well-established immunization program. In Pakistan, measles vaccines have been administered at the age 9 to 15 months to prevent the infection, however due to limited resources the vaccine efficacy was not detected. In a recent epidemic , thousands of children suffered due to measles and its complications in Punjab.The study has been designed to, Measure the level of measles antibodies in different age groups in children and to study the increase in the titre of antibodies. **Material and Method:** Children at age of six, nine, twelve and fifteen months were investigated for detection of Measles IgG level pre and post vaccination. The blood samples were drawn for their serological and haematological profiles. After initial blood sample the children were followed after one and two months like 0-1-2 months as follow-up samples. Data obtained from ELISA technique and haematological values were analyzed by one way ANOVA test. **Results:** After the administration of first dose of measles vaccine, an increase in serological titre was observed after 30 days and further 60 days of vaccination. It was observed that among the group of six month of age, 90% were found to be protected by the end of 60 days; the range of titre was 14 - 41 NTU/mL, while 10% were unprotected, however, the Protection level was low in 9 months old children group as 30% of children was found unprotected even after 60 days having a titre ranging between 2-8 NTU/mL, while 70% were protected having a range of 13-25 NTU/mL. The status of protection level in 12 months old children describes that 20 % of the children were unprotected while remaining 80% children were at the protection level having titre range 14-20 NTU/mL. The status of protection level in 15 months old children describes that 10 % of the children were observed unprotected while remaining 90% children were at the protection level having titre range 15-35 NTU/mL. **Conclusions**: The result of this research to be helpful in recommendation of a well established vaccination schedule for children in the area.

Author Disclosure Block:

T. Ijaz: None. **S. Ijaz:** None. **S. Ahmad:** None.

Poster Board Number:

MONDAY-575

Publishing Title:

Children Travelling from Athens to International Destinations: An Airport-based Study

Author Block:

H. Maltezou[1], A. Pavli[1], S. Patrinos[1], M. Theodoridou[2]; [1]Hellenic Ctr. for Disease Control and Prevention, Athens, Greece, [2]Aghia Sophia children Hosp., Athens, Greece

Abstract Body:

Background: The number of children who travel to tropical and subtropical areas has increased. The aim of the current study was to assess the preparedness of children departing from Greece to Africa and Asia, in terms of vaccination and malaria chemoprophylaxis. **Methods:** Airport-based survey conducted from November 2011 through April 2013.**Results:** 183 children were studied, of whom 122 (66.7%) had a foreign nationality. Their main destinations were the Indian subcontinent (79 children; 43.2%), South-East Asia (56 children; 30.6%), and sub-Saharan Africa (26 children; 14.2%). Children traveled mainly to visit friend and relatives (VFRs) (134 children; 73.2%) and to stay in local residences (137 children; 74.9%). Forty (21.9%) children had received pre-travel consultation. VFRs sought pre-travel consultation less frequently compared with non-VFRs (17.9% versus 32.7%; p-value=0.033) and planned to travel for longer periods (p-value <0.001). Factors significantly associated with an increased probability for seeking pre-travel consultation were female sex and having the Greek nationality (p-values=0.007 and <0.001, respectively). The rabies and the typhoid fever vaccines were administered inadequately to children travelling to endemic areas. Malaria chemoprophylaxis was generally justified.**Conclusions:** Despite the increasing number of pediatric international travelers, only one every five children travelling to Africa and Asia seeks pre-travel consultation. Travel vaccines, and especially the rabies and the typhoid fever vaccines, were provided inadequately to children travelling to endemic areas. Malaria chemoprophylaxis was generally justified. Travel medicine services for children in Greece should improve. There is a need to develop communication strategies to access VFRs.

Author Disclosure Block:

H. Maltezou: None. **A. Pavli:** None. **S. Patrinos:** None. **M. Theodoridou:** None.

Poster Board Number:

MONDAY-576

Publishing Title:

Epidemic Strains of Fmd (1998-2007 Gg.) in Armenia

Author Block:

T. Yesayan, K. Sargsyan; SSFS of the MOA RA, Yerevan, Armenia

Abstract Body:

The main objective of the single integrated system of Foot and Mouth Disease (FMD) measures in Armenia was to create buffer zones to contain exotic FMD viral types coming into the country from Turkey and Iran. Implementation of systematic mass vaccination of cattle, sheep and goats utilizing a polyvalent vaccine with a high protective activity helped to stabilize the situation by 1990. However, circulating strains have undergone antigenic drift making the vaccines ineffective. From 1998 to 2007 the following FMD virus strains were found in Armenia: A-98 (Amasia and Akhuryan regions, 1998), Asia-1 (Amasia region, 2000) and strain O (Ashotsk region, 2002). In all cases, outbreaks of FMD were caused by drifting from neighboring Turkey in the border areas of summer pasture. The highest rate of infection in 2000 was in Amasia (73.0%) and in 2002 was in Ashotsk (68.0%). Outbreaks of FMD type Asia-1 in the Shirak zone caused considerable damage initially, leading to development of a specific prevention plan. Implementation of a large-scale double dose of the vaccine strain Az-48 (for cattle), with an interval of 7-10 days and a single vaccination of sheep managed to eliminate FMD in the primary foci. Disease caused by the above strains of FMD virus have not been recorded since 2001. FMD virus type O is now the most common virus type, by all indicators of epizootic process. During the analyzed period, the number of foci and cases of FMD virus type O was significantly higher than those of the FMD types A and Asia-1. In addition, the most severe disease occurs in cows and calves up to 3-4 months age. Breakthrough infection in the repeatedly immunized population and the severity of disease in adult cattle was due to the mismatch of epizootic and production strains leading to low efficacy of the applied Antiepizootic series of vaccines. Due to the complexity of the epizootic situation of FMD, it is necessary to provide for emergency immunization of animals, in order to eliminate the disease in the event of primary foci and replace the production strains. For example, Vladimir Research Institute for Animal Health used, a polyvalent vaccine, AOAziya-1 in the farm border area of Armenia in 2003 during spring vaccinations, in the framework of the program "FMD buffer zone." Obtained from field strains, the vaccine has high immunogenic properties, thereby eliminating FMD types A, O, and Asia-1 in primary foci.

Author Disclosure Block:

T. Yesayan: None. **K. Sargsyan:** None.

Poster Board Number:

MONDAY-577

Publishing Title:

Seasonal Influenza Vaccination Coverage Improvement Through Implementation of an Immunization Strategy Bundle: A Pre-Post-Intervention Study

Author Block:

M. St-Amant, M-A. Pearson, C. Amireault, J. Beaudoin-Maitre, M. Landry, A. Chapdeleine, **S. Grandjean Lapierre**; Ctr. Hosp. Univ. de Montréal, Montreal, QC, Canada

Abstract Body:

Background: Nosocomial outbreaks of seasonal *Influenza* have been described. Increasing vaccination coverage among hospitalised patients is challenging. The objective of this study was to evaluate the efficacy of an immunization strategy bundle for the improvement of vaccination coverage among hospitalised patients. **Methods:** An immunization strategy bundle was implanted after 2012 and before 2015 *Influenza* seasons on our tertiary care institutions' internal medicine ward. The bundle included an immunisation status form, patient counselling and delegation of vaccine prescription authority to hospital pharmacists and trained nurses. Patients hospitalised in November 2012 and 2015 were included and respectively considered as the pre intervention and post intervention study groups. No impactful changes occurred among health care professional teams, hospitalisation setting or concomitant immunization strategies between both periods. Data were retrospectively collected through medical chart review.**Results:**

Table 1 - Baseline characteristics		
	Pre intervention (n=89)	Post intervention (n=108)
Average age - yr	61.4	64.5
Vaccine indication - no. (%)	72 (80.1)	99 (91.7)
Vaccine contraindication - no. (%)	1 (1.1)	0 (0)
Average no. of indications per patient	1.8	2.8
Most frequent indications - no. (%)		
Age > 60 yr	49 (55.1)	65 (60.2)
Pulmonary disease	21 (23.6)	33 (30.6)
Diabetes	20 (22.5)	41 (38.0)

Health care teams showed a compliance rate of 47.5% to proper filling of the immunization status form. As presented in table 2, rates of immunization status evaluation and rates of vaccination when indicated increased with the implementation of the bundle. The observed increase in vaccination coverage was found to be statistically significant (p=0.0362).

Table 2 - Bundle performance			
	Pre-intervention	Post-intervention	P value
Vaccination status evaluation - no (%)	15 (16.9)	49 (45.4)	< 0.001
Vaccination when indicated - no (%)	15 (20.8)	33 (33.3)	0.0362

Conclusion: Our data shows that the implementation of a standardised immunisation status evaluation form along with counselling on vaccination and delegation of vaccine prescription authority can improve seasonal *Influenza* vaccination coverage in a hospital setting.

Author Disclosure Block:

M. St-Amant: None. **M. Pearson:** None. **C. Amireault:** None. **J. Beaudoin-Maitre:** None. **M. Landry:** None. **A. Chapdeleine:** None. **S. Grandjean Lapierre:** None.

Poster Board Number:

MONDAY-578

Publishing Title:

Impact of 10-Valent Pneumococcal Conjugate Vaccine Introduction on Pneumococcal Carriage in Fiji: Results from Four Annual Cross-Sectional Carriage Surveys

Author Block:

E. M. Dunne[1], C. Satzke[1], T. Ratu[2], E. Rafai[2], M. Kama[2], R. Devi[2], K. Bright[1], L. Tikoduadua[2], J. Kado[2], C. L. Pell[1], M. L. Nation[1], J. Smyth[1], M. Habib[1], B. D. Ortika[1], S. Matanitobua[2], K. Gould[3], J. Hinds[3], K. Jenkins[4], K. Mulholland[1], F. M. Russell[1]; [1]Murdoch Childrens Res. Inst., Parkville, Australia, [2]Ministry of Hlth.and Med. Servies, Suva, Fiji, [3]St. George's, Univ. of London, London, United Kingdom, [4]Fiji Hlth.Sector Support Program, Suva, Fiji

Abstract Body:

Fiji introduced the 10-valent pneumococcal conjugate vaccine (PCV10) in 2012 using a 3+0 schedule. As part of the New Vaccine Evaluation Project, we are measuring the impact of PCV10 on nasopharyngeal carriage, invasive pneumococcal disease, and pneumonia. Cross-sectional nasopharyngeal carriage surveys were conducted annually prior to and for three years post-PCV10 introduction to measure direct and indirect PCV10 effects in infants (5-8 wk), children (12-23 mth, and 2-6 y), and adult caregivers. Nasopharyngeal swabs were collected from healthy participants (n≈500 per age group per year), stored in STGG, and frozen at -80°C until analysis. Pneumococci were detected by *lytA* qPCR and molecular serotyping performed by microarray. Results from 2012 - 2014 indicate that overall pneumococcal carriage declined following PCV10 introduction: carriage rates were significantly lower in 2014 than 2012 for all four age groups ($P < 0.05$). For 12-23 mth old children, the total pneumococcal carriage rate was 50%, 47%, and 30% in 2012, 2013, and 2014 respectively. Of the pneumococci identified, 38%, 32%, and 21% were PCV10 serotypes in 2012, 2013, and 2014, respectively. Carriage of multiple serotypes was common in this age group, with 9% of samples containing more than one pneumococcal serotype. Non-typeable pneumococci were the most common type observed, with their carriage increasing following vaccine introduction. Serotyping of other age groups is underway. This multiyear study in Fiji will provide important data on the impact of PCV10 introduction on pneumococcal carriage on both vaccinated and unvaccinated members of the population, as a decline in carriage is likely to translate to a decline in disease. Examining pneumococcal carriage is a practical means for evaluating vaccine impact in resource-limited settings, where disease surveillance is difficult.

Author Disclosure Block:

E.M. Dunne: None. **C. Satzke:** None. **T. Ratu:** None. **E. Rafai:** None. **M. Kama:** None. **R. Devi:** None. **K. Bright:** None. **L. Tikoduadua:** None. **J. Kado:** None. **C.L. Pell:** None. **M.L. Nation:** None. **J. Smyth:** None. **M. Habib:** None. **B.D. Ortika:** None. **S. Matanitobua:**

None. **K. Gould:** None. **J. Hinds:** None. **K. Jenkins:** None. **K. Mulholland:** None. **F.M. Russell:** None.

Poster Board Number:

MONDAY-579

Publishing Title:

Incidence and Serotype Variations of Invasive Pneumococcal Disease After Conjugate Vaccines Introduction in Gipuzkoa, Northern Spain

Author Block:

J. M. Marimón[1], M. Ercibengoa[2], M. Alonso[1], M. Lopez-Olaizola[2], G. Cilla[1], E. Perez-Trallero[3]; [1]CIBERES & H.U.Donostia-IIS Biodonostia, San Sebastián-Donostia, Spain, [2]H.U.Donostia-IIS Biodonostia, San Sebastián-Donostia, Spain, [3]EHU/UPV, CIBERES & H.U.Donostia-IIS Biodonostia, San Sebastián-Donostia, Spain

Abstract Body:

Background: Pneumococcal conjugate vaccines (PCV) were sequentially introduced in Spain: PCV7 in 2001, PCV10 in 2009, and PCV13 in mid-2010. Not subsidized by Government, an estimated coverage of around 70% was estimated for children <5 year-old in our region. Objective: analyze the evolution of the epidemiology of invasive pneumococcal disease (IPD) in different age groups before and after the introduction of the different PCVs. **Methods:** The study was performed between 1996 and 2014 in the University Hospital Donostia, San Sebastian, Spain that attends a population of about 400,000 people (between 15,000 and 20,000 children <5 years). All cases of IPD were recorded and pneumococcal serotypes were determined with the Quellung reaction. All incidence data were expressed as annual cases per 100,000 population. **Results:** In children <5 year-old, IPD incidence progressively decreased from 48.1 in 1996-2000 to 25.1 in 2011-2014, due to a progressively decrease in PCV7 cases (from 31.2 to 3.8) but also to a decrease between 2006-2010 and 2011-2014 in the incidence of PCV13 serotypes not included in PCV7 (1, 3, 5, 6A, 7F, 19A): from 30.4 to 10.0. The incidence of these 6 PCV13 serotypes had increased from 13.0 to 30.4 between 1996-2000 and 2006-2010. In adults (>14 year-old), incidence increased from 15.6 to 21.3 between 1996-2000 and 2001-2005, mainly due to an increase in non-PCV7 serotypes (principally serotypes 1, 3, 7F, and 19A). Since then, it progressively decreased to reach 10.0 in 2011-2014, due to a decrease in both PCV7 (from 6.6 to 0.8) and PCV13 serotypes since 2006-2010 (from 9.2 to 3.4). **Conclusions:** Despite being used only young children, PCV have contributed to a decrease in IPD in all age-groups in our region. An initial increase in the overall incidence of IPD in adult population after introduction of PCV7 was observed decreasing to a minimum between 2011-2014 after PCV13 introduction. Incidence variations of IPD in adults mimicked that of vaccinated young children, showing the great effect of PCV in carriage.

Author Disclosure Block:

J.M. Marimón: None. **M. Ercibengoa:** None. **M. Alonso:** None. **M. Lopez-Olaizola:** None. **G. Cilla:** None. **E. Perez-Trallero:** None.

Poster Board Number:

MONDAY-580

Publishing Title:

Immunogenicity And Safety Of 13-Valent Pneumococcal Conjugate Vaccine (Pcv13) In Heart Transplant Candidates And Recipients

Author Block:

H-Y. Sun[1], A. Cheng[2], C-I. Tsao[1], S-S. Wang[1], C-C. Hung[1], S-C. Chang[1]; [1]Natl. Taiwan Univ. Hosp., Taipei, Taiwan, [2]Natl. Taiwan Univ. Hosp., Hsin-Chu Branch, Hsin-Chu City, Taiwan

Abstract Body:

Background:PCV13 is recommended for patients with end stage heart disease and heart transplant recipients who are vulnerable to invasive pneumococcal diseases, but the immunogenicity and safety of PCV13 in this population is unknown. The present study aimed to assess the immunogenicity and safety of PCV13 in heart transplant candidates and recipients.**Methods:**Heart transplant candidates during transplant evaluation and recipients during follow-up were invited to receive 1-dose PCV13. Anti-capsular antibody responses against 4 serotypes (6B, 14, 19F and 23F) at baseline and 1, 3, 6, 9, and 12 months following vaccination were measured. Significant antibody responses were defined as 2-fold or greater increase of antibody levels compared with the baseline level.**Results:**From 2013/10/1 to 2015/7/3, one dose PCV13 was given to 12 heart transplant recipients (Group 1) with a median age of 54 and 23 heart transplant candidates (Group 2) with a median age of 56. Group 1 received PCV13 after a median of 1788 days (interquartile 816-2169) after transplantation. Significant responses to the 4 serotypes at 1, 3, 6 months post PCV13 developed in 45.5-72.7%, 27.3-72.7%, 28.6-85.7% of Group 1, and 66.7-85.7%, 44.4-72.2%, 46.2-53.8% of Group 2, respectively. Pain, erythema, and fever within 1 week after vaccination occurred in 16.7%, 8.3%, and 0% of Group 1 and 17.4%, 0%, 0% of Group 2, respectively. No differences in immunogenicity and safety were observed between the two groups.**Conclusions:**PCV13 is immunogenic and safe in heart transplant candidates and recipients although long-term follow-up is needed to assess the durability of its immunogenicity in this population.

Author Disclosure Block:

H. Sun: None. **A. Cheng:** None. **C. Tsao:** None. **S. Wang:** None. **C. Hung:** None. **S. Chang:** None.

Poster Board Number:

MONDAY-581

Publishing Title:

Comparison of Immunogenicity of a Next Generation Pneumococcal Conjugate Vaccine in Different Animal Models

Author Block:

J. Xie, I. Caro-Aguilar, L. Indrawati, W. J. Smith, C. Giovarelli, M. Winters, J. McNair, J. He, Y. Zhang, A. Espeseth, C. Abeygunawardana, L. Musey, M. Kosinski, J. M. Skinner; Merck & Co. Inc, West Point, PA

Abstract Body:

Several animal models have been used to evaluate pneumococcal conjugate vaccines prior to evaluation in humans. We have developed a 15-valent pneumococcal conjugate vaccine (PCV15) which includes polysaccharides (PS) from serotypes (1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 22F and 33F). All polysaccharides are conjugated to CRM197 and formulated with an aluminum adjuvant. The objective of this study is to investigate the immunogenicity of PCV15 in infant Rhesus monkey, CD1 mice, and New Zealand white rabbit.Animals were intramuscularly immunized with PCV15 vaccine (17 animals/group) and sera were collected before each immunization and after the final immunization. Functional antibodies were measured by multiplexed opsonophagocytic killing assay (MOPA). Geometric mean and 95% confidence interval were calculated and used to compare the titers between the two groups.PCV15 was immunogenic and increased functional antibodies titers by 10-1000 fold for most serotypes in all species. All serotypes except ST5 had higher OPA titers in rabbits compared to that in monkeys and mice. Serotypes 3 had very low OPA titers in monkeys. Serotypes 23F and 33F showed lower immunogenicity in mice while induced high titers in monkeys and rabbits. PCV15 also induced high anti-ST14 OPA titer in mice and protected mice from serotype 14 intraperitoneal challenge.These results demonstrate that PCV15 is immunogenic across multiple animal species; however, there are also some significant differences in some serotypes across animal species. These data will be analyzed relative to clinical immunogenicity data to determine which animal model is most predictive of human responses.

Author Disclosure Block:

J. Xie: D. Employee; Self; Merck & Co. Inc. **I. Caro-Aguilar:** D. Employee; Self; Merck & Co. Inc. **L. Indrawati:** D. Employee; Self; Merck & Co. Inc. **W.J. Smith:** D. Employee; Self; Merck & Co. Inc. **C. Giovarelli:** D. Employee; Self; Merck & Co. Inc. **M. Winters:** D. Employee; Self; Merck & Co. Inc. **J. McNair:** D. Employee; Self; Merck & Co. Inc. **J. He:** D. Employee; Self; Merck & Co. Inc. **Y. Zhang:** D. Employee; Self; Merck & Co. Inc. **A. Espeseth:** D. Employee; Self; Merck & CO. Inc. **C. Abeygunawardana:** D. Employee; Self;

Merck & Co. Inc. **L. Musey:** D. Employee; Self; Merck & Co. Inc. **M. Kosinski:** D. Employee; Self; Merck & Co. Inc. **J.M. Skinner:** D. Employee; Self; Merck & Co. Inc..

Poster Board Number:

MONDAY-582

Publishing Title:

Trivalent Pneumococcal Protein Vaccine Protects Against Acute Otitis Media in an Infant Murine Model

Author Block:

Q. Xu, K. Pryharski, M. E. Pichichero; Rochester Gen. Hosp., Rochester, NY

Abstract Body:

Background: *Streptococcus pneumoniae* (*Spn*) infections are a major public health concern. Current licensed serotype-based pneumococcal vaccines are effective in preventing invasive pneumococcal diseases, but less effective in preventing non-bacteremic pneumonia and acute otitis media (AOM). We previously reported that a pneumococcal trivalent recombinant protein vaccine (PPrV) protected against pneumonia murine model. Here we evaluated PPrV protection against AOM in an infant murine model.**Methods:** C57BL/6J mice were intramuscularly vaccinated at 1-3 weeks of age with either a monovalent pneumococcal histidine triad protein D (PhtD), pneumococcal choline binding protein A (PcpA), detoxified pneumolysin (Ply), or trivalent vaccine, and transtympanicly challenged at 7-8 weeks of age with 100 CFU of *Spn* strain BG7322 (6A) or $1x10^4$ CFU of *Spn* nontypeable strain 0702064. Serum IgG titers were determined by ELISA. At 24 and 48 hours post infection (hpi), animals were sacrificed and middle ear fluid (MEF) samples were collected to count CFU of *Spn*.**Results:** We found that intramuscular vaccination of infant mice with monovalent and trivalent pneumococcal proteins elicited significant serum IgG antibody responses to corresponding component proteins. Vaccination with PhtD led to a tendency to reduce bacterial burdens in MEF at both 24 ($p=0.05$) and 48 hpi ($p=0.16$). Vaccination with PcpA significantly reduced bacterial burdens in MEF at both 24 ($p=0.02$) and 48 hpi ($p=0.004$); with PlyD1 significantly reduced *Spn* burden in MEF at 48 hpi ($p=0.02$), but not at 24 hpi ($p=0.24$). Vaccination with trivalent significantly reduced *Spn* burdens in MEF at both 24 ($p=0.001$) and 48 hpi ($p<0.0001$). Similar reductions of bacterial burdens were found when the vaccinated animals were challenged with the nontypeable strain.**Conclusions:** Trivalent PPrV confers protection against AOM in an infant murine model.

Author Disclosure Block:

Q. Xu: None. **K. Pryharski:** None. **M.E. Pichichero:** None.

Poster Board Number:

MONDAY-583

Publishing Title:

Phtd Pneumococcal Vaccine Protects Against Invasiveness by Preventing Pneumococcal Density in the Nasopharynx from Exceeding a Pathogenic Threshold during a Viral Upper Respiratory Co-Infection

Author Block:

M. Khan, Q. Xu, M. E. Pichichero; Rochester Gen. Hosp., Rochester, NY

Abstract Body:

Background: Unlike pneumococcal conjugate and polysaccharide vaccines, it would be desirable for protein-based, non-serotype specific vaccines not to eliminate all pneumococci from the nasopharynx (NP). Rather the goal of protein based pneumococcal vaccines would be to prevent the level of pneumococcal NP carriage to exceed a pathogenic threshold as occurs during a viral co-infection.**Methods:** Using pneumococcal clinical serotype strain 6A and mouse adapted PR8 Influenza virus (H1N1), we developed a mouse co-infection model and established pneumococcal NP carriage thresholds correlated with invasion and bacteremia (pathogenic) versus thresholds correlated with non-invasiveness (non-pathogenic). Infant mice (1 week old) were vaccinated with pneumococcal histidine triad protein D (PhtD) and vaccine efficacy in protection against pneumococcal pathogenic carriage during co-infection was ascertained. In order to assess the role of PhtD specific CD4+ T cells and *antisera* in protection, vaccinated and control mice were depleted of their CD4+T cells. Anti PhtD immunity (CD4+ T cells/antisera) was further transferred into naïve infant mice to assess the vaccine efficacy in protection against pneumococcal carriage.**Results:** An immune response to PhtD prevented pneumococcal NP carriage density to exceed the pathogenic threshold and cause disease. In adult mice, antigen specific CD4+T cell responses correlated with protection associated with enhanced phagocytic function of macrophages in the NP. In an infant colonization model, neither PhtD induced CD4+ T cells or antisera alone led to a significant reduction of NP pneumococcal colonization. However, simultaneous transfer of CD4+ T cells and antisera from vaccinated mice significantly reduced the NP bacterial density.**Conclusions:** PhtD vaccination did not eliminate all pneumococci from the NP thereby averting potential adverse consequences of other pathogens occupying the emptied NP niche. We conclude that PhtD vaccination may protect against pneumococcal disease without elimination of the organism from the NP.

Author Disclosure Block:

M. Khan: None. **Q. Xu:** None. **M.E. Pichichero:** None.

Poster Board Number:

MONDAY-584

Publishing Title:

Pneumococcal Protein Vaccine Gen-004 Reduces Experimental Human Pneumococcal Carriage in Healthy Adults

Author Block:

M. Skoberne[1], D. M. Ferreira[2], **S. Hetherington**[1], R. Fitzgerald[3], S. B. Gordon[2]; [1]Genocea BioSci.s, Cambridge, MA, [2]Liverpool Sch. of Tropical Med., Liverpool, United Kingdom, [3]Royal Liverpool Univ. Hosp., Liverpool, United Kingdom

Abstract Body:

Rationale: *Streptococcus pneumoniae* remains the leading cause globally of respiratory tract infections among young children and the production of universal inexpensive vaccine a global health priority. GEN-004 vaccine, developed with Genocea's ATLAS™ technology, is based on stimulation of T cell responses to 3 universal pneumococcal protein antigens. The vaccine was designed to protect against colonization of nasopharynx, a prerequisite step to pneumococcal disease.**Objectives:** Using an experimental human pneumococcal carriage (EHPC) model, we measured the effect of GEN-004 vaccine on carriage acquisition, density and duration following intranasal challenge of healthy adult subjects.**Methods:** 96 healthy participants aged 18–50 years received either GEN-004 (n = 46) or placebo (n = 50). Following 3 intramuscular doses at 4 week intervals subjects were inoculated with 80,000 CFU of *S. pneumoniae* 6B per naris. Subjects were followed for safety throughout the study. Blood samples were collected before and after immunization to assess immunogenicity. Nasal washes were collected 2, 7, and 14 days after inoculation to measure pneumococcal carriage by microbiological culture and qPCR.**Results:** The vaccine was well tolerated and highly immunogenic as measured by antibody response. A smaller number of vaccinated subjects compared to control group developed carriage (2 days after inoculation: 18/46 vs 26/50 measured by culture and 19/46 vs 30/50 measured by PCR). When comparing across multiple time points and methods, the impact on acquisition of carriage, ranged consistently between 18 and 36 percent lower compared to placebo. None of the individual endpoints were statistically significant, as the study was powered to observe >50% reduction in acquisition. **Conclusions:** GEN-004 was well tolerated, immunogenic and showed a consistent reduction in pneumococcal colonization in this small study. Further EHPC studies of pneumococcal protein vaccines should power to detect reductions in carriage density of 20-30% in this model. The clinical importance of the observed magnitude of reduction is unknown but should be explored in future studies.ClinicalTrials.gov Identifier: NCT02116998

Author Disclosure Block:

M. Skoberne: D. Employee; Self; Genocea Biosciences. K. Shareholder (excluding diversitied mutual funds); Self; Genocea Biosciences. **D.M. Ferreira:** B. Collaborator; Self; Genocea Biosciences. I. Research Relationship; Self; Genocea Biosciences. **S. Hetherington:** D. Employee; Self; Genocea Biosciences. K. Shareholder (excluding diversitied mutual funds); Self; Genocea Biosciences. **R. Fitzgerald:** F. Investigator; Self; Genocea Biosciences. **S.B. Gordon:** F. Investigator; Self; Genocea Biosciences.

Poster Board Number:

MONDAY-585

Publishing Title:

An *in Vitro* Adherence Inhibition Assay For *bordetella Pertussis* Vaccine Antigens

Author Block:

Y. Gorantla, E. Kim, L. Choi, J. Schiffer, **G. Rajam**; CDC, Atlanta, GA

Abstract Body:

Background: Pertussis, caused by *Bordetella pertussis* (Bp) infection, is the least controlled vaccine-preventable bacterial disease despite high vaccine coverage. Acellular pertussis vaccines (aP), Tdap and DTaP, contain a range of Bp antigens including inactivated pertussis toxin (Pt), pertactin (Prn), filamentous hemagglutinin (Fha), and fimbriae 2/3 (Fim2/3). Bp infection requires nasopharyngeal (NP) colonization. CDC has developed an *in vitro* pertussis antigen adherence inhibition assay (pAAIA) to evaluate the contributions of anti-aP antibodies in preventing Bp NP adherence.**Methods:** Fluospheres covalently bound to each of four aP target antigens, Pt, Prn, Fha and Fim2/3 were tested for their adherence to Detroit 562 epithelial cells, detected fluorometrically and expressed as median fluorescence intensity (MFI). Antigen specific fluospheres were mixed with a panel of human anti-Tdap sera (n=10) and tested for reduction in adherence. The percent inhibition of adherence (IA) was calculated using the following formula: [%IA = 100-(test MFI x 100)/(mean control MFI)]. The dilution effecting a 50% IA of antigen conjugated fluospheres was reported (ED50). The reference antiserum NIBSC 06/140 was tested to determine the potency of different antibodies and expressed as the IC50 (ED50 * known antibody concentration in IU/mL).**Results:** At normalized bead count/well, max MFI (±1SD) was recorded for Fim (28066±1544 MFI) followed by Fha (17676±4810 MFI) and Prn (10725±16 MFI). Pt fluospheres exhibited poor adherence to Detroit 562 cells. Anti-Tdap sera exhibited high IA against Prn-coated fluospheres (ED50 range 10 - 270; median - 90), Fim (ED50 range 10 - 90; median - 30), and Fha (ED50 range 10 - 60; median - 30). Relative anti-aP adherence inhibition potency was anti-Fim (IC50 = 0.15 IU/ml), anti-Prn (0.55 IU/ml) and anti-Fha (2.00 IU/ml).**Conclusions:** Bp-antigen coated fluosphere adherence to Detroit 562 cells was mediated by Fim, Fha, and Prn but not by Pt. Anti-Tdap antiserum demonstrated concentration dependent IA. Pertussis AAIA is a novel *in vitro* technique to quantify antibodies inhibiting the adherence of Bp antigens to Detroit 562 cells.

Author Disclosure Block:

Y. Gorantla: None. **E. Kim:** None. **L. Choi:** None. **J. Schiffer:** None. **G. Rajam:** None.

Poster Board Number:

MONDAY-587

Publishing Title:

Characterization of Gene Expression Patterns in Pbmcs of Children Who Are Low Vaccine Responders Compared to Normal Vaccine Responders

Author Block:

R. Kaur[1], R. Kennedy[2], M. E. Pichichero[1]; [1]Rochester Gen. Hosp., Rochester, NY, [2]Mayo Clinic, Rochester, MN

Abstract Body:

Background: Low vaccine responders (LVRs) are infants who fail to respond or respond with sub-protective immunity to routine pediatric vaccines after their primary series at 2, 4, and 6 months of age. We used transcriptomic high-dimensional Next-Generation Sequencing to characterize the gene expression profile of peripheral blood mononuclear cells (PBMCs) from children who are LVR (showing a subprotective response to >50% of the 6 antigens tested) compared with normal vaccine responders (NVR).**Methods:** RNA was extracted from PBMCs of 12 children age between 9-15 months and sequenced using the Illumina HiSeq 2000 platform. Differential expression analysis comparing the LVR relative to the NVR children was done using the edgeR package in R**Results:** Results are reported as $\log_2$ of the fold change for the LVR relative to the NVR, with corresponding p-values and false discovery rates. Following normalization 13,221 genes were analyzed for differential expression between LVR and NVR subjects. Despite the relatively small sample size we were able to identify significant expression differences in 46 genes ($p < 0.0005$ and $q \leq 0.1$) in LVRs (Top genes listed in figure). A KEGG pathway enrichment analysis identified the Calcium Signaling Pathway as being differentially expressed ($p=0.000015$) in LVR subjects. This pathway controls T and B cell receptor signaling as well as cellular responses to neurotransmitter, growth factors, and hormones.**Conclusions:** LVR children are characterized by differential expression of genes mostly related to T and B cell receptor signaling.

Differential Gene Expression in LVR and NVR			
Genes	FC	P-Value	FDR
Downregulated Genes			
IFI27	0.03	<1.00E-10	<1.00E-10
CXCL10	0.06	<1.00E-10	<1.00E-10
LAMP3	0.26	5.39E-10	2.38E-06
ANKRD22	0.34	3.97E-08	8.74E-05
SERPING1	0.34	1.49E-09	4.91E-06
CXCR2P1	0.36	2.00E-09	5.28E-06
IFIT3	0.48	1.73E-07	0.00025
HBB	0.49	1.88E-06	0.0025
TNFSF10	0.54	2.02E-05	0.012
CXCL7	0.55	4.26E-05	0.02
IFI44L	0.55	6.48E-05	0.029
GBP1	0.56	6.20E-05	0.028
Upregulated Genes			
SNORA71C	20.61	4.95E-06	0.0044
SIGLEC12	8.61	2.57E-05	0.014
FOLR3	4.12	1.27E-07	0.00021
HLA-DQA1	2.13	1.17E-07	0.00021
HLA-DQB1	1.87	2.79E-05	0.015
STAB1	1.75	8.81E-05	0.035
CD163	1.74	9.23E-05	0.036

FC = fold change for the low responders relative to the normal responders. FDR= Benjamin and Hochberg false discovery rates

Author Disclosure Block:

R. Kaur: None. **R. Kennedy:** None. **M.E. Pichichero:** None.

Poster Board Number:

MONDAY-589

Publishing Title:

Naturally Acquired Mucosal Antibody Levels to Pneumococcal Protein Vaccine Candidates Prevent Acute Otitis Media Caused by *Streptococcus pneumonia* in Young Children

Author Block:

Q. Xu[1], J. Casey[2], A. Almudevar[3], M. E. Pichichero[1]; [1]Rochester Gen. Hosp., Rochester, NY, [2]Legacy Pediatrics, Rochester, NY, [3]Univ. of Rochester, Rochester, NY

Abstract Body:

Background: Acute otitis media (AOM) is a common pediatric bacterial infection often caused by *Streptococcus pneumonia* (*Spn).* We previously found that nasopharyngeal (NP) colonization by *Spn* elicits mucosal antibody responses to 3 pneumococcal protein vaccine candidates: pneumococcal histidine triad protein D (PhtD), pneumococcal choline binding protein A (PcpA), and pneumolysin (Ply). The objective of this study was to assess association of mucosal antibody levels to these three proteins with protection against NP colonization and acute otitis media (AOM) caused by *Spn.***Methods:** Healthy infants without previous episodes of AOM were enrolled in study at 6 months of age, and NP samples were collected at 6-24 months of age. Whenever a child was diagnosed with AOM, tympanocentesis was performed and middle ear fluid samples collected to confirm the diagnosis with microbiologic culture for otopathogens. NP mucosal IgG and IgA titers to PhtD, PcpA and Ply were quantified by ELISA. The correlation of antibody levels with NP colonization and AOM were analyzed using logistic regression in a generalized estimating equation model.**Results:** Compared with children who did not experience an AOM, children who experienced 1-3 episodes of AOM caused by *Spn* within 12 months had 4-fold lower IgG levels to PcpA in the NP ($p=0.0005$), 7-fold lower IgA to PhtD ($p<0.0001$); 3-folder lower IgA to PcpA ($p=0.004$), and 2-fold lower GM of IgA to Ply ($p=0.08$). NP mucosal antibody IgG level to PcpA, and IgA titers to all three proteins correlated with subsequent occurrence of AOM caused by *Spn* ($P<0.0001$). However, mucosal antibody titers to the three proteins did not correlate with protection against NP colonization with *Spn.***Conclusions:** NP mucosal IgG levels to PcpA, IgA to PhtD, PcpA and Ply, and IgG to PcpA correlated with protection of AOM in young children but not with protection against NP colonization.

Author Disclosure Block:

Q. Xu: None. **J. Casey:** None. **A. Almudevar:** None. **M.E. Pichichero:** None.

Poster Board Number:

MONDAY-590

Publishing Title:

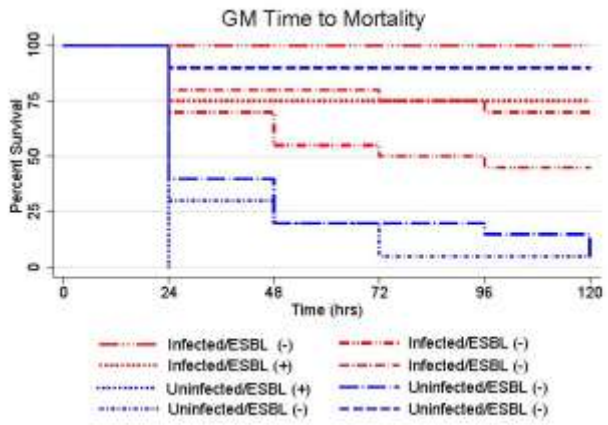
Virulence Of Ciprofloxacin Resistant *escherichia Coli* Obtained On Pre-Prostate Biopsy Rectal Screens From Infected And Non-Infected Patients In Galleria Mellonella

Author Block:

C. Miglis[1], T. Zembower[2], A. Schaeffer[2], C. Qi[2], M. Malczynski[2], K. Maxwell[2], G. Pais[1], M. Scheetz[1]; [1]Midwestern Univ., Downers Grove, IL, [2]Northwestern Mem. Hosp., Chicago, IL

Abstract Body:

Background: Postprocedural infections are a known complication of prostate biopsies and can be due to host defenses, efficacy of prophylaxis and organism virulence. We utilized a Galleria mellonella (GM) model to quantify the virulence of *E.coli* isolated from infected and non-infected patients who had undergone transrectal ultrasonography of the prostate (TRUSP) with biopsy.**Methods:** Ciprofloxacin-resistant E.coli isolates were obtained from rectal swabs of patients prior to TRUSP procedures who developed infection (n=4) and did not develop infection (n=4). Organism identification and susceptibility testing were performed according to standard procedures. GM weighing between 250-350 mg (n=20) were inoculated with 1-2 x 10^5 CFU per 10 uL aliquot into the GM hemocele for each isolate. Experimental controls consisted of GM receiving an equivalent volume of 1) normal saline and 2) no injection. GM were incubated at 37 C and observed every 24h for 5 days. Mortality was assessed with Kaplan-Meier survival curves and log rank tests.**Results:** Control GM ultimately experienced mortality in 20% of the saline and 5% of the no-injection groups respectively. GM inoculated with isolates from non-infected patients caused greater mortality compared to those from infected patients at 5 days (75% vs. 27.5%, respectively $p < 0.001$, Figure 1). Two isolates harbored ESBLs. ESBL (-) isolates killed 47.5% of GM compared with 62.5% mortality with GM injected with ESBL(+) isolates, p=0.051%.**Conclusions:** E.coli isolates that were obtained from non-infected patients and those that were ESBL + were more virulent.



Author Disclosure Block:

C. Miglis: None. **T. Zembower:** None. **A. Schaeffer:** None. **C. Qi:** None. **M. Malczynski:** None. **K. Maxwell:** None. **G. Pais:** None. **M. Scheetz:** None.

Poster Board Number:

MONDAY-591

Publishing Title:

Evaluation Of Bp82 Lps As Vaccine Adjuvant Against *burkholderia Pseudomallei* in Goats

Author Block:

L. Rice, D. Fletcher, T. Eckstein; Colorado State Univ., Fort Collins, CO

Abstract Body:

Burkholderia pseudomallei is a select agent, gram-negative bacterium that is responsible for causing melioidosis in a multitude of hosts. An auxotroph strain of this bacteria known as *Burkholderia pseudomallei ΔpurM* mutant 82 (Bp82) was developed as a select-agent list exception and is currently being investigated for its potential as a vaccine candidate. This study examined the characteristics of the lipopolysaccharide profile of Bp82 and its potential as an adjuvant in vaccine therapy in the goat model. In a proof of concept study, two goats – goats 35 and 36 – were vaccinated with Bp82. Two other goats – goats 0 and 3 – were left unvaccinated as a control. Two weeks later, all four goats were challenged with the virulent strain of *Burkholderia pseudomallei*. Serum samples were collected weekly, and the experiment was terminated two weeks after the challenge. LPS was isolated from Bp82 culture, and used in an ELISA test to evaluate the reactivity of the goat serum to the molecule. After controlling for media readings, unvaccinated goats 0 and 3 had low amounts of immunoreactivity to the LPS. Goat 35 had a significant serum response to the LPS antigen, and its ELISA reactivity was greatest one week after challenge. Goat 36 had a smaller response and its ELISA reactivity was greatest two weeks after challenge. Additionally, LPS extracted from Bp82 culture was characterized by linearizing the sugars through an alditol acetate protocol. The resulting sample was analyzed through gas chromatography – mass spectroscopy. The study implicates that the lipopolysaccharide of Bp82 may have potential as a vaccine adjuvant, though further studies are needed. Future studies may include evaluating the effectiveness of live Bp82 vaccination in causing immune responses against virulent strain LPS in BSL-3 settings, and increasing the sample size.

Author Disclosure Block:

L. Rice: None. **D. Fletcher:** None. **T. Eckstein:** None.

Poster Board Number:

MONDAY-592

Publishing Title:

Allergic Airway Sensitization Impairs Bacterial-Specific Igg Responses During *Mycoplasma pneumoniae* Infection

Author Block:

A. H. Totten, L. Xiao, D. Luo, D. M. Crabb, K. B. Waites, T. P. Atkinson; Univ. of Alabama at Birmingham, Birmingham, AL

Abstract Body:

Background: *Mycoplasma pneumoniae* (Mpn), an atypical human pathogen, has been associated with both asthma initiation and exacerbation. Asthmatics have been reported to have higher carriage rates of Mpn compared to non-asthmatic controls. We hypothesized that allergic airway sensitization would impair host immune responses to Mpn infection in a murine model.**Methods:** BALB/cJ mice were sensitized and challenged with ovalbumin (OVA) to induce allergic airway inflammation and then infected with Mpn. Immune parameters were studied by analysis of cellular profiles in bronchoalveolar lavage fluid (BALF), serum IgG and IgE antibody levels to a whole bacterial antigen preparation, recombinant Mpn P1 adhesin peptide, and OVA. Total lung RNA was used to examine cytokine transcript levels and bacterial burden was determined by detection of 16S rRNA via RT-qPCR.**Results:** Anti-Mpn and P1-specific total IgG responses were decreased in allergen-sensitized animals compared to unsensitized controls ($p < 0.01$). Decreased titers of IgG1, IgG2b and IgG3 were present in P1-specific responses but not OVA-specific IgG subclass levels. IL-4 and IL-13 mRNA levels were increased in OVA sensitized groups compared to controls. Bacterial burden was two logs lower in OVA-sensitized mice compared to unsensitized controls, and did not correlate with Mpn-specific IgG levels.**Conclusions:** Anti-Mpn IgG antibody titers were decreased in allergic animals, suggesting an inverse relationship between atopic status and humoral immunity. Due to the importance of antibody titers in controlling Mpn infections, this model may explain a reported defect in asthmatic humoral responses during infection.

Author Disclosure Block:

A.H. Totten: None. **L. Xiao:** None. **D. Luo:** None. **D.M. Crabb:** None. **K.B. Waites:** None. **T.P. Atkinson:** None.

Poster Board Number:

MONDAY-593

Publishing Title:

Examining the Prevalence of Multi-Nucleated Giant Cells (MNGCs) Using Both Inhalational and Intraperitoneal Infection Models of Melioidosis in BALB/c and C57BL/6 Mice

Author Block:

J. J. Bearss, M. Hunter, K. A. Frittspinnock, C. P. Klimko, J. L. Dankmeyer, R. G. Toothman, C. H. Weaver, C. W. Schelhase, J. A. Bozue, K. Amemiya, P. L. Worsham, S. L. Welkos, **C. K. Cote**; USAMRIID, Frederick, MD

Abstract Body:

Burkholderia pseudomallei, the etiologic agent of melioidosis, is a gram negative bacterium designated a category B threat agent by the CDC. This bacterium is endemic in Southeast Asia and northern Australia and can infect humans and animals by several routes of infection. Inhalational melioidosis has been associated with monsoonal rains in endemic areas and is also a primary concern in the biodefense community. There are currently no effective vaccines for B. pseudomallei and antibiotic treatment can be hampered by non-descript symptomology and also the high rate of naturally occurring antibiotic resistant strains. Multinucleated giant cells (MNGCs) arise when monocytes or macrophages fuse forming phenotypically distinct giant cells. MNGCs are associated with the pathogenesis and progression of some bacterial infections, and their presence has sometimes been referred to as a "hallmark of melioidosis". MNGCs are well established in Burkholderia-infected in vitro cell cultures; however, there have been limited demonstrations of in vivo MNGCs in the literature. To date they have been observed in mouse and Madagascar hissing cockroach models, and in human post-mortem autopsy findings. However the significance and prevalence of the MNGCs in vivo is still questioned. Female BALB/c and C57BL/6 mice were infected with low doses of B. pseudomallei K96243 delivered via intraperitoneal injection or exposure to aerosolized bacteria. Mice were euthanized on selected days for pathological analyses. We characterized the differences in the prevalence of MNGCs found in BALB/c and C57BL/6 mice which had been infected with similarly low infectious doses of B. pseudomallei. Additional evidence is provided which clearly demonstrates that infection with B. pseudomallei can induce the formation of multi-nucleated giant cells (MNGCs) in vivo.

Author Disclosure Block:

J.J. Bearss: None. **M. Hunter:** None. **K.A. Frittspinnock:** None. **C.P. Klimko:** None. **J.L. Dankmeyer:** None. **R.G. Toothman:** None. **C.H. Weaver:** None. **C.W. Schelhase:** None. **J.A. Bozue:** None. **K. Amemiya:** None. **P.L. Worsham:** None. **S.L. Welkos:** None. **C.K. Cote:** None.

Poster Board Number:

MONDAY-595

Publishing Title:

Urinary Tract Infection Symptoms In Women Relate To Uropathogens While False Negatives Of Standard Urine Cultures May Delay Patient Treatment

Author Block:

T. Dune, E. Hilt, T. Price, C. Brincat, C. Fitzgerald, L. Brubaker, A. Wolfe, P. Schreckenberger, E. Mueller; Loyola Univ., Maywood, IL

Abstract Body:

Background: Urinary tract infections (UTIs) are often conceptualized as a single uropathogen inappropriately growing in an otherwise sterile bladder. Compelling evidence argues against this dogma, revealing urinary microbiota within the typical female bladder. UTIs rarely lead to systemic infection, thus patient symptoms may guide treatment. The goals of this study were to compare 2 urine culture methods for uropathogen detection, relate uropathogens to classic UTI symptoms, and assess standard urine culture for its effect on patient care.**Methods:** Following IRB approval, we enrolled 150 urogynecologic patients and dichotomized them on their perception of the presence of a UTI (YES/NO). Both groups also rated severity and bothersome nature of 7 classic UTI symptoms on a Likert-type response scale using a validated UTI symptom assessment (UTISA) questionnaire. The YES group was later contacted to complete a follow-up UTISA survey. Each patient's catheterized urine sample was processed with standard urine culture (SC) and enhanced quantitative urine culture (EQUC) methods. MALDI-TOF mass spectrometry identified bacteria. Women were treated clinically as needed.**Results:** In the YES group (N=75), dysuria, urinary frequency and urgency were the most common symptoms. When SC was positive, EQUC was always positive. Of the SC+ women, 72% (54/75) were assessed clinically; the majority [81% (44/54)] was treated with antibiotics. EQUC detected uropathogens in 92% (69/75) of the YES group, but 20% (15/75) were SC negative. Of the 15 SC-/EQUC+ women, only 1 was treated with antibiotics. More SC-/EQUC+ women [66.6%] reported the same or worsening UTI symptoms than SC+/EQUC+ women [38.5%].**Conclusions:** EQUC detected uropathogens more often than SC. While UTI symptoms improved in SC+ women with antibiotic treatment, SC-/EQUC+ women were not treated despite uropathogen presence. These women often had no symptom improvement. Coupling the UTISA questionnaire with EQUC highlights the troublesome false negative rate of SC. Clinicians can improve patient care with the additional information available from EQUC.

Author Disclosure Block:

T. Dune: None. **E. Hilt:** None. **T. Price:** None. **C. Brincat:** None. **C. Fitzgerald:** None. **L. Brubaker:** None. **A. Wolfe:** N. Other; Self; Research funding from Astellas Scientific and Medical Affairs. **P. Schreckenberger:** None. **E. Mueller:** None.

Poster Board Number:

MONDAY-596

Publishing Title:

Transcriptional Profiling of the Host Immune Response to Virulent and Attenuated Vaccine Strains of Mycoplasma Gallisepticum

Author Block:

J. Beaudet, E. Tulman, K. Pflaum, X. Liao, L. Silbart, S. Geary; Univ. of Connecticut, Storrs, CT

Abstract Body:

Additional in-depth information regarding the mechanism leading to the dysregulated host response to *M. gallisepticum* is needed to understand this complex immune process and to enable us to develop a more efficacious vaccine that would limit inflammation and aid in the clearance of the bacteria. Previous *in vivo* studies analyzing the host immune response to R_{low} infection through qRT-PCR have shown an upregulation in expression of pro-inflammatory chemokine and cytokine genes such as lymphotactin, CXCL13, -14, RANTES, and MIP-1β between days one and eight post infection. Studies exposing tracheae *ex vivo* and cultured tracheal epithelial cells *in vitro* to *M. gallisepticum* revealed a similar yet transient upregulation of chemokine and cytokine gene expression profiles at 1.5, 6, and 24 hours post infection using qRT-PCR and microarray analysis.In an effort to gain a broader understanding of the molecular events that underpin the host immune response to *M. gallisepticum* we are investigating the complete transcriptomic profile of chickens infected with *M. gallisepticum* R_{low} compared to uninfected chickens. In preliminary studies, RNA from infected and uninfected white leghorn chickens seven days post inoculation were analyzed using Illumina RNA sequencing. This high-throughput analytical method allowed us to map over 21 thousand genes to the chicken genome and to identify over three thousand genes with statistically significant differential expression, of which 122 encode immune-related functions. Many of these genes are active in signaling pathways that may contribute to chronic inflammation, immune dysregulation and disease.To expand on these data we have conducted a *M. gallisepticum* R_{low} time course infection study to assess the transcriptional changes in host genes that occur day-to-day over the first 7days post-infection. Key steps in the pathways leading to the maladaptive immune response to *M. gallisepticum* may prove to be important targets for future vaccine constructs.

Author Disclosure Block:

J. Beaudet: None. **E. Tulman:** None. **K. Pflaum:** None. **X. Liao:** None. **L. Silbart:** None. **S. Geary:** None.

Poster Board Number:

MONDAY-597

Publishing Title:

Host Cytokine Response in *Staphylococcus aureus* Bacteremia Specific to Source of Infection and Its Impact on Outcomes

Author Block:

N. Bui[1], E. Minejima[2], P. Nieberg[1], K. Trinh[2], A. Wong-Beringer[2]; [1]Huntington Hosp., Pasadena, CA, [2]Univ. of Southern California Sch. of Pharmacy, Los Angeles, CA

Abstract Body:

Background: Sources of *Staphylococcus aureus* bacteremia (SAB) have been associated with mortality risk, with rates ranging from 5% for low risk up to 45% for high risk sources (i.e. endovascular). Our study aims to determine if host cytokine response varies with source of infection and thus impacts outcome of SAB. **Methods:** A prospective observational cohort study was conducted at three medical centers on adults hospitalized for SAB during 7/12 to 4/15. Plasma was obtained at onset of SAB and 72h after start of effective therapy to measure pro- (TNFα, IL6, IL8) and anti-inflammatory (IL10) cytokine levels (pg/mL) by multiplex Luminex assays. Medical records were reviewed to obtain relevant demographics, laboratory, and clinical details for comparison between patients grouped based on source of infection: high risk source (HRS: endovascular, pneumonia, intraabdominal), intermediate (IRS: skin soft tissue or osteoarticular infection, unknown), and low (LRS: catheter-related).**Results:** 237 patients were included; 53 HRS, 126 IRS, and 58 LRS. Median age was 59 y with MRSA causing 1/3 of the cases. More HRS patients were admitted from a healthcare facility (44% vs 2% vs 19%, p=0.0039) and presented with septic shock (22% vs. 6% vs 12%, p=0.0078) at initial presentation compared to IRS and LRS patients, respectively. Cytokine levels were similarly elevated for HRS and LRS groups with TNF, IL8, and IL-10 at both timepoints, but lower for IRS group. Compared to IRS and LRS, HRS patients had higher rates of clinical failure on day 4 (45% vs 34% vs 19% , p=0.012) respectively but 30-day mortality was not statistically different between groups (15% vs 10% vs 10%). Regardless of source of infection, high IL10/TNF ratio at 72h was significantly associated with risk of death when comparing non-survivors to survivors (2.26 vs 0.7, p=0.0001).**Conclusions:** Our results indicate that patients with HRS of SAB were more similar to LRS than IRS patients in severity of clinical presentation and cytokine response. A high IL10/TNF ratio predicted mortality while source of infection did not.

Author Disclosure Block:

N. Bui: None. **E. Minejima:** None. **P. Nieberg:** None. **K. Trinh:** None. **A. Wong-Beringer:** None.

Poster Board Number:

MONDAY-598

Publishing Title:

Investigating the Role of Traf3 in T Cell Biology

Author Block:

T. White[1], A. Wallis[2], L. Stunz[3], G. Bishop[3]; [1]Towson Univ., Towson, MD, [2]Univ. of Iowa, University of Iowa, IA, [3]Univ. of Iowa, Iowa City, IA

Abstract Body:

T lymphocytes carry out important functions in systemic immunity. They utilize several receptors to allow them to sense their environment and respond accordingly. Understanding how T cells regulate their activation through these receptors is important to further clarifying T cell-mediated immune responses. Our lab focuses on an adapter protein called TNF Receptor-Associated Factor 3 (TRAF3) which has recently been shown to positively regulate the T cell receptor (TCR) signaling pathway. One goal of this project was to explore the role of TRAF3 in TCR signaling. The adaptor molecules GRB2/GADS are recruited to the T cell receptor complex upon activation. Analysis of the amino acid sequences of these proteins predicted potential novel associations between the carboxy terminal domain of TRAF3 and the SH3 domains of GRB2/GADS. We used Human embryonic kidney cell transfection, immuno-precipitation, and western blotting techniques to investigate whether TRAF3 associates with, and thus potentially regulates, the TCR-associated adapter proteins GRB2/GADS.A second goal of this project was to explore the role of TRAF3 in Toll-like receptor (TLR) signaling in T cells. TRAF3 has been previously shown to regulate TLRs in other cell types. Thus, we explored the role of TRAF3 in TLR signaling in T cells by stimulating T cells with various TLR ligands, then examined cytokine production and cell activation marker regulation using the techniques of enzyme-linked immunosorbent assay and Flow cytometry. We hypothesized that TRAF3 positively regulates signals via TLRs 2/5, which predicted that in the absence of TRAF3, TLR-induced cytokine production and expression of cell surface activation molecules would decrease. Our research has shown that TRAF3 associates with GRB2/GADS. Also TRAF3 affects GRB2/GADS recruitment to the cell membrane upon TCR stimulation. Our research has also shown that TRAF3-deficient T cells show an increase in surface expression of the adhesion molecule CD54. Loss of TRAF3 in T cells also increases MHC class II surface molecule abundance before and after TL2 simulation. Our preliminary studies showed that TRAF3 does interact with GADS/GAD upon TCR stimulation, and this broadens our understanding of the ways that TRAF3 may regulate TCR signaling. Furthermore, our preliminary evidence is consistent with TRAF3 regulation of TLR2/5 signaling.

Author Disclosure Block:

T. White: None. **A. Wallis:** None. **L. Stunz:** None. **G. Bishop:** None.

Poster Board Number:

MONDAY-599

Publishing Title:

Recent Thymic Emigrants and Chronic Salmonellosis: How New T Cells Combat an Old Infection

Author Block:

J. A. Goggins, J. R. Kurtz, J. B. McLachlan; Tulane Univ. Sch. of Med., New Orleans, LA

Abstract Body:

Typhoid fever, caused by infection with *Salmonella* bacteria affects approximately 21 million people annually, killing nearly 200,000 and yet the immunological response to this infection is poorly understood. Recent thymic emigrants (RTEs) are the youngest subset of peripheral T cells, which are known to differ in how they functionally contribute to the naïve T cell pool. This distinctive cell population is known to contribute to the maintenance of T cell receptor diversity in the peripheral T cell pool, but their involvement in combating chronic bacterial infections, such as typhoid fever, has not been explored. In the present study, we hypothesized that RTEs are essential contributors to the CD4 T cell response during persistent *Salmonella* Typhimurium (ST) infection, which predominantly relies on helper T cell immunity to control bacteria. Here, we show that thymectomy of adult mice prior to oral infection with ST leads to increased mortality in addition to significantly increased bacterial burdens in the spleen, mesenteric lymph nodes and liver 30 days post-infection compared to sham surgical controls, suggesting *Salmonella*-specific RTEs may contribute to bacterial clearance. Surprisingly, thymectomized animals also demonstrated expanded numbers of peripheral *Salmonella*-specific T cells, compared to control animals, implying that these existing cells are somehow incapable of controlling infection in the absence of RTEs. The results of this study provide insight into how new pathogen-specific T cells leaving the thymus participate in combatting a persistent bacterial infection.

Author Disclosure Block:

J.A. Goggins: None. **J.R. Kurtz:** None. **J.B. McLachlan:** None.

Poster Board Number:

MONDAY-600

Publishing Title:

Purification and Characterization of a Rabbit Serum Factor That Kills *Listeria* &Lt Other Foodborne Bacterial Pathogens

Author Block:

A. Datta; FDA/CFSAN, Laurel, MD

Abstract Body:

In an *in-vitro* assay, rabbit serum, but not human serum, killed *Listeria monocytogenes*, a foodborne pathogen that causes human listeriosis. The listericidal activity of rabbit serum, termed listericidin, was purified to homogeneity in a single-step by ion-exchange chromatography with DEAE-Sepahdex A-50. The purified listericidin is a protein with a molecular weight of 9 kDa and an isoelectric point of 8.1. It is effective in killing *L. monocytogenes* at 4, 25 and 37^{o} C, but the rate of killing is faster at the highest temperature. It is resistant to acidic conditions (< pH 2), but is sensitive to proteolytic enzymes subtilisin, trypsin and chymotrypsin. The listericidin is heat-stable at 56^{o}C and loses only 50% of its activity when boiled for 30 min at 100^{o}C. Its activity is inhibited in presence of NaCl in a dose-dependent fashion and by various bacterial growth media. The listericidal activity is neutralized by monoclonal antibodies to human complement C3a, but its reaction with the antibodies in an ELISA is very weak. Its activity is not affected by iron but is enhanced by calcium chloride. The first 33 N-terminal amino acid residues of the sequence (SVQLTEKRMDKVGQYTNKELRKXXEDGMRDNPM) show a high degree of homology to various mammalian complement C3a components, but there is no sequence from rabbits in the database that is similar to the listericidin amino acid sequence. In addition to killing selected members of all the major serotypes of *L. monocytogenes*, it is effective in killing other *Listeria* spp. and various foodborne pathogens belonging to the *Vibrio*, *Salmonella*, *Escherichia*, *Cronobacter*, and *Bacillus* genera. However, many of the serovars of *Salmonella enterica* are resistant to listericidin. Results of the study indicate that the rabbit listericidin is constitutively present in rabbit serum and is a C3a-like heat-stable and acid-stable peptide which shares many of the properties of the C3a component of the C3 complement family of proteins, but also exhibits properties that are unique and different from C3a.

Author Disclosure Block:

A. Datta: None.

Poster Board Number:

MONDAY-601

Publishing Title:

Antibody Pretreatment of *Mycoplasma gallisepticum* Does Not Impact Vaccination or Experimental Infection in Chickens

Author Block:

S. A. Leigh, S. Collier, S. Branton, J. Evans; USDA-ARS, Mississippi State, MS

Abstract Body:

Mycoplasma gallisepticum infection in poultry leads to chronic respiratory disease and decreased egg production in chickens and infectious sinusitis in turkeys. The pathological effects of *M. gallisepticum* infection can be prevented by vaccination with one of the live attenuated *M. gallisepticum* vaccines. While these vaccines exhibit varying levels of protection and pathology, the host immune response that provides protection is little understood. Therefore, studies were performed to determine the impact that exposure to anti-*M. gallisepticum* serum prior to vaccination or infection would have on the response of the chicken host to the serum treated mycoplasma. At 8 weeks of age (woa), chickens were vaccinated with either a commercial F-strain vaccine, or the vaccine mixed with hyperimmune anti-*M. gallisepticum* chicken serum for 60 minutes prior to vaccination. Six weeks following vaccination (14 woa), chickens were infected with *M. gallisepticum* strain R-low, with one group being infected with *M. gallisepticum* strain R-low mixed with hyperimmune anti-*M. gallisepticum* chicken serum for 60 minutes prior to infection. At one week post infection (15 woa), chickens were bled to obtain serum, killed, and examined for airsacculitis. The presence of anti-*M. gallisepticum* chicken serum had no impact on either the vaccination results or the presence of airsacculitis following infection. This provides further evidence that the humoral immune response likely has a minimal role in host protection from *M. gallisepticum* colonization and infection.

Author Disclosure Block:

S.A. Leigh: None. **S. Collier:** None. **S. Branton:** None. **J. Evans:** None.

Poster Board Number:

MONDAY-602

Publishing Title:

Protective Efficacy of Antibody to Poly-N-Acetyl Glucosamine (Pnag) Against *Streptococcus pneumonia* and *Staphylococcus aureus* Conjunctivitis

Author Block:

T. S. Zaidi[1], T. H. Zaidi[1], Z. Ge[2], G. B. Pier[1]; [1]Brigham and Women's Hosp. and Harvard Med. Sch., Boston, MA, [2]Shandong Eye Inst., Qingdao City, China

Abstract Body:

Introduction: The ocular conjunctiva is highly susceptible to infectious agents, of which ~80% are viral. The remaining causes are bacterial, with *Streptococcus pneumonia* and *Staphylococcus aureus* being common causes. Little is known about the innate and adaptive immune effectors in this component of the eye and whether local or systemic antibody can gain access to ocular pathogens in this tissue and mediate effective immunity. We evaluated whether opsonic antibody to the conserved surface/capsular polysaccharide, poly-N-acetyl glucosamine (PNAG) was protective against bacterial conjunctivitis. **Methods:** To induce infection, *S. aureus* or *S. pneumoniae* was injected directly into the conjunctiva of 6-7 week old A/J mice. Four, 24 and 32 hours post-infection a human IgG1 monoclonal antibody (MAb) to PNAG or control IgG1 MAb were either injected into the conjunctiva or applied topically. Forty-eight hours post-infection eyes were scored for pathology daily, using a 0 to 4 scale, evaluating palpebral and bulbar conjunctivae for erythema, edema, and exudation. After 48 h animals were euthanized and CFU/conjunctiva determined. **Results:** Both topical and injected antibody to PNAG markedly reduced bacterial levels in the conjunctivae and reduced conjunctival pathology. Histopathologic analysis of infected eyes showed severe edema and lymphocyte infiltration into eyes of mice treated with control MAb, while the conjunctiva of the anti-PNAG treated MAb showed only very mild lymphocyte infiltration. **Conclusions:** Antibody to PNAG demonstrated therapeutic efficacy in models of *S. pneumonia* and *S. aureus* conjunctivitis, and this particular set of animal experiments further validated the capacity of such antibody to protect against divergent microbial species.

Author Disclosure Block:

T.S. Zaidi: None. **T.H. Zaidi:** None. **Z. Ge:** None. **G.B. Pier:** None.

Poster Board Number:

MONDAY-603

Publishing Title:

The Effect of Coinoculaton with *Bradyrhizobium japonicum* USDA 110 and *Pseudomonas putida* NUU8 on Nitrogen and Phosphorus Availability to Soybean Growth

Author Block:

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Abstract Body:

We studied the effect of *Bradyrhizobium japonicum* USDA 110 alone and in combination with *Pseudomonas putida* NUU8 on nutrient contents of nitrogen and phosphorous uptake efficiency of soybean (*Glycine max* L.) plants grown under hydroponic conditions in a greenhouse. Plants were grown for 45 days in a greenhouse with an average temperature of 23/20^{o}C (day/night), and relative humidity of 48/83% v/v (day/night). At harvest, soybean shoots, roots and nodules were separated. Shoots, roots and nodules were oven-dried to a constant weight at 75^{o}C for 48 hours. The total nitrogen content in plant tissues was determined following the semi-micro Kjedahl procedure using a nitrogen analyzer (Kjedahl 2300; FOSS, Hoganas, Sweden). The phosphorous content was determined spectrophotometrically using the classic method. The effect of bacterial inoculation on nitrogen (N) and phosphorus (P) was determined under non saline and saline conditions. In general, the nitrogen content in soybean tended to increase in response to bacterial and salt treatments. The results showed that when soybean was inoculated with *B. japonicum* USDA 110 strain alone up to 25% of the amount of root N content, the amount of P in the root and shoot the amount of N and P content increased to 20% compared to control. When the symbiont *B. japonicum* USDA 110 was co-inoculated with *P. putida* NUU8, the nitrogen and phosphorus contents of soybean were as significantly increased compared to uninoculated. The increase in nodule P and N contents was significant for soybean grown in LN (Low Nitrogen) and LP (Low phosphorus) solutions and co-inoculated with *B. japonicum* USDA 110 and *P. putida* NUU8 compared that plants inoculated with *B. japonicum* USDA 110 alone. According to the analysis of the results obtained, giving a soybean processing plant B.japonicumUSDA110 food nutrition has improved compared to the control elements of the shadows. Especially soybean plants co-inoculated with *B. japonicum* USDA 110 and *P. putida* NUU8 strains N, P nutrients were found to be relatively high.

Author Disclosure Block:

D. Jabborova: None.

Poster Board Number:

MONDAY-604

Publishing Title:

Lactic Acid Bacteria Reduce *salmonella* Javiana-Induced Epithelial Cell Cytotoxicity and Decrease Pathogen Virulence Gene Expression

Author Block:

L. Gileau, D. Fletcher, R. Camire, S. Goudreau, K. Burkholder; Univ. of New England, Biddeford, ME

Abstract Body:

Salmonella enterica is a foodborne bacterial pathogen that causes severe gastrointestinal infections. An emerging strain of *Salmonella enterica*, *S.* enterica Javiana, has recently surfaced as a leading cause of deadly Salmonellosis, but little is known of mechanisms by which *S.* Javiana interacts with host cells, or of potential methods for ameliorating the bacterial-host interaction. Using the mucus-producing HT29-MTX human intestinal epithelial cell line, we examined the adhesion, invasion and cytotoxic effect of *S.* Javiana on host cells. We also evaluated the potential inhibitory effect of probiotic lactic acid bacteria (LAB), including *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *Lactobacillus casei*, *Lactobacillus plantarum* and *Lactobacillus mesenteroides*, on *S.* Javiana-induced infection. Using adhesion, invasion and lactate dehydrogenase release assays, we observed that *S.* Javiana exhibits modest adhesion and invasion, but has a strong cytotoxic effect on intestinal epithelial cells. When host cells were pre-treated with *L. rhamnosus*, *L. acidophilus* or *L. casei* prior to infection, *S.* Javiana-induced cytotoxicity was significantly reduced. We further sought to determine the mechanism by which the LAB decreased *S.* Javiana-induced cytotoxicity, and found that none of the LAB tested affected *S.* Javiana viability, or *S.* Javiana adhesion to or invasion of host cells. However, *S.* Javiana exposed to *L. acidophilus* and *L. rhamnosus* did exhibit decreased expression of toxin genes *pltA* and *cdtB*, as well as the gene *invA*, which facilitiates toxin secretion via a Type III Secretion System. Data suggest that *L. acidophilus* and *L. rhamnosus* might be useful if taken as prophylactics to reduce incidence or severity of *S.* Javiana Salmonellosis, and that these probiotics may work by preventing *S.* Javiana from secreting key toxins that would otherwise damage host intestinal cells.

Author Disclosure Block:

L. Gileau: None. **D. Fletcher:** None. **R. Camire:** None. **S. Goudreau:** None. **K. Burkholder:** None.

Poster Board Number:

MONDAY-605

Publishing Title:

Inflammasome Activation of Macrophages Co-Infected with *Mycobacterium tuberculosis* and HIV-1

Author Block:

M. B. Huante[1], R. J. Nusbaum[1], V. Calderon[2], P. Sutjita[1], J. K. Actor[3], J. Aronson[1], R. L. Hunter[3], J. J. Endsley[1]; [1]The Univ. of Texas Med. Branch, Galveston, TX, [2]The Univ. of Texas El Paso, El Paso, TX, [3]The Univ. of Texas Hlth.Sci. Ctr., Houston, TX

Abstract Body:

Mycobacterium tuberculosis (*M.tb*), the etiological agent of tuberculosis (TB), is the leading cause of death in people living with Human Immunodeficiency Virus (HIV). Treatment of co-infected persons is challenging due to the aggressive course of both individual diseases, and often is further complicated by drug interaction and malabsorption issues when combining antiretroviral treatment and TB chemotherapy. The molecular basis for pathogen synergy that leads to an aggressive course of TB in those with HIV-1 infection, however, is poorly defined. This is especially true at earlier stages of disease prior to the immune suppression that occurs due to $CD4^+$T cell loss. Using our newly developed humanized mouse model of TB/HIV co-infection, we find that initial acute co-infection in the lung is characterized by excessive inflammation. We observed greater pathology, inflammatory influx, and increased pro-inflammatory cytokines (e.g. IL-1β, TNFα) in co-infected HuMouse lung. Using primary human monocyte-derived macrophages (MDM) and an *in vitro* co-infection system, we further observed greater cell death and increased expression of pro-inflammatory cytokines in co-infected MDMs compared to mono-infection with either HIV-1 or *M.tb*. Analysis of cellular lysates demonstrates that increased caspase-1 activation due to HIV, and increased production of pro-IL-1β due to *M.tb*, may promote the overall increase in active IL-1β and cell death observed in co-infected MDMs. These studies suggest that convergence of pathogen pattern recognition signaling in macrophages may promote an exacerbated pro-inflammatory response and tissue damage in the *M.tb*/HIV co-infected lung.

Author Disclosure Block:

M.B. Huante: None. **R.J. Nusbaum:** None. **V. Calderon:** None. **P. Sutjita:** None. **J.K. Actor:** None. **J. Aronson:** None. **R.L. Hunter:** None. **J.J. Endsley:** None.

Poster Board Number:

MONDAY-606

Publishing Title:

Role of Systemic Inflammation in Hiv, Tb and Hiv Tb Co Infected Individuals

Author Block:

S. N. Shaukat[1], E. Eugenin[2], S. Kazmi[1]; [1]karachi uni, Karachi, Pakistan, [2]PHRI, NJ, NJ

Abstract Body:

The dysregulation of T-helper 1 and T-helper 2 immune subsets is a feature of TB/HIV co-infection. The geographic and immune overlapping effects of *M.tb* and HIV enable both pathogens to spread and synergize the pathogenesis of both organisms. *M.tb* is confined by Type 1 immune responses, mainly IFN-γ, whereas, HIV infection drives the immune shift to Type 2 responses such as IL-10 & 1L-4. Several studies have shown the predominant occurrence of signature cytokines in the context of *M.tb* and HIV infection, however, the comprehensive assessment of cytokines pool remain uncharacterized, particularly in the co-infection of *M.tb* & HIV. Therefore, in order to understand theTH1 & TH2 immune profile in the microenvironment of TB /HIV, we have performed a detailed transcriptomic analysis of various TH1 & TH2 inflammatory cytokines. Messenger RNA was obtained from PBMCs of infected individuals i.e. 31 TB patients, 44 HIV-1 Patients, 16 TB/HIV-1 co-infected individuals and 25 uninfected healthy controls. After screening the RNA quality, an appropriate amount of RNA was transcribed into cDNA. Gene arrays were customized with 84 immune markers related to TH1 & TH2 subsets. Sequentially, RT2 profiler PCR array was carried out on total of 60cDNA samples. Significantly, more than 2 fold up regulated (TH1) genes are IL-27 (3.5) & IL-12 (7.8) in monoinfection of TB & HIV respectively. In contrast, the significant down regulated genes are CXCR3 (-7.6) & CD 28 (-2.5) in TB, whereas CCR2 (-2.02) in co-infected group. In TH2 profile, the significant 2 folds down modulation was seen in IL4, IL-24 in HIV/TB group, 1L-24 in HIV group & IL-24, PTGDR2 in TB group, whereas IL-10, showed significant up-regulation(4.4) only in HIV group. Similarly, 2 folds enhancement in gene expression was also noted in CD4 Cell markers such as CTLA4 (HIV) & LAG 3 (HIV& HIV/TB) while CD40 (-1.8) was found to be under expressed only in HIV/TB group. Most notably, the combine effects of down modulation of CCR2, CD40 & over expression of LAG 3 indicate high level of immune inhibition in co-infected (HIV/TB) group. In addition, IL-27 partially, and IL24 predominantly recognized as common immune modulator in our study. Therefore, we conclude that cumulative role of IL-24 in TB, HIV and its co-infection, suggests a unique perspective to further investigate IL-24 marker as therapeutic target in the management of HIV/TB co-infection..

Author Disclosure Block:

S.N. Shaukat: None. **E. Eugenin:** None. **S. Kazmi:** None.

Poster Board Number:

MONDAY-607

Publishing Title:

Release of Invasive *Streptococcus pneumoniae* Is Stimulated by *Staphylococcus aureus* Biofilms on Human Pharyngeal Cells

Author Block:

G. L. Matzkin, F. Khan, F. Sakai, **J. E. Vidal**; Emory Univ., Atlanta, GA

Abstract Body:

Background: *Streptococcus pneumoniae* (Spn) and *Staphylococcus aureus* (Sau) colonize the human nasopharynx during early childhood forming bacterial aggregates often referred as biofilm structures. Epidemiological studies from my laboratory, and others, demonstrate a negative association between Spn and Sau when colonizing the nasopharynx of healthy children. In vitro studies have shown that planktonic cultures of Spn kills planktonic Sau cells which provided an explanation for the observed effect. However, lysis of Sau by Spn has not been characterized in colonizing (biofilms) structures. In this work colonization dynamics of mixtures of Spn and Sau, and release of planktonic (invasive) cells, was studied using life-like models. **Methods:** Biofilm mixtures containing Spn strain TIGR4 (serotype 4, vaccine type), or D39 (serotype 2, vaccine escape type), and Sau (Newman strain) were produced on polystyrene microplates, on immobilized human pharyngeal Detroit 562 cells, or in a biofilm bioreactor (biBio) mimicking conditions found in the human nasopharynx. Viable counts were obtained by dilution and plating, and confocal micrographs were obtained. **Results:** When biofilms were produced on polystyrene both Spn strains induced a reduction of Sau biofilms, 8 h post-inoculation, but only TIGR4 eradicated most Sau cells. Confocal microscopy images demonstrated that Spn D39, or TIGR4, was structurally linked to Sau cells while forming biofilm mixtures. Micrographs also confirmed reduction induced by D39 and almost complete eradication produced by TIGR4 8 h post-inoculation. On immobilized human pharyngeal cells, however, TIGR4 and D39 killed planktonic cells, as expected, but Sau biofilms were protected as no significant reduction of the Sau biomass was observed. Accordingly, experiments in the biBio showed similar Sau planktonic or biofilm cells when incubated with Spn for 8 or 24 h. Experiments revealed, however, a significant 6-fold increase (p=0.01) of released Spn planktonic cells when co-incubated with Sau (median, $3.2x10^6$ cfu/ml) in comparison to biBio chambers containing only Spn (median, $5.2x10^5$ cfu/ml). **Conclusion:** Our experiments demonstrate survival of Sau biofilms when co-incubated with Spn on human pharyngeal cells. Release of invasive Spn cells was stimulated by the presence of Sau in a life-like microenvironment.

Author Disclosure Block:

G.L. Matzkin: None. **F. Khan:** None. **F. Sakai:** None. **J.E. Vidal:** None.

Poster Board Number:

MONDAY-608

Publishing Title:

Role of Alveolar Macrophages and Inflammatory Monocytes in *Streptococcus pneumoniae* (*spn*) Super Infection during an Influenza Co-Infection

Author Block:

M. Khan, M. E. Pichichero; Rochester Gen. Hosp., Rochester, NY

Abstract Body:

Background: *Spn*-Influenza lung coinfection is a major cause of global mortality. However, pulmonary mechanisms and the immune environment correlated with protection against influenza induced *Spn* pathogenesis remains largely enigmatic.**Methods:** We developed lung mono-infection (*Spn* strain 6A or PR8 Influenza) and co-infection (PR8-Spn) mouse models to study immune events in lung leading to resolving pneumonia or non-resolving pneumonia and bacteremia. Lung immunophenotyping was done to discern the differences in immune cell recruitment during mono Spn/PR8 or co-infection and lung homogenate was used to investigate cytokines and chemokines. To understand the role of recruited monocytes on CD4 T cell proliferation, monocytes from PR8 or control mice were FACS sorted and mixed with CFSE labeled naïve CD4 T cells in the presence CD3 antibody. To understand the role of alveolar macrophages in the restoration of lung anti-bacterial immunity during co-infection, PR8 infected mice were re-constituted with alveolar macrophages (FACS sorted) before pneumococcal infection.**Results:** We found that *Spn*/influenza co-infection depleted lung alveolar macrophages and simultaneously led to a robust recruitment of inflammatory monocytes that correlated with *Spn* invasiveness and bacteremia. Accumulation of monocytes was associated with increased vascularization and lung pathology. Re-constitution of influenza infected lungs with alveolar macrophages significantly reduced *Spn* invasiveness during co-infection. Pulmonary monocytic lymphopoesis during co-infection also correlated with increased levels of chemokines CCL-1, CCL-2, CCL-3, CCL-7 and CCL-9. Compared to control (un-infected) or *Spn* infected lungs, the levels of cytokines such as IL-1β, IL-6, MCP-1 and M-CSF were higher in PR8 infected lungs and *Spn* co-infection further increased their levels. However, TNF-alpha and IL-10 were the only two cytokines that were upregulated during co-infection.**Conclusions:** We conclude that inflammatory monocytes and alveolar macrophages in lung inversely correlate with susceptibility to *Spn* super infection during an influenza co-infection and a balance of these two immune subsets in lung is indispensable for protection against influenza induced *Spn* pathogenesis.

Author Disclosure Block:

M. Khan: None. **M.E. Pichichero:** None.

Poster Board Number:

MONDAY-609

Publishing Title:

Morphological and Physiological Changes Induced by Contact-Dependent Interaction between *Candida albicans* and *Fusobacterium nucleatum*

Author Block:

B. Bor, L. Cen, M. Angello, W. Shi, **X. HE**; UCLA, Los Angeles, CA

Abstract Body:

Candida albicans and *Fusobacterium nucleatum* are two well-known oral commensal microbes with pathogenic potential that are involved in various oral polymicrobial infectious diseases. However, the biologically relevant interactions between these two clinically important microbes remain elusive. In a recent study, we demonstrated that *F. nucleatum* ATCC 23726 coaggregates with *C. albicans* SN152, a process mainly mediated by RadD and FLO9, two membrane proteins from *F. nucleatum* and *C. albicans*, respectively. This study was aimed at investigating the potential biological impact of this inter-kingdom interaction. We showed that *F. nucleatum* ATCC 23726 inhibits growth and hyphal morphogenesis of *C. albicans* SN152 in a contact-dependent manner. Further analysis revealed that the observed contact-depended inhibition of *Candida* hyphal morphogenesis is mediated via the membrane protein FLO9 in *C. albicans* and RadD in *F. nucleatum*, the same protein pair that is involved in facilitating physical attachment between these two microorganisms. Using mouse macrophage cell line as an *in vitro* model system, we demonstrated that *F. nucleatum*-induced inhibition of hyphal morphogenesis in *C. albicans* increased survival rate of *C. albicans* when exposed to RAW macrophage cells. Furthermore, *F. nucleatum* negatively impacted *C. albicans'* macrophage-killing capability; while yeast form of *C. albicans* repressed *F. nucleatum*-induced MCP-1 and TNFα production in the same macrophages. Our study suggested that by inhibiting each other's virulence, *C. albicans* and *F. nucleatum* might promote a commensal life-style to achieve long-term fitness within oral cavity. This finding could impact our understanding of inter-kingdom signaling communications and affect clinical treatment strategy.

Author Disclosure Block:

B. Bor: None. **L. Cen:** None. **M. Angello:** None. **W. Shi:** None. **X. He:** None.

Poster Board Number:

MONDAY-610

Publishing Title:

***Vibrio cholerae In Vivo* Horizontal Gene Transfer and Antagonistic Attack to Neighboring Bacteria Cells Mediated by Cell-Cell Contact During Infection**

Author Block:

Y. Fu, B. T. Ho, J. J. Mekalanos; Harvard Med. Sch., Boston, MA

Abstract Body:

Vibrio cholerae, a gram-negative pathogenic organism, is the causative agent of the severe diarrheal disease cholera. Whether the success of the current (7^{th}) pandemic El Tor O1clade is driven by its improved environmental fitness or properties related to its interaction with the human host remains a poorly answered question. We have previously used transposon mutagenesis sequencing analysis (Tn-Seq) and competition assays to investigate *V. cholerae* El Tor C6706 strain intestinal colonization in the modified infant rabbit model. Several strong pieces of evidence that *V. cholerae* cell-cell competition occur *in vivo* are provided, including phenotypes related to the Type VI Secretion System (T6SS) activation *in vivo* and the intestinal colonization deficiency exhibited by multiple metabolic mutants was exacerbated by competing these mutants against the prototrophic parental strain. Because the T6SS is thought to deliver toxic effectors to neighboring cells only through direct cell-cell contact, we investigated whether plasmid-based DNA conjugation *in vivo* might be used to indirectly measure the extent of cell-cell contact in the rabbit model. We show that cell-cell contact occurs early during infection in the distal small intestine. We also provide evidence that *V. cholerae* can deploy its T6SS to attack neighboring cells within this microenvironment very early during the infection process. Our data also revealed that *in vivo* horizontal genes transfer can also occur by cell-cell contact mediated processes such as conjugation. Finally, we have observed that there are a limited number of intestinal niches suitable for initial colonization of *V. cholerae* and another enteric bacteria, EHEC *E. coli*. The infant rabbit animal model and assays we described in this work could be potentially be used for the development of probiotics and novel anti-virulence therapeutics that can be delivered by transmissible elements *in vivo*.

Author Disclosure Block:

Y. Fu: None. **B.T. Ho:** None. **J.J. Mekalanos:** None.

Poster Board Number:

MONDAY-611

Publishing Title:

The Human Gut Microbiota Predicts Susceptibility to *Vibrio cholerae* Infection

Author Block:

F. S. Midani[1], A. A. Weil[2], F. Chowdhury[3], A. Khan[3], Y. Begum[3], R. Charles[2], S. B. Calderwood[2], E. T. Ryan[2], J. B. Harris[2], F. Qadri[3], R. Larocque[2], L. A. David[1]; [1]Duke Univ., Durham, NC, [2]Massachusetts Gen. Hosp., Boston, MA, [3]Intl. Ctr. for Diarrheal Disease Res., Bangladesh, Dhaka, Bangladesh

Abstract Body:

Susceptibility to *Vibrio cholerae* infections is known to be influenced by epidemiological factors of age, blood type, and pre-existing immunity. Yet, infection is likely also affected by microbial ecological forces, as *V. cholerae* must outcompete gut microbiota for resources and epithelial attachment sites. Here, we test whether gut microbiota composition predicts susceptibility to cholera infection within humans. We studied the gut microbiota of a cohort of 76 subjects from Dhaka, Bangladesh: 22 later developed infections and 54 remained uninfected. Using machine learning techniques, we identified a subset of operational taxonomic units (OTUs) that are predictive of human susceptibility to infection (cross-validated ROC with area of 0.72). Further analysis demonstrated that predictive bacterial taxa tend to be rare among susceptible individuals. In contrast, the gut microbiota of the unsusceptible cohort is distinguished by higher abundance of both rare and common subset of OTUs. These results suggest that cholera infection is associated with a gut microbiota that facilitates presence of rare gut bacterial taxa. This study also suggested interactions between select OTUs and *V. cholerae* that we have tested experimentally. We showed that growth of *V. cholerae* is enhanced in media conditioned with *Paracoccus aminovorans.* Finally, discriminatory OTUs may be associated with several epidemiological factors such as host diet, physiology, and immunity. These findings have implications for prophylactic measures for prevention and control of cholera infections.

Author Disclosure Block:

F.S. Midani: None. **A.A. Weil:** None. **F. Chowdhury:** None. **A. Khan:** None. **Y. Begum:** None. **R. Charles:** None. **S.B. Calderwood:** None. **E.T. Ryan:** None. **J.B. Harris:** None. **F. Qadri:** None. **R. Larocque:** None. **L.A. David:** None.

Poster Board Number:

MONDAY-612

Publishing Title:

Dynamic Interaction between Epiparasitic TM7x and Its Bacterial Host *A. odontolyticus*

Author Block:

B. Bor, X. He, L. Cen, W. Shi; UCLA, Los Angeles, CA

Abstract Body:

Recently, we characterized a unique and intimate association between two oral commensal bacterial species, a TM7 phylotype (TM7x) and *Actinomyces odontolyticus* subsp. *actinosynbacter* (XH001). TM7x is the first cultivated representative of the TM7 phylum. It is characterized as an obligate epibiotic parasite that lives on the surface of its basibiont, XH001. In the current study, the TM7x/XH001 interaction was further analyzed by investigating morphological and physiological changes during symbiotic growth. TM7x and its basibiont XH001 were cultivated under various gas and nutrient conditions to determine the optimal and stressed growth conditions. The morphological changes in TM7x and XH001 were analyzed by FISH fluorescence microscopy, phase contrast microscopy, and SEM. Stress response in XH001 induced by environmental changes as well as TM7x association was determined by targeted qRT-PCR. XH001 cells manifested as short rods in monoculture, but displayed elongated and hyphal morphology when physically associated with TM7x. These morphological changes in XH001 were also induced by depletion of oxygen in the absence of TM7x. Both the physical association with TM7x and oxygen depletion triggered up-regulation of key stress response genes in XH001. Under nutrient-replete conditions, TM7x and XH001 co-exist well with relatively uniform cell morphologies. However, upon nutrient depletion, TM7x-associated XH001 displayed a variety of cell morphologies, including swollen cell body, clubbed ends and even cell lysis; while a large portion of TM7x cells transformed from ultra-small cocci to larger filamentous cells. Our study demonstrates a highly dynamic interaction between epibiont TM7x and its basibiont XH001 in response to physical association as well as environmental cues, as reflected by their morphological and physiological changes during symbiotic growth. Our TM7x/XH001 pair provides an ideal model system to better understand this new type of inter-bacterial interaction.

Author Disclosure Block:

B. Bor: None. **X. He:** None. **L. Cen:** None. **W. Shi:** None.

Poster Board Number:

MONDAY-613

Publishing Title:

Cross-Kingdom Interactions Between *C. albicans* and *S. oralis* Mediated by Bacterial and Fungal Glucan Synthases

Author Block:

M. Bertolini[1], H. Xu[1], T. Sobue[1], A. Thompson[1], J. Mansfield[2], M. Vickerman[2], A. Dongari-Bagtzoglou[1]; [1]Univ. of Connecticut Hlth.Ctr., Farmington, CT, [2]Univ. at Buffalo, Buffalo, NY

Abstract Body:

The commensal *Streptococcus oralis* interacts with the opportunistic pathogen *Candida albicans* to form complex biofilm communities on the oroesophageal mucosa, promoting disseminated infection and exacerbating host inflammatory responses. However the mechanisms of cross-kingdom interactions which lead to pathogenic biofilms remain to be elucidated. The objective was to explore the role of streptococcal α-glucans and fungal β-glucans in early or late mixed bacterial-fungal biofilm development. For this *C. albicans* reference strain and a heterozygous deletion mutant, with reduced β-1,3 glucan biofilm matrix biosynthetic capacity (*FKS1/fks1−*, kindly provided by Dr. D. Andes), were used. A *gtfR* deletion mutant in the *S. oralis* 34 strain background, that produces no α-glucans, was constructed by allelic replacement of an internal segment of *gtfR* with an aad9 gene encoding spectinomycin resistance. A complemented strain with a plasmid-borne *gtfR* gene, was used as control. Microorganisms were grown together in biofilms on poly-L-lysine coated chamber slides for 6 or 16 hours. In adhesion assays *C. albicans* was allowed to germinate for 6 hours on chamber slides and *S. oralis* was then added for additional incubation periods of up to 2h, followed by washing. Biofilm media consisted of RPMI, 10% FBS, 10%BHI. Immunofluorescence combined with FISH staining was used to simultaneously visualize both microorganisms. We found reduced co-aggregation interactions between *C. albicans* and the *gtfR* deletion mutant, compared to the parental and complemented S. oralis strains in both early (6h) and late (16h) biofilms, especially along hyphal filaments. Adhesion of the *gtfR* mutant on *C. albicans* hyphae was also diminished, compared to parental and complemented strains. The FKS1/*fks1* heterozygous *C. albicans* deletion mutant did not aggregate with the *S. oralis gtfR* deletion mutant, the parent or the complemented strain. Our data suggested that the biosynthetic enzymes for alpha 1,3 glucans in *S. oralis* and β-1,3-glucan in *C. albicans* strongly affect cross-kingdom interactions between the two organisms and enhance the adhesive interactions and aggregation of *S. oralis* on the hyphae surface.

Author Disclosure Block:

M. Bertolini: None. **H. Xu:** None. **T. Sobue:** None. **A. Thompson:** None. **J. Mansfield:** None. **M. Vickerman:** None. **A. Dongari-Bagtzoglou:** None.

Poster Board Number:

MONDAY-614

Publishing Title:

No Evidence of Synergistic or Antagonistic Effects During Experimental Co-infection Between *Salmonella, Campylobacter* and Apec in Broiler Chickens

Author Block:

P. Wigley, Williams, N.J, Humphrey, S., Chaloner, G., Humphrey T.J., Lacharme Lora, L.; Univ. of Liverpool, Neston, United Kingdom

Abstract Body:

Background: *Campylobacter* and *Salmonella* are important foodborne zoonotic pathogens in poultry production. Recently Avian Pathogenic *Escherichia coli* (APEC) has emerged as an increasingly important pathogen of broiler chickens . A number of epidemiological studies in the UK have found an association between APEC and *Campylobacter*. Here we develop an experimental approach to determine how interactions between bacterial species in the chicken influence both disease and pathogen carriage . **Methods:** 80 Ross 308 broiler chickens were reared under biosecure conditions and divided into 8 groups of 10. At 22 days of age birds were infected by oral gavage with 5×10^6 CFU in a volume of 0.2ml in phosphate buffered saline (PBS) or mock infected with PBS alone Isolates used: *Salmonella enterica* serovar Typhimurium 4/74, *Campylobacter jejuni* M1 and an O78 Avian Pathogenic *Escherichia coli* isolate χ7122. 48h later birds were challenged with a secondary infection or mock infected . At 3d post secondary challenge, birds were killed. Heart, liver, spleen, lung and cecal samples taken aseptically. Bacterial load of *S.* Typhimurium, *C. jejuni* and APEC in these samples was determined on BG, mCCDA and EMB selective agar respectively. Birds were examined for any gross pathology and samples taken for histopathology.**Results:** Co-infection does not affect bacterial numbers in the liver, spleen or lungs. No bacteria were isolated from the heart samples. Numbers of *Salmonella* in the ceca were unaffected by APEC or *C. jejuni* co-infection, though numbers of *C. jejuni* were reduced by prior *S.* Typhimurium infection, though not statistically significant. No changes in pathology were observed in birds co-infected with two bacterial species over single infections.**Conclusions:** Experimental co-infection with two enteric bacterial species has little effect on the progression of either. Only *Salmonella* infection prior to C*ampylobacter* challenge appears to have any effect upon the numbers able to colonise the ceca, probably though a competitive exclusion effect in the same niche. Whilst field studies show an association between APEC and *C. jejuni*, there are more variables in a production environment that could impact on both host and pathogen such as stress that are absent in a controlled laboratory experiment.

Author Disclosure Block:

P. Wigley: None.

Poster Board Number:

MONDAY-615

Publishing Title:

***Streptococcus oralis* and *Candida albicans* Synergistically Activate μ-Calpain to Degrade E-cadherin from Oral Epithelial Junctions**

Author Block:

H. Xu, T. Sobue, M. Bertolini, A. Thompson, A. Dongari-Bagtzoglou; UConn Hlth.Ctr., Farmington, CT

Abstract Body:

Background: *Streptococcus oralis*, an oral commensal found in the majority of healthy humans, forms a robust biofilm with *Candida albicans*. Studies have shown that when both organisms form a biofilm on the oral mucosa, fungal invasion and PMN infiltration are exaggerated, which may be enhanced by epithelial cell junction dissolution.To investigate whether co-infection with *S. oralis* and *C. albicans* affects mucosal cell junctions and dissect related mechanisms.**Methods:**Organotypic mucosal constructs and a mouse model were used for infection with *C. albicans* and *S. oralis* 34. qPCR was performed for gene expression analysis. Immunofluorescence and FISH staining were used to visualize junction proteins and microorganisms. ELISA is to quantify soluble E-cadherin and western blots to detect E-cadherin and calpain proteins. A fluorogenic assay was performed to measure calpain activity. Data were analyzed for statistical differences using the Graph-Pad Prism® software. **Results:** Using organotypic mucosal constructs and a mouse model of co-infection, we demonstrated that in the presence of *S. oralis* 34, *C. albicans* penetrated through oral epithelial intercellular junctions, while E-cadherin protein levels were reduced. We also showed that *C. albicans* SC5314 and *S. oralis* 34 decreased E-cadherin protein levels in oral epithelial cell lysates and increased soluble E-cadherin release, which was inhibited by a calpain activity inhibitor. Similarly, in the mouse oral co-infection model, reduced E-cadherin protein levels in tongue tissues were accompanied by increased levels of μ-calpain protein, increased fungal invasion in the oral mucosa and kidney dissemination. To directly rule out involvement of fungal secreted aspartyl proteases (sap) 4, 5 and 6 in this process a *sap456* triple mutant was tested. Co-infection of *the* mutant with *S. oralis*34 increased μ-calpain protein levels in organotypic mucosal constructs and triggered fungal invasion into the mucosal layer. There were similar findings were in mice infected with the *sap456* mutant where *S. oralis* 34 triggered increased calpain expression and fungal kidney dissemination. **Conclusions:** *C. albicans* and *S. oralis* synergize to activate host enzymes that cleave tight junction proteins. This may play a role in fungal mucosal invasion and systemic dissemination.

Author Disclosure Block:

H. Xu: None. **T. Sobue:** None. **M. Bertolini:** None. **A. Thompson:** None. **A. Dongari-Bagtzoglou:** None.

Poster Board Number:

MONDAY-616

Publishing Title:

Metabolite Exchange Drives Antimicrobial Activity of *Pseudomonas aeruginosa* against *Staphylococcus aureus*

Author Block:

A. T. Nguyen, J. W. Jones, M. A. Kane, A. G. Oglesby-Sherrouse; Univ. of Maryland, Baltimore, Baltimore, MD

Abstract Body:

Pseudomonas aeruginosa is a Gram negative opportunistic pathogen that affects people with compromised immune systems, such as cystic fibrosis (CF) patients. Early infection of the CF lung with *Staphylococcus aureus* is common, while infection with *P. aeruginosa* occurs as disease progresses. This shift in microbial population is associated with increased exacerbation and a decline in pulmonary function. Emergence of *P. aeruginosa* as the dominant CF lung pathogen is likely multifactorial, and includes the production and secretion of 2-alkyl-4-(*1H*)-quinolones (AQs), which have antimicrobial activity against *S. aureus*. We recently showed that AQ-dependent antimicrobial activity against *S. aureus* is enhanced by depletion of iron, an essential and limiting nutrient in the CF lung. We hypothesize that iron-regulated antimicrobial activity against *S. aureus* allows *P. aeruginosa* to outcompete *S. aureus* in iron limiting environments. Here we show that iron-regulated antimicrobial activity is observed in numerous laboratory strains and CF isolates of *P. aeruginosa,* including strains that do not produce AQs in mono-culture. We previously found that co-culture with *S. aureus* can restore AQ production to *P. aeruginosa* strains that are deficient for AQ production in mono-culture. We show here that precursors in the AQ metabolic pathway restore AQ production to these strains in a manner similar to co-culture with *S. aureus,* suggesting a metabolic exchange between these two species in polymicrobial environments. This work demonstrates the importance of considering how virulence traits exhibited by bacteria in pure culture may be altered in polymicrobial environments that are relevant to human disease.

Author Disclosure Block:

A.T. Nguyen: None. **J.W. Jones:** None. **M.A. Kane:** None. **A.G. Oglesby-Sherrouse:** None.

Poster Board Number:

MONDAY-617

Publishing Title:

Nasopharyngeal Colonization with *Streptococcus pneumoniae* Exacerbates Pneumonia Following Influenza Virus Infection in a Mouse Model

Author Block:

S. Kimura[1], K. Mimura[1], M. A. Schaller[2], Y. Ishii[1], S. L. Kunkel[2], K. Tateda[1]; [1]Toho Univ. Sch. of Med., Tokyo, Japan, [2]Univ. of Michigan, Ann Arbor, MI

Abstract Body:

Background: Secondary bacterial infections after influenza virus are an important cause of morbidity and mortality worldwide. It is considered that these bacteria infected in lung endogenously derived from nasopharyngeal colonization with bacteria in patients. However, the most common mouse model of secondary bacterial infection has been infected with *S. pneumoniae* exogenously after influenza virus infection. In this study, we constructed and analyzed by using mouse colonized with *S. pneumoniae* in nasopharynx. **Methods:** C57BL/6J mice (Charles River Laboratories Japan) were used in this study. For colonization model with *S. pneumoniae* (ATCC 6303), a 10-µL bacterial suspension (2.0 x 10^5 CFU/mouse) was intranasally administered to anesthetized mice by slow delivery over several minutes. These mice were intranasally challenged with influenza A virus (A/PR/8/34, 10^3 PFU/mouse) 7 days after colonization. Whole lungs were homogenized in 1.0 mL of saline using a tissue homogenyizer. Nasal passages were washed with 400 µL of saline. Serial dilutions of the lung homogenates and nasal washes were plated on 5% sheep blood agar plate. Cytokine/chemokine production in the lung was evaluated by quantitative RT-PCR.**Results:** Nasally colonized mice consistently harbored 10^4 to 10^5 CFU of bacteria within the nasal cavity throughout day 28 post colonization. Under this condition, drastic decreases in survival were observed in *S. pneumoniae*-colonized mice infected with influenza A virus, whereas no mortality was observed in *S. pneumoniae*-colonized mice with saline or influenza A virus infected mice ($P<0.001$). On the 5 days post influenza A virus infection, bacteria was detected in lung (10^8 CFU) in *S. pneumoniae*-colonized mice. However, no bacterial burden was observed in lung in *S. pneumoniae*-colonized mice treated with saline instead of influenza A virus.**Conclusions:** We successfully constructed secondary pneumonia mouse model after influenza A virus infection by using mouse colonized with *S. pneumoniae* in nasopharynx. This model may provide a clinically acceptable approach for pneumonia derived from nasopharyngeal colonization with bacteria and also may be useful for evaluating mechanisms of secondary bacterial infection.

Author Disclosure Block:

S. Kimura: None. **K. Mimura:** None. **M.A. Schaller:** None. **Y. Ishii:** None. **S.L. Kunkel:** None. **K. Tateda:** None.

Poster Board Number:

MONDAY-618

Publishing Title:

Efg1 Regulates Interactions Between *C. Albicans* and *S. Oralis* in Mucosal Polymicrobial Infection Models

Author Block:

T. Sobue[1], H. Xu[1], M. Bertolini[1], A. Thompson[1], C. J. Nobile[2], A. Dongari-Bagtzoglou[1]; [1]UCONN Hlth., Farmington, CT, [2]UC Merced, Merced, CA

Abstract Body:

Background: *C. albicans* and *S. oralis* are oral commensal organisms, which form hypervirulent polymicrobial biofilms in oropharyngeal mucosal infection, characterized by increased fungal mucosal invasion and dissemination. *C. albicans* biofilm formation is controlled by 6 master transcription regulators: Bcr1, Brg1, Efg1, Tec1, Ndt80, and Rob1. The objective of this work was to identify the biofilm regulators involved in the invasive response of *C. albicans* to *S. oralis* and the downstream genes under control of this regulators. **Methods:** *C. albicans* wild type, *BCR1*, *BRG1*, *EFG1*, *TEC1*, *NDT80*, and *ROB1* deletion mutants and revertants strains were used for biofilm growth with *S. oralis*. Biofilm interactions with these strains were initially examined on plastic wells. Organotypic mucosal constructs and a mouse model of oropharyngeal infection were used to further analyze biofilm growth, *C. albicans* gene expression and fungal invasion. Data were analyzed for statistical differences using Graph-Pad Prism® software. **Results:** By screening the 6 *C. albicans* biofilm transcription regulator reporter strains in biofilms growing on plastic we identified *EFG1* as a regulator strongly activated by interaction with *S. oralis* in late biofilms. None of the biofilm regulators were activated by *S. oralis* in early biofilms. Real time qRT-PCR showed that *EFG1* gene expression was increased in polymicrobial biofilms after 24h growth on abiotic surfaces and mucosal constructs. None of the other regulators were upregulated in response to *S. oralis*. These findings were confirmed in a mixed infection model where expression of *EFG1* was increased at days 5 and 6 post-infection. The *EFG1* gene was required for the induction of a more invasive phenotype in *C. albicans* by *S. oralis*, since invasion of mucosal constructs was enhanced in the *EFG1*-revertant strain but not the *efg1* deletion mutant. We also found that *ALS1* gene expression was increased by *S. oralis* in the *EFG1*-revertant strain but not the *efg1* mutant strain in mucosal biofilms. **Conclusion:** We conclude that *S. oralis* modulates *C. albicans* virulence by increasing *EFG1* expression in late stages of biofilm growth. This, in conjunction with upregulation of downstream adhesions such as *ALS1*, is responsible for the increased fungal mucosal invasion phenotype in the presence of *S. oralis*.

Author Disclosure Block:

T. Sobue: None. **H. Xu:** None. **M. Bertolini:** None. **A. Thompson:** None. **C.J. Nobile:** None. **A. Dongari-Bagtzoglou:** None.

Poster Board Number:

MONDAY-619

Publishing Title:

Targeted Therapies are Effective at Preventing Polymicrobial Pneumonia

Author Block:

J. J. Hilliard, O. Jones-Nelson, C. Stover, B. R. Sellman; MedImmune, Gaithersburg, MD

Abstract Body:

Background: *S. aureus* (Sa) is frequently present with Gram-negative organisms in polymicrobial lung infections. The potential interplay between these opportunistic bacteria is of interest for developing strategies to prevent or treat serious drug-resistant infections. We therefore investigated the effect *S. aureus* has on infection with Gram-negative opportunists in a co-infection model. The protective capacity of pathogen specific antibodies was also explored in this context..**Methods:** : Mice were challenged intranasally with a sub-lethal dose of *Sa* along with *P. aeruginosa* (*Pa*), *A. baumannii* (*Ab*) or *K. pneumoniae* (*Kp*). Post-challenge survival and bacterial lung burdens were monitored and compared to mice infected with a single pathogen. Additionally, survival and bacterial CFU in the lungs were assessed in mice passively immunized with pathogen specific antibodies relative to an isotype control. **Results:**Intranasal co-infection with a sub-lethal dose of *Sa* with *Pa*, *Kp* or *Ab* resulted in dramatically increased proliferation ($p<0.002$) of the Gram-negative pathogen and reduced survival ($p<0.0001$) of the mice relative to infection with the individual bacteria. Although the co-infection did not increase *Sa* proliferation, it did reduce the rate of *Sa* clearance in the lung ($p<0.0001$). Additionally, prophylaxis with a pathogen-specific antibody protected from death ($p<0.0001$) and significantly reduced *Pa* ($p=0.002$), *Kp* ($p=0.005$) or *Ab* ($p=0.001$) in the lungs of co-infected mice.**Conclusions:** *Sa* and Gram-negative co-infection resulted in enhanced disease and increased proliferation of the Gram-negative pathogen in this model. Targeting *Sa* or the Gram-negative bacteria in a mixed infection afforded protection and reduced the bacterial burden of *Pa*, *Kp* and *Ab*. These findings suggest that co-colonization or co-infection with *Sa* may potentiate or increase the risk of Gram-negative pneumonia and that a pathogen-specific prophylactic or treatment approach targeting *Sa* or the Gram-negative organism may prevent or limit the severity of mixed infection.

Author Disclosure Block:

J.J. Hilliard: D. Employee; Self; MedImmune. **O. Jones-Nelson:** D. Employee; Self; Medimmune. **C. Stover:** D. Employee; Self; Medimmune. **B.R. Sellman:** D. Employee; Self; Medimmune.

Poster Board Number:

MONDAY-620

Publishing Title:

Delineating the Role of Enhanced Urease Activity in Tissue Damage and Bacteremia During Polymicrobial Urinary Tract Infection

Author Block:

C. E. Armbruster, S. N. Smith, K. Eaton, V. S. DeOrnelles, H. L. T. Mobley; Univ. of Michigan Med. Sch., Ann Arbor, MI

Abstract Body:

Catheter-associated urinary tract infections (CAUTIs) are among the most common healthcare-associated infections and are frequently polymicrobial, yet few studies experimentally address the consequences of polymicrobial infection. *Proteus mirabilis* and *Providencia stuartii* are two urease-positive uropathogens that co-colonize catheters, and infection with either can result in urinary stones (urolithiasis) and potentially bacteremia. We previously observed that coinfection in a murine model of urinary tract infection results in an increased incidence of urolithiasis, tissue damage, and bacteremia, without a corresponding increase in the total bacterial burden within the urinary tract. We also observed enhanced total urease activity during co-culture of *P. mirabilis* and *P. stuartii*, which requires production of catalytically active urease from *P. mirabilis*, but not *P. stuartii*. Our present work utilizes *P. mirabilis* and *P. stuartii* urease mutants to define the role of enhanced urease activity in the pathogenesis of polymicrobial urinary tract infection, and to unmask additional virulence factors that contribute to infection severity. Mice were transurethrally inoculated with *P. mirabilis* HI4320 and *P. stuartii* BE2467 or their respective urease mutants, and urine pH was measured at 6, 24, 48, 72, and 96 hours post infection. Mice coinfected with the parental isolates exhibited the highest pH values, mice coinfected with *P. mirabilis ureF* and the parental *P. stuartii* isolate exhibited lower urine pH values, and mice coinfected with *P. stuartii ure*$^{-}$ and the parental *P. mirabilis* isolate exhibited intermediate values, indicating that enhanced urease activity occurs during experimental coinfection and requires urease activity from *P. mirabilis* but not *P. stuartii*. Similarly, *P. stuartii* urease was not required for development of urinary stones, tissue damage, and dissemination to the bloodstream during coinfeciton. Together, these data indicate that *P. stuartii* urease is dispensable for development of the severe consequences of experimental coinfection. Ongoing work focuses on the molecular mechanism of enhanced urease activity and identifying additional virulence factors that contribute to coinfection severity.

Author Disclosure Block:

C.E. Armbruster: None. **S.N. Smith:** None. **K. Eaton:** None. **V.S. DeOrnelles:** None. **H.L.T. Mobley:** None.

Poster Board Number:

MONDAY-621

Publishing Title:

***Pseudomonas aeruginosa* Benefits from Respiratory Viral Infection in Cystic Fibrosis**

Author Block:

J. A. Melvin, M. R. Hendricks, J. M. Bomberger; Univ. of Pittsburgh, Pittsburgh, PA

Abstract Body:

Chronic *Pseudomonas aeruginosa* infection is the primary cause of morbidity and mortality in cystic fibrosis (CF) patients. *P. aeruginosa* enters a biofilm mode of growth during interaction with the CF lung, which displays vastly increased resistance to clearance by the immune system and antimicrobials. Clinical studies have found a correlation between respiratory viral infection and pulmonary colonization with *P. aeruginosa*, though the mechanism underlying this phenomenon is unknown. Using a unique model of the initial interaction of *P. aeruginosa* with respiratory epithelium, we have demonstrated that respiratory viral infection of airway epithelium stimulates rapid biofilm biogenesis by *P. aeruginosa*, providing a potential mechanism by which respiratory viral infection supports establishment of *P. aeruginosa* infection. Unexpectedly, the primary source of the biofilm stimulatory effect is extracellular vesicles secreted by the airway epithelium during viral infection. We are investigating the mechanism by which *P. aeruginosa* is able to take advantage of the pulmonary environment following viral infection, characterizing the nature of biofilms produced during viral co-infection, and defining the interaction of *P. aeruginosa* with the extracellular vesicles secreted by the airway epithelium. Preliminary data suggest that *P. aeruginosa* utilizes multiple iron uptake pathways to scavenge increased iron in apical secretions from airway epithelial cells and alters its metabolism to adapt to the conditions created by the viral co-infection. The results of these studies will aid in the development of novel therapeutic agents and treatment strategies to prevent and clear chronic *P. aeruginosa* infections in CF patients.

Author Disclosure Block:

J.A. Melvin: None. **M.R. Hendricks:** None. **J.M. Bomberger:** None.

Poster Board Number:

MONDAY-622

Publishing Title:

The Role of *S. aureus* in Establishment and Maintenance of *P. aeruginosa* Biofilms in Cystic Fibrosis

Author Block:

T. W. Beaudoin, Y. Yau, V. Waters; Hosp. for Sick Children, Toronto, ON, Canada

Abstract Body:

Background: The primary cause of morbidity and mortality amongst CF patients arises from pulmonary inflammation secondary to bacterial infections. It is well documented that *Staphylococcus aureus* (SA) is the most common pathogen seen in young children with CF, and *Pseudomonas aeruginosa* (PA) transitions to become the predominant pathogen during young adulthood. This transition is associated with PA biofilm formation and phenotypic changes. It is unclear how bacterial interactions within the CF lung microbiome can affect this transition and influence antibiotic treatment.**Hypothesis and Aims**: We hypothesize that secreted bacterial products from SA will impact the formation and maintenance of PA biofilms. This study aims to determine the effects of SA on initial PA biofilm formation and maintenance of established biofilms with regards to antimicrobial resistance and phenotypic diversity.**Methods**: Multiple SA clinical isolates (n=10) were obtained from children with cystic fibrosis that uninfected with PA. Bacterial filtrates were derived during planktonic and biofilm growth by separation of secreted material from bacterial cells. Newly acquired PA isolates were used to represent initial acquisition and interaction with SA. The effects of filtrates on PA biofilms grown in a slide chamber were measured using confocal microscopy to visualize biofilm viability and thickness and CFU counts were performed.**Results**: The presence of most SA filtrates did not impact the formation of *P. aeruginosa* biofilms grown in a slide chamber as no difference in thickness or architecture was observed. However, the presence of SA filtrates led to increased aggregation in established PA biofilms, resulting in an average of 50% reduction in the thickness of the biofilm. Interestingly, PA biofilms grown in the presence of filtrates were more resistant to tobramycin killing (60% kill at 1000g/mL in media only, 30% kill in 10% filtrate). Additionally, we have identified a filtrate of SA obtained from one patient that is able to inhibit PA biofilm formation, in fact killing PA biofilms.**Conclusions:** Clinical SA strains can impact PA biofilm growth and its resistance to antibiotics. Thus, microbial interactions play a key role in bacterial susceptibility to antibiotics and impact patient treatment.

Author Disclosure Block:

T.W. Beaudoin: None. **Y. Yau:** None. **V. Waters:** None.

Poster Board Number:

MONDAY-623

Publishing Title:

An *in Vitro* Model Of The Subgingival Microbiome

Author Block:

A. Hoare[1], P. D. Marsh[2], P. I. Diaz[1]; [1]Univ. of Connecticut Hlth.Ctr., Farmington, CT, [2]Univ. of Leeds, Leeds, United Kingdom

Abstract Body:

Background: Periodontitis is associated with shifts in microbiome composition characterized by a decrease in health-associated species and an increase in periodontitis-associated taxa, with a group known as "core species" remaining at a constant relative abundance. Considering the need to understand the nature of the interactions among these species during shifts, we developed batch and continuous culture subgingival community models under oral-like conditions, including prevalent species from the three groups. **Methods:** For the batch model, individual cultures were grown in appropriate media and normalized aliquots were inoculated in hog gastric mucin medium enriched with human serum and hemin. Community growth was monitored for 10 days by viable counts on selective media or via qPCR. Frozen inocula were used for the continuous culture model and two different chemostat protocols were tested: (i) one inoculation, dilution rate (D) of 0.1 h^{-1} (T_d= 6.7h), or (ii) three inoculations, D=0.046 h^{-1} (T_d=15h). Viable counts and qPCR were also used to monitor cultures, which reached steady-state after 15 generations. **Results:** A 6-species community formed by the health-associated and core species *Actinomyces oris*, *Veillonella parvula*, *Fusobacterium nucleatum*, *Streptococcus sanguinis*, *Rothia dentocariosa* and the periodontitis-associated *Porphyromonas gingivalis* was successfully grown in both models. In batch, health-associated and core species reached maximum biomass 1 day after inoculation. In contrast, *P. gingivalis* reached its maximum biomass at day 5 or 6. A comparison of the two chemostat protocols tested showed that health and core species were not affected by changes in D, reaching ~10^8 CFU/mL. In contrast, *P. gingivalis* yield was 10^4 cells/mL at D=0.046 h^{-1}, compared to ~10^7 cells/mL obtained at D=0.1 h^{-1}. **Conclusion:** We established a 6-species community model with some of the most prevalent subgingival species. Work is currently underway to increase the complexity of the inoculum to better resemble the diversity of the subgingival microbiome. This defined-inoculum model will allow the study of inter-species interactions and evaluation of the effect of environmental conditions on the community for a better understanding of microbiome shifts.

Author Disclosure Block:

A. Hoare: None. **P.D. Marsh:** None. **P.I. Diaz:** None.

Poster Board Number:

MONDAY-624

Publishing Title:

Amino Acid Labeling, Detection and Characterization of Respiratory Pathogens

Author Block:

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Abstract Body:

The genetic disease cystic fibrosis (CF) is characterized by chronic polymicrobial lung infections, which have been implicated as the major cause of morbidity and mortality. However, there is relatively little known about how the bacterial community within the lung is causing severe disease. Previous studies characterizing the bacteria using 16S sequencing and metabolomics have been unable to find a trend that associates microbial activity with disease state. Conventional proteomics have also been unable to yield this information as it is difficult to detect microbial-derived proteins among the background noise of host derived proteins. Furthermore, these studies reveal little about the timing of protein synthesis. To examine the link between microbes and disease, in this study we used click chemistry to label newly synthesized proteins and identify metabolically active bacterial populations within sputum derived from CF patients.Sputum was obtained from patients during routine clinic visits, and subjected to labeling via bioorthogonal noncanonical amino acid tagging (BONCAT) during 1-hour incubations. Samples were then subjected to LC-MS/MS and fluorescence microscopy to identify proteins that were synthesized and bacteria that were translationally active immediately post-expectoration. In a subset of patients, we have been able to identify actively growing bacteria by their morphological characteristics. In particular, cocci and rod shaped bacteria have been identified which we propose are the species of *Porphyromonas* and *Streptococcus*. In addition, our preliminary data obtained from mass spectrometry has identified proteins from actively growing bacteria including the 50S ribosomal protein of *Staphylococcus aureus* and a hypothetical protein found in *Veillonella* spp., and we are currently using these techniques to follow patients through the course of their disease.Our findings suggest that BONCAT could be a crucial tool in understanding chronic respiratory infections. Using this technique, we are able to examine the proteomics of the community over a relatively short period of time such that specific activities can be linked to changes in disease, and ultimately can be used to help inform therapeutic strategies.

Author Disclosure Block:

K. Binder: None. **S. Lucas:** None. **R. Hunter:** None.

Poster Board Number:

MONDAY-625

Publishing Title:

Differentiating Combinations of Microorganisms in Polymicrobial Bacteremia

Author Block:

A. Sato[1], I. Nakamura[1], T. Matsumoto[2]; [1]Tokyo Med. Univ. Hosp., Tokyo, Japan, [2]Tokyo Med. Univ., Tokyo, Japan

Abstract Body:

Background: We sometimes detect cases of polymicrobial bacteremia in blood culture. The objectives of our study were to describe the clinical characteristics of polymicrobial bacteremia and to determine which organs were affected according to the combination of bacteria.**Methods:** We retrospectively analyzed infectious diagnosis, combinations of microorganisms, and mortality of cases becoming bacteremia from January 1, 2012 to December 31, 2015 at Tokyo Medical University Hospital and Tokyo Medical University Hachioji Medical Center.**Results:** Of 44786 bottles, 5958 (13.3%) were positive and 473 (1.1%) (129 cases) were polymicrobial. One hundred and two (79.1%) patients had some sort of immunodeficiency disorder as an underling disease, such as malignancy (35.7%), diabetes mellitus (13.2%), or hematological malignancy (7.8%). Fifteen (11.6%) patients died within 7 days after the blood culture became positive, and 10 (66.7%) had Gram-positive cocci (GPCs). Among the 129 polymicrobial cases, the diagnosis of bacteremia was catheter-related bloodstream infection (CRBSI) in 34 (26.4%), complicated urinary tract infection (UTI) in 24 (18.6%), biliary tract infection (BTI) in 19 (14.8%), unknown focus in 15 (11.6%), and perforation of the digestive tract in nine (7.0%). Twenty-seven cases of CRBSI had GPCs and six cases of digestive tract perforation had anaerobic bacteria. There were 13 combinations of *Enterococcus* and Gram-negative rods (GNRs); six (46.2%) were in UTI and five (38.5%) in BTI. Of the 16 combinations containing GNRs, six (37.5%) each were in UTI and BTI. Of 10 cases containing *Candida*, 8 (80.0%) were CRBSI. **Conclusions:** Polymicrobial bacteremia was seen most frequently in patients with immunodeficiency. Combination of GPCs and *Candida* raises the possibility of CRBSI, and including GNRs in polymicrobial bacteremia, raises the possibility of UTI and BTI. It is possible to infer the diagnosis of infection by the combinations of microorganisms. Our results suggest a diagnostic aid in cases of polymicrobial bacteremia.

Author Disclosure Block:

A. Sato: None. **I. Nakamura:** None. **T. Matsumoto:** None.

Poster Board Number:

MONDAY-626

Publishing Title:

The Effects of Inhaled Aztreonam on the Cystic Fibrosis Lung Microbiome

Author Block:

A. Heirali[1], M. Workentine[1], N. Acosta[1], A. Nguyen[1], D. Storey[1], H. Rabin[1], R. Somayaji[1], F. Whelan[2], M. Surette[2], M. Parkins[1]; [1]Univ. of Calgary, Calgary, AB, Canada, [2]McMaster Univ., Hamilton, ON, Canada

Abstract Body:

Aztreonam lysine solution for inhalation (AZLI) is an inhaled antibiotic, cycled in a 28-day "on/off" fashion, used to treat chronic *Pseudomonas aeruginosa* (PA) infections in CF[1]. AZLI achieves high sputum concentrations which may extend its spectrum of activity to a wide range of organisms compared to its IV formulation [2]. We hypothesize that AZLI is active against a diverse number of organisms within the CF lung microbiome thereby exerting its clinical benefit. Accordingly, we sought to determine if the initiation of AZLI induced changes within the CF lung microbiome.The Calgary Adult CF Clinic prospectively collects and stores sputum samples from all patients. 24 patients with chronic PA who had ≥2 sputum samples collected within one year before and after initiation of AZLI were included. DNA was extracted from 162 sputum samples and the V3 region of the 16S rRNA gene was sequenced using Illumina MiSeq. Alpha-diversity measurements of species richness and evenness were determined using Shannon indices. Beta-diversity measurements were conducted using Bray-Curtis distance and visualized with principal coordinate analysis. Patients experiencing a median increase of 3% in lung function as measured by FEV_1 values after AZLI initiation were classified as responders. Differential abundance of OTU levels between responders and non-responders was calculated using the DESeq2 package in R.There was no significant difference in alpha and beta diversity measures before and after AZLI therapy indicating the microbiome was resilient to AZLI perturbation. However, changes were detected when the patients were on systemic antibacterial therapies. Patients classified as responders (14/24) had higher levels of *Pseudomonas* (p=1.0E-2), *Capnocytophaga* (p=2.3E-5), *Veillonella* (p=5.0E-3) and reduced levels of *Fusobacterium* (p=1.5E-19), *and Gemella* (p=9.0E-3) species.Significant changes in microbiome diversity were not induced following initiation of AZLI. However, certain microbiome constituents were associated with response to AZLI suggesting the microbiome may be linked to patient-treatment responsiveness. Analysis of the CF microbiome and its association with patient treatment response may enable personalization of CF chronic antibacterial therapies.

Author Disclosure Block:

A. Heirali: None. **M. Workentine:** None. **N. Acosta:** None. **A. Nguyen:** None. **D. Storey:** None. **H. Rabin:** E. Grant Investigator; Self; Gilead Sciences. **R. Somayaji:** None. **F. Whelan:**

None. **M. Surette:** E. Grant Investigator; Self; Gilead Sciences. **M. Parkins:** E. Grant Investigator; Self; Gilead Sciences.

Poster Board Number:

MONDAY-627

Publishing Title:

The *B. bronchiseptica* Type III Secretion System Does Not Negatively Affect the Protective Immunity Induced by Influenza A Virus Vaccines

Author Block:

T. L. Nicholson[1], A. L. Vincent[1], S. L. Brockmeier[1], D. S. Rajão[1], E. J. Abente[1], P. C. Gauger[2]; [1]Natl. Animal Disease Ctr., Agricultural Res. Service, USDA, Ames, IA, [2]Dept. of Vet. Diagnostic and Production Animal Med., Iowa State Univ., Ames, IA

Abstract Body:

B. bronchiseptica is a widely prevalent respiratory bacterial pathogen that infects a variety of wild and domesticated animals, including swine. Infection results in long-term colonization of the upper respiratory tract resulting in a range of clinical outcomes from asymptomatic carriage to lethal pneumonia. *B. bronchiseptica* expresses many virulence factors a type III secretion system (T3SS). The *B. bronchiseptica* T3SS is required for maximal disease severity and persistence throughout the lower swine respiratory tract. Additionally, the T3SS dampens the adaptive immune response by hindering the development of serum anti-*Bordetella* antibody levels and inducing an IL-10 response, enabling *B. bronchiseptica* to persistently colonize the respiratory tract. Influenza A virus (IAV) is an economically important zoonotic pathogen that causes an acute respiratory infection in swine and is considered a public health concern. Vaccination is the main strategy used to control and diminish disease burden. The hemagglutinin (HA) protein is the primary target of protective immune responses and the major component in swine (IAV) vaccines. Given the ability of the *B. bronchiseptica* T3SS to diminish adaptive immune responses, we hypothesized that T3SS would negatively affect the protective immunity in pigs vaccinated against IAV while concurrently colonized with *B. bronchiseptica*. To test this hypothesis, pigs were intranasally inoculated with a wild-type *B. bronchiseptica* strain or a T3SS *B. bronchiseptica* mutant. After 14 days, pigs were vaccinated against H3N2 as a live-attenuated influenza virus (LAIV) vaccine or as a whole inactivated virus (WIV) vaccine. To maximize the detection of any decrease in vaccine strain efficacy, pigs were subsequently challenged with a H3N2 strain from a separate antigenic cluster. No differences in clinical disease, virus titers, or lung lesions were observed among pigs colonized with wild-type *B. bronchiseptica* or the T3SS *B. bronchiseptica* mutant regardless of the vaccine platform used. Our results demonstrate that the *B. bronchiseptica* T3SS does not negatively affect the protective immunity induced by influenza A virus vaccines.

Author Disclosure Block:

T.L. Nicholson: None. **A.L. Vincent:** None. **S.L. Brockmeier:** None. **D.S. Rajão:** None. **E.J. Abente:** None. **P.C. Gauger:** None.

Poster Board Number:

MONDAY-628

Publishing Title:

Partners In Crime: Enhanced Urease Activity During Co-Culture Of Bacterial Uropathogens

Author Block:

A. O. Johnson[1], C. E. Armbruster[1], S. N. Smith[1], A. Yep[2], V. S. DeOrnellas[1], H. L. T. Mobley[1]; [1]Univ. of Michigan, Ann Arbor, MI, [2]California Polytechnic State Univ., San Luis Obispo, CA

Abstract Body:

Catheter associated urinary tract infections (CAUTIs) are common health-care associated infections, which in some cases, progress to sepsis. These infections are often polymicrobial, with urealytic organisms being common culprits. These organisms increase urine pH, causing the formation of painful bladder or kidney stones, and an overall more severe infection. Previous studies have indicated that the urealytic bacteria, *Proteus mirabilis* and *Providencia stuartii*, frequently co-colonize, and experimental infections indicate that co-infection increases disease severity due to enhanced urease activity. We developed an *in vitro* assay to assess urease activity of co-cultures of at least 17 isolates of these bacterial species. Clinical isolates of *P. mirabilis* and *P. stuartii* were cultured in LB medium to mid-log phase. Cultures were centrifuged and the bacteria were resuspended in filter sterilized human urine (pH 6) and incubated either separately, or in a 50:50 mixture of both species. Urease activity was assessed at 30, 60 and 90 minutes. Our data show that increased urease activity occurs with many different isolates when co-cultured. Furthermore, using urease negative mutants of both bacterial species indicates that *P. mirabilis* urease is required to enhance overall activity, whereas *P. stuartii*'s urease is not required. Co-culture of *P. mirabilis* with additional CAUTI causing species such as *Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Acinetobacter baumanii* also enhances activity, indicating that this is a broad phenomenon. Future work will assess enhanced urease activity involving co-cultures of additional species and define the mechanism responsible. As well, we will use this *in vitro* data to guide experimental infection studies. As neither *P. stuartii* nor *P. mirabilis* have "canonical" quorum sensing systems, our data may also uncover a novel form of microbe-microbe communication, in addition to increasing the understanding of mechanisms that influence the severity of polymicrobial infections.

Author Disclosure Block:

A.O. Johnson: None. **C.E. Armbruster:** None. **S.N. Smith:** None. **A. Yep:** None. **V.S. DeOrnellas:** None. **H.L.T. Mobley:** None.

Poster Board Number:

MONDAY-629

Publishing Title:

Expression Pattern of Kir3ds1/ Kir3dl1 Gene Markers in HIV-1/Tb Co-Infection

Author Block:

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Abstract Body:

Natural Killer cells are the major arm of the early host defense system . NK has been well studied, due to its ability to act spontaneously in clearing the microbial infection as well as cancerous cells. The complex pathway of regulatory genes that are involved in the modulation of NK cells, are the potential investigating spot for researchers nowadays. Recently, the members of Killer cell immunoglobulin-like receptor (KIR) family has been evaluated in context with HIV infections and overall activity of NK cells are reported to be down regulated in HIV milieu. Altered expression of NK Cells is influenced by various activating and inhibitory receptors (KIR2DLI, KIR2DL2, KIR3DL1). Since, KIR3DS1 (activating receptor) and KIR3DL1 (inhibitory receptor) have interesting attributes in HIV -1 infection, whereas the utmost complication of HIV-1 infection is likely to be a co-infection state with Tuberculosis, we invetigated the expression pattern of these selected genes(KIR3DS1/ KIR3DL1) in HIV, TB and their co-infection settings. PBMCs were isolated from 44 HIV-1 infected individuals, 31 TB patients, 16 HIV-1/TB Co-infected patients and 25 uninfected healthy controls. RNA extraction was performed from PBMCs, followed by reverse transcription process into cDNA and finally, these cDNA samples were analyzed through RT-PCR. Fold changes were calculated using ▲▲CT method. Significantly, higher expression of inhibitory receptor (KIR3DL1) was observed in co- infected patients with fold regulation 2.7 (p=0.016), whereas in case of mono infection of HIV and TB, alike pattern of up-regulation was observed with 1.7 (p=0.29) and 1.6 (0.33) folds respectively, which is low compared to co-infection. In contrast the activating marker, KIR3DS1 was dramatically found to be down-regulated, particularly in HIV mono-infection cases (-240 fold regulation, p=0.17), followed by TB (-88 fold regulation, p=0.30) and HIV/TB co-infected cases (-56 fold regulation,p=0.54). Our findings indicate that enhanced expression of inhibitory receptor (KIR3DL1) may reflect impaired functional activity of NK cells particularly in co-infected (HV-1/TB) settings. Secondly, huge differences in the expression level of KIR3DS1 in TB and HIV-1alone, further state the importance of causative agents of infections in altering the NK response.

Author Disclosure Block:

F. Nasir: None. **S.N. Shaukat:** None. **S.U. Kazmi:** None.

Poster Board Number:

MONDAY-631

Publishing Title:

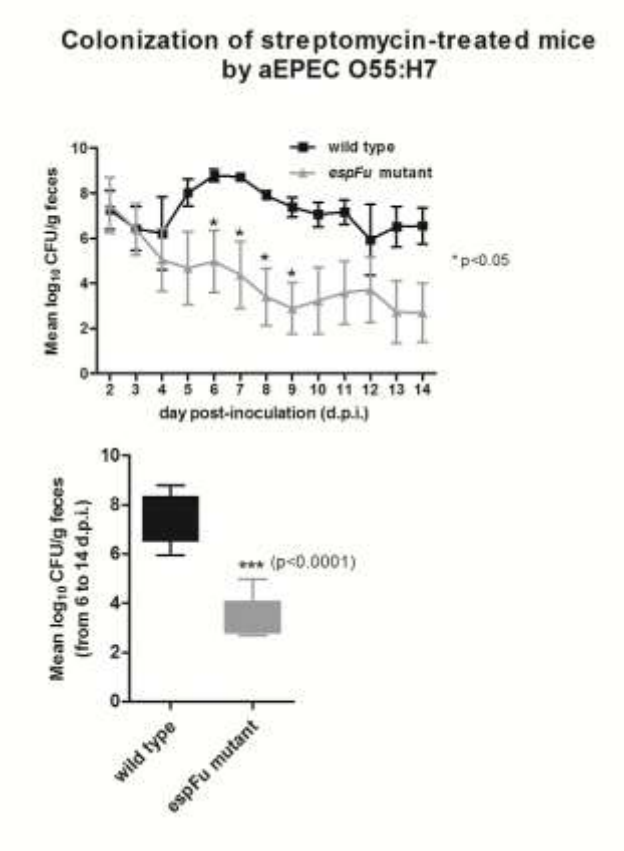
Role of Espfu in an O55:H7 Atypical Enteropathogenic *Escherichia coli* Strain

Author Block:

F. H. Martins, C. M. Abe, W. P. Elias; Inst. Butantan, Sao Paulo, Brazil

Abstract Body:

Atypical enteropathogenic *Escherichia coli* (aEPEC) is amongst the major etiological agent of diarrhea worldwide. The hallmark of aEPEC pathogenesis is the ability to induce attaching and effacing (A/E) lesions on intestinal epithelium. The proteins involved in A/E phenotype are encoded by a chromosomal pathogenicity island called locus of enterocyte effacement (LEE). In addition, non-LEE encoded proteins as EspFu may contribute to aEPEC pathogenesis, although this has not been extensively investigated. Thus, the aim of this study was to assess the role played by EspFu on aEPEC pathogenesis. For this purpose, an aEPEC strain (serotype O55:H7) and its isogenic *espFu* mutant were compared regarding the motility, interaction to cultured epithelial cells and intestinal colonization in a murine model. The deletion of the *espFu* gene did not affect the motility and adherence of aEPEC O55:H7, but impaired the ability to induce actin polymerization and pedestal formation on HeLa cells. Complementation with an EspFu-Myc-producing plasmid restored actin polymerization activity, indicating that the ability of aEPEC O55:H7 to promote A/E lesions is dependent of EspFu. Both wild type and *espFu* mutant strains established colonization in the intestine of streptomycin-treated mice. However, wild type strain showed higher efficiency than *espFu* mutant on colonization from 6 to 14 days post inoculation ($p<0.0001$), suggesting that EspFu is involved on the latest stages of this process. Together, these data suggest that EspFu protein contributes to relevant aspects of the aEPEC O55:H7 pathogenesis such as pedestal formation and late-stage intestinal colonization.



Author Disclosure Block:

F.H. Martins: None. **C.M. Abe:** None. **W.P. Elias:** None.

Poster Board Number:

MONDAY-632

Publishing Title:

Induction and Signaling of the Immuno-regulatory Receptor B7-H1 in Oral Epithelial Cells

Author Block:

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Abstract Body:

Background: Up-regulation of the immuno-regulatory receptor B7-H1 on host cells may contribute to the deterioration of immune response which may promote the development of human cancers. Inflammation induces this receptor on normal epithelial cells that negatively regulate T-cell responses while tumor-associated expression of B7-H1 is associated with mechanisms of immune escape. *P. gingivalis* is known as one of the keystone pathogens in chronic periodontal infections and exhibits various virulence factors. The aim of this study was to investigate if bacterial protein fractions, e.g. membrane proteins, of *P. gingivalis* up-regulate B7-H1 on oral squamous cell carcinoma cells and primary human gingival keratinocytes and which signaling pathways may be involved. **Methods:** Squamous cell carcinoma cells (SCC-25) and primary human gingival keratinocytes (PHGK) were seeded (1 x 10^6 cells) in cell culture wells and infected with *P. gingivalis* W83 total, inner and outer membrane fractions, cytosolic proteins, as well as LPS and peptidoglycans. After 48h cells were harvested and prepared for Western blot and immuno-staining analysis. Up-regulation of B7-H1 RNA was analyzed using quantitative real time PCR.**Results:** It was demonstrated that the total membrane fraction induced the highest up-regulation in B7-H1 expression, 7-fold in SCC-25 cells and 3-fold in PHGK after 48h ($p<0.05$). The outer and inner membrane caused 3-fold induction in SCC-25 cells whereas cytosolic proteins and LPS caused no regulation of B7-H1. PD98059, an inhibitor of ERK, attenuated the *P. gingivalis* membrane induced B7-H1 expression. B7-H1 RNA in SCC-25 cells was up-regulated 10.3 fold by the total membrane fraction ($p < 0.01$), 7.5 fold by the inner membrane fraction and 6.5 fold by the outer membrane fraction ($p < 0.05$). In PHGK RNA was up-regulated 7.9 fold by the total membrane fraction ($p < 0.01$), 10.1 fold by the inner membrane (IM) and 5.3 fold by the outer membrane ($p < 0.05$).**Conclusions:** The results of this study demonstrate that the membrane fraction of *P. gingivalis* is responsible for up-regulation of B7-H1 in oral epithelial cells which may help to elucidate the possible role of the immunoregulatory B7-H1 in the immune evasion of this pathogen.This study was supported by a grant of the von Behring-Röntgen-Foundation

Author Disclosure Block:

F. Jarzina: None. **S. Gröger:** None. **U. Mamat:** None. **J. Meyle:** None.

Poster Board Number:

MONDAY-633

Publishing Title:

Neurological Tick-Borne Lyme Disease: Investigating the Role of a *Borrelia burgdorferi* Gene Important for Invasion of the Blood Brain Barrier

Author Block:

L. Hritzo[1], M. Lichay[2], A. Smith[3], X. Yang[3], V. Culotta[4], U. Pal[3], J. Dumler[5], D. Grab[2]; [1]Wheeling Jesuit Univ./Johns Hopkins SOM, Wheeling, WV, [2]Johns Hopkins SOM, Baltimore, MD, [3]Univ. of Maryland, College Park, MD, [4]Johns Hopkins Univ. Bloomberg Sch. of Publ. Hlth., Baltimore, MD, [5]Univ. of Maryland, Baltimore, MD

Abstract Body:

Lyme disease, caused by the spirochete *Borrelia burgdorferi* (*Bb*) that is transmitted by *Ixodes* species ticks, is the most common vector-borne illness in the United States and Europe. Newly revised estimates from the Center for Disease Control (CDC) suggest that there are likely to be over 300,000 new cases per year in the United States. The infection can be difficult to diagnose and a substantial fraction of untreated patients develop neurological complications known as neuroborreliosis, although how the spirochetes invade the brain and central nervous system remains enigmatic.Using an in-vitro model of the human blood brain barrier (BBB) of immortalized human brain endothelial cells (HBMEC), we compared gene expression of *Bb* both before and after their transmigration across the BBB. Of 92 *Bb* genes known to be expressed when interacting with mammalian cells, one gene, *lmp1*, displayed an average 41-fold increase in gene expression among the transmigrated spirochetes vs. those that did not.To confirm *lmp1's* role, wild type (WT) *Bb* (B31-A3 clonal strain), Δ*lmp1* mutants (KO) and in *Bb* KO in which *lmp1* was complemented, were compared for their ability to transmigrate across confluent HBMEC monolayers grown on Transwell inserts. Dark-field microscopy and quantitative polymerase chain reaction (qPCR) was used to assess spirochete number and viability after transmigration. Compared to WT, the Δ*lmp1 Bb* mutants showed a decreased HBMEC crossing phenotype, while complementing *lmp1* to the *Bb* Δ*lmp1* strain restored the WT *Bb* BBB crossing phenotype. In conclusion, these studies suggest a role for *Bb lmp1* as a virulence factor in neuroborreliosis.

Author Disclosure Block:

L. Hritzo: None. **M. Lichay:** None. **A. Smith:** None. **X. Yang:** None. **V. Culotta:** None. **U. Pal:** None. **J. Dumler:** None. **D. Grab:** None.

Poster Board Number:

MONDAY-634

Publishing Title:

Chlamydial Infection Alters Mhc Class I Antigen Presentation in a Human Lymphoblastoid Cell Line

Author Block:

A. Palmer, E. Cram, D. Rockey, B. Dolan; Oregon State Univ., Corvallis, OR

Abstract Body:

Incidences of chlamydial infection are increasing and although the current method of treatment is still effective, cases of tolerant infections are ongoing. As an obligate intracellular microbe, Chlamydia relies on host cell processes to complete its life cycle. Additionally, Chlamydia must evade the adaptive immune response of the host, specifically the cytotoxic $CD8^+$ T cell responses which eliminate cells infected with intracellular pathogens. The ability for Chlamydia to alter host MHC I antigen presentation is important for Chlamydia immune evasion, but the mechanism remains unknown. In this study, we investigated the ability of different species of Chlamydia to alter the presentation of self-peptides derived from a model protein. Additionally, we investigated how Chlamydia infection altered levels of poly-ubiquitinated proteins. An increase in peptide presentation was observed following infection, but interestingly, this increase in presentation only occurred when the peptide source was limited to host-derived Defective Ribosmal Products or DRiPs. Western blot analysis determined that poly-ubiquitin protein levels differed between mock and bacterial infection as well as levels of ubiquitin-like protein NEDD8 demonstrating perturbations to the ubiquitin proteasome system, an essential pathway for generating antigenic peptides coupled to MHC I molecules. The capability to alter host antigen presentation can potentially help Chlamydia subvert the immune system lending to the idea of a mechanism for tolerance. Ongoing experiments will identify candidate gene products secreted by Chlamydia during infection that can affect this host system in an effort to further understand chlamydial disease progression.

Author Disclosure Block:

A. Palmer: None. **E. Cram:** None. **D. Rockey:** None. **B. Dolan:** None.

Poster Board Number:

MONDAY-635

Publishing Title:

The Leptospiral Adhesion Lsa23 Inhibits the Lytic Activity of the Human Alternative & Its Classical Complement Pathways

Author Block:

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Abstract Body:

Background: Pathogenic *Leptospira* are the etiological agent of leptospirosis, a disease of human and veterinary concern. It has been previously described that pathogenic *Leptospira* are resistant to normal human serum (NHS) due to their ability to evade the complement immune system by interacting with factor H (FH) and C4- binding protein (C4BP) regulators. Moreover, plasmin generating on leptospiral surface diminishes C3b and IgG deposition, decreasing opsonophagocytosis by immune competent cells. We have earlier reported that Lsa23 (LIC11360) is capable of binding purified human FH, C4BP and plasminogen (PLG)/plasmin in the presence of PLG activators. We decided to assess the consequences of Lsa23 on the activity of complement system **Methods:** The effect of Lsa23 on classical pathway of complement was assessed by hemolytic assay using antibody-coated sheep erythrocytes, while the effect on alternative pathway was evaluated measuring the deposition of C5b9 on zymosan. Interaction with factor H (FH), C4BP and plasminogen was evaluated by ELISA. Cofactor activity of complement regulators and proteolytic activity of plasmin were assessed by immunoblotting. **Results:** We show that Lsa23 has the ability to acquire FH, C4BP and PLG from NHS. The binding with the complement regulators FH and C4BP preserve factor I (FI) activity, leading to C3b and C4b degradation products, respectively. C3b and C4b alpha chains cleavage were also observed when Lsa23 bound to PLG generate plasmin, an effect blocked by the protease inhibitor aprotinin. Lsa23 also inhibited lytic activity by NHS mediated by both classical and alternative pathways. Furthermore, NHS-treated with Lsa23 confers partial serum resistance phenotype to *L. biflexa*, whereas blocking this protein with anti-Lsa23 renders pathogenic *L. interrogans* more susceptible to complement mediated-killing. **Conclusions:** The reduction of C3b and C4b amount might obstruct MAC formation by classical and alternative pathways, as observed by hemolytic inhibition and decrease C5b-9 deposition, respectively. The complement-inhibitory activity mediated by plasmin bound to Lsa23 leading to C3b and C4b degradation offers an interesting strategy to understand how this resourceful pathogen uses PLG/plasmin to evade the host immune system.

Author Disclosure Block:

A.L. Nascimento: None. **G. Siqueira:** None. **S. Vasconcellos:** None.

Poster Board Number:

MONDAY-636

Publishing Title:

Two Lytic Transglycosylases in *Neisseria gonorrhoeae* Impart Resistance to Killing by Lysozyme and Human Neutrophils

Author Block:

S. Ragland[1], R. Schaub[2], K. Hackett[2], J. Dillard[2], A. Criss[1]; [1]Univ. of Virginia, Charlottesville, VA, [2]Univ. of Wisconsin-Madison, Madison, WI

Abstract Body:

Symptomatic infection by the obligate human pathogen *Neisseria gonorrhoeae* (Gc) induces a potent inflammatory response involving a neutrophil-rich exudate. Although neutrophils readily detect and kill microbes, a population of Gc resists neutrophil killing. Gc differs from most Gram-negative bacteria by releasing extracellular monomeric peptidoglycan (PG), which is dependent on the activity of two nonessential and nonredundant lytic transglycosylases (LTs), LtgA and LtgD. PG released by LtgA and LtgD is a pathogen-associated molecular pattern that stimulates immune responses, but LTs in other bacteria can contribute to envelope integrity. To test how PG turnover and monomer release affects Gc survival from neutrophils, we infected interleukin 8-treated, adherent primary human neutrophils with *ltgA ltgD* mutant Gc. In the presence of neutrophils, *ltgA ltgD* was significantly decreased in survival compared with the parent or double complement. Resistance to neutrophil killing required LT activity, but addition of PG monomer failed to rescue the survival of *ltgA ltgD*, indicating that LtgA and LtgD protect Gc from neutrophil killing independently of monomer release. We found two reasons for increased sensitivity of *ltgA ltgD* to neutrophils. First, *ltgA ltgD* was more sensitive to two neutrophil antimicrobial proteins, lysozyme and neutrophil elastase (NE). Lysozyme sensitivity was linked to decreased envelope integrity, shown by increased permeability to propidium iodide, increased extracellular ATP, and increased vancomycin sensitivity. Second, neutrophils exposed to *ltgA ltgD* had increased secondary and primary granule exocytosis by flow cytometry and increased secondary granule fusion to the Gc-containing phagosome by immunofluorescence. Notably, secondary granules contain lysozyme, and primary granules contain lysozyme and NE. We conclude that LtgA and LtgD protect Gc from lysozyme and neutrophils by maintaining membrane integrity and limiting neutrophil activation that could increase bacterial exposure to antimicrobials. This work underscores the importance of envelope integrity in Gc as a means to avoid neutrophil killing and adds to a growing literature linking bacterial degradation by lysozyme to increased immune activation.

Author Disclosure Block:

S. Ragland: None. **R. Schaub:** None. **K. Hackett:** None. **J. Dillard:** None. **A. Criss:** None.

Poster Board Number:

MONDAY-637

Publishing Title:

Immune System Modification by *Salmonella typhimurium*

Author Block:

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Abstract Body:

Background: Intracellular bacterial pathogens are capable of causing a diverse array of diseases in humans and represent a significant threat to global health. These pathogens have evolved sophisticated strategies to interfere with host cell functions and to perturb immune responses. Of major importance, are pathogen proteins termed virulence factors, which manipulate key molecular checkpoints and signaling pathways in host cells and provide the pathogen with a tailored advantage for survival within the host, making them a desirable area for investigation. Furthermore, the increased emergence of acquired antibiotic resistant strains of pathogenic bacteria has greatly increased the demand for the discovery of alternative drug targets.**Methods:** Here, we investigated immune system modifications by the gram negative, pathogenic bacteria, *Salmonella typhimurium*, in macrophage host cells. To provide a comprehensive view of the pathogen and host prior to infection, we performed intracellular proteome and secretome profiling of the *S. typhimurium* wild-type (SL1344) and type 3 secretion system (T3SS) mutant (dspi-1 and dspi-2) strains, and proteome profiling of the host macrophage cells. Next, we performed temporal proteomic profiling of the macrophage cells following co-culturing with *S. typhimurium* at 0.5, 1.0, 2.0, 6.0, and 24.0 hours post infection.**Results:** Bioinformatic analyses for post-translational modifications showed changes to host proteins and signal transduction pathways during infection. Our approach highlighted modifications of known host cell targets of the *S. typhimurium* T3SS, in addition to the identification of novel host cell targets. Furthermore, we report the first comprehensive proteomic profiling of the *S. typhimurium* wild-type and T3SS mutant strains, emphasizing the extensive role of the T3SS in the release of virulence factors and subsequent host infection.**Conclusion:** Overall, proteomic profiling of the host immune system during infection identified important pathogen-related modifications associated with the host defense response and highlighted an opportunity for novel target discoveries and for the development of innovative therapeutic agents to combat infection.

Author Disclosure Block:

J. Geddes-McAlister: None. **M. Mann:** None. **F. Meissner:** None.

Poster Board Number:

MONDAY-638

Publishing Title:

Molecular Interactions of Bacterial Tir Domain Proteins Tcpb and Tcpc with Human Adapter Proteins Tirap and Myd88

Author Block:

G. Snyder; Univ. of Maryland, Baltimore, Baltimore, MD

Abstract Body:

Microbial pathogens have evolved mechanisms to regulate and evade host responses. One mechanism involves the subversion of innate immune Toll/IL-1 receptor (TIR) signaling proteins by bacterial TIR mimics. Bacterial TIRs are thought to function by direct disruption of host TIR signaling pathways. For example, the TIR proteins TcpB from *Brucella and* TcpC from uropathogenic *E. coli* inhibit TIR signaling through direct interaction with host adapter TIR proteins TLR4, TIRAP and MyD88. We have previously reported the crystal structure of human MyD88 and characterized its interactions with TcpC for developing TcpC peptides capable to inhibit TLR4 and MyD88 signaling. We now report the X-ray crystal structure of *Brucella* TIR protein TcpB and characterize its interactions with TIRAP using hydrogen/deuterium (H/D) exchange mass spectrometry. The crystal structure of the TcpB reveals the BB loop microtubule-binding site as well as a symmetrical dimer involving the DD and EE loops. This interface is identifies a set of candidate TcpB blocking peptides. A comparison between the microbial TcpB, TIRAP and MyD88 crystal structures reveal differences in the region that encompasses the BB loop. These findings lend insight into the molecular mechanisms of microbial mimicry of human host signaling adapter proteins and provide a framework for identification and development of novel microbial based therapeutics.

Author Disclosure Block:

G. Snyder: None.

Poster Board Number:

MONDAY-639

Publishing Title:

Identification of *Klebsiella pneumoniae* Virulence Factors Necessary to Withstand Interactions with the Innate Immune System in the Lungs Using Tnseq

Author Block:

M. Paczosa, J. Mecsas; Tufts Univ., Boston, MA

Abstract Body:

K. pneumoniae is a leading cause of nosocomial diseases both in the USA and internationally, and causes a range of infections, including pneumonias, sepsis and UTIs. In order to identify virulence factors that *K. pneumoniae* uses to successfully infect the lungs of healthy hosts, as well as those needed to protect against neutrophils, we used TnSeq. Specifically, we screened an arrayed K. pneumoniae library consisting of ~13,000 transposon mutants through a lung infection model in both immunocompetent and neutrophil-depleted mice, and then used deep sequencing to quantify and compare the frequency of each mutant before and after infection of wildtype and neutropenic mice. Using this approach, we identified 151 genes required by K. pneumoniae to cause pneumonia in wildtype mice. Of these, 86 genes were specifically required to promote infection by *K. pneumoniae* in the presence of neutrophils. In order to confirm a number of these hits, we retrieved ~60 *K. pneumoniae* transposon insertions mutants from our ordered array and evaluated their fitness in this lung infection model. We also used this smaller library of transposon mutants to evaluate whether there is a role for these genes in *K. pneumoniae* growth in nutrient poor environments, protection against neutrophil effectors, such as hydrogen peroxide and nitric oxide, and non-specific immune effectors, such as complement. In summary, we found several distinct subsets of genes, where some genes are critical for *K. pneumoniae* infection regardless of the immune status of the host and others are critical for responding to specific bactericidal effectors of innate immunity.

Author Disclosure Block:

M. Paczosa: None. **J. Mecsas:** None.

Poster Board Number:

MONDAY-640

Publishing Title:

Molecular Analysis of the Interaction of Leptospiral LigB and Tropoelastin

Author Block:

C-L. Hsieh, C. Ptak, E. Chang, R. Oswald, **Y. Chang**; Coll. of Veterianry medicine, Cornell Univ., Ithaca, NY 14850, Ithaca, NY

Abstract Body:

Leptospira deploy surface adhesins for host binding, the first step of bacterial infection and colonization. LigB, a surface adhesin, mediates leptospiral attachment to the host by binding to a number of extracellular matrix (ECM) components such as fibronectin and elastin. LigB contains 12 Ig-like domains arranged like a string of beads with certain flexibility extending from the pathogenic bacterial surface. Tropoelastin, the building block of elastin, confers resilience and elasticity to lung, blood vessels, uterus and other tissues. It has been shown that the terminal LigB Ig-like domain (LigB12) interacts with human tropoelastin (HTE). The ability of LigB12 to interact with HTE is likely to promote Leptospira adhesion to lung tissue, the main cause of pulmonary hemorrhage in cases of severe leptospirosis.The aim of this study is to develop a comprehensive understanding of the molecular details that contribute to the LigB12-HTE binding interface. Using truncated HTE constructs, we fine-mapped the LigB12 binding site on HTE by ELISA and characterized the binding kinetics of the LigB12-HTE interaction by surface plasmon resonance (SPR). A LigB12 binding site on HTE with a sub-micromolar binding affinity was identified on the N-terminal region of the 20th exon of HTE (HTE20N). Alanine mutants of specific basic and aromatic residues on HTE20N significantly reduced binding to the LigB12. The conformations of complexes of LigB12 with various HTE constructs were investigated using both small-angle X-ray scattering (SAXS) and nuclear magnetic resonance (NMR) spectroscopy. With the high-resolution solution NMR structure of LigB12 in hand, we plan to use NMR-based chemical shift perturbation experiments to further study the LigB12-HTE20N complex. We have begun to decipher the molecular details of the LigB12-HTE20N binding interface, which should expand our understanding of leptospiral pathogenesis and also assist in the future design of alternative therapeutics for leptospirosis.

Author Disclosure Block:

C. Hsieh: None. **C. Ptak:** None. **E. Chang:** None. **R. Oswald:** None. **Y. Chang:** None.

Poster Board Number:

MONDAY-641

Publishing Title:

HCV Proteins Modulate TLR8 Mediated NF-κB Signaling in THP-1 Cells

Author Block:

T-M. Lin[1], C-H. Huang[2], Y-Y. Hsu[3], H-L. Eng[3]; [1]E-DA Hosp./I-shou Univ., Kaohsiung, Taiwan, [2]Natl. Cheng Kung Univ., Tainan, Taiwan, [3]Kaohsiung Chang Gung Mem. Hosp. and Chang Gung Univ. Coll. of Med., Kaohsiung, Taiwan

Abstract Body:

Hepatitis C virus (HCV) infection is a worldwide threat to public health. Monocytic cells, key players in the immune response to infection, represent one possible site of HCV replication. Toll-like receptors (TLRs) play pivotal roles in the innate immune system, particularly, TLR8 that senses HCV ssRNA to initiate the production of a variety of nuclear factor (NF)-κB-mediated cytokines in monocytes. In our preliminary study, we demonstrated that monocytes of HCV infection patients produced higher TLR8 mRNA expression and increased four-fold IL-12p40 mRNA in response to CL075 stimulation. HCV might thus modulate TLR8 signaling in monocytes to induce inflammation and enhance liver damage. In the present study, the modulatory effects of HCV proteins on TLR8 mediated NF-kB signaling in THP-1 cells were investigated. Enhanced NF-κB activation was observed by HCV core protein, but suppressed by HCV E2 protein (115±17.5%, p=0.03 and 77±9.4%, p< 0.001; respectively) in THP1-XBlue cells (a human TLR reporter monocytic cell line expressing an NF-κB-inducible secreted embryonic alkaline phosphatase reporter gene), that transfected with plasmids of HCV proteins. However, after stimulation of the transfected cells with TLR8 agonists, 5 μM CL075, both HCV E1 and E2 proteins were able to enhance TLR8 induced NF-κB signaling (125±5.9%, p=0.002 and 131±9%, p=0.004; respectively). In contrast, TLR8 induced NF-κB signaling was down-regulated by HCV core, NS2 and NS4 proteins to 70±3.3%, 62±10.7%% and 68±10.5%, respectively (p< 0.001). Our results indicate that HCV proteins modulate TLR8 mediated NF-κB signaling in monocytes during HCV infection.

Author Disclosure Block:

T. Lin: None. **C. Huang:** None. **Y. Hsu:** None. **H. Eng:** None.

Poster Board Number:

MONDAY-642

Publishing Title:

***Histophilus somni* Survives Within Bovine Macrophages Through Inhibition of Lysosome-Phagosome Fusion**

Author Block:

T. J. Inzana[1], Y. Pan[1], A. Champion[1], Y. Tagawa[2]; [1]Virginia Tech, Blacksburg, VA, [2]Natl. Inst. of Animal Hlth., NARO, Tsukuba, Japan

Abstract Body:

Background: *Histophilus somni* is a gram-negative bacterial pathogen of cattle that can survive within phagocytic cells. The fic motif within the IbpA surface protein is toxic for phagocytes, but it is not clear whether *H. somni* persists within phagocytic cells through an evasive mechanism or due to cell toxicity. Mutants with deletions in *ibpA* and following intracellular trafficking were used to determine the mechanism of *H. somni* intracellular survival.**Methods:** Disease and commensal isolates, and strains with mutations within *ibpA* were used with bovine peripheral blood monocytes and macrophage cell line BM to determine intracellular survival and trafficking following incubation of bacteria with phagocytic cells. Intracellular trafficking of bacteria was determined using confocal microscopy and Alexa Fluor 488 or 546-labelled monoclonal antibodies to early phagosome and late lysosomal markers. Phagosome acidification was determined using Lysotracker.Results: Incubation of *H. somni* strain 2336, and other IbpA-positive strains¸ with BM cells caused the cells to round up, but the bacteria survived within these cells for at least 72 h. In contrast, commensal strain 129Pt, and other IbpA-negative strains, were not toxic for macrophages and did not survive 24 h within BM cells. Transposon and allelic exchange mutants of strain 2336 containing mutations in *ibpA* outside the fic motif remained toxic for BM cells and survived intracellularly. However, mutants with the entire *ibpA* gene, or only the fic motif, deleted were no longer toxic for BM cells, but still survived intracellularly as well as the parent. Early phagosomal marker EEA-1 co-localized with both strains 2336 and 129Pt. However, strain 2336 prevented acidification of phagosomes and did not co-localize with late lysosomal marker LAMP-2 ($P<0.05$).**Conclusions:** *H. somni* strains expressing IbpA containing the fic motif were toxic to bovine macrophages and survived within these cells. Deletion of the fic motif from IbpA eliminated toxicity, but these mutants remained capable of surviving within macrophages. The failure of strain 2336 to co-localize with LAMP-2 and lack of phagosome acidification suggests that intracellular survival was in part due to prevention of phagosome-lysosome fusion, and that *H. somni* may be a permissive intracellular pathogen.

Author Disclosure Block:

T.J. Inzana: None. **Y. Pan:** None. **A. Champion:** None. **Y. Tagawa:** None.

Poster Board Number:

MONDAY-643

Publishing Title:

Recombinant Variant of *Fasciola hepatica* Fatty Acid Binding Protein Impairs the NF-κB Activation in Response to Toll-Like Receptors Agonists

Author Block:

M. J. Ramos-Benítez, C. Ruiz-Jimenez, A. M. Espino; Univ. of Puerto Rico - Med. Sci. Campus, San Juan, Puerto Rico

Abstract Body:

Background: In recent years, it has been demonstrated that a strong Th2 immune response is able to suppress the inflammatory type or Th1 response; this Th1 response is linked to septic shock and some autoimmune diseases. *Fasciola hepatica* modulates the host immune system by promoting a Th2 response during chronic infections, hence the production of anti-inflammatory and regulatory cytokines (1). Our research group demonstrated that fatty acid biding protein (FABP) purified in native form (Fh12) from *F. hepatica* adult worm impairs the inflammatory cytokines storm from macrophages typically induced by exposure to Lipopolysaccharides (LPS) (2). The current work aimed to determine whether a recombinant variant of FABP could have a similar anti-inflammatory effect than Fh12. **Methods:** We optimized the expression in *Escherichia Coli* of an isoform of FABP named Fh15. We also performed western blot analysis to confirm an immunological similarity between Fh12 and Fh15. Human monocytes cell line THP1-CD14 treated with Fh15 were stimulated separately with specific activation ligands for TLR 2, 4, 5 and 8 to determine the capacity of Fh15 to block the NF-κB activation in comparison to Fh12. We used Quanti-Blue medium to detect the inhibition. **Results:** Fh15 was purified with a high degree of purity. Western blot analysis using an anti-Fh12 antibody demonstrated that both proteins are immunologically similar. Also, that Fh15 was able to significantly block the NF-κB activation induced by specific TLR ligands in a dose dependent manner up to 95%. **Conclusions:** The results indicate that Fh15 exhibits similar capacity as Fh12 to suppress the NF-κB activation. The use of bacterial expression system lead us to conclude that the potential glycosylation sites described for Fh12 are not required for the effect. Based on these results we can anticipate that Fh15 has the potential to exhibit a strong capability of suppressing inflammatory cytokines, as proved for Fh12. Also, both proteins impairing the NF-κB activation induced by different TLR-ligands suggest a broad spectrum of action. Studies are in progress to elucidate the mechanisms for this effect and examine the potential of Fh12 and Fh15 as anti-inflammatory molecules in animal models for experimental septic shock and autoimmune diseases.

Author Disclosure Block:

M.J. Ramos-Benítez: None. **C. Ruiz-Jimenez:** None. **A.M. Espino:** None.

Poster Board Number:

MONDAY-644

Publishing Title:

Degradation of Incretin by the Prokaryotic Dipeptidyl-Peptidase 4 from *Porphyromonas gingivalis*

Author Block:

Y. Shimoyama[1], Y. Ohara-Nemoto[2], T. K. Nemoto[2], M. Nakasato[1], T. Yaegashi[1], T. Ishikawa[1], M. Sasaki[1], S. Kimura[1]; [1]Iwate Med. Univ. Sch. Dent., Morioka, Japan, [2]Nagasaki Univ., Nagasaki, Japan

Abstract Body:

The incretins, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), are the major regulators of post-prandial insulin secretion, which are rapidly degraded to inactive forms with the cleavage of Ala^2-Glu^3 bond by dipeptidyl-peptidase 4 (DPP4). Recent studies demonstrated that *Porphyromonas gingivalis*, one of the major causative organisms of chronic periodontitis in human, possesses various exopeptidases such as four DPPs (DPP4, DPP 5, DPP 7 and DPP 11) to incorporate nutritional amino acids as di- and tri-peptides. Since the prokaryotic DPP4 is a structural homologue to mammalian DPP4, it is possible that *P. gingivalis* infection could lead to the onset/progression of type 2 diabetes mellitus through the degradation of incretins by *P. gingivalis* DPP4 (PgDPP4). In this study, we assessed the degradation of GLP-1 and GIP by PgDPP4 using bacterial whole cells (*P. gingivalis* ATCC 33277 and a *dpp4*-disruped mutant strain) and recombinant PgDPP4 (rPgDPP4), by means of MALDI-TOF MS in vitro. The results indicated that both GLP-1 and GIP were cleaved Ala^2-Glu^3 bond and degraded to the inactive forms by the incubation with the wild type of *P. gingivalis* cells and/or rPgDPP4 in dose and time dependent manners. Accordingly, the present findings indicate that *P. gingivalis* DPP4 can possibly function to degrade incretins as does human DPP4, and thus the periodontitis with *P. gingivalis* infection could be an important risk factor for type 2 diabetes mellitus.

Author Disclosure Block:

Y. Shimoyama: None. **Y. Ohara-Nemoto:** None. **T.K. Nemoto:** None. **M. Nakasato:** None. **T. Yaegashi:** None. **T. Ishikawa:** None. **M. Sasaki:** None. **S. Kimura:** None.

Poster Board Number:

MONDAY-645

Publishing Title:

Regulation of Intestinal Epithelial Cell Serotonin Transporter by *Lactobacillus* and *Bifidobacterium spp.*

Author Block:

C. Visuthranukul, M. Engevik, B. Ganesh, B. Luk, M. Esparza, J. Versalovic; Baylor Coll. of Med., Houston, TX

Abstract Body:

Background: The metabolite and neurotransmitter serotonin (5-HT) has a diverse physiological repertoire. In the gut, 5-HT can be produced by the conversion from dietary tryptophan by commensal bacteria. Lactic acid-producing bacteria (LAB), particularly the probiotic *Lactobacillus* and *Bifidobacterium* groups, have hypothesized to beneficially influence the serotonergic system. To address the potential role of LAB in 5-HT regulation, we sought to define 5-HT production by *Lactobacillus* strains and examine how the presence of these species may affect serotonin transporter (SERT) expression *in vitro* and *in vivo*. We hypothesized that select *Lactobacillus* and *Bifidobacterium* strains would stimulate 5-HT production and upregulate SERT.**Methods:** 24 *Lactobacillus* strains were cultured anaerobically in a fully defined media (LDM4) and serotonin production measured by MS/MS analysis. The human intestinal cell lines, HT29, were exposed to LAB conditioned media for 24 hrs and SERT expression was analyzed by qRT-PCR. To address serotonin production *in vivo*, germ-free mice were colonized with *B. dentium* or *L. reuteri* as well as ileum and colon luminal serotonin levels were examined via ELISA. Mouse intestinal SERT expression was examined by qRT-PCR.**Results:** Of the 24 *Lactobacillus* strains screened for serotonin production, none of the strains secreted detectable serotonin levels. However, addition of *L. reuteri* conditioned media to HT29 epithelial cells resulted in significant upregulation of SERT expression after 24 hrs. Likewise, germ-free mice mono-associated with *B. dentium* exhibited increased ileal serotonin levels demonstrating that LAB colonization can promote 5-HT production.**Conclusions:** These data demonstrate that the commensal microbes, *L. reuteri* and *B. dentium*, are capable of regulating key components of the intestinal serotonergic system. As downregulation of SERT has been implicated in the pathophysiology of several functional gut disorders, our data supports the consideration of next generation probiotics as therapies for serotonin-associated disorders.

Author Disclosure Block:

C. Visuthranukul: None. **M. Engevik:** None. **B. Ganesh:** None. **B. Luk:** None. **M. Esparza:** None. **J. Versalovic:** None.

Poster Board Number:

MONDAY-646

Publishing Title:

***Yersinia* Overcomes Reactive Oxygen Species in Tissues through the Action of the Nucleoid Associated Protein Fis**

Author Block:

E. R. Green, S. Clark, J. Mecsas; Tufts Univ. Sch. of Med., Boston, MA

Abstract Body:

All three pathogenic *Yersinia* species share a conserved virulence plasmid that contributes to growth and virulence in mammalian infection. However, in the absence of this plasmid, strains of the enteric pathogen *Yersinia pseudotuberculosis* (*Yptb*) retain the ability to colonize and grow within host organs. Because studies of chromosomal factors that contribute to *Yptb* infection have been limited, we previously utilized TnSeq to uncover chromosomal factors that contribute to pathogenicity of *Yptb* in the absence of the virulence plasmid. This approach identified nearly 50 genes, including several that were previously uncharacterized, that contribute to *Yptb* infection. In a follow-up to this work, we utilized a deep sequencing-based approach to validate and further characterize the phenotype of 18 of these genes by infecting mixed pools of in-frame knockouts into immunocompetent mice, as well as into mice lacking phagocytic cells. Using this approach, we identified a role for the nucleoid associated protein Fis in *Yptb* infection. Specifically, we found that mutants containing deletions of the *dusB-fis* operon were attenuated for virulence in mice following systemic infection. Interestingly, this virulence defect was apparent at late time-points, and was ablated when mice lacked phagocytic cells, suggesting that Fis may be protecting *Yptb* from restriction by the innate immune response, potentially by initiating a global transcriptional response that enables bacterial survival during these conditions. To identify potential stress(es) Fis may be responding to, we monitored the sensitivity of Δ*dusB-fis* and WT bacteria to several conditions that reflect mechanisms used by phagocytes to restrict bacterial survival, and found that Fis is crucial for resistance to oxidative stress *in vitro*. In agreement with this result, we found that the Δ*dusB-fis* mutant was fully virulent in mice unable to produce ROS, suggesting that Fis is critical for promoting resistance to oxidative stresses imposed by phagocytic cells during mammalian infection.

Author Disclosure Block:

E.R. Green: None. **S. Clark:** None. **J. Mecsas:** None.

Poster Board Number:

MONDAY-647

Publishing Title:

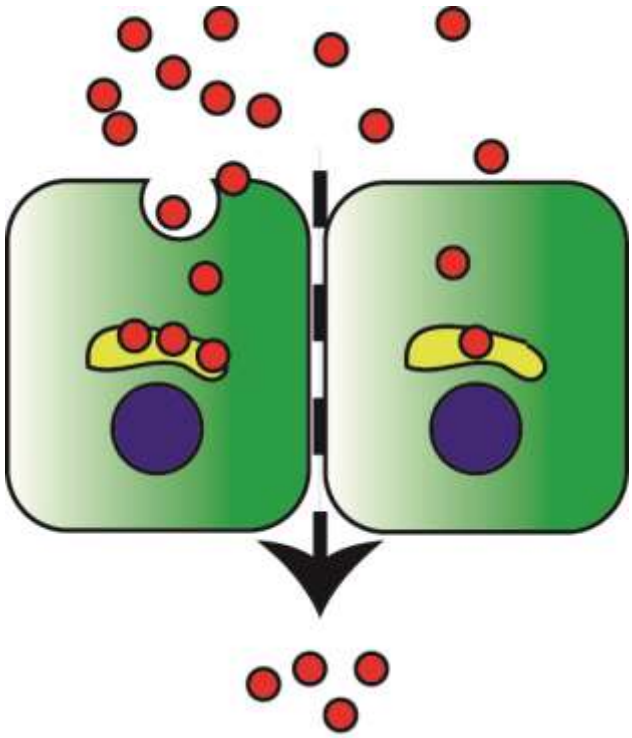
***Bacteroides thetaiotaomicron* Outer Membrane Vesicles Traverse and Alter the Intestinal Epithelial Barrier**

Author Block:

C. A. Hickey[1], M. Wegorzewska[1], D. Donermeyer[1], R. Glowacki[2], E. Martens[2], P. Allen[1], T. Stappenbeck[1]; [1]Washington Univ., St. Louis, MO, [2]Univ. of Michigan, Ann Arbor, MI

Abstract Body:

Background: *Bacteroides thetaiotaomicron* (*B. theta*) produces abundant outer membrane vesicles (OMVs) that engage host cells and potentially contributes to inflammatory bowel disease. We previously found OMV antigens in host intestinal macrophages after *B. theta* triggered colitis in a genetically susceptible mouse model. How OMVs traverse the epithelium and access macrophages is unclear. We hypothesize that OMVs enter the intestinal epithelium and alter paracellular permeability, thereby enhancing their access to macrophages.**Methods:** Primary murine colonic epithelial stem cells were plated on either Matrigel coated slides or Transwells, and the subsequent monolayers were treated apically with pre-labeled fluorescent *B. theta* OMVs. A time course of cellular entry and pharmacological and genetic manipulation of the epithelial cells to determine the mode of OMV entry were measured. Epithelial monolayer barrier function and OMV ability to pass through the monolayer were also evaluated.**Results:** *B. theta* OMVs were internalized by colonic epithelial cells, and this process was disrupted by inhibitors of lipid raft formation. OMV antigens were localized in scattered cytoplasmic foci and concentrated within lysosomes. By 4 hours, tight junctions at cell-cell borders opened, and *B. theta* OMVs were found in the media below the Transwell, confirming passage through this layer.**Conclusion:** *B. theta* OMVs enter epithelial cells, are targeted for degradation, and disrupt tight junctions, thereby potentially allowing OMVs to pass through this barrier. These results further elucidate how OMVs access and alter host cells, a key factor in how commensal bacteria may trigger disease in the host.



Cartoon of *B. theta* OMV (red) internalization into host epithelial cells (green) then degradation in lysosomes (yellow). Tight junctions are affected and OMVs traverse the epithelial barrier.

Author Disclosure Block:

C.A. Hickey: None. **M. Wegorzewska:** None. **D. Donermeyer:** None. **R. Glowacki:** None. **E. Martens:** None. **P. Allen:** None. **T. Stappenbeck:** None.

Poster Board Number:

MONDAY-648

Publishing Title:

Role of *Neisseria gonorrhoeae* Lytic Transglycosylases in Mediating Resistance to Host Phagocytic Killing

Author Block:

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Abstract Body:

Background: *Neisseria gonorrhoeae* is a gram negative bacteria that most commonly infects the urogenital tract causing either urethritis or cervicitis. *N. gonorrhoeae* has evolved mechanisms to modify the host response to the presence of the bacteria. Host cells can recognize *N. gonorrhoeae* by detecting the presence of peptidoglycan (PGN), the primary building block of the cell wall. PGN polymers are broken down and released from the remodeling cell wall during bacteria growth. Most bacteria recycle released PGN limiting host detection. The activity of lytic transglycosylases, *LtgA* and *LtgD*, causes *N. gonorrhoeae* to release large amounts of biologically active monomeric PGN containing 1,6-anhydro muramic acid moiety, rather than recycling PGN. In previous studies, *N. gonorrhoeae* with genetic deletions of *ltgA* and *ltgD* were found to release multimers instead of monomers of PGN. Duncan lab found that *N. gonorrhoeae* lacking *ltgA/D* cause increased host cytokine production when compared to the parental strain. Furthermore, conditioned media released by this mutant strain stimulates increased activation of NOD2, an intracellular receptor for PGN, in reporter cell lines. These data suggest that *LtgA* and *LtgD* many provide immune - evasive activities to *N. gonorrhoeae*. In this study we investigated whether *N. gonorrhoeae* lytic transglycosylases allow the bacteria to evade host phagocyte induced killing. **Methods:** Bone Marrow Derived Dendritic (BMDC) cells were generated from both C57BL6 mice and *Nod2-/-* mice. *N. gonorrhoeae* strain FA1090 and an isogenic derivative lacking both *ltgA* and *ltgD (ΔltgA/ltgD*) were inoculated into the BMDC cultures and the quantity of surviving bacteria colonies was enumerated over time.**Results:** *ΔltgA/ltgD* was killed more rapidly by BMDC than the isogenic parental strain FA1090. Under the same culture media conditions with no BMDC the strains survived equally, although they did not grow. There was no difference in killing of *N. gonorrhoeae* between C57BL6 and *Nod2-/-* under the conditions we tested.**Conclusions:** *LtgA* and *LtgD* appear to increase *N. gonorrhoeae* viability against the pressure of host cell phagocytic killing. Though *ΔltgA/ltgD N. gonorrhoeae* are more readily recognized by host NOD2, their enhanced susceptibility to BMDC killing is not NOD2 dependent.

Author Disclosure Block:

C. Weng: None. **K. Knilans:** None. **J. Dillard:** None. **J. Duncan:** None.

Poster Board Number:

MONDAY-649

Publishing Title:

Immunosuppressive *Yersinia* Effector YopM Binds Dead Box Helicase DDX3 to Control Ribosomal S6 Kinase in the Nucleus of Host Cells

Author Block:

L. Berneking[1], M. Schnapp[1], M. Alawi[2], M. Aepfelbacher[1]; [1]Univ. Med. Ctr. Hamburg-Eppendorf (UKE), Hamburg, Germany, [2]Heinrich-Pette-Inst., Hamburg, Germany

Abstract Body:

YopM is a crucial immunosuppressive effector of the plaque agent Yersinia pestis and other pathogenic Yersinia species. YopM enters the nucleus of host cells but neither the mechanisms governing its nucleocytoplasmic shuttling nor its intranuclear activities are known. Here we identify the DEAD-box helicase DDX3 as a novel interaction partner of Y. enterocolitica YopM. Knockdown of DDX3 or inhibition of the exportin CRM1 increased the nuclear level of YopM suggesting that YopM exploits DDX3 to exit the nucleus via the CRM1 export pathway. Increased nuclear YopM levels caused enhanced phosphorylation of Ribosomal S6 Kinase 1 (RSK1) in the nucleus. In Y. enterocolitica infected primary human macrophages YopM increased the level of Interleukin-10 mRNA which required interaction of YopM with RSK. We propose that the DDX3/CRM1 mediated nucleocytoplasmic shuttling of YopM determines phosphorylation of RSK in the nucleus to control transcription of immunosuppressive cytokines.

Author Disclosure Block:

L. Berneking: None. **M. Schnapp:** None. **M. Alawi:** None. **M. Aepfelbacher:** None.

Poster Board Number:

MONDAY-650

Publishing Title:

Mutations Inactivating the *Streptococcus pyogenes* Multiple Gene Regulator Decrease Host Inflammatory Responses and Promote Persistent Mucosal Carriage

Author Block:

K. M. Jacob, I. L. Laczkovich, S. Ahmed, S. R. Dawid, **M. E. WATSON, Jr**; Univ. of Michigan Med. Sch., Ann Arbor, MI

Abstract Body:

Streptococcus pyogenes is a human bacterial pathogen causing inflammatory infections at multiple mucosal tissues, including pharyngitis and vulvovaginitis. The Multiple Gene Regulator (Mga) controls transcription of up to 10% of the genome, including activation of adhesins and virulence factors. Spontaneous mutations resulting in the inactivation of Mga have been identified among asymptomatic carriage strains isolated from humans and mouse models, suggesting that Mga may represent a key switch from virulence to an avirulent state. This study examined the ability of *S. pyogenes* lacking Mga to persist in a murine model of mucosal carriage and the associated host inflammatory responses. An in-frame deletion mutation of the *mga* gene was constructed in virulent serotype M28 *S. pyogenes* (MEW123), producing a ΔMga mutant strain. A murine vaginal colonization model was used to assess mucosal carriage. Murine Bone-Marrow Dendritic Cells (mBMDCs) were examined for cytokine production via ELISA after exposure to streptococcal strains. Characterization of the ΔMga strain demonstrated it had no growth defect in broth culture and lacked SpeB protease expression in comparison to the parent strain, suggesting Mga-dependent activation of SpeB expression in this strain. Mice vaginally-colonized with the ΔMga strain exhibited a longer duration of streptococcal mucosal carriage compared with mice inoculated with the parent strain. Examination of vaginal smears showed a significant neutrophil recruitment in mice colonized with the parent strain, whereas those colonized by the ΔMga mutant exhibited no significant inflammatory response, appearing similar to non-inoculated controls. Stimulation of mBMDCs by the ΔMga strain produced significantly less pro-inflammatory cytokine IL-1β and IL-6 secretion compared to the parent strain. In summary, inactivation of the *mga* gene led to prolonged mucosal carriage and an absence of any significant inflammatory response typically observed following inoculation with the virulent parent strain. These results support the hypothesis that Mga is a central determinant in streptococcal virulence and that loss of Mga function results in a switch to an avirulent persister-like state that may allow for prolonged carriage.

Author Disclosure Block:

K.M. Jacob: None. **I.L. Laczkovich:** None. **S. Ahmed:** None. **S.R. Dawid:** None. **M.E. Watson:** None.

Poster Board Number:

MONDAY-651

Publishing Title:

Effect of 3-(2-Aminoethyl)-5-Hydroxy-(2,3-Benzopyrrole) on EHEC Virulence

Author Block:

A. Mishra[1], V. Sperandio[2]; [1]UT Southwestern MEdical Ctr., Dallas, TX, [2]UT Southwestern Med. Ctr., Dallas, TX

Abstract Body:

Host bacterial associations have a profound impact in health and disease. Prokaryotes and eukaryotes have co-existed for millions of years. The gastrointestinal (GI) tract is one of the most prominent sites in the human body where host/microbial associations are paramount. The GI tract is inhabited by trillions of commensal bacteria that comprise the microbiota, which aid in the digestion of food, vitamin production, and play crucial roles in human physiology and development. Both the host and microbiota produces cohorts of signaling molecule which can regulate the virulence expression of a pathogen. We assessed the role of molecule 3-(2-Aminoethyl)-5-hydroxy-(2,3-Benzopyrrole) produced in the mammalian GI tract in the virulence expression of Enterohemorrhagic *E.coli*(EHEC). EHEC has a pathogenicity island, the locus of enterocyte effacement(LEE) that is essential for virulence. EHEC was grown in low glucose DMEM at 37°C till mid log phase either in presence or absence of 3-(2-Aminoethyl)-5-hydroxy-(2,3-Benzopyrrole). The presence of 3-(2-Aminoethyl)-5-hydroxy-(2,3-Benzopyrrole) in the media changed the levels of LEE gene expression as measured by quantitative real time PCR, and western blot. To assess the effect of 3-(2-Aminoethyl)-5-hydroxy-(2,3-Benzopyrrole) as a signaling molecule regulating LEE *in-vivo* studies involving mice that are able to accumulate increased levels of mucosal 3-(2-Aminoethyl)-5-hydroxy-(2,3-Benzopyrrole) in the GI tract were performed. Mice having normal levels of mucosal 3-(2-Aminoethyl)-5-hydroxy-(2,3-Benzopyrrole) and those with increased levels of mucosal 3-(2-Aminoethyl)-5-hydroxy-(2,3-Benzopyrrole) were infected with *C.rodentium*, which is used as a surrogate model for EHEC infection. Levels of CFU/ml, survival and histology assays were performed. The mice with increased levels of mucosal 3-(2-Aminoethyl)-5-hydroxy-(2,3-Benzopyrrole) were more susceptible to *C.rodentium* infection. Thus molecule 3-(2-Aminoethyl)-5-hydroxy-(2,3-Benzopyrrole) present in the mammalian GI tract seems to serve as a signaling molecule for EHEC, and affect its virulence by regulating the LEE.

Author Disclosure Block:

A. Mishra: None. **V. Sperandio:** None.

Poster Board Number:

MONDAY-652

Publishing Title:

Impact of Amoebal Endosymbiont *Neochlamydia* on Host Defense against Harmful *Legionella* Infection and Its Defense Mechanism

Author Block:

C. Maita[1], T. Yamazaki[1], J. Matsuo[1], S. Nakamura[2], T. Okubo[1], H. Nagai[3], H. Yamaguchi[1]; [1]Hokkaido Univ., Sapporo, Japan, [2]Juntendo Univ., Tokyo, Japan, [3]Osaka Univ., Suita, Japan

Abstract Body:

We have previously established an amoeba strain (S13WT amoebae) from environmental soil harboring obligate intracellular symbiont *Neochlamydia*, an environmental chlamydia; the endosymbiont has an amoebal infection rate of 100%, but does not cause amoebal lysis with lacking transferability to other host amoebae (Environ Microbiol Rep, 2: 524-33, 2010; Microbes Environ, 27: 423-9, 2012). Also, our recent work intriguingly has shown that the amoebal endosymbiont has a critical role into host amoebal defense against harmful *Legionella* infection (PLoS ONE, 18: e95166, 2014). Here, to explore the defense mechanism, we performed the following experiments with three distinct GFP-expressing *Legionella pneumophila* [JR32 ($T4ASS^+/T4BSS^+/Tra^-$), Lp01 ($T4ASS^-/T4BSS^+/Tra^+$), Lp02 ($T4ASS^-/T4BSS^-/Tra^+$)], and S13WT ameobae and its aposymbiotic amoebae (S13RFP amoebae) in addition to C3 reference amoebae. Uptake experiment revealed that S13WT amoebae could hardly ingest not only all *Legionella* strains also FITC-latex beads when compared to the aposymbiotic S13RFP or C3 amoebae. The CFU count assay showed that the *Legionella* minimally could infect and grow into S13WT amoebae. The 2D-DIGE showed that major spots into S13WT amoebae significantly decreased in these of aposymbiotic S13RFP amoebae; the LC/MS identified the proteins to be actin. Meanwhile, the DNA microarray revealed that JR32 infection dramatically increased the *Neochlamydia* gene expression to high levels, although the gene expression levels minimally changed at any time regardless of Lp01 or Lp02 infection. Thus, the host defense mechanism is very complicated. However, we found a crucial piece that amoebal endosymbiotic *Neochlamydia* specifically could sense *Legionella* T4ASS (Lvh) but not T4BSS (Dot/Icm), presumably connecting to the host defense against harmful *Legionella* infection.

Author Disclosure Block:

C. Maita: None. **T. Yamazaki:** None. **J. Matsuo:** None. **S. Nakamura:** None. **T. Okubo:** None. **H. Nagai:** None. **H. Yamaguchi:** None.

Poster Board Number:

MONDAY-653

Publishing Title:

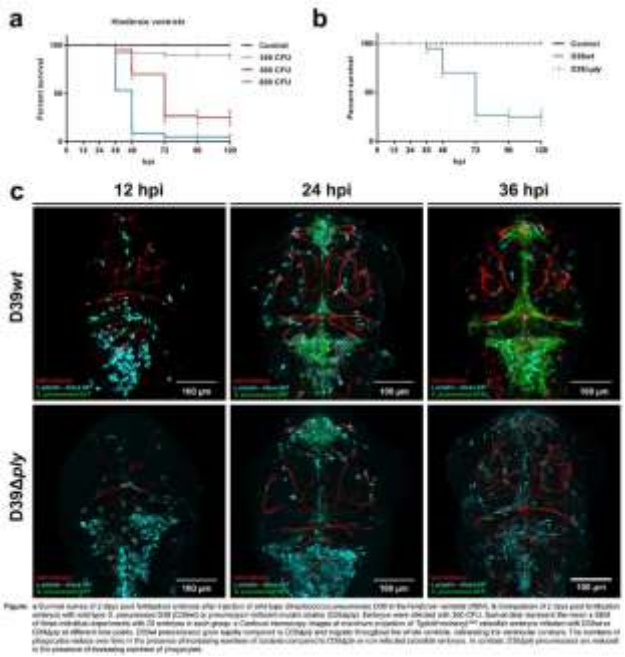
Zebrafish Embryo Infection Model of Pneumococcal Meningitis

Author Block:

K. Jim[1], A. M. van der Sar[2], A. van der Ende[1], M. C. Brouwer[1], D. van de Beek[1], W. Bitter[2], C. M. J. E. Vandenbroucke-Grauls[2]; [1]Academic Med. Ctr., Amsterdam, Netherlands, [2]VU Univ. Med. Ctr., Amsterdam, Netherlands

Abstract Body:

Background*Streptococcus pneumoniae* is the most important cause of meningitis, with high morbidity and mortality. The aim of our study was to develop a pneumococcal meningitis model in the zebrafish embryo, to study host-microbe interactions.**Methods**Zebrafish embryos (60 per group) were infected in the bloodstream or hindbrain ventricle with green fluorescent *S. pneumoniae* D39*wt* or pneumolysin-deficient D39 (D39*ply*). A *kdrl:mCherry* transgenic zebrafish line was used to visualize blood vessels. Phagocytic cells were visualized by anti-L-plastin or by using double labeled *mpo:gfp* x *mpeg:mCherry* zebrafish to visualize neutrophils and macrophages individually. Embryos were imaged using confocal and time-lapse microscopy.**Results**Inoculation of *S. pneumoniae* in bloodstream and hindbrain ventricle induced dose-dependent mortality (fig. a). Whereas bloodstream injection resulted in fulminant systemic disease with meningitis, hindbrain ventricle injection caused local brain infection. The D39*ply* mutant was attenuated (fig. b) and difference in phagocyte recruitment was observed (fig. c). Time-lapse imaging showed that upon infection the initial innate immune response consists mainly of neutrophils without involvement of macrophages.**Conclusions**Pneumococcal meningitis in zebrafish embryos occurs both after bloodstream or hindbrain ventricle injection. This meningitis model permits detailed visualization of meningitis pathogenesis in real time and appears as a powerful tool to study pneumococcal host-microbe interactions, thereby providing the opportunity to study host and pathogen factors that play a role in the pathogenesis of this infection.



Author Disclosure Block:

K. Jim: None. **A.M. van der Sar:** None. **A. van der Ende:** None. **M.C. Brouwer:** None. **D. van de Beek:** E. Grant Investigator; Self; Diederik van de Beek. **W. Bitter:** None. **C.M.J.E. Vandenbroucke-Grauls:** None.

Poster Board Number:

MONDAY-654

Publishing Title:

Simultaneous Rna-Seq Analysis of Gram Negative Pathogen and Host Transcriptomes During *In Vitro* And *In Vivo* Infections Using a Capture Based Technique

Author Block:

A. LaBauve, Kunal Poorey, Zachary W. Bent, Anupama Sinha, Steven S. Branda, and Robert J. Meagher; Sandia Natl. Lab., Livermore, CA

Abstract Body:

Detailed global understanding of the mechanisms utilized by bacteria over the course of an infection could aid in the discovery of novel targets for much needed therapeutics for emerging and drug-resistant bacterial pathogens. Bacteria adapt rapidly with concerted shifts in gene expression in response to changing conditions: from external environment or host organism to a new host, or between different cell or tissue microenvironments within the host. However, most studies of pathogenesis focus primarily on a handful of genes at a time, or conversely only focus on the response of the host. RNAseq analysis is a promising approach to understanding the global shifts in bacterial gene expression patterns over the course of an infection, but applying RNAseq techniques to *in vitro* or *in vivo* bacterial infection models is technically challenging because bacterial transcripts make up an exceedingly small fraction of the total RNA in an infected sample containing both host and bacteria. To address this issue, we have developed a method to separate bacterial transcripts from host transcripts prior to sequencing. Using this unbiased capture-based technique we are able to enrich for all possible bacterial transcripts leading to gene level analysis of the entire bacterial transcriptome while host transcriptomes from the same sample can also be analyzed. This enrichment strategy was used in conjunction with RNA-seq to further our understanding of host-pathogen interactions by analyzing both host and pathogen transcriptomes of *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* in the context of acute murine lung infections. Combining this powerful tool with host transcriptome analysis simultaneously will provide details about host-pathogen interactions that have not previously been possible and could provide insight into targetable bacterial pathways which could circumvent the ever-growing problem of resistance to conventional broad spectrum antibiotics.

Author Disclosure Block:

A. LaBauve: None.

Poster Board Number:

MONDAY-655

Publishing Title:

Using Capture Based Enrichment And Rna-Seq To Study Host Pathogen Interaction Dynamics In Emerging Superbugs

Author Block:

K. Poorey; Sandia Natl. Labs, Livermore, CA

Abstract Body:

To develop early diagnostics, new treatment strategies, and new antibiotics drugs it is necessary to understand the biology of both host and pathogen in detail. We studied the gene expression profile of host and pathogen simultaneously for the infection time course of different pathogens with RNA-seq. For pathogen transcriptomics we have developed a solution-phase hybridization-based capture approach to enrich specifically for pathogen transcripts and significantly increasing the number of pathogen reads by >100-fold. This enabled the study pathogen transcription in much greater detail. We have demonstrated the application of the capture technique to in vitro tissue culture infections with *Yersinia enterocolitica* and *NDM-1 Klebsiella pneumomiae*, as well as a detailed study the dynamics of *Burkholderia* infection in primary cells over the whole time course. We have developed new RNA-seq analysis pipeline called MAnTra which is a parallel & multithreaded software which uses existing and novel tools to perform RNA-seq and profile transcription fidelity. Through the detail transcriptomics analysis we discover new active virulence pathways in *Yersinia*, how superbug *NDM-1 Klebsiella pneumomiae* and multidrug resistant *Burkholderia* behaves in infection. Also from the host transcriptomics time series we characterized the immune response to infections to these bugs over the period of infection. Further we want to discover biomarkers of infection in the host data and new targets in pathogens to tackle antibiotic resistance.

Author Disclosure Block:

K. Poorey: None.

Poster Board Number:

MONDAY-656

Publishing Title:

Exploiting the Zebrafish Embryo Model to Characterize the Divergent Virulence Potential Among *Cronobacter* spp

Author Block:

A. K. Eshwar[1], B. D. Tall[2], J. Gangiredla[3], G. R. Gopinath[4], I. R. Patel[4], R. Stephan[1], **A. Lehner**[1]; [1]Univ. Zurich, Zurich, Switzerland, [2]U. S. Food and Drug Admin., Laurel, MD, [3]U. S Food and Drug Admin., Laurel, MD, [4]U.S. Food and Drug Admin., Laurel, MD

Abstract Body:

Background: Bacteria belonging to the genus *Cronobacter* spp. have been recognized as causative agents of life-threatening systemic infections primarily in premature, low-birth weight and immune-compromised neonates. Apparently not all *Cronobacter* spp. are linked to infantile infections and it has been proposed that virulence varies among strains. In a previous study, the zebrafish embryo model was successfully established to study the pathogenesis of these organisms. In the current study, we combined data obtained from *in silico* analysis of the genome content of strains with the ability of the strains to cause infections *in vivo*.**Materials and Methods**: *Cronobacter* type strains (9), two RepFIB cured strains, the *C. sakazakii* BAA894 *cpa* mutant and *E. coli* complemented with *cpa* were included in this study. The strains were characterized to the genome level using a recently developed pan genome DNA microarray. For infection studies, bacteria were injected into the yolk sac of zebrafish embryos (2 days post fertilization, 30 embryos per strain) and pathogenicity was assessed by determining mortality rates over 96 hours post infection (hpi).**Results:** Pathogenicity varied among *Cronobacter* spp. type strains with *C. sakazakii, C. turicensis* showing 100 % mortality rate within 36 hpi whereas a 100 % survival rate was observed for all embryos injected with *C. muytjensii.* The latter strain does not harbour the RepFIB plasmid which has been proposed as a „virulence plasmid", thus being most likely the reason for the apathogenic behaviour of this strain. Infection experiments using RepFIB plasmid cured derivatives of the highly pathogenic strains *C. turicensis and C. sakazakii* supported this hypothesis. In addition, the involvement of one of the genetic determinants located on this plasmid, the *Cronobacter* plasminogen activator (*cpa*) in pathogenicity was confirmed in infection experiments using a *cpa* knock out mutant and an apathogenic *E. coli* strain complemented with this determinant.**Conclusion:** The combination of genome content related data with *in vivo* pathogenicity data enables a molecular dissection of the virulence spectrum of these organisms.

Author Disclosure Block:

A.K. Eshwar: None. **B.D. Tall:** None. **J. Gangiredla:** None. **G.R. Gopinath:** None. **I.R. Patel:** None. **R. Stephan:** None. **A. Lehner:** None.

Poster Board Number:

MONDAY-657

Publishing Title:

Assessment of Early Events in *E. coli* o157:H7 Infection of Human Cells at the Single Cell Level Using a Novel Acoustic Wave Device

Author Block:

R. Katani, P. Li, A. M. Pedley, L. Li, T. J. Huang, S. J. Benkovic, V. Kapur; Pennsylvania State Univ., University Park, PA

Abstract Body:

Adherence to host cells by a pathogen is often regarded as the first and key step in pathogenesis. However, relatively little is known about the changes that occur within the microbe and the host cell during the very early time points (seconds to minutes) of host - pathogen interactions. Acoustic wave technology holds considerable promise in enabling single cell bacteria-host interactions to be controlled and monitored with high spatio-temporal resolution. We have sought to examine the effects of acoustic wave on bacteria (*Escherichia coli* O157:H7) and host cells (Hela and Caco2), and identify conditions that result in minimal perturbation to their normal cellular processes. The results suggest that acoustic wave coupled with RNA-seq may enable a precise temporal resolution of the very early (seconds to minutes) biochemical events that occur during the interaction of microbes with their host cells at the single cell level. In the long-term, studies such as these are likely to provide key missing insights on the very early stages of microbial pathogenesis, and aid in the identification of potential novel targets for the development of the next generation of therapeutics and vaccines.

Author Disclosure Block:

R. Katani: None. **P. Li:** None. **A.M. Pedley:** None. **L. Li:** None. **T.J. Huang:** None. **S.J. Benkovic:** None. **V. Kapur:** None.

Poster Board Number:

MONDAY-658

Publishing Title:

Coevolution of the Hingut Microbiota of Xylophagous Termites & Cockroaches

Author Block:

M. Berlanga[1], R. Duro[2], R. Guerrero[2]; [1]Univ. of Barcelona, Barcelona, Spain, [2]Barcelona Knowledge Hub-Academia Europaea, Barcelona, Spain

Abstract Body:

Background: The rapid growth in microbiome research has revealed the crucial contributions of microbial communities to numerous physiological functions in animals, including digestion, immunity and reproduction. Fundamental physiological processes, such as tissue development, nutrient absorption, immunity, and circadian regulation, are emergent properties of the interactions between the host and its microbiota. Those emergent properties involve multiple microbial species and genotypes, reflecting the microbe composition at the level of community rather than individual microbial taxa.**Methods:** In this study, high-throughput 454 pyrosequencing of 16S rRNA was used to investigate the diversity of metagenomic hindgut bacterial communities of *Cryptocercus punctulatus* and *Reticulitermes grassei*.**Results:** Our results revealed that the hindgut prokaryotic communities of both xylophagous Dictyoptera are dominated by members of four *Bacteria* phyla: Bacteroidetes, Firmicutes, Proteobacteria, and Actinobacteria. In the case of *Reticulitermes*, Spirochaetes was also a dominat phyla. Phylogenetic analysis based on the characterized clusters of Bacteroidetes, Spirochaetes, and *Deltaproteobacteria* showed that many OTUs present in *Cryptocercus* and *Reticulitermes* clustered with sequences previously described in other termites and cockroaches, but not with those from other animals or environments.**Conclusions:** One of the most important factors in the fitness of the organisms is nutrition. Insect symbionts have provided the enzymatic capabilities that enable the insect to feed on recalcitrant plant polymers (cellulose, hemicellulose, pectin or lignin). This work describes the relationships between the hindgut microbiotas of two xylophagous Dictyoptera (namely *Reticulitermes*, a lower termite, and *Cryptocercus*, a cockroach), to understand the characteristics that have determined bacterial fidelity over generations and throughout evolutionary history. Symbiotic interactions have probably played a central role in the evolutionary success of these insects, allowing their adaptation to unexploited ecological niches that are nutritionally deficient and/or unbalanced.

Author Disclosure Block:

M. Berlanga: None. **R. Duro:** None. **R. Guerrero:** None.

Poster Board Number:

MONDAY-659

Publishing Title:

Brain Meta-transcriptomics from Harbor Seals to Infer the Role of the Microbiome and Virome in a Stranding Event

Author Block:

S. M. Rosales; Oregon State Univ., Corvallis, OR

Abstract Body:

Marine diseases are becoming more frequent, and tools for identifying pathogens and disease reservoirs are needed to help prevent and mitigate epizootics. Meta-transcriptomics provides insights into disease etiology by cataloguing and comparing sequences from suspected pathogens. This method is a powerful approach to simultaneously evaluate both the viral and bacterial communities, but few studies have applied this technique in marine systems. In 2009 seven harbor seals, Phoca vitulina, stranded along the California coast from a similar brain disease of unknown cause of death (UCD). We evaluated the differences between the virome and microbiome of UCDs and harbor seals with known causes of death. Here we determined that UCD stranded animals had no viruses in their brain tissue. However, in the bacterial community, we identified Burkholderia and Coxiella burnetii as important pathogens associated with this stranding event. Burkholderia were 100% prevalent and ~2.8 log2 fold more abundant in the UCD animals. Further, while C. burnetii was found in only 35.7% of all samples, it was highly abundant (~94% of the total microbial community) in a single individual. In this harbor seal, C. burnetii showed high transcription rates of invading and translation genes, implicating it in the pathogenesis of this animal. Based on these data we propose that Burkholderia taxa and C. burnetii are potentially important opportunistic neurotropic pathogens in UCD stranded harbor seals.

Author Disclosure Block:

S.M. Rosales: None.

Poster Board Number:

MONDAY-660

Publishing Title:

Genome-Wide Identification Of Fitness Genes In Carbapenem-Resistant St131 *escherichia Coli* During Urinary Tract Infection

Author Block:

J. Marzoa, S. N. Smith, S. Crepin, H. T. L. Mobley; Univ. of Michigan Med. Sch., Ann Arbor, MI

Abstract Body:

The *E. coli* pandemic multidrug resistant clone ST131, the major etiologic agent of urinary tract infections internationally, is responsible for spreading the β-lactamase CTX-M-15 worldwide. Carbapenem-resistant *Enterobacteriaceae* are an emerging threat to public health worldwide because these agents are often the last line of effective therapy available for the treatment of serious infections. Recently, the *H*30-R ST131 clone has been reported as the principal *E. coli* clone harboring the carbapenemase (KPC)-encoding gene *bla*KPC. Because of the unprecedented global spreading of ST131 clone, *bla*KPC-positive ST131 strains are of particular concern and new targets must be identified for antimicrobial therapies. Using a Tn-seq approach, we are defining essential genes required for infection in a murine model of UTI and evaluating their suitability as therapeutic targets. To address this, we first characterized the *bla*KPC positive O25b:H4-B2-ST131 clinical isolate UMCRE19. UMCRE19 belongs to the subclone *H*30-R and the virotype C, and displayed reduced susceptibilities to ertapenem, cefazolin, cefotaxime, ampizilin/sulbactam, aztreonam, ciprofloxazin, levofloxacin and gentamycine. UMCRE19 was sequenced using PacBio sequencing (100X coverage, P6-C4 chemistry). The chromosome of UMCRE19 contains a 5,148,820 bp, 5,334 coding sequences and 110 RNA features. At least two plasmids are present in strain UMCRE19: pUMCRE19_1 and pUMCRE19_2, of 113 and 51 kb, respectively. Female CBA/J mice were transurethrally inoculated and at 6 h and 24 h post-inoculation, the urine, bladder, kidneys and spleen were harvested, homogenized and plated for CFU enumeration. At 6 h post-inoculation the urine, bladder, kidneys and spleen were efficiently colonized and the bacterial burden in those organs was comparable to the produced by the prototypic uropathogenic *E. coli* strain CFT073. Secondly, to determine the genes required for UTI, a transposon library of 50,000 mutants was constructed. Transposon mutants with fitness defects in bladder will be identified by sequencing and comparing the frequency of transposon insertion sites from the inoculum (input) and bacteria that colonized the bladder (output). These fitness factors may represent novel targets to develop antimicrobial therapy against carbapenem-resistant *E. coli*.

Author Disclosure Block:

J. Marzoa: None. **S.N. Smith:** None. **S. Crepin:** None. **H.T.L. Mobley:** None.

Poster Board Number:

MONDAY-661

Publishing Title:

Identification Of *in Vivo* Fitness Genes For Opportunistic Pathogens Of The *enterobacteriaceae* Family

Author Block:

M. T. ANDERSON, L. A. Bradford, H. L. T. Mobley; Univ. of Michigan Med. Sch., Ann Arbor, MI

Abstract Body:

Serratia marcescens and *Citrobacter freundii* are opportunistic human pathogens that are capable of causing severe infections and are frequently resistant to antibiotic treatment. Risk factors for these infections include extended hospital stays, prolonged antibiotic treatment, and a compromised immunological state. In contrast to other members of the *Enterobacteriaceae*, the genetic factors that facilitate survival and replication within the mammalian host have not been thoroughly characterized for these two organisms. To identify fitness factors on a genome-wide scale, human bacteremia isolates were subjected to transposon insertion sequencing analysis. Using input populations of 50,000 random transposon mutants of each species, a total of 127 *S. marcescens* and 187 *C. freundii* genes were found to encode fitness factors important for bacterial survival in a mouse model of bacteremia. Further analysis of the insertion sites in the initial transposon mutant populations identified an additional >600 putative essential genes for each organism. Among the mutated *S. marcescens* genes conferring a significant fitness defect, 11 were located within a large 18-gene cluster that has predicted function in the production and assembly of an extracellular polysaccharide. Further investigation of this locus through comparative analysis of 11 newly determined *S. marcescens* genomic sequences revealed a high degree of heterogeneity, indicating the genetic potential for surface-antigen diversity within this species. For *C. freundii*, one of the most highly represented (12 genes) categories of fitness factors were genes encoding homologous recombination and DNA repair functions. Although the significance of this finding is under investigation, one interpretation of this result is that the inability to repair DNA damage within the host is detrimental to *C. freundii* survival. *S. marcescens* and *C. freundii* were also found to share several homologous fitness factors, leading to the hypothesis that these organisms have conserved *in vivo* survival strategies. Together, these newly identified fitness genes of *S. marcescens* and *C. freundii* provide a more complete understanding of the genetic requirements for bacterial survival in the mammalian host and provide a framework for the development of alternative therapeutic interventions.

Author Disclosure Block:

M.T. Anderson: None. **L.A. Bradford:** None. **H.L.T. Mobley:** None.

Poster Board Number:

MONDAY-662

Publishing Title:

Development of Bioluminescent *Salmonella* Strains for *In Vivo* Chicken Pathogenesis Studies

Author Block:

G. Chaloner, L. Lacharme-Lora, P. Wigley; Univ. of Liverpool, Neston, United Kingdom

Abstract Body:

Background:*Salmonella enterica* subspecies *enterica* infection remains a serious problem in a wide range of animals and in man. Poultry meat is the main source of human infection for serovars such as *S.* Enteritidis whilst *serovars such as S.* Gallinarum, the causative agent of fowl typhoid, are an important cause of disease in poultry. These two serotypes behave quite differently in the chicken, S. Gallinarum is invasive, non-colonising and produces typhoid whereas *S.* Enteritidis is invasive, able to colonise and non-typhoidal. The aim of this study was to develop bioluminescent *Salmonella* strains, which could be used for *in vivo* pathogenesis studies.**Methods:**Plasmids carrying the luminescence genes (*luxABCDE*) were inserted into *S.* Gallinarum 287/91 and *S.* Enteritidis PT4 P125109 to construct bioluminescent strains of these pathogens. Day old chicks were then orally infected with these strains. Birds were sacrificed at 1 and 3 days post-infection. Birds were first imaged whole using the IVIS Spectrum machine at the Centre for Preclinical Imaging of the University of Liverpool to determine whether *lux* signal could be detected in the organs and gastro-intestinal sites before removing the gastro-intestinal tract, liver and spleen to image *ex vivo*. Bacteria were also enumerated from liver, spleen, ileal and caecal contents.**Results:**Limited bacterial *lux* signal was detected in the birds when the gastro-intestinal tract and organs were imaged i*n vivo* at 1 day post infection. However at 3 days post-infection bioluminescence was detected in the ceca of birds infected with both *S.* Enteritidis P125109 and *S.* Gallinarum 287/91 *ex vivo*.**Conclusions:**This study demonstrates for the first time the potential to image bioluminescent *Salmonella ex vivo* in the gastro-intestinal tract of chickens and allow more detailed studies of the interactions between *Salmonella* and the avian host. By using IVIS technology we are able to study these interactions while reducing animal numbers and morbidity.

Author Disclosure Block:

G. Chaloner: None. **L. Lacharme-Lora:** None. **P. Wigley:** None.

Poster Board Number:

MONDAY-663

Publishing Title:

***galleria Mellonella* Applied In The Study Of *paracoccidioides Spp* - Host Interaction**

Author Block:

L. Scorzoni, A. de Paula e Silva, J. Singulani, F. Sangalli-Leite, H. de Oliveira, C. Maria Marcos, R. Moraes da Silva, A. Fusco-Almeida, M. Mendes-Giannini; UNESP- Univ. Estadual Paulista, Araraquara, Brazil

Abstract Body:

Background: Paracoccidioidomycosis is an endemic fungal disease in Latin American, caused by dimorphic fungi *P. brasiliensis* and *P. lutzii.* Alterations in fungi characteristics are described after successive subcultures; the recovery of these characteristics can be re-established after passage in mammalian animals. Because of ethical issues with mammalian, alternative animal has been investigated. Invertebrates, as *G. mellonella,* represent a good alternative for *in vivo* assays. In this study, we compared the virulence of *P. brasiliensis and P. lutzii* in *G. mellonella* model.**Methods:** We evaluated survival curves, phagocytosis, hemocyte density (flow cytometer), hemocyte fungal interaction (flow cytometer) and adhesins gene expression (real time PCR). Additionally, we compare the interaction profile by flow cytometry and adhesin genes expression profile by real time PCR of a subcultivated *P. brasiliensis* culture and after passage in mice and in *G. mellonella*. **Results:** The survival curves of larvae infected with *P. brasiliensis* or *P. lutzii* showed a similar virulence (p=0.3051), and both species were able to reduce the hemocyte number in 3 times after 1 and 3 hours. Additionally, the phagocytosis rate was 5% for both species, but when we analyzed hemocyte-*Paracoccidioides* spp. interaction, *P. lutzii* showed higher interactions with hemocytes (48%), while in *P. brasiliensis* the interaction was 14% after 3 hours. The gene expression of gp43 was higher for *P. lutzii,* and this expression may contribute to a greater interaction to hemocytes. Moreover, *P. brasiliensis* passage to *G. mellonella* was able to increase the interaction to pneumocyte cells in a similar way that occurs after passage in mice (p=0.2926) and the expression of the enolase, gp43, 14-3-3 and triose phosphate isomerase adhesins were also similar.**Conclusions:** These results helped us evaluate the behavior of *Paracoccidioides* spp. and indicate that *G. mellonella* is a suitable model to reactivate one of the most studied virulence mechanism of *P. brasiliensis*, the adhesion, beside the different behavior of *P.lutzii* as gp43 expression.

Author Disclosure Block:

L. Scorzoni: None. **A. de Paula e Silva:** None. **J. Singulani:** None. **F. Sangalli-Leite:** None. **H. de Oliveira:** None. **C. Maria Marcos:** None. **R. Moraes da Silva:** None. **A. Fusco-Almeida:** None. **M. Mendes-Giannini:** None.

Poster Board Number:

MONDAY-664

Publishing Title:

Novel Insights Into *Salmonella* Typhi Pathogenesis from *Ex Vivo* Human Tissue Models

Author Block:

K. P. Nickerson[1], S. Senger[1], M. B. Sztein[2], A. Fasano[1], M. R. Fiorentino[1]; [1]Massachusetts Gen. Hosp., Boston, MA, [2]Univ. of Maryland, Baltimore, MD

Abstract Body:

Salmonella enterica serovar Typhi is the causative agent of Typhoid fever, a disease with an estimated 22 million cases annually, resulting in 200,000 deaths. In some areas of the globe, the incidence of Typhoid fever is as high as 500 cases out of every 100,000 children. At present, much remains to be uncovered concerning the host responses to *S.* Typhi infection. Available vaccines are moderately protective. Current therapeutic strategies include antibiotic treatment; however the frequency of antibiotic-resistant serovars is increasing worldwide. Additionally, in some individuals chronic *S.* Typhi colonization of the gall bladder culminates in gall bladder cancer, therefore highlighting the significant short and long-term consequences of infection. To design alternative therapeutic strategies, there is an immediate need to better understand *S.* Typhi infection and pathogenesis. To interrogate human responses to *S.* Typhi infection, terminal ileum biopsies were collected from donors for direct infection *in vitro* or generation of organoids. Terminal ileum derived organoids give rise to a diversity of epithelial cells, including goblet, Paneth and M cells, which are grown as a monolayer *in vitro*. Use of the epithelial monolayer and whole biopsy models identified specific contributions of the epithelium in response to *S.* Typhi infection as assessed by RNA-sequencing, qPCR, and cytokine secretion. Differences in cellular association of bacteria were assessed using immunofluorescence and transmission electron microscopy. To identify how the gut mucosa responds to infection, apical and basolateral culture supernatants were collected for ELISA analysis. These studies demonstrated differences in interleukin (IL)-8, IL-1β and IL-12p70 cytokine production depending on the infection model and the secretion location. Interestingly, infected monolayers showed the highest levels of basolateral cytokine release. Taken together, our data characterizes key aspects of terminal ileum response to *S.* Typhi infection addressing a critical gap in our current understanding of Typhoid fever pathogenesis.

Author Disclosure Block:

K.P. Nickerson: None. **S. Senger:** None. **M.B. Sztein:** None. **A. Fasano:** C. Consultant; Self; General Mills, Inc; Crestovo, LLC; Pfizer, Inc.. K. Shareholder (excluding diversitied mutual funds); Self; Alba Therapeutics. N. Other; Self; Mead Johnson Nutrition. **M.R. Fiorentino:** None.

Poster Board Number:

MONDAY-665

Publishing Title:

Manual Collection, Ontological Representation, and Bioinformatics Analysis of over 5000 Microbial Virulence Factors and Related Host-Pathogen Interactions

Author Block:

Y. He[1], S. Sayers[1], S. Deng[1], G. Fu[1], Y. Li[2], B. Yang[1], S. Zhang[1], Y. Lin[1], B. Zhao[1], Z. Xiang[1]; [1]Univ. of Michigan, Ann Arbor, MI, [2]Inst. for Animal Husbandry and Vet. Sci., Beijing, China

Abstract Body:

Background: Virulence factors are those components of microbes that are necessary to overcome host defenses and cause disease in a host. It is critical to systematically collect and analyze virulence factors and how they interact with host.**Methods:** Manual curation from peer-reviewed journal articles was used to identify virulence factors. The mutants of these virulence factors were experimentally found to be attenuated inside host (including human and animals) or host cells. These virulence factors and how they interact with host were also studied using bioinformatics approaches.**Results:** We identified 5,173 virulence factors in total, including 4644 virulence factors for 50 bacteria, 179 for 54 viruses, 105 for 13 parasites, and 245 for 8 fungi. The data of these virulence factors and their interactions with host are stored in the web-based database Victors (http://www.phidias.us/victors). All Victors virulence factor data are queryable through a user-friendly web interface. The virulence factors can also be searched by a customized BLAST sequence similarity searching program. A Clusters of Orthologous Groups (COG) analysis method has been developed for functional annotation of these virulence factors. Furthermore, to support data sharing and integration, we have developed an Ontology of Host-Pathogen Interactions (OHPI, http://www.ontobee.org/ontology/OHPI) that ontologically represents the virulence factor data using machine-readable Web Ontology Language (OWL) format. The ontology information is queryable using a web-based SPARQL query program. As a use case demonstration, the *Brucella* virulence factors and their interactions with host were analyzed with the support of the Victors and OHPI systems. *Brucella* is a facultative intracellular bacterium that causes brucellosis, one of the most common zoonotic diseases in the world in humans and a variety of animal species. Victors stores 439 *Brucella* virulence factors.**Conclusions:** Virulence factors demonstrate their special patterns on how to interact with host and host cells. Overall, Victors and OHPI provide a good platform for integrated studies of microbial virulence factors and host-pathogen interactions.

Author Disclosure Block:

Y. He: None. **S. Sayers:** None. **S. Deng:** None. **G. Fu:** None. **Y. Li:** None. **B. Yang:** None. **S. Zhang:** None. **Y. Lin:** None. **B. Zhao:** None. **Z. Xiang:** None.

Poster Board Number:

MONDAY-666

Publishing Title:

Investigations Into The Life Cycle Of *drepanocephalus Auritus* In The Double-Crested Cormorant *phalacrocorax Auritus*

Author Block:

N. R. Alberson[1], T. G. Rosser[1], D. T. King[2], L. H. Khoo[3], D. J. Wise[3], E. T. Woodyard[1], L. M. Pote[1], M. J. Griffin[3]; [1]Mississippi State Univ. Coll. of Vet. Med., Mississippi State, MS, [2]United States Dept. of Agriculture Wildlife Services, Mississippi State, MS, [3]Thad Cochran Natl. Warmwater Aquaculture Ctr., Mississippi State Univ., Stoneville, MS

Abstract Body:

Drepanocephalus auritus, a parasite of the double-crested cormorant (DCC) *Phalacrocorax auritus* and the marsh ramshorn snail *Planorbella trivolvis,* has been shown to infect channel catfish *Ictalurus punctatus*. In a recent longevity study, metacercariae were seen in catfish 7 days post infection but a marked reduction in parasite burden was observed by day 21, with infection failing to persist much past 70 days. This called for investigations whether channel catfish can serve as a true intermediate host in the *D. auritus* life cycle. A total of 2,807 *P. trivolvis* snails were collected from a catfish production pond in Mississippi, U.S.A., of which 389 snails (13.9%) were actively releasing *D. auritus* cercariae. Juvenile channel catfish (2-4 cm) were individually exposed to ~150 cercariae/fish. At 5 days post-challenge, a sub-sample of fish was examined histologically to confirm infection. Seven DCC were live-captured, housed indoors in individual pens, and administered praziquantel to clear any residual gastrointestinal helminths. Experimental birds (n=3) were fed *D. auritus* parasitized catfish. Bird fecal samples were collected daily and sedimentations and egg counts performed. Few trematode eggs were observed 6 days post infection. However, at 8 days post-infection adult trematodes were found in the feces. Experimental and control birds were euthanized 18 days post infection. A total of 245 adult trematodes morphologically consistent with *D. auritus* were recovered from the intestines of birds fed *D. auritus* infected fish. No adult *D. auritus* were recovered from control birds. Mitochondrial cytochrome *c* oxidase subunit 1 (CO1) sequences from recovered adults shared 99.6-100% identity with other North American *D. auritus* isolates. This study demonstrates that channel catfish can serve as an intermediate host in the life cycle of *D. auritus* and is the first experimental elucidation of the *D. auritus* life cycle in DCC.

Author Disclosure Block:

N.R. Alberson: None. **T.G. Rosser:** None. **D.T. King:** None. **L.H. Khoo:** None. **D.J. Wise:** None. **E.T. Woodyard:** None. **L.M. Pote:** None. **M.J. Griffin:** None.

Poster Board Number:

MONDAY-667

Publishing Title:

Pathogenesis of the Novel Species *Streptococcus tigurinus* in a *G. mellonella* Bacteremic Model

Author Block:

A. Fadil, N. Tullos; Mississippi Coll., Clinton, MS

Abstract Body:

Background: *Streptococcus tigurinus* is a novel oral viridans species recently separated from *Streptococcus mitis*. Though *S. tigurinus* has been shown to cause lethal bacteremic disease, to date there has only been one study to examine the virulence of this opportunistic pathogen. Therefore we determined to analyze the pathogenesis of *S. tigurinus* (ATCC 15914) in an invertebrate model of bacteremia using the greater wax moth (*Galleria mellonella*). Our hypothesis is that *S. tigurinus* will exhibit a high level of virulence in this model. **Methods:** Fifth instar larva were infected with 5, 6 or 7 LogCFU/larva via injection into the hemocoel. Larva were observed for a 24 hour period at which point they were homogenized in PBS. Lethality was determined by calculating the LD_{50} of *S. tigurinus* using Probit analysis. Bacterial survival was calculated at 24 hours using the CFU plate count method. Immune induction of the melanin deposition pathway was measured by photographic analysis of larval pigmentation using the Gray Scale Value (GSV) assay and phenoloxidase activity (PO) at 24 hours post infection (p.i.). Statistical analysis was performed using a Student's T test. **Results:** The 24 hour LD_{50} of *S. tigurinus* 15914 was calculated as $3.98x10^5$ per larva (n = 150). Bacterial survival was unchanged in larva infected with 5 LogCFU/larva at 24 hours, though larva infected with 6 LogCFU and 7 LogCFU showed significant growth compared to the 5 LogCFU group (6 LogCFU group = 8.96 LogCFU/larva , 7 LogCFU group = 8.41 LogCFU/larva ; $n \geq 4$ per group, $P \leq 0.05$). All three groups showed a significant immune activation as demonstrated by increased larval pigmentation compared to mock infected larva (Mock = 105.3 gsv, 5 LogCFU/larva = 89.2 gsv, 6 LogCFU/larva = 53.81 gsv, 7 LogCFU/larva 47.31 gsv; $n \geq 12$ per group, $P \leq 0.01$). Interestingly all three groups showed significant decreases in PO activity at 24 hours compared to mock infected larva (Mock = 1.842 abs , 5 LogCFU/larva 0.997 abs , 6 LogCFU/larva = 0.814 abs, 7 LogCFU/larva = 0.756 abs ; $n \geq 4$ per group . $P \leq 0.041$).**Conclusions:** This study is the first to examine *S. tigurinus* pathogenesis in a *G. mellonella* model and only the second virulence study *in vivo*. *S. tigurinus* demonstrated significant lethality, survivability and induced a robust immune response in this bacteremic model. Therefore this study provides new information regarding the bacteremic pathogenesis of this novel Streptococcus species.

Author Disclosure Block:

A. Fadil: None. **N. Tullos:** None.

Poster Board Number:

MONDAY-668

Publishing Title:

A Novel Method For Proteomic Profiling Of *burkholderia* grown Under Diverse Environmental Conditions

Author Block:

M. Franco, M. Liou, Y. Haider, J. Pena, B. Segelke, S. El-Etr; Lawrence Livermore Natl. Lab., Livermore, CA

Abstract Body:

Burkholderia sp is a diverse gram-negative genus that includes pathogenic species that are of great medical importance (e.g. *B. mallei* and *B. pseudomallei*, the causative agents of glanders and melioidosis, respectively). Although the advancement of 'omics' technologies made headway in expanding our understanding of *Burkholderia* sp. biology, proteomic methods for studying bacterial responses to various growth environments or for identifying secreted bacterial effectors are lacking. To address this need, our study takes advantage of orthogonal amino acid labeling method, which allows for an easy purification of newly synthesized proteins expressed by bacteria growing in virtually any condition. The expression of mutant variant of methionyl-tRNA synthetase (NLL-MetRS) in *B. thailandensis* (a nonpathogenic model strain) allows for the substitution of azidonorleucine (Anl) in the place of Methionine (Met) in newly synthesized bacterial proteins (1). NLL-MetRS gene from *E.coli* was codon optimized for optimal expression in *Burkholderia* sp. and stably expressed in *B. thailandensis* using MiniTn7 transposon insertion method. In this study, Anl-tagged proteins are then affinity purified using alkyne-functionalized biotin and identified by mass spectrometry. We believe that this 'pulse-chase' proteomic method represents a new powerful approach in the *Burkholderia* field to quickly purify bacterial proteins and to study the changes that occur at the proteomic level in response to different growth environments (e.g. extracellular or while replicating within an eukaryotic host). The results from this study will be presented.

Author Disclosure Block:

M. Franco: None. **M. Liou:** None. **Y. Haider:** None. **J. Pena:** None. **B. Segelke:** None. **S. El-Etr:** None.

Poster Board Number:

MONDAY-669

Publishing Title:

Pathogenesis of *Pseudomonas aeruginosa* in Microgravity

Author Block:

E. PAUL, A. Shresta; Southwestern Oklahoma State Univ., Weatherford, OK

Abstract Body:

BackgroundStressors during space flights take their toll on the human body, particularly the immune system, increasing the susceptibility of astronauts to opportunistic pathogens like *Pseudomonas aeruginosa*. *P. aeruginosa*, a bacterium found in soil, water and on the skin, can cause urinary tract, lung, and kidney infections especially under microgravity conditions encountered during space flights.**Methods**It would be interesting to study if the microbe adapts to microgravity and changes its virulence profile. To undertake this study *P. aeruginosa* was grown in conditions mimicking microgravity using a rotating wall vessel reactor for a period of 48 hours and compared to cultures grown at normal gravity conditions. The cultures were then studies for motility characteristics using 0.3% Tryptic soy agar plates. The morphology of the colonies produced was recorded. In addition bioassays using lettuce leaves were conducted by measuring the zones of necrosis. All experiments were conducted more than 5 times and were reproducible. Previous research in our lab optimized conditions required to mimic microgravity conditions.**Results**Twitching assays were conducted to examine if microgravity conditions enhance twitching and disease establishment. After growth periods of 48hours, *P. aeruginosa* grown under microgravity conditions showed a significant difference in the spreading or twitching growth on 0.3% agar motility plates. The microgravity grown cultures showed an average increase of 12% in colony size compared to normal cultures in multiple replicates. In addition these microgravity cultures exhibited differences in colony morphology; microgravity conditions microbes grew 120% taller than cultures grown under normal gravity conditions.**Conclusions**We also observed that microgravity cultures caused greater necrotic zones in bioassays using lettuce leaves compared to the culture grown in normal conditions. These results led us to believe that *P. aeruginosa* displays increased virulence under micro-gravity conditions. We have identified three potential proteins involved in increased twitching/motility. We plan to further examine the function of the three proteins.

Author Disclosure Block:

E. Paul: None. **A. Shresta:** None.

Poster Board Number:

MONDAY-670

Publishing Title:

A Virulence Comparison of Two Temporally Separated *Mycoplasma gallisepticum* Strains

Author Block:

K. Pflaum, E. R. Tulman, J. Beaudet, X. Liao, S. J. Geary; Univ. of Connecticut, Storrs, CT

Abstract Body:

Since 1994, *Mycoplasma gallisepticum*, a respiratory pathogen of poultry, has emerged as a significant pathogen of the American house finch (*Carpodacus mexicanus*), causing severe conjunctivitis in the eyes of the birds. Over the course of the ensuing epizootic event, the house finch-related *M. gallisepticum* (HFMG) strains demonstrated rapid spread and increased virulence in the house finch host. In this current study, we assessed two temporally separated HFMG isolates also differing in virulence for house finches - VA1994, the virulent index isolate of the epizootic, and highly virulent VA2013. To compare virulence of these isolates in domestic poultry hosts, chickens were experimentally challenged and assessed pathologically and for bacterial recovery. Here we report a significant difference between these two HFMG isolates for virulence in chickens. Interestingly, VA2013, more virulent in the house finch, demonstrated a significant reduction in virulence in chickens relative to VA1994, displaying decreased pathology and recovery from the respiratory tract. Genomic sequencing of VA2013 and comparison to VA1994 indicated genomic changes potentially affecting observed phenotypic differences. Overall, these data indicate a reciprocal evolution of HFMG virulence in house finch and poultry hosts, and provide insight to bacterial adaptation and HFMG virulence evolution in a novel host.

Author Disclosure Block:

K. Pflaum: None. **E.R. Tulman:** None. **J. Beaudet:** None. **X. Liao:** None. **S.J. Geary:** None.

Poster Board Number:

MONDAY-671

Publishing Title:

Establishing *Dugesia japonica* as a Model System to Study *Mycobacterium avium* Subspecies *Avium* Pathogenesis

Author Block:

I. A. Adekola Estinville, R. J. Major, V. Irani; Indiana Univ. Of PA, Indiana, PA

Abstract Body:

Mycobacterium avium is an opportunistic environmental pathogen causing disseminated and respiratory infections in healthy and immunocompromised humans. It has been determined that the host gut is one of the portals of entry for this bacterium, leading to the development of bacteremia and systemic disease. Through our studies using host murine macrophage (mø) cell line, J774A.1, we have demonstrated that *M. avium* serovar 8 glycopeptidolipid (ssGPL) is responsible for modulation of a variety of host innate immune responses (pro- and anti-inflammatory cytokine production, MAPK signaling, phagosome-lysosome fusion) and *M. avium* survival. To determine if these host immune responses are linked to *M. avium* survival, it is important to have a host model system so as to dissect these immune pathways in a sequential fashion. We have selected *Dugesia japonica*, a planarian, as our host model organism-it offers a means to characterize the innate immune responses conserved from yeast to man. In addition, (i) the planarian gastrovascular cavity branches throughout the body, which is useful to study the effect of *M. avium* colonization on host gut and (ii) unlike a "one type of cell" host system of mammalian møs, being multicellular and having the ability to live indefinitely is a powerful tool to study long term *M. avium* infection in a host. Using green fluorescent protein-expressed *M. avium* serovar-8 wild type and the serovar-null (no ssGPL) mutant, we are currently establishing the *M. avium* infectious process in *D. japonica*. With the help of bacterial (survival) counts, whole in situ hybridization (WISH) technology and pharmacological inhibitors, we will start the process of finally understanding and piecing together the complex *M. avium* infection process and modulation of host innate responses in *D. japonica*. This could provide an effective strategy for manipulating host innate responses to counteract bacterial survival in diseases beyond *M. avium*.

Author Disclosure Block:

I.A. Adekola Estinville: None. **R.J. Major:** None. **V. Irani:** None.

Poster Board Number:

MONDAY-672

Publishing Title:

A New Player in Bioluminescence Quorum Sensing in *Vibrio fischeri*

Author Block:

I. Ster[1], R. Foxall[1], K. L. Visick[2], C. A. Whistler[1]; [1]Univ. of New Hampshire, Durham, NH, [2]Loyola Univ. Med. Ctr., Maywood, IL

Abstract Body:

Bacteria use phosphorelay systems to sense and respond to environmental stimuli, and to direct cell density quorum sensing (QS). The phosphorelay system that controls QS bioluminescence by *Vibrio fischeri* transduces signal from the hybrid-histidine kinases (HHK) LuxQ and AinR, which use kinase activity to phosphorylate a histidine phosphotransferase protein (HPT) LuxU, which in turn phosphorylates a response regulator (RR) LuxO, a repressor of bioluminescence. When LuxQ and AinR accept QS signals at high cell density they act as phosphatases on LuxU, relieving repression of bioluminescence by dephosphorylation of LuxO. Following experimental evolution of squid-naïve *V. fischeri* strain in the squid *Euprymna scolopes*, a previously uncharacterized HHK (VFMJ11_A0397) was identified as an activator of bioluminescence in strain MJ11. Here, we investigated how this regulator interfaces with the known QS machinery using native and mutant forms of VFMJ11_A0397. We hypothesized that this HHK is prototypical and requires a C-terminal REC domain to interact with bioluminescence-specific HPT and RR partners. To identify downstream RR partners, the wild-type allele was overexpressed in a series of RR null mutants of the well-characterized strain ES114. The VFMJ11_A0397 gene did not significantly increase bioluminescence in a *luxO* null mutant or in the presence of a LuxO allele locked in the phosphorylated state, suggesting that LuxO is a downstream partner. Expression of a truncated VFMJ11_A0397 lacking the REC domain did not increase bioluminescence by wild-type strain ES114 to the same extent as the full-length copy, suggesting loss of activity. Interestingly, the REC domain alone increased bioluminescence. Together, these results suggest that VFMJ11_A0397 promotes bioluminescence like the known *lux* HHKs LuxQ and AinR in a REC-dependent manner, likely by phosphatase activity. Future work will further elaborate how this new player integrates with the well-insulated *lux* regulatory pathway and provide insight into the evolution of the QS pathway as a key player in symbiosis.

Author Disclosure Block:

I. Ster: None. **R. Foxall:** None. **K.L. Visick:** None. **C.A. Whistler:** None.

Poster Board Number:

MONDAY-673

Publishing Title:

The Effect of an Anti-Virulence Therapy on Virulence, Pathogen Fitness, Pathogen Behavior and Host Responses

Author Block:

M. Weigert[1], A. Ross-Gillespie[1], G. Pessi[1], S. P. Brown[2], R. Kümmerli[3]; [1]Univ. of Zurich, Zürich, Switzerland, [2]Georgia Inst. of Technology, Georgia, GA, [3]Univ. of Zürich, Zürich, Switzerland

Abstract Body:

Anti-virulence treatments aim to disarm rather than kill pathogens, and that is why selection for drug resistance should be relatively weak. However, in order to develop effective and evolutionarily robust anti-virulence treatments we need a detailed understanding of how anti-virulence drugs affect pathogen fitness and virulence. Additionally, we also need to understand potential side effects of these drugs, including changes in pathogen behavior (through altered gene expression) and host defense mechanisms.In this study we address these questions using the human pathogen *Pseudomonas aeruginosa* and the insect host *Galleria mellonella* as a model system. In particular, we are focusing on pyoverdine, an iron scavenging siderophore, which is an important virulence factor. We reduced pyoverdine availability by using gallium (an iron mimic), which quenches pyoverdine outside the cell, but also studied the effect of increased pyoverdine availability by adding purified pyoverdine to infections. We examined how these treatments affected host survival (as a proxy for virulence), bacterial fitness, the expression of genes linked to pyoverdine, and host responses.We found that virulence scales linearly with pyoverdine availability, which indicates that anti-virulence drugs can efficiently and predictively reduce virulence. Pathogen fitness, however, peaked at intermediate pyoverdine availability, showing that this anti-virulence therapy does not curb bacterial growth in a predictable way. Next, we used qPCR to show that pyoverdine quenching also reduced the availability of two other virulence factors (exotoxin A and protease IV), highlighting that anti-virulence therapy can affect multiple traits. Finally, we found that pyoverdine availability significantly affected host immune responses, which in turn might modulate pathogen and host fitness.Our findings show that gallium in particular and anti-virulence treatments in general could become important tools against nosocomial infections caused by multi-resistant bacteria. However, our results also reveal that a deeper engagement with the intrinsic complexity of host-pathogen systems is required to realize effective and evolutionarily robust anti-virulence therapies.

Author Disclosure Block:

M. Weigert: None. **A. Ross-Gillespie:** None. **G. Pessi:** None. **S.P. Brown:** None. **R. Kümmerli:** None.

Poster Board Number:

MONDAY-674

Publishing Title:

Examining the Nodulation Pathway in *Burkholderiatuberum*

Author Block:

A. Arnell, H. Abdulla, M. Lum; Loyola Marymount Univ., Los Angeles, CA

Abstract Body:

Nitrogen is the most limiting macronutrient for plant growth despite its abundance in the Earth's atmosphere, as plants are incapable of harnessing atmospheric N_2. Some bacteria are capable of fixing N_2 into ammonia (NH_3), a form usable by plants. Nitrogen-fixing bacteria known as rhizobia engage in a symbiotic relationship with legumes and trigger the formation of nodules on roots within which the bacteria are housed and fix nitrogen for the plant in exchange for carbohydrates. The bacterial *nod* genes are induced by flavonoids secreted by plants experiencing nitrogen stress, which results in the production of Nod factor, a critical signal triggering nodule formation. In the well-studied α-rhizobia, *nodD* encodes a constitutively expressed regulatory protein sensitive to these flavonoids and induces the expression of the *nod* genes, It is thus far believed that β-rhizobia function similarly to α-rhizobia both genotypically and phenotypically, however few studies have addressed the regulatory mechanisms in the β-rhizobia. In order to shed light on the nature of the nodulation pathway in *B. tuberum*, we are investigating the role of *nodD* in nodulation and regulation of the *nod* genes by characterizing a *nodD* deletion mutant and expression of a *nodC* transcriptional fusion to the *lacZ* reporter gene. The *nodC* gene was selected as the target of a promoter-reporter fusion due to its regulation by NodD in α-rhizobia and its importance in the nodulation pathway. Nodulation assays indicate that the deletion of *nodD* does not negatively affect the bacteria's ability to interact with the host plant, as functional nodules are observed in plants inoculated with the mutant. Through a series of Miller assays we discovered a significant increase (>400%, $p<0.01$) in the expression of *nodC* in *B. tuberum* in the presence of genistein and daidzein, flavonoids known to induce the nodulation response in α-rhizobia.

Author Disclosure Block:

A. Arnell: None. **H. Abdulla:** None. **M. Lum:** None.

Poster Board Number:

MONDAY-675

Publishing Title:

Investigating Partner Choice of *Medicago polymorpha* as It Evolves in Non-Native Regions

Author Block:

K. J. Wozniak[1], M. L. Friesen[1], S. S. Porter[2]; [1]Michigan State Univ., East Lansing, MI, [2]Washington State Univ., Vancouver, WA

Abstract Body:

The *Medicago polymorpha-Ensifer medicae* mutualism increases legume growth due to symbiotic nitrogen fixation in nodules and has evolved from the Mediterranean coast to all continents except Antarctica. This legume-rhizobia symbiosis has agricultural and ecological importance and is a model system of how mutualisms operate in diverse ranges. To determine whether the legume's ability to choose bacteria has evolved as a consequence of invading new habitats, *M. polymorpha* from California (invasive) and Portugal (native) was inoculated with single and mixed strains of co-evolved and naive *E. medicae.* We hypothesized that if native legume genotypes exhibit a specialist behavior, they would have increased biomass when inoculated with single strains of co-evolved rhizobia. Conversely, increased fitness would be observed in invasive legume genotypes inoculated with a mixed range of rhizobia due to relaxed selection on host partner choice. After legumes reached reproductive maturity, dry weights were taken of nodules, shoots, and roots to determine how partner choice influenced fitness according to region. The Portugal legumes outperformed California legumes in overall biomass and had slightly higher biomass when inoculated with a single co-evolved strain. California legumes had higher shoot and root biomass when given a mixed inoculation of co-evolved strains. Additionally, invasive *M. polymorpha* genotypes appear to have relaxed symbiont selection because they performed better with mixed inoculation and native genotypes maintain their specialist behavior when given co-evolved *E. medicae*. Taken together, these findings suggest that both native and invasive *M. polymorpha* perform well when interacting with co-evolved strains.

Author Disclosure Block:

K.J. Wozniak: None. **M.L. Friesen:** None. **S.S. Porter:** None.

Poster Board Number:

MONDAY-676

Publishing Title:

A Multi-Omics Investigation of the Response of *Setaria viridis* to Growth Promoting *Azospirillum brasilense* Rhizobacteria

Author Block:

A. P. Ferrieri[1], C. K. Ansong[1], E. M. Zink[1], A. Dohnalkova[1], R. K. Chu[1], J. B. Cliff III[1], C. R. Anderton[1], L. Paša-Tolić[1], M. Schueller[2], G. Stacey[2], R. Ferrieri[2]; [1]Environmental and Molecular Sci. Lab., Richland, WA, [2]Univ. of Missouri, Columbia, MO

Abstract Body:

Plant growth-promoting rhizobacteria are considered to have a beneficial effect on host plants; however, it is controversial whether biological nitrogen fixation from associative interaction contributes to growth promoting effects. This project uses S*etaria viridis* as a model C4 grass to explore the mechanisms underpinning biological nitrogen fixation in association with the plant growth promoting rhizobacteria, *Azospirillum brasilense*. Using a systems biology approach, *S. viridis* plants have been extensively studied at the physiological and biochemical levels using compositional and flux analysis with radioactive (^{11}C, ^{13}N) tracers. Here we highlight new results using a high-throughput, isobaric tags for relative and absolute quantitation (iTRAQ)-based, quantitative proteomic approach to analyze changes in protein profiles of *S. viridis* roots inoculated with an ammonium-excreting strain of *A. brasilense* (Hm053). General features of the *S. viridis* root proteome included the up-regulation of proteins related to amino acid biosynthesis (e.g. glutamate and glutamine metabolic processes), carbon transport and metabolism, and protein turnover in the presence of *A. brasilense.* These findings provided direction for multi-scale mass spectrometry imaging (MALDI, nanoSIMS) studies aimed to understand the trafficking of ^{13}C- and ^{15}N-labeled compounds between bacteria and roots. Together these studies offer insight into the mechanisms by which rhizobacteria establish themselves as symbionts within their hosts during early stages of infection, and a deeper understanding of the mechanism for their promotion of plant growth through associative nitrogen fixation and other phytostimulatory actions. Knowledge gained from this effort will directly translate to next-generation bioenergy cropping systems fostering more robust plants.

Author Disclosure Block:

A.P. Ferrieri: None. **C.K. Ansong:** None. **E.M. Zink:** None. **A. Dohnalkova:** None. **R.K. Chu:** None. **J.B. Cliff III:** None. **C.R. Anderton:** None. **L. Paša-Tolić:** None. **M. Schueller:** None. **G. Stacey:** None. **R. Ferrieri:** None.

Poster Board Number:

MONDAY-677

Publishing Title:

Developing a Genetic System for *Frankia*

Author Block:

R. Oshone, S. Hurst, C. Pesce, L. Tisa; Univ. of New Hampshire, Durham, NH

Abstract Body:

Background: *Frankia* are soil-dwelling actinobacteria that form a nitrogen-fixing symbiotic association with 8 different families of angiosperms. Despite over two decades of research in the area, the genetics of *Frankia* is still in its infancy. There are no known systems for gene transfer in *Frankia*. For an increased understanding of *Frankia* physiology and plant-microbe interactions, it is essential that transformation and targeted mutagenesis protocols are established. Here, we report the introduction of a plasmid (pHTK1) that could stably replicate and express both an antibiotic resistance gene and the green fluorescent protein marker (GFP) in *Frankia*. **Methods**: *Frankia* sp. CcI3 was conjugated with *Escherichia coli* BW29427 (DAP^-) containing the pHTK1 plasmid. The conjugants were plated on minimal growth medium containing tetracycline (20 µg/ml) and kanamycin (50 µg/ml). After three weeks, single colonies of transformants were inoculated into broth growth medium containing both antibiotics. The plasmid was extracted using a modified alkaline lysis method and visualized on an agarose gel. A PCR approach was used to confirm the plasmids by amplifying the REP and the *tetA* genes. The Transformed *Frankia* cells expressing the GFP protein were examined under UV light. **Results:** The filter mating between *Frankia* sp. CcI3 and *E. coli* BW29427 containing pHTK1 produced a conjugation frequency of 10^{-4} to 10^{-5} transconjugants/recipients. Single colonies of transformed *Frankia* cells were isolated and propagated in minimal growth medium containing tetracycline and kanamycin. Isolated transconjugants were stably maintained in culture suggesting that the pHTK1 plasmid was replicating in *Frankia* and the *tetA* gene on the plasmid was being expressed. The plasmid was re-isolated from *Frankia* transconjugants and visualized on an agorose gel. PCR amplification of the *tetA* and REP regions confirmed the presence of the plasmid in transconjugants. The copy number of the plasmid in *Frankia* was determined to be two per genome. Transconjugants fluoresced under UV light indicating the expression of the GFP protein in *Frankia*. **Conclusion:** This is the first report on the induction of a plasmid that could stably replicate in *Frankia* and is a major breakthrough in *Frankia* genetics. We are currently trying to use the pHTK1 plasmid to engineer a codon-optimized CRISPR/Cas9 system for site specific gene disruption in *Frankia*.

Author Disclosure Block:

R. Oshone: None. **S. Hurst:** None. **C. Pesce:** None. **L. Tisa:** None.

Poster Board Number:

MONDAY-678

Publishing Title:

Single Molecule Real-Time Sequencing of Two *Bradyrhizobium* genomes Reveals Differences That May Account for Symbiotic Effectiveness and Viral Predation

Author Block:

N. Place; Delaware Biotechnology Inst., Newark, DE

Abstract Body:

Ensuring crop production is vital to food security and is a pressing issue in light of predicted world population increase. Legumes, which are rich in protein and critical to the diets of humans and animals, require a steady supply of nitrogen, provided through root symbiosis with nitrogen-fixing bacteria, such as those in the genus *Bradyrhizobium*. This work sought to provide broad-based information on bradyrhizobia biology through whole genome sequencing and analysis of two strains differing in their symbiotic effectiveness with soybean.Using Pacific Biosciences single molecule real-time (SMRT) sequencing, >100X coverage of the genomes of *B. elkanii* USDA 76 and *B. diazoefficiens* USDA 122 was obtained and the genomes were closed through sequence assembly (ca. 9.1 Mbps each). The Rapid Annotation using Subsystem Technology (RAST) was used to predict and annotate genes. These genomes were found to be novel showing only distant homology to publically available complete genomes. One intact prophage was found in USDA 76 by PHAST (PHAge Search Tool). As no *Bradyrhizobium* phage genome sequences are currently available, this and other prophage segments provide a first glimpse into the potential impact of viral predation on the host. SMRT sequencing indicated a m6A modification in GANTC motif in both strains; this methylation is different from whole genome to prophage-like regions in USDA 122, 0.73% and 0.83% respectively.The location of genes related to symbiosis have been found in symbiotic islands and plasmids. Rhizobitoxine, a protein necessary for nodulation can be detrimental if overproduced. Using reference rtx operon genes, these genes were found in the sequenced genomes. USDA 76 continues to support this theory of an operon, but USDA 122's genes matching rtx genes were spread across the chromosome. Comparing the arrangement of these genes in the two strains divulges information about gene regulation affecting symbiosis. Analyzing the genomes of bradyrhizobia and their constitutive viruses discloses the symbiotic effectiveness due to population levels.

Author Disclosure Block:

N. Place: None.

Poster Board Number:

MONDAY-679

Publishing Title:

Dictyostelium discoideum* as a Model Organism to Identify Virulence and Virulence Regulatory Factors in *Escherichia coli

Author Block:

M. Weichseldorfer, M. Qureshi, P. Phan, B. Leicht, D. Parker, T. White, M. Hemm, N. Alkharouf, M. Snyder; Towson Univ., Towson, MD

Abstract Body:

Escherichia coli (*E. coli*) is normally found as part of the human gut microbiota, however a number of diarrheagenic strains have emerged, such as enterohemorrhagic *E. coli*. Identification and disruption of the virulence factors that promote infection could lead to a reduction in infection period of these *E. coli* strains and potentially species with homologous means of virulence. In order to identify *E. coli* strains with certain characteristics of virulence, we have turned to *Dictyostelium discoideum* (*D. discoideum*), a social amoeba that phagocytizes bacteria for nutrition and shares similar properties with mammalian macrophages. Using random mutagenesis, we created two mutant stocks of *E. coli*, each exposed to a different concentration of mutagen. These stocks were individually allowed to be phagocytized by *D. discoideum* over a period of time, enriching the culture and selecting for mutants resistant to the intracellular methods that *D. discoideum* employs to kill. After several rounds of selection, highly resistant mutants were isolated and we found that some of the resistant strains showed increased survival when compared to the wild-type strain. Once isolated, the full genomes of our resistant mutant strains were sequenced using Illumina's next generation MiSeq technology. While analyzing the sequencing data from these mutant strains, we found several potential genes of interest, including genes related to flagella, oxidoreductases, and acid resistance. We have begun to characterize genes of interest through various qualitative and quantitative assays using individual gene knockout strains. These studies should allow for identification of novel factors associated with regulation of virulence in *E. coli*. In addition, given that the development of mechanisms to resist *D. discoideum* predation may have contributed to the selection and maintenance of bacterial virulence factors against mammalian hosts, these studies may provide insight on the evolution of host-pathogen interactions.

Author Disclosure Block:

M. Weichseldorfer: None. **M. Qureshi:** None. **P. Phan:** None. **B. Leicht:** None. **D. Parker:** None. **T. White:** None. **M. Hemm:** None. **N. Alkharouf:** None. **M. Snyder:** G. Member; Self; Michelle Snyder.

Poster Board Number:

MONDAY-680

Publishing Title:

Dna Inversion Regulates Outer Membrane Vesicle Production in *Bacteroides fragilis*

Author Block:

H. Nakayama-Imaohji, T. Kuwahara; Kagawa Univ., Faculty of Med., Kagawa, Japan

Abstract Body:

Background: Phase changes in *Bacteroides fragilis*, a member of the human colonic microbiota, mediate variations in a vast array of cell surface molecules, such as capsular polysaccharides and outer membrane proteins through DNA inversion. Three master DNA invertases that globally control promoter inversions at many distant regions have been identified in *B. fragilis*: Mpi, Tsr0667 and Tsr19 (corresponding to the BF2766-encoded DNA invertase in *B. fragilis* YCH46). BF2766 inverts two distantly localized promoters, IVp-I and IVp-II. In this study, these BF2766-regulated invertible promoters of *B. fragilis* were further analyzed to determine the role of their associated proteins in surface structure modification.**Methods:** A series of BF2766 mutants were constructed in which the two promoters were locked in different configurations (IVp-I/IVp-II=ON/ON, OFF/OFF, ON/OFF or OFF/ON). Cell surface morphology of the four mutants were analysed by scanning electron microscopy and transmission electron microscopy. DNA microarray analysis was performed with the four locked mutants to identify genetic regions whose alterations in expression were specifically associated with a hypervesiculating ON/ON. To examine the role of outer membrane vesicle (OMV) production in adaptation to cell envelope stress, we compared the survival of the four mutants following contact with antimicrobial compounds (bile, LL-37 and human defensins).**Results:** ON/ON *B. fragilis* mutants exhibited hypervesiculating, whereas the other mutants formed only a trace amount of OMVs. By comparing the transcriptomes of the four BF2766 deletion mutants, we found that the transcription of the genes downstream of IVp-II markedly elevated in a hypervesiculating ON/ON strain. The ON/ON mutants showed higher resistance to treatment with bile, LL-37, and human β-defensin 2. Incubation of wild-type cells with 5% bile increased the population of cells with the ON/ON genotype.**Conclusions:** These results indicate that *B. fragilis* regulates the formation of OMVs through DNA inversions at two distantly related promoter regions in response to membrane stress, although the mechanism underlying the interplay between the two promoters remains unknown.

Author Disclosure Block:

H. Nakayama-Imaohji: None. **T. Kuwahara:** None.

Poster Board Number:

MONDAY-681

Publishing Title:

Pseudomonas Quinolone Signal: A Cross-Species Outer Membrane Vesicle Inducer

Author Block:

A. M. Horspool, J. W. Schertzer; Binghamton Univ., Binghamton, NY

Abstract Body:

Abstract: Microbial communities in nature rely on cellular communication and transport for regulating essential processes governing microbial growth, viability, and pathogenesis. Utilization of quorum sensing molecules by bacteria to monitor cell density within an environment is necessary to establish synchronized expression of virulence factors required for pathogenesis. Previously, quorum sensing molecules and virulence factors have been discovered to be transported within vesicles produced from the outer membrane of *Pseudomonas aeruginosa*. These Outer Membrane Vesicles (OMVs) are capable of delivering virulence factors to host cells and can induce cytotoxicity.It has been shown that the quorum-sensing molecule 2-heptyl-3-hydroxy-4-quinolone (PQS) is capable of inducing the formation of OMVs in *P. aeruginosa* in a manner independent of its receptor or *de novo* protein synthesis. We showed that PQS was capable of inducing membrane curvature in model membranes and developed the Bilayer-Couple model to describe this phenomenon. We further hypothesized that biophysical control of OMV formation by small molecules may play an important role in the physiology of other Gram-negative organisms. To test this hypothesis, we exposed other species of bacteria known to produce OMVs to exogenous PQS and quantified the resulting OMV production. The rationale was that if PQS exerts a biophysical effect on membranes, OMV production would be increased in the other species when exposed to exogenous PQS. Here we show that introduction of PQS to cultures of *P. aeruginosa, Escherichia coli, Proteus mirabilis, Proteus hauseri*, or *Klebsiella pneumoniae* results in increased OMV formation in all cases. Consistent with our work using model membranes, this suggests that PQS has a biophysical effect on the outer membranes of many common Gram-negative organisms and that membrane curvature-induction by small molecules may be a feature involved in OMV formation across species.

Author Disclosure Block:

A.M. Horspool: None. **J.W. Schertzer:** None.

Poster Board Number:

MONDAY-682

Publishing Title:

Enteroaggregative *Escherichia coli* Antiaggregation Protein Shields Multiple Surface Proteins Involved in Autoaggregation

Author Block:

L. J. Bowers[1], C. O. Hale[1], L. V. Blanton[1], L. T. Wang[1], J. Hofmann[1], J. W. DuBow[1], I. N. Okeke[2]; [1]Haverford Coll., Haverford, PA, [2]Univ. of Ibadan, Ibadan, Nigeria

Abstract Body:

Background: Enteroaggregative *Escherichia coli* (EAEC) cause diarrhea and show exceptional autoaggregation and biofilm-formation abilities. Among the surface factors contributing to autoaggregation of EAEC strain 042 are aggregative adherence fimbriae II (AAF/II) and the heat-resistant agglutinin 1 (Hra1), which is sufficient but not necessary for autoaggregation. We hypothesized that double mutations in these genes and the antiaggregation protein, Aap, would identify autoaggregation factors that are masked by Aap. **Methods:** Single and double mutants in the *aafA, hra1* and *aap* genes were constructed by allelic exchange. Autoaggregation was quantified in settling assays and the ability of strains to initiate biofilms on a vertical glass surface was monitored qualitatively. **Results:** We confirm that *aap* mutants autoaggregate faster and to a greater extent than wild type and that an *hra1* mutant is not deficient in autoaggregation. Deletion of *aafA* reduced autoaggregation but autoaggregation was actually enhanced in the *aap* and *aafA* double mutant, suggesting that other adhesins were unmasked by removal of these factors. Autoaggregation phenotypes largely predicted the formation of aggregates in a vertical biofilm, with the important exception that the *hra1* mutant, but not the *hra1-aap* double mutant, was defective in this phenotype. We additionally observed autoaggregation mediated by an unidentified phase-variable factor in the *hra1-aap* double mutant. **Conclusions:** Autoaggregation and biofilm formation are robust in 042 because of contributions from different adhesins. AAF/II and Hra1 make subtly separate contributions to these phenotypes. Aap sterically masks afimbrial surface proteins involved in autoaggregation including Hra1 and an as yet unknown, potentially phase-variable factor.

Author Disclosure Block:

L.J. Bowers: None. **C.O. Hale:** None. **L.V. Blanton:** None. **L.T. Wang:** None. **J. Hofmann:** None. **J.W. DuBow:** None. **I.N. Okeke:** None.

Poster Board Number:

MONDAY-683

Publishing Title:

Enteroaggregative *Escherichia coli* Surface Proteins Modulating Intercellular Interactions

Author Block:

M. A. Levine[1], J. Hofmann[1], I. N. Okeke[2]; [1]Haverford Coll., Haverford, PA, [2]Univ. of Ibadan, Ibadan, Nigeria

Abstract Body:

Background: Enteroaggregative *Escherichia coli* (EAEC) are diarrheal pathogens defined by the characteristic 'stacked brick' or aggregative adherence pattern they produce on epithelial cells. EAEC are exceptional colonizers and express several factors involved adherence to eukaryotic cells. We studied adherence of EAEC strain 042, focusing on aggregative adherence fimbriae II (AAF/II), antiaggregation protein (Aap) and heat-resistant agglutinin 1 (Hra1). All three have separately been shown to contribute to eukaryotic cell adherence, and Aap and AAF/II are transcriptionally regulated by AggR. We hypothesized that Aap, Hra1 and AAF/II interact to optimize adherence. **Methods:** We qualitatively studied hemagglutination and epithelial cell adherence of EAEC strain 042 as well as its single and double isogenic mutants in the *aap, aafA* and *hra1* genes. **Results:** The *aggR* master regulator gene was required for all phenotypes and *aafA* was required for hemagglutination. However, all other single deletions produced no or modest changes in eukaryotic cell adherence. The *aap* mutant did not elicit true aggregative adherence at one hour but the phenotype was similar to wild type after three hours incubation. Deleting *hra1* or *aafA* produced a similar slowing of stacked-brick formation, unless *aap* was also deleted, in which case aggregative adherence occurred at wild type levels by one hour. **Conclusions:** Aap and AAF/II both appear to interact with Hra1 and/or another adhesin, likely by steric inhibition. Deletion of *aap* and *hra1* impedes stacked brick formation, suggesting that these might be important early-stage factors in EAEC adherence.

Author Disclosure Block:

M.A. Levine: None. **J. Hofmann:** None. **I.N. Okeke:** None.

Poster Board Number:

MONDAY-684

Publishing Title:

Detergent-Based Purification of *Shigella* Invasion Plasmid Antigen C Provides Superior Stability and Facilitates Direct Characterization of Membrane Interaction

Author Block:

A. Bernard[1], P. Kumar[2], N. E. Dickenson[1]; [1]Utah State Univ., Logan, UT, [2]Univ. of Kansas, Lawrence, KS

Abstract Body:

Shigella spp. infection results in shigellosis, a severe form of bacillary dysentery. *S. flexneri* utilizes a Type III Secretion System (T3SS) to provide a direct conduit between the bacterial and host cell cytoplasm and allow direct transfer of effector proteins into the host cell cytoplasm. Invasion Plasmid Antigen C (IpaC) is a critical component of the secretion system as it not only serves to polymerize the host cell cytoskeleton and promote pathogen uptake, but it also localizes to the tip of the T3SS where it is believed to play a crucial role in membrane interaction and translocon insertion. While the precise roles of IpaC within the context of the T3SS are not fully understood, *Shigella's* reliance on IpaC for virulence and its localization on the pathogen surface make it a particularly attractive target for anti-infective therapeutics. Progress on this front has proven challenging, however, as difficulties obtaining recombinant soluble IpaC have prevented a full understanding of the mechanism and driving forces behind the IpaC host membrane interaction. Traditionally, purification of IpaC has relied on either collection from *Shigella* culture supernatant or use of the chaotropic agent urea, imposing certain limitations on *in vitro* characterization. Here, we have developed alternative purification methods to obtain purified recombinant IpaC and present data finding that these conditions provide IpaC in an appropriate state for further *in vitro* characterization. Specifically biophysical characterization including liposome flotation assays, encapsulated dye release and circular dichroism found that the developed methods provide significantly higher levels of active protein with superior stability characteristics compared to traditional methods. Additionally, recent findings made possible by these advancements in IpaC purification are presented, providing new insight into the IpaC/host membrane interaction.

Author Disclosure Block:

A. Bernard: None. **P. Kumar:** None. **N.E. Dickenson:** None.

Poster Board Number:

MONDAY-686

Publishing Title:

Inhibition of Pilus Assembly by the Small Molecule Nitazoxanide

Author Block:

J. J. Psonis, P. N. Chahales, D. G. Thanassi; Stony Brook Univ., Stony Brook, NY

Abstract Body:

Uropathogenic *Escherichia Coli* (UPEC) is the primary causative agent of urinary tract infections (UTIs), which afflict more than 50% of women and 15% of men. Critical to the establishment of UTIs by UPEC are pili. The pili (or fimbriae) expressed by UPEC are virulence-associated surface structures that are assembled by the chaperone/usher (CU) pathway and mediate bacterial adhesion to host cells. We have discovered that the small molecule nitazoxanide (NTZ) inhibits pilus biogenesis via the CU pathway in UPEC. We have determined that the inhibitory effect of NTZ on pilus biogenesis is due to interference with proper maturation of the usher protein in the bacterial outer membrane (OM). The usher is required for assembly of the pilus fiber and secretion of the fiber across the OM to the cell surface. The usher is folded and inserted into the OM by the ß-barrel assembly machinery complex (Bam), which in *E. coli* consists of five proteins, BamA-E. We are using a variety of approaches to determine the mechanism by which NTZ interferes with usher folding, including measuring binding of NTZ to the usher, to individual Bam proteins, or to the intact Bam complex. In addition, we are using lipid nanodisc technology to reconstitute the folding pathway of the usher in the outer membrane. Lipid nanodiscs are self-assembled, discoidal, nanoscale particles of lipid bilayer stabilized in solution by two encircling amphipathic helical proteins, termed membrane scaffold proteins. As soluble and stable membrane mimetics, nanodiscs represent an attractive model system for biophysical and biochemical studies of membrane proteins such as the usher. We have successfully reconstituted the Bam complex into lipid nanodiscs and are using these to compare the ability of nanodisc-reconstituted Bam complex to fold urea-denatured PapC, in the presence or absence of NTZ. These studies will help to identify the specific target of NTZ and its mechanism of action, and will facilitate the design of new therapeutic agents that target bacterial adhesion. In addition, these studies will reveal new details by which proteins such as the usher fold in the outer membrane.

Author Disclosure Block:

J.J. Psonis: None. **P.N. Chahales:** None. **D.G. Thanassi:** None.

Poster Board Number:

MONDAY-687

Publishing Title:

Homo-Trimeric Structure of Type IV Minor Pilin CofB of CFA/III from Human Enterotoxigenic *Escherichia coli*

Author Block:

H. Oki, K. Kawahara, S. Fukakusa, T. Yoshida, T. Maruno, Y. Kobayashi, D. Motooka, T. Iida, T. Ohkubo, S. Nakamura; Osaka Univ., Suita, Japan

Abstract Body:

In gram-negative bacteria, the assembly of type IV pilus (T4P) and its evolutionally related pseudopilus of type II secretion (T2S) system, involving characteristic structural proteins called pilins or pseudopilins respectively, is dynamically regulated for enhancing bacterial functions, such as protein transport, adherence, and motility. Despite their importance for bacterial pathogenesis, the question of how (pseudo) pilins are assembled to form such filamentous appendage is still remains to be answered. Previous studies suggested that the structural "tip" like ternary complex formed through the interaction of minor (pseudo) pilins, for example GspK, GspI, and GspJ from the T2S system of enterotoxigenic *Escherichia coli*, plays an important role in this process, while some members of the pathogenetic type IVb subfamily are known to have only one such minor subunit whose function is unknown. Here we determined the crystal structure of the type IVb minor pilin CofB of CFA/III from human enterotoxigenic *Escherichia coli* at a resolution of 1.88 Å. The structure, in conjunction with physicochemical analysis in solution, clearly showed the homo-trimeric arrangement of CofB. Each CofB monomer in the trimer has unique three-domain architecture, in which C-terminal lectin-like domain could effectively initiate the trimer association of its pilin-like N-terminal domain by extensive hydrophobic interactions followed by domain-swapping occurred at middle domain. Coupled with the result of *cofB* deletion mutant of *Escherichia coli* showing phenotype with no pili formation, we found by the molecular modeling study that the minor pilin CofB of CFA/III does promote the T4P assembly by forming homo-trimetric structural "tip" like complex. This study further enhances our understanding of the conserved mechanism of (pseudo) pilus assembly among these systems.

Author Disclosure Block:

H. Oki: None. **K. Kawahara:** None. **S. Fukakusa:** None. **T. Yoshida:** None. **T. Maruno:** None. **Y. Kobayashi:** None. **D. Motooka:** None. **T. Iida:** None. **T. Ohkubo:** None. **S. Nakamura:** None.

Poster Board Number:

MONDAY-688

Publishing Title:

Characterization of a Novel K88-/Cs13-Like Pili of a Biofilm-Forming Enteroinvasive *Escherichia coli* (Bf-Eiec) Emergent Strain

Author Block:

C. Zhang, D. R. Leal-Barrera, 37232, O. G. Gomez-Duarte, 37232; VANDERBILT Univ., Nashville, TN

Abstract Body:

Biofilm-forming enteroinvasive *Escherichia coli* (BF-EIEC) is an emergent pathotype identified from a child with moderate to severe diarrhea in Bucaramanga, Colombia. Transposition mutagenesis of the isolate designated BC/Ac52.1 resulted in a mutant negative for biofilm formation. Preliminary sequencing analysis revealed that the transposition event occurred at a fimbrial operon homologous to K88 and CS13 pili. The objective of the study was to characterize the K88-/CS13-like pili at the genetic and phenotypic levels. Based on whole genome DNA sequence BLAST analysis, this BF-EIEC pili operon, putatively designated as *epl* (*E. coli* pili locus), is composed of seven structural genes including four fimbrial minor subunits C (504 bp), F (492 bp), H (798bp), and I (753 bp); one major subunit G (825 bp); one usher protein D (2397bp) and one chaperone protein E (783bp). Potential transcriptional regulators were found upstream and downstream of the operon in close proximity. The C, F, H, I minor subunits have the highest protein sequence homology with aalA of CS23 (90%), faeF, faeH, faeI of K88 (100%) of ETECs, respectively. The G major subunit has highest protein sequence homology with cshE of CS13 (ETEC) (99%) but low homology to major subunits in other closely related pili operons (23% to 37%). The operon contains eight overlapped nucleotides between C and D genes and three atypical initiation codons (ATT, TTG, and GTG) in three genes. Transmission Electron Microscopy (TEM) imaging showed the presence of the pili-like structure on the cell surface of the wild-type strain but not the transposition mutant strain. We cloned, expressed, and purified the pili major subunit *epl* G *in vitro*, indicating that the major subunit gene is intact and may likely be expressed in the wild type strain. This study provides preliminary evidence that the *epl* operon may be expressed in BF-EIEC that it may be implicated in adherence and gut colonization.

Author Disclosure Block:

C. Zhang: None. **D.R. Leal-Barrera:** None. **O.G. Gomez-Duarte:** None.

Poster Board Number:

MONDAY-689

Publishing Title:

Fima2 Fimbriae are Required for *Acinetobacter baumannii* Biofilm Formation and Motility *In Vitro*

Author Block:

S. Singh, S. O'Dell, C. N. McQueary, J. L. Kessler, J. Luka, D. V. Zurawski; Walter Reed Army Inst. of Res., Silver Spring, MD

Abstract Body:

Acinetobacter baumannii is an important nosocomial bacterial pathogen due to its virulence, multidrug resistance (MDR), and prolonged survival in a desiccated state. *A. baumannii* has been a significant militarily-relevant pathogen in past wars such as Vietnam as well as the recent conflicts of Iraq and Afghanistan. *A. baumannii* is also responsible for ≥10% infections acquired in U.S. hospitals, and poses a formidable threat to post-operation patients. There are some strains that have been classified as extensively drug resistant and even pandrug-resistant, which is fueling the current research to develop novel therapeutics. Factors that may contribute to *A. baumannii* pathogenicity include biofilm formation, motility, dissemination, secreted toxins, and adherence. Fimbriae have been previously implicated in both motility and biofilm formation, qualities that are thought to contribute to *A. baumannii* colonization, and ultimately virulence. After sequencing and annotation of our model strain, AB5075, we have identified two other fimbrial operons, which we are now calling FimA1 and FimA2 because of their homology to the FimA-like fimbriae from other bacterial species. We hypothesized that a disruption in either of these two systems may impact motility and/or biofilm formation. Using a sequenced, Tn5 transposon library of AB5075, we analyzed mutants of the major fimbrial subunit in each system, fimA1::Tn*5* and fimA2::Tn*5* (fimA1- and fimA2-) and a pilA- mutant as a control. We found that the fimA2- mutant made less biofilm in comparison to wild-type AB5075 and to the other mutants tested. Further, a fimA2- mutant had decreased twitching motility when compared to its wild-type parent and pilA-. Upon analysis of proteins being secreted during late log/stationary phase, we identified the FimA2 protein by mass spectrometry as being one of five major proteins secreted into the extracellular milleu in minimal media. FimA2 protein was overexpressed, purified, and monoclonal antibodies were generated. We are currently testing the antibody as a possible treatment and comparing wild-type, mutant, and complemented strains in virulence models of infection. Overall, these results suggest that FimA2 is the major fimbrial subunit of a previously unidentified fimbriae that is required for biofilm and motility in *A. baumannii*.

Author Disclosure Block:

S. Singh: None. **S. O'Dell:** None. **C.N. McQueary:** None. **J.L. Kessler:** None. **J. Luka:** None. **D.V. Zurawski:** None.

Poster Board Number:

MONDAY-690

Publishing Title:

The Genomic Sequence of a Ciprofloxacin-Resistant Strain of *Francisella tularensis* Identified an LPS Mutation Which Leads to a Severe Attenuation in Murine Models of Tularemia

Author Block:

J. A. Bozue, R. Toothman, J. Chua, T. Kijek, J. Ladner, C. Cote, G. Koroleva, S. Lovett, F. Biot, S. Mou, G. Palacios, N. Vietri, P. Worsham; USAMRIID, Fort Detrick, MD

Abstract Body:

Background: *Francisella tularensis* is a gram-negative facultative intracellular bacterial pathogen that can infect many mammalian species, including humans. Because of its ability to cause a lethal infection, its low infectious dose, and aerosolizable nature, *F. tularensis* subspecies *tularensis* (*Ft*) is considered a potential bioterrorism agent. The potential of bacteria to become resistant to antibiotics through natural or intentional genetic alteration increases our risk to biothreat agents. The fluorinated quinolone, ciprofloxacin is one of the antibiotics of choice for treating many of these infections. However, there are several examples of laboratory derived resistant *Francisella* species in the literature.**Methods:** A naturally selected ciprofloxacin resistant mutant of the Schu4 strain was further characterized, including whole genome sequencing.**Results:** Alterations to the *gyrA* and *parE* genes were confirmed, most likely leading the ciprofloxacin resistance. Most other mutations were missense mutations. However, a frame shift to *kdsD* which encodes for arabinose phosphate isomerase was identified. This enzyme catalyzes the conversion of the pentose pathway intermediate D-ribulose 5-phosphate into D-arabinose 5-phosphate (A5P). A5P is a precursor of KDO, an integral part of the LPS. A *kdsD* mutation in Schu4 *Ft* was constructed using a modified TargeTron mutagenesis system and characterized. In addition, a transposon mutant in the homologous gene (*kpsF*) of the subspecies *F. novicida* (*Fn*) U112 strain was also examined. The virulence of these mutant strains was characterized through in vitro macrophage assays and various murine models of tularemia. Both mutant strains were affected in their ability to infect macrophages and to cause disease in mice. Virulence could be restored via genetic complementation.**Conclusions:** The A5P isomerase is essential for tularemia, in murine models of infection. This enzyme appears to be an attractive target for future therapeutic studies to combat antimicrobial resistance for biothreat agents.

Author Disclosure Block:

J.A. Bozue: None. **R. Toothman:** None. **J. Chua:** None. **T. Kijek:** None. **J. Ladner:** None. **C. Cote:** None. **G. Koroleva:** None. **S. Lovett:** None. **F. Biot:** None. **S. Mou:** None. **G. Palacios:** None. **N. Vietri:** None. **P. Worsham:** None.

Poster Board Number:

MONDAY-691

Publishing Title:

Adherent to a New Set of Rules: Adhesin Gene Maintenance and Expression in *Shigella flexneri* Infection

Author Block:

R. B. Chanin, K. P. Nickerson, D. K. Vijaya Kumar, C. S. Faherty; Massachusetts Gen. Hosp., Boston, MA

Abstract Body:

Shigella flexneri is a Gram-negative, facultative intracellular pathogen that invades the colonic epithelium and causes significant diarrheal disease in children of the developing world. Based on the current infection paradigm, *Shigella* transits M cells in the colon prior to invasion, but it remains unknown how contact is initiated with these cells. *Shigella* is closely related to *Escherichia coli* and maintains many of the same fimbrial gene operons; however, at least one critical gene component in each operon is mutated. These mutations, coupled with the immunostimulatory nature of adherence factors, have led the field to hypothesize that *Shigella* is unable to produce fimbriae. With previous data suggesting that *S. flexneri* can adhere to epithelial cells, our goal is to determine if the pathogen utilizes adhesins to initiate infection. Using RT-PCR, we have detected expression of conserved Type 1 *fim* genes and five additional adherence gene clusters. Expression of these genes was further enhanced by *S. flexneri* growth in glucose, as determined by quantitative RT-PCR. Furthermore, bacterial adherence to colonic HT-29 cells was enhanced 10-fold following glucose exposure. Interestingly, transmission electron microscopy of *S. flexneri* grown in the presence of glucose revealed mostly afimbriated bacteria. However, in a related project, imaging of bacteria grown in the presence glucose and bile salts revealed highly adhesive bacteria with fimbrial-like structures. Since we have previously shown that bile salts induces protein secretion in *S. flexneri*, we hypothesize that the coordinated exposure to glucose combined with a secretion signal, such as host cell contact or bile salts, is required for the proper assembly and expression of *S. flexneri* adhesins. We are currently performing targeted mutagenesis and protein interaction studies to identify the specific genes required for producing these structures. Our work demonstrates that conditions present endogenously in the host, such as glucose and bile salts, offer key stimuli needed for proper adherence factor expression in *Shigella*. This work may translate to other enteric pathogens; and importantly, these fimbrial-like structures could become novel vaccine targets for *Shigella.*

Author Disclosure Block:

R.B. Chanin: None. **K.P. Nickerson:** None. **D.K. Vijaya Kumar:** None. **C.S. Faherty:** None.

Poster Board Number:

MONDAY-692

Publishing Title:

The *Shigella flexneri* OspE1 and OspE2 Proteins Form a Bile Salt-Induced Complex to Facilitate Adherence

Author Block:

K. P. Nickerson[1], R. B. Chanin[1], J. R. Sistrunk[2], D. A. Rasko[2], C. S. Faherty[1]; [1]Massachusetts Gen. Hosp., Boston, MA, [2]Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract Body:

Globally, the *Shigella* species cause watery diarrhea and bacillary dysentery, resulting in significant pediatric morbidity and mortality in developing countries. Attempts to develop an effective vaccine remain unsuccessful, possibly due to a limited understanding of early steps in *Shigella* pathogenesis. Following oral ingestion and transit through the gastrointestinal tract, which includes bile exposure in the small intestine, *Shigella* invades the colonic epithelium to establish infection. We have previously demonstrated that bile salts promote adherence to colonic epithelial cells through the induced expression and localization of the homologous OspE1 and OspE2 proteins to the bacterial outer membrane. In this study, we have continued to characterize the mechanism of OspE1/2-mediated adherence. First, we have demonstrated that pre-treatment of bacteria with an OspE1/2 antibody inhibited bile salt-induced adherence to colonic epithelial cells. Furthermore, bile salts promoted bacterial adhesion to several extracellular matrix components including laminin, fibronectin and mucin. These interactions were dependent on OspE1/2 since a Δ*ospE1/2* mutant was unable to reproduce the phenotype. We hypothesize that OspE1/2 adhesion to ECM components is a mechanism by which *Shigella* identifies the colonic epithelium prior to infection. Finally, through co-immunoprecipitation and protein purification experiments, we have identified bacterial protein candidates that bind OspE1/2. RNA-sequencing analysis has demonstrated that the genes encoding some of these proteins are also induced by bile salts exposure. We are currently performing additional mutational analyses to determine which candidates interact with OspE1/2. We hypothesize that the interacting proteins form an adhesion complex with OspE1/2 to facilitate bile salt-induced adherence. In all, our data demonstrate that bile salts exposure represents a critical gastrointestinal signal that coordinates *Shigella* OspE1/2-dependent adhesion during infection.

Author Disclosure Block:

K.P. Nickerson: None. **R.B. Chanin:** None. **J.R. Sistrunk:** None. **D.A. Rasko:** None. **C.S. Faherty:** None.

Poster Board Number:

MONDAY-693

Publishing Title:

***Aliivibrio salmonicida* Requires O-Antigen for Optimal Virulence in Its Salmonid Host**

Author Block:

S. F. Nørstebø, H. Sørum, A. M. Bjelland; Norwegian Univ. of Life Sci., Oslo, Norway

Abstract Body:

Aliivibrio salmonicida is the causative agent of coldwater vibriosis, a hemorrhagic septicemia of farmed salmonids and cod. In the early years of Norwegian aquaculture, the disease caused major losses for the industry. Today, it is being well controlled by the use of vaccines. However, the virulence factors of *A. salmonicida* are still poorly understood. Several authors have reported the presence of VS-P1, an outer membrane complex comprised of rough-type LPS and a protein moiety, in the serum of moribund fish. A role in virulence for VS-P1 through modulation of the host immune response has been postulated.To investigate the role of LPS in the pathogenesis, we have constructed in-frame deletion mutants for O-antigen ligase, *waaL*. As this gene exists in two copies in the genome of *A. salmonicida* as part of a 29 kb duplication, we have constructed single and double mutants to explore potential effects of the gene duplication. Virulence was assessed by challenging Atlantic salmon parr (*Salmo salar* L.) through intraperitoneal injection (i.p.) of mutant or wild type bacteria.The Δ*waaL* mutant did not differ significantly from WT in terms of mortality. For Δ*waaL*Δ*waaL* on the other hand, mortality was reduced five-fold. In addition, bacterial colonization of the host blood stream was delayed for the double, but not the single mutant.The results suggest that *A. salmonicida* requires O-antigen for optimal disease development in Atlantic salmon. Even though two copies of the *waaL* gene is present, one is sufficient for function in virulence. Also, O-antigen does not seem to be necessary for survival *in vivo*, as some mortality was still seen after challenge with the double mutant.

Author Disclosure Block:

S.F. Nørstebø: None. **H. Sørum:** None. **A.M. Bjelland:** None.

Poster Board Number:

MONDAY-694

Publishing Title:

Delineating Protein-Protein Interactions between Components of the Cpi-2 Encoded Type-III Secretion System from the Opportunistic Pathogen *Chromobacterium violaceum*

Author Block:

K. Zoccola, S. James, **H. Betts-Hampikian**; Clarion Univ. of Pennsylvania, Clarion, PA

Abstract Body:

Virulent bacteria are equipped with proteins capable of disrupting host cells. In order for many species to be able to infect a host, the pathogen must be able to transport and translocate virulence factors from the bacterial cytosol to the host cell cytoplasm. The evolution of bacterial secretion systems have allowed for this type of movement. Out of the six known secretion pathways, our focus is the type-III secretion system (T3SS). T3SSs are found only in Gram negative bacteria and resemble a molecular syringe, consisting of approximately 25 proteins housed within the cell envelope, collectively forming a hollow conduit. Distal to this basal apparatus is an extracellular needle-like structure which facilitates interaction with the host cell plasma membrane, and allows for the translocation of anti-host effector proteins directly into the eukaryotic cell. Recently an emerging opportunistic pathogen of humans and mammals, *Chromobacterium violaceum*, has been shown to harbor gene clusters encoding two T3SSs. A comprehensive bioinformatics analysis assigned these clusters as Chromobacterium pathogenicity islands 1 and 2 (Cpi-1 and Cpi-2 respectively). Genes on the loci of both Cpi-1 and Cpi-2 are evolutionary related to those in the Spi-1 and Spi-2 encoded T3SSs of *Salmonella enterica*. Based on this evidence, it has been proposed that Cpi-1 may function early in infection, and Cpi-2 may be required for survival within host cells. While some research pertaining to Cpi-1 has been accomplished, very little is known about Cpi-2. We have begun delineating the structure and function of the Cpi-2 apparatus by investigating protein-protein interactions between 11 putative inner membrane and needle apparatus components. Using the yeast two-hybrid (Y2H) system and assaying pair-wise for direct interactions, we have found the following: Interactions between CsaQ-U indicate they likely comprise the inner membrane ring structure, CV2588 interacts with itself in a manner consistent with its putative role as the needle protein, CseB1-4 may be capable of forming an extra-cellular filament, and CV2578 (putative class-II chaperone) interacts with the putative translocator proteins CV2576 and CV2676. We are currently in the process of performing further analyses to confirm our Y2H data.

Author Disclosure Block:

K. Zoccola: None. **S. James:** None. **H. Betts-Hampikian:** None.

Poster Board Number:

MONDAY-695

Publishing Title:

***clostridium Sordellii* Exosporial Proteins Play A Role In Spore Viability And Gut Colonization**

Author Block:

R. Rabi, M. Awad, S. Larcombe, D. Lyras; Monash Univ., Clayton, Australia

Abstract Body:

Clostridium sordellii causes a range of often fatal diseases in humans and animals. Crucial to the infectious cycle of *C. sordellii* is its ability to form spores, the outermost layer of which, the exosporium, is the initial contact point between the spore and the host or environment. Despite the importance of spores in the clostridial life cycle, the *C. sordellii* spore structure remains largely unknown. In particular, its exosporium has not been investigated.The *C. sordellii* exosporial proteome was determined using mass spectrometry and two uncharacterised proteins, Protein A and Protein B, were identified in high abundance. Genes encoding Protein A and Protein B were insertionally inactivated and spore morphology was characterised by electron microscopy. Mutant A spores had a polar exosporium compared to the central, uniformly distributed exosporium seen in wild-type spores. Mutant B spores lacked an attached exosporium, however, exosporial fragments were seen in the spore preparation. Thus, both Protein A and B may play a role in correct attachment of the exosporium to the spore. Sporulation assays determined that mutant A produced 10% less viable spores than the wild-type strain. Treating spores to a range of harsh conditions identified that mutant A had up to four times less resistance to acids and bases compared to wild-type spores and were therefore less hardy.*C. sordellii* has been identified as the cause of enteric disease in many animals. We therefore developed a mouse gut infection model and used this model to study the phenotypic effects of the exosporial mutations. Mice ($n \geq 5$) were orally gavaged with spores of each strain and faecal spores from each mouse were enumerated for four days post infection. Mice infected with wild-type and mutant A strains showed a steady decrease in faecal spore numbers by about one log per day. However, mice infected with mutant B did not show a decrease in faecal spore numbers, with spore numbers remaining high for the duration of the experiment. This phenotype was restored to that of the wild-type upon complementation. These results suggest that either the absence of Protein B or an exosporium, resulted in the persistence of spores in the gut. This is in line with *Clostridium difficile*, where research has shown that spores lacking an exosporium resulted in increased adherence to gut epithelial cells.

Author Disclosure Block:

R. Rabi: None. **M. Awad:** None. **S. Larcombe:** None. **D. Lyras:** None.

Poster Board Number:

MONDAY-696

Publishing Title:

Regulation of the Enterotoxin Gene Cluster and Role in *Staphylococcus aureus* Infective Endocarditis

Author Block:

A. M. Martini[1], C. S. Stach[2], J. M. King[1], W. Salgado-Pabon[1]; [1]Univ. of Iowa, Iowa City, IA, [2]Univ. of Minnesota, St. Paul, MN

Abstract Body:

Infective endocarditis (IE) is a microbial infection of the heart characterized by inflammation and deposition of host factors on the endocardium. Left-sided, native valve IE caused by *Staphylococcus aureus* (SAIE) is notably aggressive, resulting in frequent cardiac, neurological, and renal complications and carries a mortality rate of 30-40%. Among the myriad virulence factors produced by *S. aureus*, Staphylococcal enterotoxins (SEs) (e.g., SEA, SEB, and SEC) and non-emetic superantigens (SAgs) (e.g., TSST-1) are well-established agents of diseases such as food poisoning and toxic shock syndrome. The enterotoxin gene cluster (*egc*) is a locus of five to six SE-like genes with previously indirect association with disease and high prevalence in nasal and toxemia isolates of *S. aureus*. Previous work performed in our laboratory has demonstrated a critical role for SEC in a rabbit model of IE, suggesting *egc* SEs may also play a role in the development of SAIE. We utilize a rabbit model of left-sided, native valve IE and heterologous expression constructs to demonstrate the involvement of both TSST-1 and the *egc* in vegetation formation. Concomitant deletion of *tstH* and *egc* results in a significant decrease in vegetation size, while heterologous expression of either *tstH* or *egc* from a non-pathogenic strain produces vegetations. To investigate potential regulatory mechanisms of the *egc*, promoter-GFP fusions, immunoblotting, qPCR, and *in vitro* DNA-binding assays were performed. The global regulator MgrA was identified by EMSA to specifically bind upstream regions of *selo* and *selu*; however, transcriptional fusions in MgrA and an array of global regulator mutants known to modulate other virulence factors did not show alterations in promoter activation, suggesting a more complex level of control beyond global regulators. In contrast to other characterized SEs and SAgs, qPCR suggests expression of *egc* during exponential phase. Finally, immunoblotting suggests proteases do not impact the ability to detect native *egc* proteins from culture supernatant. Our data demonstrate a significant role for the *egc* in development of SAIE. Molecular mechanisms controlling *egc* expression appear distinct from those of the well-characterized SEs and SAgs, suggesting a novel mechanism of regulation for this locus.

Author Disclosure Block:

A.M. Martini: None. **C.S. Stach:** None. **J.M. King:** None. **W. Salgado-Pabon:** None.

Poster Board Number:

MONDAY-697

Publishing Title:

Humanized Non-obese Diabetic Severe Combined Immunodeficient Common Gamma Chain-deficient (Nsg) Mice as Novel Models to Study the Acute and Chronic Effects of *Staphylococcal superantigens* on the Human Immune System *In Vivo*

Author Block:

A. Krogman, **G. Rajagopalan**; Mayo Clinic Coll. of Med., Rochester, MN

Abstract Body:

Background: Superantigens (SAg) are a family of potent exotoxins produced by *Staphylococcus aureus*. We systematically investigated the impact of SAg on the human immune system from the *in vivo* perspective using immunodeficient NSG mice reconstituted with human immune cells. Materials: Eight to ten-week old NSG mice were adoptively transferred with 50 million peripheral blood mononuclear cells isolated from healthy donors through intravenous route (n=6-8 mice/group). For the acute exposure model, mice were challenged with 10 μg of staphylococcal enterotoxin B (SEB), bled at 3 hours and sacrificed at 72 hours. For chronic exposure model, mice were subcutaneously implanted with mini osmotic pumps capable of releasing 10 μg of SEB or saline over a 7-day period and sacrificed on day 8. Results: In the acute model, unreconstituted NSG mice challenged with SEB as well as mice reconstituted with human PBMC but challenged with saline had undetectable levels of 26 human cytokines/chemokines at 0, 3 as well as 72 hours measured using a multiplex assay. However, NSG mice reconstituted with human PBMC and challenged with SEB had significantly elevated levels of Th1, Th2 as well as Th17 families of cytokines, with some elevated at 3 hours and some at 72 hours. Notable among them were IL-2 (10778±1886 pg/ml), IL-17A (330±20 pg/ml) and IFN-γ (9296±850 pg/ml) at 3 hours. Lungs, livers and kidneys only from this group of mice had significant infiltration with human CD4+ and CD8+ T cells mimicking classical multi-organ failure. Serum AST/ALT levels were also elevated in this group. In the chronic exposure model, SEB group had splenomegaly (Total splenocytes count = 79±11 million) compared to PBS group (Total splenocytes count = 21±6.5 million). The CD4+ as well as CD8+ T cell numbers were also increased in the SEB group (21±2 and 9.5±0.1 million) compared to PBS group (1.8±0.8 and 0.4±0.2 million). This was also accompanied by severe infiltration of various organs with T cells in mice from SEB group compared to PBS group. Summary: Overall, using humanized NSG mouse model we demonstrate for the first time that both acute as well as chronic exposure to staphylococcal SAg can elicit significant immunopathology and our observations could have significant clinical implications.

Author Disclosure Block:

A. Krogman: None. **G. Rajagopalan:** None.

Poster Board Number:

MONDAY-698

Publishing Title:

Report of the *egc* Cluster and Exotoxin Genes in a Blood Culture Strain of *Staphylococcus epidermidis* by Whole-Genome Sequencing

Author Block:

L. Pinheiro, V. C. Pereira, J. P. Araújo Jr, M. R. S. Cunha; Inst. de Biociencias de Botucatu, UNESP, Botucatu - SP, Brazil

Abstract Body:

Despite being considered as avirulent organisms for decades, the Coagulase-Negative Staphylococci (CoNS) have emerged as important cause of opportunistic infections, mainly associated to medical devices. The objective of this study was to investigate the toxigenic features of a *Staphylococcus epidermidis* strain (H-1006/91) isolated from blood culture of a patient hospitalized at a teaching hospital in Brazil. By PCR, the enterotoxin genes *sec*, *seg* and *sei* were identified in the strain. The isolate lacked the *sea*, *seb*, *sed*, *see*, and *seh* genes. Using the RT-PCR, it was found that H-1006/91 expressed both *seg* and *sei* mRNAs. The whole genome sequencing was performed using Illumina technology. Eighteen contigs contained parts or the complete sequence of enterotoxin genes of the *egc* cluster, including *seo*, *sem*, *sei*, *sen* and *segL29P* (*seg*), non-coding regions and integrase sequences, besides 89% and 99% of identity, respectively, to the *selv* and *selu* genes, and part of the pseudogenes *φent1* and *φent2*. The sequences were very similar (>70%) to the *S. aureus egc* cluster; *sem* and *seo* sequences presented the highest identities to the *S. aureus* genes. An 84-bp region of plasmid replication, very similar to the pETB (exfoliatin B plasmid) replication protein was also observed. There were identified very similar sequences to other exotoxins of *S. aureus* (*set8*, *set20*, *set13*, *set14*) and recombination genes were found. A large portion of the sequenced plasmid showed high relatedness to phage genes and a truncated transposase of the SCC*mec* type IV. In fact, the strain was *mecA*-negative. To our knowledge, this is the first report of the presence of all the coding sequences of the *egc* cluster by whole-genome sequencing in a strain of *S. epidermidis*. The *egc* is considered as an enterotoxin gene nursery, which could form new superantigen genes by recombination. In *S. aureus*, several allelic variants of this operon have been found, but its presence in CoNS is controversial. Here we show that the *egc* cluster may also confer selective advantage for *S. epidermidis* and, although possessing identity to the *S. aureus egc*, it contains several regions of recombination and SNPs. These events may lead to misidentification of these toxin genes and also cause generation of new superantigens in CoNS species.

Author Disclosure Block:

L. Pinheiro: None. **V.C. Pereira:** None. **J.P. Araújo Jr:** None. **M.R.S. Cunha:** None.

Poster Board Number:

MONDAY-699

Publishing Title:

Sphingomyelinase and Dna Biofilm Ligase Activities of *Staphylococcus aureus* β-Toxin Promote Infective Endocarditis and Sepsis

Author Block:

A. HERRERA[1], B. G. Vu[1], C. S. Stach[2], J. A. Merriman[3], A. R. Horswill[1], W. Salgado-Pabon[1], P. M. Schlievert[1]; [1]THE Univ. OF IOWA, Iowa City, IA, [2]Univ. of Minnesota, Minneapolis, MN, [3]Washington Univ., St. Louis, MO

Abstract Body:

Background: Infective Endocarditis (IE) is a life threatening infection of native/prosthetic valves and lining of the heart caused by bacteria. IE is characterized by the formation of vegetations composed of bacteria and host factors. Each year there are as many as 100,000 cases of IE in the United States. *Staphylococcus aureus* is the most commonly identified pathogen (up to 40%) in patients with IE. β-toxin is an important virulence factor in the arsenal of *S. aureus*, contributing to its ability to colonize and cause disease. This cytolysin has two distinct mechanisms of action: sphingomyelinase activity and DNA biofilm ligase activity. While a role for β-toxin has been demonstrated in IE, the mechanism of action that it uses to cause its effects has not been elucidated. **Methods:** Considering the active site of the DNA biofilm ligase activity of β-toxin is unknown, we began its characterization by examining deficiencies in site-directed mutants by use of *in vitro* DNA precipitation and biofilm formation assays. Possible conformational changes in mutant structure compared to wild type toxin were assessed via trypsin digestion analysis, retention of SMase activity, and a predicted model structure. We addressed the significance of each mechanism of action in producing IE *in vivo* during infections in a rabbit model of IE and sepsis.**Results:** We determined mutants T149A, H162A, and D163A were deficient in a DNA precipitation assay, and mutants T149A and H162A were no longer able to form biofilms comparable to wild type β-toxin in biofilm formation assay. All mutants were still able to lyse sheep erythrocytes and maintained the predicted folding conformation. Mutants H162A and D163A had similar stability to heat, and trypsin treatment as wild type. The β-toxin mutant, H289N, lacking sphingomyelinase activity, was decreased in lethality and vegetation formation compared to wild type. β-toxin mutants disrupted in biofilm ligase activity did not decrease lethality, but were deficient in vegetation formation compared to wild type.**Conclusions:** Our study begins to characterize the DNA biofilm ligase active site of β-toxin and demonstrates β-toxin plays an important role in IE via both mechanisms of action.

Author Disclosure Block:

A. Herrera: None. **B.G. Vu:** None. **C.S. Stach:** None. **J.A. Merriman:** None. **A.R. Horswill:** None. **W. Salgado-Pabon:** None. **P.M. Schlievert:** None.

Poster Board Number:

MONDAY-700

Publishing Title:

Bacillus thuringiensis* Secreats Some Virulence Factors to Disrupt Intestinal Epithelial Junctions and Accelerate the Invasion in *Caenorhabditis elegans

Author Block:

J. Lin, D. Peng, C. Pang, H. Du, L. Wan, S. Ju, L. Ruan, **M. Sun**; Huazhong Agricultural Univ., Wuhan, China

Abstract Body:

Bacillus thuringiensis (Bt), which is widely accepted as an entomopathogen, has been used to control agriculture pests. Some *B. thuringiensis* strains have high toxicity to nematodes and crystal (Cry) proteins are commonly known as the main virulence factors. It has been proposed that the culture mixtures of Cry proteins and spores (spore/crystal mixtures) can invade the nematodes, leaving the undigested cuticle which serves as a bag to form the "Bob" or bag-of-bacteria phenotype. To date, no study reveals the details about this significant phenomenon. Here we describe spores play an important role in the formation of "Bob" phenotype. We found that spore/crystal mixtures are able to disrupt intestinal cells in *C. elegans* to accelerate the invasion of *Bt* cells, leading eventually to the death of the worms, whereas the crystal proteins alone could only damage the gut, causing the shrinkage of the intestinal cells. The transcriptional regulation factors in *Bt* such as PlcR, NprR, CodY, AbrB and LuxS, which were reported to be responsible for the expression of multiply virulence factors, proved to be involved in the formation of "Bob". To deeply analysis the differences between the nematicidal mechanisms between crystal proteins and spore/crystal mixtures, we proposed that the epithelial junction of intestinal cells may serve as the underlying target of virulence factors regulated by these transcriptional regulation factors in *Bt* spores. The metalloproteinase ColB and Bmp1, which were proved to be regulated by PlcR in our previous work, were found capable of disrupting epithelial junctions directly. On the other hand, when these transcriptional regulation factors were knocked out in *Bt*, the disruption of intestinal epithelial junctions would decline rapidly, as well as the toxicity of these bacteria, indicating that the presence of the transcriptional regulation factors could accelerate the invasion of *Bt* in *C. elegans*, leading to the more effective nematicidal activity. Our findings present a novel perspective of the interaction between pathogenic bacteria and its nematode hosts and provide a convenient method to identify new virulence factors against nematodes, thus constructing the more efficient products of *B. thuringiensis*.

Author Disclosure Block:

J. Lin: None. **D. Peng:** None. **C. Pang:** None. **H. Du:** None. **L. Wan:** None. **S. Ju:** None. **L. Ruan:** None. **M. Sun:** None.

Poster Board Number:

MONDAY-701

Publishing Title:

Screening Anthrax Toxin Inhibitors from Natural Plant Extracts

Author Block:

P. Jacques, K. Borrego, R. Skouta, **J. Sun**; Univ. of Texas at El Paso, El Paso, TX

Abstract Body:

Bacillus anthracis, the etiologic agent of anthrax diseases, is a gram-positive, spore-forming, rod-shaped bacterium. While naturally occurring anthrax is rare in United States, *B. anthracis* is classified by the Centers for Disease Control and Prevention (CDC) as a category A select agent, representing the biological agents most at risk of being weaponized. The major symptoms of anthrax diseases are caused by anthrax toxin action. Therefore, development of anti-toxin inhibitor is of great importance for biodefense and national security.Plant-derived natural compounds are a rich potential source of compounds that treat infectious diseases. Successes like artemisinin and a recently reported antimicrobial remedy with anti-staphylococcal activity as well as many others encourage us to screen anthrax toxin inhibitors from a group of antioxidant-enriched herb extracts. We discovered that the extract of rosemary, even at a very low concentration, had a very strong inhibitory effect on anthrax toxin action, thus rescued mammalian cells (mouse and human macrophage cell lines) from anthrax lethal toxin-induced cytotoxicity in a dose-dependent manner, while the extract alone had no cytotoxic effect on the cells. Further, we found that the extract strongly inhibited pore-formation of anthrax protective antigen on the liposomal membranes. Identification and characterization of the active component in the extract is currently in progress. This study has the potential to develop a novel natural compound inhibitor against anthrax toxin.

Author Disclosure Block:

P. Jacques: None. **K. Borrego:** None. **R. Skouta:** None. **J. Sun:** None.

Poster Board Number:

MONDAY-702

Publishing Title:

Inhibition of Cholera Toxin and Other AB Toxins by Polyphenolic Compounds

Author Block:

P. Cherubin[1], M. C. Garcia[1], D. Curtis[1], C. B. T. Britt[1], J. W. Craft, Jr.[2], H. Buress[1], C. Berndt[1], S. Reddy[1], J. M. Briggs[2], K. Teter[1]; [1]Univ. of Central Florida, Orlando, FL, [2]Univ. of Houston, Houston, TX

Abstract Body:

All AB-type protein toxins have intracellular targets despite an initial extracellular location. These toxins use different methods to reach the cytosol and have different effects on the target cell. Broad-spectrum inhibitors against AB toxins are therefore hard to develop because the toxins use different surface receptors, entry mechanisms, enzyme activities, and cytosolic targets. We have found grape seed extract provides resistance to five different AB toxins: cholera toxin (CT), Shiga toxin (ST), ricin, diphtheria toxin, and exotoxin A. To identify individual compounds in grape extract that can inhibit the activities of these AB toxins, we used a cell culture system to screen twenty common phenolic compounds of grape extract for anti-toxin properties. Twelve compounds inhibited CT, one inhibited ricin, three inhibited diphtheria toxin, and four inhibited exotoxin A. No individual compound generated resistance against ST, although a cocktail of all 20 compounds conferred partial resistance to ST. Additional studies were performed to determine the mechanism of inhibition against CT. Two compounds inhibited CT binding to the cell surface and even stripped CT from the plasma membrane of a target cell. Two other compounds inhibited the enzymatic activity of CT, and four blocked cytosolic toxin activity without directly affecting the enzymatic function of CT. We have thus identified individual toxin inhibitors from grape extract and some of their mechanisms of inhibition against CT. This work will help formulate a defined mixture of polyphenolic compounds that could potentially be used as a therapeutic against a broad range of AB toxins.

Author Disclosure Block:

P. Cherubin: None. **M.C. Garcia:** None. **D. Curtis:** None. **C.B.T. Britt:** None. **J.W. Craft:** None. **H. Buress:** None. **C. Berndt:** None. **S. Reddy:** None. **J.M. Briggs:** None. **K. Teter:** None.

Poster Board Number:

MONDAY-703

Publishing Title:

Purification And Identification Of Two Novel Toxins Implicated In Mucormycosis Pathogenesis

Author Block:

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Abstract Body:

Background: Mucormycosis, caused by *Mucorales* fungi, is a life-threatening infection, occurring predominantly in immunocompromised patients and characterized by extensive vessel thrombosis and tissue necrosis. We previously discovered that killed *Mucorales* cause considerable damage to host cells, suggesting that a toxin(s) might be operative during infection. We sought to purify these toxins and characterize their role in damaging host cells and causing disease.**Methods:** *Rhizopus* mat was separated from the supernatant by filtration. The supernatant was extracted with ethyl acetate, dehydrated with Na_2SO_4 anhydrous, and evaporated before reconstituting in host cell medium. The separated fungal mat was ground in liquid nitrogen, extracted with either sterile water or host cell medium. Both extracts were filter sterilized prior to assessing their role in causing damage to alveolar epithelial (A549) cells by ^{51}Cr-release assay. The sterile extracts were also assessed for their ability to cause disease in CD-1 neutropenic mice injected i.v. qod with both extracts for a total of 3 doses. To further purify the extracts, fractionation-based A549 cell damaging assay was performed using size exclusion, PAGE, capillary force, silica and cellulose chromatography. Fractions with A549 cell damaging activity were trypsinized and the peptide sequenced by LC-MS.**Results:** Both crude extracts caused damage to A549 cells. Injection of the concentrated crude extracts in mice resulted in behavior highly suggestive of sudden circulatory shock within 30 min of injection. Further, mice injected with the toxin-like extracts lost weight (~30% drop in 5 d vs. water injected mice) and ultimately required euthanasia, similar to mice infected i.v. with live spores (70% and 100% mortality for extracts and live spores injected mice, respectively). LC-MS data of the purified active fractions from supernatant and hyphae extracts revealed a peptide that had sequence similarity to type VI bacterial toxin secretion system (T6SS) and ricin-like toxin, respectively.**Conclusions:** We have purified and characterized two novel *Mucorales* toxins that have similarities to (T6SS) and ricin-like toxins. These two toxins are likely to play a major role in the pathogenesis of mucormycosis and further investigations into this role are warranted.

Author Disclosure Block:

S. Soliman: None. **M. Liu:** None. **J. Edwards, Jr.:** None. **A.S. Ibrahim:** None.

Poster Board Number:

MONDAY-704

Publishing Title:

The Toxin Pneumolysin Promotes Transmission of *Streptococcus pneumoniae*

Author Block:

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Abstract Body:

Streptococcus pneumoniae serially and sequentially colonizes the mucosal surface of the human nasopharynx beginning in the early childhood (the carrier state). Transmission occurs from carriers to non-carriers and is most frequent in settings of close contact, such as among siblings. Both colonization and transmission are more common in the setting of recent viral infection. Our previous work established an infant mouse model for pup to pup transmission of pneumococci using influenza A co-infection, which led to transit of the bacterium from inoculated index mice to uninfected contact mice. Here we show that without influenza A co-infection infant mice still shed pneumococci at moderate levels in a strain dependent manner. By employing a well-shed isolate, we showed that shedding was age dependent as four day old mice inoculated with pneumococci shed the organism from the nares at an increased level compared to mice inoculated at either eight or twelve days of age. Using this isolate in four day old mice, we established that this moderate level of shedding correlated with bacterial transmission, albeit at lower level when compared to the influenza A co-infection model where pneumococci are shed in higher amounts. Expression of its pore-forming toxin, pneumolysin (Ply) was necessary for transmission in the absence of influenza A co-infection. Intranasal administration of purified toxin stimulated a dose dependent effect on bacterial shedding. This elevated level of shedding was not caused by altered colonization density. Rather shedding correlated with an increased inflammatory response as determined by numbers of neutrophils and level of pro-inflammatory cytokines in nasal lavages. Furthermore, PdB, which contains a single amino acid mutation and lacks the ability to oligomerize to form pores, did not elicit an inflammatory response or promote shedding. Our results suggest that Ply promotes inflammation to enhance shedding and facilitate host to host transmission. Taken together, this work provides a mechanistic understanding of how *S. pneumoniae* takes advantage of the host response during carriage.

Author Disclosure Block:

M.A. Zafar: None. **Y. Wang:** None. **S. Hamaguchi:** None. **M. Kono:** None. **J.N. Weiser:** None.

Poster Board Number:

MONDAY-705

Publishing Title:

High Glucose Sensitizes Host Cells to Necroptosis in Response to Bacterial Pore-Forming Toxins and Inflammatory Signals

Author Block:

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Abstract Body:

Necroptosis is a RIP1-dependent programmed cell death (PCD) pathway in host cells that is relevant to bacterial and viral diseases as well as inflammatory conditions including sepsis. Additionally, necroptosis is an inflammatory PCD that is distinct from apoptosis. Damaging agents in necroptosis include advanced glycation end products (AGEs) and reactive oxygen species (ROS), both of which depend on glycolysis. This suggests that increased cellular glucose may sensitize cells for necroptosis. Here we show that exposure to hyperglycemic levels of glucose enhances necroptosis in primary RBCs, Jurkat T cells, and U937 monocytes in response to bacterial pore-forming toxins and inflammatory cytokines including TNF and CD178 (Fas ligand). Pharmacologic (nec-1s) or siRNA inhibition of RIP1 prevented the enhanced death, confirming it as RIP1-dependent necroptosis. Hyperglycemic enhancement of necroptosis depends upon host cell glycolysis with AGEs and ROS playing a role. Total levels of RIP1, RIP3, and MLKL increased following treatment with high levels of glucose during necroptosis and was not due to transcriptional regulation. The observed increase in RIP1, RIP3, and MLKL protein levels suggests a potential positive feedback mechanism that drives hyperglycemic enhancement of necroptosis. Extrinsic apoptosis was inhibited by exposure to high levels of glucose suggesting a PCD shift to necroptosis under these conditions. Moreover, hyperglycemia resulted in increased infarct size in a mouse model of brain hypoxia-ischemia injury. The increased infarct size was prevented by treatment with nec-1s, strongly suggesting that increased necroptosis accounts for exacerbation of this injury in conditions of hyperglycemia. This work reveals that hyperglycemia represents a condition in which cells are extraordinarily susceptible to necroptosis, that local glucose levels alter the balance of PCD pathways, and that clinically relevant outcomes may depend on glucose-mediated effects on PCD. As diabetes increases the risk for bacterial infections, this work suggests that necroptosis may emerge as the principal PCD in response to bacterial pathogens in diabetic individuals. A shift from non-inflammatory apoptosis to necroptosis during bacterial infections may explain the exacerbation of bacterial infections and associated pathologies in diabetes.

Author Disclosure Block:

T.J. Larocca: None.

Poster Board Number:

MONDAY-706

Publishing Title:

Induction of Shiga Toxin-encoding Bacteriophage is Required for Lethal Disease in a Mouse Model of Enterohemorrhagic *Escherichia coli* Infection

Author Block:

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Abstract Body:

Enterohaemorrhagic *Escherichia coli* comprise lysogenic bacteria that colonize the colonic mucosa and can produce phage-encoded Shiga toxin (Stx), resulting in life-threatening hemolytic uremic syndrome. Stx2 genes, located in a "late" phage operon, are repressed in the lysogenic state. Induction of the SOS response leads to lytic phage growth and Stx2 production *in vitro* and in a **germ-free** mouse model, but the role of Stx2 phage induction during infection of a host with a complete microbiome has not been determined. For example, do released phage lysogenize or lytically infect other enteric bacteria, potentially amplifying Stx production?We used a derivative of the mouse pathogen *Citrobacter rodentium* lysogenized by Stx2-encoding *E. coli* lambdoid phage Φ1720-2a. This strain colonizes C57BL/6J mice to high levels, causing lethal disease featuring colitis, weight loss, proteinuria and renal pathology. Deletions in Cr(Φ1720-2a) genes were generated, and Stx2 levels and free phage were measured by ELISA and qPCR, respectively. Lysogenization of gut bacteria was assessed by deep sequencing.Phage-linked DNA sequences from feces of Cr(Φ1720-2a)-infected and diseased mice showed no integration into the chromosomes of other gut strains or species, suggesting such integration and subsequent induction is not required in this disease model. In addition, secondary lytic infection of other such strains by phage induced from Cr(Φ1720-2a) was not required for disease, as Cr(Φ1720-2a) mutant lysogens unable to package phage particles still caused lethal infection. Phage integrase, required for phage excision and integration, and phage lysis genes S and R, were not required for Stx2 production *in vitro* or for mouse lethality. In contrast, phage anti-terminator protein Q, required for late gene transcription, was essential for both functions. Consistent with the hypothesis that the host SOS response is required for phage induction leading to Stx2 production, disruption of Cr(Φ1720-2a) *recA* abrogated the ability to cause disease. Phage production by the various mutants is being quantified *in vitro* and *in vivo*. The mechanisms and extent of prophage induction required for disease is under study using *C1* repressor mutants.

Author Disclosure Block:

S. Balasubramanian: None. **M.S. Osburne:** None. **H.K. BrinJones:** None. **J.M. Leong:** None.

Poster Board Number:

MONDAY-707

Publishing Title:

***Helicobacter pylori* Vaca-mediated Mitochondrial Dysfunction Induces Global Disruption of Host Cell Metabolic Homeostasis**

Author Block:

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Abstract Body:

The consequences of pathogen-mediated disruption of host cell metabolic homeostasis on the infection microenvironment are poorly understood. Here, we evaluated the hypothesis that infection of gastric epithelial cells with the human gastric pathogen *Helicobacter pylori* disrupts host cell metabolism in a manner that impedes the capacity of host cells to appropriately respond to infection. *H. pylori* induce mitochondrial dysfunction in host cells through the action of the organism's only secreted cytotoxin, VacA. In these studies, we addressed a major gap in knowledge, which is the extent to which localized organelle dysfunction induced by VacA results in global cellular consequences. These studies revealed that VacA induced mitochondrial stress leads to inhibition of mammalian target of Rapamycin (mTOR), a master cellular metabolic regulator that integrates a variety of cellular stress signals. VacA-mediated mTOR inhibition was detected at low nM concentrations of VacA, and detected within the first several hours of VacA intoxication. mTOR inhibition was dependent on the capacity of VacA to form ion-conducting membrane channels. Consistent with the idea that mTOR inhibition occurs concurrently with a global cellular switch from anabolic to catabolic metabolism, protein synthesis was suppressed in VacA intoxicated cells. Proteomic analysis revealed decreased levels of key regulatory components in VacA intoxicated cells versus control cells that are important for maintaining cellular function. Unexpectedly, our data also revealed that VacA-induced protein synthesis inhibition resulted in derepression of the master inflammatory regulator, nuclear factor kB (NF-κB) by inhibiting the synthesis of the inhibitor of NF-κB (IκB), a short-lived repressor of NF-κB. Together, these results support a model that VacA manipulation of host metabolic homeostasis directly affects development of the *H. pylori* infection microenvironment.

Author Disclosure Block:

I. Kim: None. **M. Hamad:** None. **S. Kim:** None. **S.R. Blanke:** None.

Poster Board Number:

MONDAY-708

Publishing Title:

Causal Or Independent? Evaluating The Interrelatedness Of Host Cell Alterations Induced By The Vacuolating Cytotoxin Of Helicobacter Pylori

Author Block:

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Abstract Body:

Secreted bacterial exotoxins modulate host cell physiology without requiring direct pathogen contact. Pore-forming toxins form ion-conducting channels and/or disrupt host membranes, resulting in a plethora of consequences associated with the loss of membrane integrity. Here, we evaluated the causal relationships between several well-characterized consequences of the vacuolating cytotoxin (VacA), a pore-forming toxin secreted by the human gastric bacterium *Helicobacter pylori*. Once internalized in a host cell, VacA causes the formation of large membrane bound vacuoles and mitochondrial dysfunction. Although current literature suggests these two cellular phenotypes are independent consequences of VacA intoxication, a major gap in knowledge is understanding the mechanism by which one population of toxin is able to induce two apparently disparate outcomes. To evaluate the hypothesis that the internalization of VacA and subsequent mitochondrial damage precedes and is directly linked to vacuolation, cellular systems previously shown to be important in vacuolation were evaluated for a role in VacA mediated mitochondrial dysfunction. Our studies revealed that vacuolation and mitochondrial dysfunction are both dependent on the same cell surface receptor, suggesting a model of linear and independent targeting of VacA. VacA-mediated vacuolation, but not mitochondrial dysfunction, was shown to be an actin dependent phenotype. The requirement of actin for vacuole formation supports a model of VacA-mediated mitochondrial dysfunction preceding cellular vacuolation, or alternatively, vacuolation and mitochondrial dysfunction are two independent consequences of toxin action. Studies to evaluate a causal relationship revealed both localization of VacA to and induction of mitochondrial dysfunction precede vacuolation and localization to vacuoles. Cumulatively, these data support a linear model of VacA trafficking where mitochondrial dysfunction occurs upstream of vacuolation. Demonstrating that VacA mediated mitochondrial dysfunction precedes and is directly linked to vacuolation not only provides insight into the consequences of VacA intoxication, but highlights the potential interrelatedness of cellular activities associated with the larger class of pore-forming bacterial toxins.

Author Disclosure Block:

K.D. Bosi: None. **R.L. Holland:** None. **S.R. Blanke:** None.

Poster Board Number:

MONDAY-709

Publishing Title:

Subversion of Host Cell Autophagy by Genotoxic Pathogenic Bacteria

Author Block:

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Abstract Body:

During infection, the host employs several strategies in an attempt to maintain physiological homeostasis. An important strategy for relieving pathogen-induced stress is the induction of cellular autophagy, a catabolic process for engulfment and degradation of intracellular components within specialized vacuoles. Here, we report the unexpected finding that host cell autophagy is down regulated in response to genotoxic stress induced by clinically prominent human mucosal bacterial pathogens secreting the cytolethal distending toxins (CDTs). CDTs comprise an unusual class of protein toxins that are taken up into host cells, localize to the nucleus, and activate DNA damage repair responses. Autophagy is regulated in part by the cellular pool of LC3-I, which must be converted to LC3-II as an obligate step in the formation of autophagic vesicles. In epithelial cells exposed to potent inducers of autophagy, the formation of autophagosomes, and the concurrent increase in cellular LC3-II levels, were both inhibited in the presence of CDT. Unexpectedly, a significant decrease in cellular LC3-I levels was observed in CDT-exposed cells. The decrease in cellular LC3-I was dependent on CDT-dependent DNA damage, as autophagy was not inhibited in cells exposed to either mutant forms of CDT unable to induce DNA damage or inhibitors of the eukaryotic DNA damage repair response. Cellular depletion of LC3-I was not due to decreases in gene expression, as LC3 transcript levels were identical in CDT-treated and untreated cells. Instead, LC3-I depletion was correlated with increased proteasome activity, as proteasome inhibitors rescued both cellular LC3-I, as well as autophagosome formation. Our data support a here-to-fore unrecognized strategy by which some pathogens may subvert the host autophagic response, and suggest a new role for CDTs during establishment of the infection microenvironment.

Author Disclosure Block:

D.J. Lieu: None. **J. Kryza:** None. **Z.P. Schaefer:** None. **B. Tamilselvam:** None. **H. Mok:** None. **H. Chen:** None. **H. Phillips:** None. **S.R. Blanke:** None.

Poster Board Number:

MONDAY-710

Publishing Title:

Identification of a New *Helicobacter pylori* vaca Motif Important for Receptor Specificity

Author Block:

S. J. Oh, P. Mahinthichaichan, M. Perisin, E. Tajkhorshid, S. R. Blanke; Univ. Illinois,, Urbana, IL

Abstract Body:

The cellular tropism of intracellular-acting bacterial exotoxins is largely dictated by specific receptors that couple surface bound toxins to existing cellular uptake pathways. *Helicobacter pylori* secrete VacA, which causes intracellular vacuole formation, mitochondrial depolarization, and ultimately cell death. The abundant membrane sphingolipid, sphingomyelin (SM), is a VacA receptor important for binding the toxin to the surface of sensitive cells. In this study, we evaluated the molecular details underlying the poorly understood interactions of VacA with the phosphorylcholine headgroup of SM. Based on previous work suggesting that tryptophan (W) side chains stabilize the positively charged amine head group of SM via pi-cation interactions, alanine scanning of the eleven VacA W residues identified a specific W residue in the carboxyl-terminal p55 domain important for both the cellular activity and plasma membrane binding of VacA. Computational modeling of VacA-SM binding indicated close interactions of the SM headgroup with the VacA p55 W residue, as well as 2 arginine (R) residues, which we propose to interact with the negatively charged phosphate moiety of SM. Solid state SM binding assays indicated that alanine substitutions for the three residues comprising the WRR structural pocket significantly reduced toxin-SM binding. Analysis of VacA binding to mammalian cells, which previous work indicated has both specific and non-specific components, indicated that a mutant form of the toxin lacking the proposed WRR-SM-binding pocket no longer specifically bound to a component on the cell surface of epithelial cells, although non-specific binding was retained. Taken together, our data suggest that VacA-SM interactions confer specific binding of the toxin to the surface of host cells required for toxin activity. These results provide the framework for understanding the molecular basis of VacA cellular tropism.

Author Disclosure Block:

S.J. Oh: None. **P. Mahinthichaichan:** None. **M. Perisin:** None. **E. Tajkhorshid:** None. **S.R. Blanke:** None.

Poster Board Number:

MONDAY-711

Publishing Title:

Effect of Environmental Salt Concentration on Composition of the *Helicobacter pylori* Exoproteome

Author Block:

R. R. Caston, B. J. Voss, C. A. Snider, T. L. Cover; Vanderbilt Univ. Sch. of Med., Nashville, TN

Abstract Body:

Background*: Helicobacter pylori* infection and a high salt diet are each associated with an increased risk of gastric cancer. In response to changes in environmental salt concentration, specific *H. pylori* proteins such as CagA undergo changes in relative abundance. In a previous study, we used mass spectrometry-based methodology to identify proteins that were selectively released by *H. pylori* into the extracellular space. In the current study, we tested the hypothesis that the composition of the *H. pylori* exoproteome is dependent on the salt concentration of the culture medium. **Methods**: *H. pylori* strain 26695 was grown in Brucella broth filtrate (containing components < 3 kDa plus supplemental cholesterol) overnight, and then subcultured into medium containing various sodium chloride concentrations (0.25%, 0.5%, 1.0%, or 1.25%). Aliquots were removed at 24 h and 36 h, centrifuged to yield supernatant and bacterial pellets, bacteria were fractionated, and samples were analyzed by single dimensional LC-MS/MS. Analysis was based on merged results from two independent experiments. **Results:** By comparing the proportional abundance of proteins in culture supernatants with abundance in cytoplasmic/periplasmic fractions, we identified proteins that were selectively released into the culture supernatant under each of the tested conditions. We then evaluated the effect of salt concentration on release of these proteins. At both 24 h and 36 h time points, VacA was enriched in the supernatant of cultures grown in media containing high salt conditions (1.0% and 1.25%), but was not enriched in supernatant of cultures grown in media containing lower salt concentrations. At the 36 h time point, the proportional abundance of VacA in the supernatant of cultures grown with 1.25% salt was about 8-fold higher than in cultures grown with 0.5% salt. **Conclusions:** The proportional abundance of VacA in the extracellular space increases in response to an elevation in environmental sodium chloride concentration. This change may be attributable to several factors, including increased *vacA* transcription as well as increased secretion and release of VacA. Salt-induced changes in *H. pylori* protein production or secretion may contribute to the increased risk of gastric cancer associated with a high salt diet.

Author Disclosure Block:

R.R. Caston: None. **B.J. Voss:** None. **C.A. Snider:** None. **T.L. Cover:** None.

Poster Board Number:

MONDAY-712

Publishing Title:

A T6ss Immunity Factor Dictates Social Recognition During Intraspecies Warfare

Author Block:

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Abstract Body:

Gram-negative type VI secretion systems (T6SS) function to deliver a lethal payload of proteins into a target cell during interbacterial competition. Many studies have shown that protection against a single, lethal T6SS effector exclusively resides with a cognate antidote immunity protein, which are often located together in two-gene operons. In contrast, we have previously identified an immunity protein called PefE in *Proteus mirabilis* that is necessary and sufficient for immunity and is necessary but not sufficient for killing. Here, we describe a new class of T6SS immunity effectors which differ from the conventional model of effector-immunity pairs. The unique composition of this newly identified effector group is its occurrence as a large operon containing 8 genes that are all required for killing, including *hcp* and *vrgG*, and a single immunity gene. To investigate the relationship between immunity and social recognition, we utilized two swarming *P. mirabilis* isolates that contain identical effector operons that differ only in their immunity genes. Interestingly, despite containing identical effector genes, the less conserved immunity proteins, PefE and PefE2, only provide protection against their native effector operons. We hypothesize that specificity and recognition are dependent on variation within a single dual-function immunity factor. To determine residues within PefE important for specificity, a total of 6 full-length chimeras were designed and constructed using gene fragments of varying lengths from *pefE* and *pefE2*. The chimeras were tested during multicellular behavior using the Dienes line phenomenon and immunity was quantified using a killing assay. All chimeras were functional and each chimera provided immunity in a strain-specific manner. Upon further investigation, the region required for strain-specific immunity was localized. Sequence analysis of PefE from multiple strains indicates the presence of five less conserved variable regions, which in turn create modules that differ between strains. Together, these data demonstrate that 11 residues located within two of the five variable regions are specificity determinants. These findings help clarify the relationship between immunity against lethal T6SS attack and social recognition in bacteria.

Author Disclosure Block:

C.J. Alteri: None. **S.D. Himpsl:** None. **N. Musili:** None. **K. Zhu:** None. **J.E. Miller:** None. **H.L.T. Mobley:** None.

Poster Board Number:

MONDAY-713

Publishing Title:

Diversity and Distribution of Type VI Secretion Substrates in Epidemic Multidrug-Resistant *Acinetobacter baumannii* Isolates

Author Block:

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Abstract Body:

Background: *Acinetobacter baumannii* is an opportunistic bacterial pathogen in humans. Since 2000, multidrug-resistant *A. baumannii* (MDRAB) has emerged and spread worldwide. *A. baumannii* isolates belonging to international clone II (IC2), the most successful epidemic lineage, are predominantly associated with multidrug resistance carried on mobile gene elements, such as plasmids, and often cause nosocomial outbreaks. So far, it remains unclear how such bacterial high-risk clones emerge and spread. Here we show that type VI secretion system (T6SS) that directly translocates antibacterial proteins into neighboring bacterial cells could be involved in the clonal transmission mechanism of MDRAB epidemic isolates. **Methods:** Whole genome sequencing of 152 isolates of *Acinetobacter baumannii* was performed using the Illumina HiSeq 2500/HiSeq 4000 and/or Pacific Biosciences PacBio RSII platforms. Protein-coding genes, pseudogenes, and non-coding RNAs were annotated using the RASTtk scripts, and prophage regions were predicted using the PhiSpy algorithm. Comparative sequence analysis was performed using the BLAST Ring Image Generator (BRIG), Artemis Comparison Tool (ACT), and Mauve. **Results and Conclusions:** Most of MDRAB IC2 isolates conserved intact T6SS gene clusters for the secretion machine and substrates, whereas drug-susceptive non-IC2 isolates frequently lacked the gene clusters. Whole-genome single nucleotide polymorphism-based phylogenetic analysis revealed that IC2 isolates are further divided into five distinct genetic clades. Comparative genomic analysis between IC2 isolates identified that each IC2 clade harbors own specific antibacterial T6SS substrates encoded within the conserved T6SS gene clusters. Given that T6SS-mediated bacterial competition is known to affect conjugative transfer of drug resistance genes-carrying plasmids, T6SS could be an important key factor for development of MDRAB high-risk clones.

Author Disclosure Block:

M. Suzuki: None. **M. Matsui:** None. **S. Suzuki:** None. **K. Shibayama:** None.

Poster Board Number:

MONDAY-714

Publishing Title:

Developing an Innate Immunity Based High Throughput Screen for Small Molecule Inhibitors of the Type III Secretion System

Author Block:

H. Lam, J. M. Morgan, T. Simowitz, W. Bray, R. Linington, V. Auerbuch; UC Santa Cruz, Santa Cruz, CA

Abstract Body:

Antibiotic resistant bacteria are an emerging threat to global public health. New classes of antibiotics and tools for antimicrobial discovery are urgently needed. The type III secretion system (T3SS), which is required by dozens of Gram-negative bacteria for virulence, but is largely absent from non-pathogenic bacteria, is a promising virulence blocker target. The ability of mammalian cells to recognize the presence of a functional T3SS and trigger NFκB activation provides a rapid and sensitive method to identify chemical inhibitors of T3SS activity.Previously, we used host NFκB-driven luciferase as a readout for *Yersinia* T3SS activity in a pilot T3SS inhibitor screen and identified a family of compounds called piericidins. While this pilot screen was successful in identifying T3SS inhibitors, the Z score of the screen was poor. In order to improve our screening strategy in preparation for high throughput screening, we examined 83 different NFκB-driven promoters to identify sequences that provide the most robust signal in response to a functional *Yersinia* T3SS. We generated a HEK293 stable cell line expressing Green Fluorescence Protein (GFP) driven by the most optimal NFκB promoters of those tested. Use of this stable cell line improves our previous screening strategy by eliminating the need for to use a luciferase reagent (which increases cost) or for transient transfection of an NFκB reporter (which increases variability),but with the added ability to measure GFP fluorescence over time. We have screened the natural products library used in our original pilot, confirmed the ability to detect piericidin as a T3SS inhibitor, and report a Z score >0.4, validating our improved screening strategy. In addition, we detect a small portion of compounds from 2880 extracts that can reduce NFκB driven GFP upon *Yersinia* infection. None of the hits have antibiotic activity against *Yersinia.* The strongest hits will be tested for secretion inhibition of *Yersinia* effectors.

Author Disclosure Block:

H. Lam: None. **J.M. Morgan:** None. **T. Simowitz:** None. **W. Bray:** None. **R. Linington:** None. **V. Auerbuch:** None.

Poster Board Number:

MONDAY-715

Publishing Title:

A *Vibrio anguillarum* Isocitrate Dehydrogenase Mutant is Highly Attenuated and Immunogenic in Rainbow Trout (*Oncorhynchus mykiss*)

Author Block:

E. Spinard, X. Mou, D. R. Nelson; Univ. of Rhode Island, Kingston, RI

Abstract Body:

We have been investigating the regulation and roles of the hemolysin/cytotoxin genes in pathogenesis by *V. anguillarum*, the causative agent of vibriosis in fish. We have shown that there are three major hemolysin genes (*vah1*, *plp* and *rtxA*) and have created an avirulent strain by knocking out *rtxA*. Currently, we aim to create an avirulent, immunogenic *V. anguillarum* strain that can be used as a live vaccine for fish without knocking out any of the hemolysin genes. Previously, we constructed several strains of *V. anguillarum* M93Sm (serotype O2a) with mutations in TCA cycle genes and showed that the isocitrate dehydrogenase (*icd)* mutant was highly attenuated in virulence against juvenile rainbow trout (*Oncorhynchus mykiss*) that were challenged via IP injection or immersion. The *icd* mutant grew to a cell density lower than wild type in LB20 and Nine Salt Solution supplemented with 200 µg/ml of salmon intestinal mucus (NSSM). Expression levels of the three hemolysin genes were determined by qRT-PCR and the *icd* mutant exhibited either the same or higher expression levels compared to the wild type during exponential phase growth. Further, rainbow trout that were pre-treated by immersion with the *icd* mutant were protected from a subsequent challenge of *V. anguillarum* M93Sm. When *icd* was restored, virulence against rainbow trout and growth in LB20 returned to wild type levels. In this study we examined the cause of the *icd* mutant growth limitation in rich media. The *icd* mutant showed wild type levels of cell density when the LB20 was supplemented with 2% glutamic acid. We also created and characterized a citrate synthase (*gltA*) mutant. Like the *icd* mutant, the *gltA* mutant grew to a lower cell density compared to the wild type in two forms of rich media (LB20 and NSSM), exhibited either the same or higher expression levels of the three hemolysin genes compared to the wild type during exponential phase growth, and showed wild type levels of cell density when LB20 was supplemented with 2% glutamic acid. Our data indicate that *icd* is necessary for virulence and growth to a wild type level in rich media.

Author Disclosure Block:

E. Spinard: None. **X. Mou:** None. **D.R. Nelson:** None.

Poster Board Number:

MONDAY-716

Publishing Title:

Functional Analysis of HlyU, a Transcriptional Regulator of *Vibrio vulnificus*

Author Block:

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Abstract Body:

An opportunistic pathogen *Vibrio vulnificus* produces a large pore-forming toxin, RtxA, which triggers necrotic and apoptotic cell death. Previous studies reported that a transcriptional regulator HlyU upregulates *rtxA* expression by direct binding to the promoter region of the *rtxHCA* operon. To examine the function of HlyU, the *hlyU* mutant was constructed and its virulence was evaluated. Compared with the wild type, the *hlyU* mutant exhibited low cytotoxicity toward mucin-secreting HT-29 MTX cells and reduced mortality in mice, suggesting that HlyU is essential for the virulence of *V. vulnfiicus*. To further identify the genes regulated by HlyU, transcriptomic profiles of the wild type and the *hlyU* mutant were analyzed by RNA-seq. In addition to *rtxA*, genes encoding virulence factors such as hemolysin and phospholipase were down-regulated in the *hlyU* mutant, implying that HlyU contributes to the *V. vulnificus* pathogenesis by regulating various virulence genes. To elucidate the mechanism of HlyU in *rtxA* regulation, the levels of *rtxA* and *hlyU* transcripts in *V. vulnificus* cells grown in different conditions were determined. Induction of the *rtxA* expression occurred in the wild-type cells either exposed to the INT-407 human epithelial cells or grown under anaerobic conditions, but not in the *hlyU* mutant, indicating that HlyU mediated the induction. Since the levels of *hlyU* transcript in the wild-type cells were not significantly changed in those conditions, the *rtxA* activation might be attributed to altered activity rather than amounts of HlyU. To better understand this activity alteration of HlyU, C30, C96, and M87, which were predicted as essential amino acids from structural analysis, were mutated by site-directed mutagenesis. Among the mutants, *V. vulnificus* expressing HlyU-C30S showed a decreased *rtxA* level and cytotoxicity toward the HT-29 MTX cells than the wild type, suggesting that C30 is a critical residue of HlyU for *rtxA* activation. Taken together, HlyU might act as a regulator for the various virulence genes and induce *rtxA* under host environment by altering its activity.

Author Disclosure Block:

Z. Lee: None. **Y. Bang:** None. **S. Choi:** None.

Poster Board Number:

MONDAY-717

Publishing Title:

Characterization of the Quorum Sensing Transcriptional Regulator Sdia From Ehec

Author Block:

D. Knowles, V. Sperandio; Univ. of Texas Southwestern Med. Ctr., dallas, TX

Abstract Body:

Enterohemorrhagic E. coli (EHEC) is a Gram-negative pathogen responsible for outbreaks of bloody diarrhea and hemolytic uremic syndrome. To colonize its bovine host, EHEC relies on its LuxR-type acyl homoserine lactone (AHL) sensing transcription factor, SdiA, to coordinate virulence gene expression in response to AHL signaling molecules produced by the host ruminal microbiota. SdiA controls expression of acid resistance genes and the Ler master regulator of the EHEC pathogenicity island, the locus of enterocyte effacement (LEE), necessary for host colonization. Here we study folding, ligand, and promoter-binding of SdiA from EHEC. Purification, denaturation and extensive dialysis was used to generate apo-SdiA, which was subsequently refolded in the absence of ligand. Using circular dichroism (CD) and fluorescence spectroscopy, we show that even in the absence of exogenous ligand, and over a >100-fold protein concentration range, SdiA is able to refold to a fully "native" conformation. We use analytical ultracentrifugation and CD spectroscopy to show that ligand binding affects the monomer-dimer equilibrium, however, in contrast to all other LuxR-type transcription factors studied to date, does not induce large scale folded<->unfolded-type conformational changes in the protein. We use isothermal titration calorimetry (ITC) and intrinsic Trp fluorescence to directly characterize the binding of a set of AHL's spanning a broad range of acyl chain lengths and chemical modifications to apo-SdiA, and the effect of these ligands on SdiA binding to the ler promoter. These observations account for gene-regulation by SdiA in the absence of AHL, and raise the possibility that AHL-sensing LuxR transcription factors may function in roles outside AHL sensing.

Author Disclosure Block:

D. Knowles: None. **V. Sperandio:** None.

Poster Board Number:

MONDAY-718

Publishing Title:

Effects of Anaerobic Culture on *In-Vitro* and *In-Vivo* Virulence of *Streptococcus pneumoniae*

Author Block:

K. Nagaoka, S. Konno, M. Nishimura; Hokkaido Univ. Sch. of Med., Sapporo, Japan

Abstract Body:

Background: *Streptococcus pneumoniae*, a facultative anaerobic bacterium, is a leading cause of respiratory infection worldwide. Its pathogenicity, which is thought to be mediated by pneumolysin or autolysin, is examined usually under condition of aerobic culture. Considering its facultative anaerobic nature, we wondered whether the culture condition of either aerobic or anaerobic might be influential to the virulence of this bacterium. **Methods:** In vitro, we incubated *S. pneumoniae* under aerobic or anaerobic condition, and quantitatively examined the production of pneumolysin by Western blot analysis. In vivo, we infected mice by intra-tracheal injection of *S. pneumoniae*, which had been grown until late log phase under either aerobic or anaerobic condition. We then compared survival rates and bacterial loads in the lungs as well as in blood between the two conditions.**Results:** In vitro, the productions of pneumolysin were apparently reduced in both late log phase (8 -10 h) and stationary phase (12-15 h) when culture was done under anaerobic condition compared with aerobic condition (Fig 1 A). In vivo, however, survival rate was lower and bacterial loads both in the lungs and in blood were higher in mice which had been inoculated with bacteria pre-cultured under anaerobic condition compared with under aerobic condition. In particular, significantly higher loads of bacteria in blood were seen even at an earlier phase of infection in anaerobic group (Fig 1 B).**Conclusions:** We here demonstrate that the virulence of *S. pneumoniae* is highly enhanced in vivo when pre-cultured under anaerobic condition compared with aerobic condition. However, it is not likely that pneumolysin mediates the enhancement of virulence.

Figure 1A

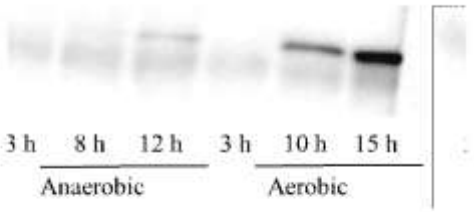
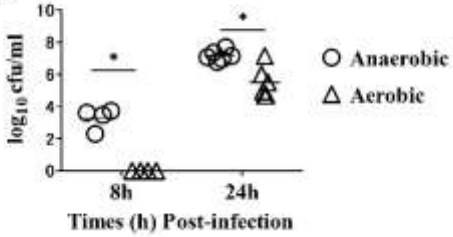


Figure 1B



Author Disclosure Block:

K. Nagaoka: None. **S. Konno:** None. **M. Nishimura:** None.

Poster Board Number:

MONDAY-719

Publishing Title:

Virulence in and Molecular Characteristics of Pvl-Negative, Tsst-1 Positive Ca-Mrsa Circulating in Japan

Author Block:

T. Yamaguchi, S. Sonoda, K. Aoki, D. Ono, A. Sato, Y. Ishii, K. Tated; Toho Univ., Tokyo, Japan

Abstract Body:

Background: PVL-positive Community-associated MRSA (CA-MRSA) is now endemic in the United States. However, in the nationwide survey of CA-MRSA in Japan that we have conducted, PVL-positive strains were only 10.3% and TSST-1-positive strains were 31.2% among total 858 CA-MRSA strains. Especially, ST8/SCC*mec* IV/TSST-1+ clone (ST8/IV/T) is isolated from severe skin and soft tissue infections and rarely from sepsis or pneumonia. The virulent potential of PVL-negative CA-MRSA strains has remained controversial. In the present study, to gain an understanding of virulence in the PVL-negative CA-MRSA, we investigated the virulence in ST8/IV/T clone using sepsis mouse model. **Methods:** TUM14604 (ST8/IV/T isolates from necrotizing pneumonia: PVL-, TSST1+) and two reference strains (BAA1556, USA300/ST8/SCC*mec* IV/PVL+; N315, USA100/ST5/SCC*mec* II/TSST-1+) were used. In sepsis model, six week-old female BALB/c mice (n = 6) were injected intravenously with 2-4X10^7 CFU/mouse and were monitored for survival and body weight change over 21 days. In addition, Staphylococcal load in tissues (blood, liver, spleen, kidney, and lung) and cytokine responses in serum and spleen were analyzed on day1 and day3. The virulence genes of TUM14604 strain were detected by whole genome sequence using a MiSeq sequencer (Illumina), and transcriptional levels of several virulence factors (ex. *tst-1*) were examined by real time PCR. **Results:** In sepsis model, TUM14604 strain demonstrated significantly stronger virulence than that of N315 strain (mortality rate of TUM14604 60% vs BAA1556 0% vs N315 0%). The levels of bacterial burdens in kidney and cytokines (IL6, IL1β, and TNFα) were higher in injected mice by TUM14604 strain and BAA1556 strain than N315 strain. High transcriptional levels of *psmα*, *tst-1*, *agrA*, and *rnaIII* in TUM14604 strain were observed, and it may contribute to the high virulence of sepsis model. **Conclusions:** We confirmed the high virulence in PVL-negative ST8/IV/T clone. The activation of agr system and the elevated production of TSST-1 may influence the severity of sepsis. Our study high lights strain-dependent differences, understanding of virulence in PVL-negative CA-MRSA warrants further investigation.

Author Disclosure Block:

T. Yamaguchi: None. **S. Sonoda:** None. **K. Aoki:** None. **D. Ono:** None. **A. Sato:** None. **Y. Ishii:** None. **K. Tated:** None.

Poster Board Number:

MONDAY-720

Publishing Title:

Characterization of the *Borrelia burgdorferi bb0168* Deficient Mutants Indicate *bb0168*-Encoded DnaK Suppressor Protein is a Global Regulator of Gene Expression

Author Block:

W. K. Boyle[1], J. A. Shaw[1], A. Groshong[2], J. S. Blevins[3], F. C. Gherardini[4], T. J. Bourret[1]; [1]Creighton Univ., Omaha, NE, [2]Univ. of Connecticut Hlth.Ctr., Farmington, CT, [3]Univ. of Arkansas for Med. Sci., Little Rock, AR, [4]NIAID, Hamilton, NE

Abstract Body:

Borrelia burgdorferi, the causative agent of Lyme disease, must sense and respond to diverse environmental challenges throughout its natural infectious cycle in *Ixodes spp.* ticks and various mammalian hosts including shifts in temperature, pH, osmolarity, nutrient availability, as well as oxidative and nitrosative stresses. In this study, we set out to characterize the role of the *bb0168*-encoded DnaK suppressor protein (DksA) on *B. burgdorferi* gene expression and infectivity. Microarray analysis comparing the transcriptomes of a wild-type *B. burgdorferi* strain and a *dksA*-deficient mutant revealed that DksA impacts the expression of more than 500 genes in cultures grown under microaerobic conditions (5% CO_2, 3% O_2) in BSK II medium, pH 7.6 at 34 degrees C. When wild-type and *dksA*-deficient *B. burgdorferi* strains were subjected to nutrient limitation by shifting cultures from BSK II medium to RPMI medium lacking serum, the expression of at least 315 plasmid encoded genes were impacted in the wild-type cultures, whereas only 76 plasmid encoded genes were affected in the *dksA*-deficient strain. Additionally, the *dksA*-deficient *B. burgdorferi* show reduced expression of genes required for infectivity in the murine model including *ospC* (30-fold), *dbpA* (52-fold), and *dbpB* (31-fold) compared to wild-type controls. Surprisingly, the expression of the alternative sigma factor S (*rpoS*), which helps coordinate the expression of *ospC*, *dbpA*, and *dbpB*, was unaffected by the loss of *dksA*. Moreover, induction of the *Borrelia* RpoS regulon by shifting cultures from pH 7.6 to pH 6.8, and subsequent immunoblot of the cell lysates showed reduced production of OspC in the *dksA*-deficient mutants compared to wild-type controls. The reduced expression of virulence-associated genes corresponded to the inability of the *dksA*-deficient *B. burgdorferi* strain to infect Swiss Webster mice following subcutaneous injection with inoculums of 10^3 or 10^5 spirochetes/mouse. Collectively, the results indicate that DksA is a global regulator of gene expression in *B. burgdorferi*, and is required for infectivity in a murine model of infection.

Author Disclosure Block:

W.K. Boyle: None. **J.A. Shaw:** None. **A. Groshong:** None. **J.S. Blevins:** None. **F.C. Gherardini:** None. **T.J. Bourret:** None.

Poster Board Number:

MONDAY-721

Publishing Title:

Characterization Of The Role Of Transcriptional Regulator Arac Of Francisella Tularensis

Author Block:

D. K. Marghani[1], Z. Ma[2], M. Malik[2], C. S. Bakshi[1]; [1]New York Med. Coll., Valhalla, NY, [2]Albany Coll. of Pharmacy and Hlth.Sci., Albany, NY

Abstract Body:

Background: *Francisella tularensis* is the causative agent of a deadly human disease tularemia. *F. tularensis* has been used in bioweapon programs in the past and now it is feared to be used as a potential biothreat agent. The CDC has classified *Francisella* as Category A Select Agent. *Francisella* possesses very few transcription regulators. A majority of these have been shown to regulate genes involved in virulence and cellular functions. *Francisella* also possesses a transcriptional regulator known as AraC. In several Gram-negative bacteria, AraC is transcribed divergently from, and control *araBAD* operon involved in arabinose utilization. In contrast, in *F. tularensis araC* is transcribed divergently from an operon encoding EmrA multidrug efflux pump and is not required for arabinose utilization, indicating a unique role for AraC. This study is characterizing the role of AraC as a transcriptional regulator of *F. tularensis*.**Methods:** A deletion mutant of *FTL_689* gene encoding AraC (Δ*araC*) of *F. tularensis* LVS and its transcomplemented strain were generated. The Δ*araC* mutant was characterized for its ability to grow in the presence of carbohydrates, sensitivity to oxidants and antibiotics, virulence attributes, and identification of regulated target genes.**Results:** AraC has two conserved helix_turn_helix domains that show homology to DNA binding domain of SoxS of *E. coli*. The Δ*araC* mutant grows similar to wild type *F. tularensis* LVS in the presence of arabinose, indicating that AraC does not regulate arabinose utilization genes. Δ*araC* mutant exhibits enhanced sensitivity towards hydrogen peroxide and Tert-butyl hydroperoxide. The Δ*araC* mutant is not sensitive to tetracycline, nalidixic acid, chloramphenicol and streptomycin. Macrophage cell culture invasion assay shows a 5-fold reduction in the number of Δ*araC* mutant bacteria recovered from infected macrophages as compared to *F. tularensis* LVS indicating a partial attenuation of intramacrophage growth.**Conclusions:** AraC of *F. tularensis* is required for resistance against oxidative stress and not for arabinose utilization. Since SoxS, an oxidative stress transcriptional regulator is absent in *F. tularensis*, results from this study indicate that AraC may assume its role in this pathogen. Studies elucidating the role of AraC in gene regulation are currently underway.

Author Disclosure Block:

D.K. Marghani: None. **Z. Ma:** None. **M. Malik:** None. **C.S. Bakshi:** None.

Poster Board Number:

MONDAY-722

Publishing Title:

Stringent Response Regulation Of *vibrio Cholerae* Virulence Factors

Author Block:

D. RASKIN; Marian Univ., Indianapolis, IN

Abstract Body:

Cholera is an epidemic diarrheal disease caused by the gram-negative bacterium *Vibrio cholerae*. In order to colonize a host, *V. cholerae* must express the toxin-coregulated pilus (TCP), and diarrheal symptoms are primarily produced by the virulence factor cholera toxin (CT). CT and TCP expression is regulated by the ToxR regulon. Components of the ToxR regulon include ToxT, a transcription factor that directly initiates transcription of the CT and TCP genes. Upstream regulators include the integral membrane proteins TcpP and ToxR, transcription factors necessary for expression of *toxT*. TcpP and ToxR transcription initiation require TcpH and ToxS, respectively. ToxR may also directly initiate transcription of the CT genes independently of ToxT. It is thought that TCP is required early in infection to colonize the small intestine and that CT is expressed later in order to produce diarrhea and escape the host. It is not fully understood how the differential timing of CT and TCP expression occur. We found that stringent response, the low nutrient stress response, was necessary for colonization of the infant mouse small intestine. We made deletions in the stringent response genes and tested stringent response-defective mutants for expression of *ctxA* and *tcpA*, genes coding for components of CT and TCP respectively. Stringent response-defective strains showed greatly reduced expression of *tcpA* and a small but significant reduction in expression of *ctxA*. We tested whether stringent response regulated components of the ToxR regulon. We found that stringent response induced *toxT* and *tcpP* expression, while repressing *toxR*. The gene expression data was consistent with the requirement for stringent response-dependent colonization of the mouse. Stringent response is required for TCP expression, with TCP required for colonization. The gene expression data showing that stringent response had differential effects on components in the ToxR regulon may explain how CT and TCP may be differentially regulated despite being controlled by the same transcriptional activators. We are continuing to investigate how stringent response affects the ToxR regulon and how differential regulation of CT and TCP occurs.

Author Disclosure Block:

D. Raskin: None.

Poster Board Number:

MONDAY-723

Publishing Title:

Two Novel Regulators of Capsule and Virulence in *Klebsiella pneumoniae*

Author Block:

M. Palacios, K. A. Walker, V. L. Miller; Univ. of North Carolina, Chapel Hill, Chapel Hill, NC

Abstract Body:

In addition to having a reputation as the causative agent for several types of hospital-acquired infections, *Klebsiella pneumoniae* has gained widespread infamy as a pathogen with a propensity for developing antibiotic resistance. It is capable of causing a range of infections including UTIs, pneumonia, and sepsis. The list of identified virulence factors has mostly been limited to production of capsule, fimbriae, and siderophores. Due to the rapid emergence of resistance among *Klebsiella* strains to the last resort antibiotic, Carbapenem, there is a dire need for a better understanding of virulence mechanisms and identification of new drug targets. MarR (*m*ultiple *a*ntibiotic *r*esistance *r*egulator) is present in several pathogenic bacteria. Interestingly, *Klebsiella* has more copies of genes encoding MarR-like proteins than any other bacterial genera. Given the high number of these proteins in *Klebsiella*, these regulators likely contribute to the ability of this bacterium to respond to, and survive in, a wide variety of environmental conditions such as the human body. In an attempt to expand our understanding of *Klebsiella* virulence determinants, our lab focused on screening potential regulators of virulence by generating mutations in *marR*-like genes and screening them for attenuation in the lungs and spleens of mice. Our lab uses a 'classical' strain, KPPR1, and a mouse model that successfully recapitulates human infection by establishing bacterial colonization of the respiratory tract followed by the development of a systemic infection. While most of the mutations did not affect virulence in this model, we identified two previously uncharacterized regulators of *Klebsiella* virulence and have named these KvrA and KvrB. KvrA and KvrB were found to be required for expression from the *cpsB* promoter (one of the promoters in the capsule locus). Additionally, when testing the corresponding promoter of a 'hypervirulent (*hv*)' strain (liver-abscess derived), KvrA and KvrB also contributed to *cpsB* expression in this *hv* strain suggesting the contribution of our regulators is conserved among pathogenic *Klebsiella* strains. Further work on characterizing the relationship of our novel *Klebsiella* regulators of capsule with other previously identified regulators is under way. Our data demonstrate for the first time the *in vivo* contribution of a MarR homologue to *Klebsiella pneumoniae* virulence.

Author Disclosure Block:

M. Palacios: None. **K.A. Walker:** None. **V.L. Miller:** None.

Poster Board Number:

MONDAY-724

Publishing Title:

Spxa2 Coordinates Stress-response and Virulence in *Streptococcus pyogenes*

Author Block:

G. C. PORT, Z. T. Cusumano, P. R. Tumminello, M. G. Caparon; Washington Univ. Sch. of Med., St Louis, MO

Abstract Body:

For *Streptococcus pyogenes* and other pathogens, it is generally assumed that resistance to thermal and oxidative stresses is essential for pathogenesis. In investigating this question, we recently identified the multi-stress regulator ClpX as a regulator of toxin expression, particularly for the secreted SpeB cysteine protease. Although much is known about the conditions that stimulate SpeB expression, including stationary growth phase, low salinity, and low pH, little is known regarding the genetic circuitry that integrates these various signals and its intersection with stress resistance. For many gram-positive bacteria, ClpX regulates stress resistance via interactions with two orthologs of the Spx transcriptional regulator, SpxA1 and SpxA2. Analysis of Spx mutants revealed that while ΔSpxA1 had a defect only for growth under aerobic conditions, ΔSpxA2 was severely attenuated for multiple stresses, including thermal and oxidative stress. When examined in a murine model of soft-tissue infection, ΔClpX and ΔSpxA1 were highly attenuated. In contrast, the highly stress-sensitive ΔSpxA2 was hyper-virulent with higher tissue damage and bacterial burdens than the wild type strain. Furthermore, while the attenuation of ΔClpX and ΔSpxA1 was associated with reduced SpeB expression, ΔSpxA2 virulence was associated with SpeB hyper-expression and ΔSpxA2 virulence could be restored to wild-type levels by deletion of *speB*. Taken together, these data demonstrate that: 1. *S. pyogenes'* ability to damage tissue is combinatorial, involving fine-tuning of toxin expression of and 2. there is no direct correlation between ClpX-mediated multi-stress resistance and virulence. Further examination of ClpX's influence on virulence will provide insight into the genetic circuits that control the fine-tuning of toxin expression.

Author Disclosure Block:

G.C. Port: None. **Z.T. Cusumano:** None. **P.R. Tumminello:** None. **M.G. Caparon:** None.

Poster Board Number:

MONDAY-725

Publishing Title:

Changes in Expression of Genes Involved in O-Antigen Chain-Length Regulation and Lipid A Covalent Modification Are Regulated by Fnr in *Salmonella* Enteritidis

Author Block:

P. Fernandez[1], C. A. Silva[2], F. Velásquez[1], F. Amaya[1], H. Garcias[1], C. A. Santiviago[1], S. A. Álvarez[1]; [1]Facultad de Ciencias Químicas, Univ. de Chile, Santiago, Chile, [2]Tufts Univ., Boston, MA

Abstract Body:

The lipopolysaccharide (LPS) is a major component of the outer membrane of Gram-negative bacteria and plays an important role in *Salmonella* virulence. Previously we showed that the LPS of *Salmonella* Enteritidis undergoes changes in both the degree of polymerization of the O-antigen and covalent modifications in the lipid A-core region, in response to oxygen availability. The adaptation of *Salmonella* from aerobiosis to anaerobiosis involves changes in the expression of numerous genes, and the transcription factor FNR is a major regulator of this process. The aim of this work was to determine if the expression of *wzzB* and wzz_{fepE} genes, encoding O-antigen chain-length regulators, and *eptA, lpxO, lpxR* and *pagP* genes, encoding proteins involved in the covalent modification of the lipid A-core, is regulated by the oxygen availability in *S.* Enteritidis, and the involvement of FNR regulator in this process. We determined by qRT-PCR that the expression of these genes is affected by the oxygen availability in *S.* Enteritidis NCTC13349. This differential expression pattern was not observed in a Δ*fnr* mutant strain, indicating that these changes would be dependent on this transcriptional factor. These results are in agreement with bioinformatic analysis that showed the presence of putative FNR boxes in the promoters of these genes. Finally, electrophoretic mobility shift assay (EMSA) revealed that FNR directly interacts with the promoter regions of these genes. In conclusion, FNR directly binds to *wzzB*, wzz_{fepE}, *eptA, lpxO, lpxR* and *pagP* promoter regions in *S.* Enteritidis and this would account for the oxygen-dependent changes observed in the expression of these genes.

Author Disclosure Block:

P. Fernandez: None. **C.A. Silva:** None. **F. Velásquez:** None. **F. Amaya:** None. **H. Garcias:** None. **C.A. Santiviago:** None. **S.A. Álvarez:** None.

Poster Board Number:

MONDAY-726

Publishing Title:

The Role of Msab in the Nutrient-Dependent Regulation of Capsule Production in *Staphylococcus aureus*

Author Block:

J. L. Batte, G. Ghag, V. Rangachari, M. O. Elasri; The Univ. of Southern Mississippi, Hattiesburg, MS

Abstract Body:

Capsule production in *Staphylococcus aureus* is controlled by several regulators in response to various environmental stimuli. We have identified a new regulator, MsaB, which specifically binds to its target site upstream of *cap* operon. Furthermore, we have found that MsaB may sense nutrients availability. In this study, we examine the mechanism of capsule regulation by MsaB in response to nutrients. To examine the role of MsaB nutrient-dependent regulation, we examined MsaB sequence to predict putative ligands/nutrients to use in this study. We used nutrient-depleted in the early/mid-phases of growth and nutrient-replenished conditions in the late/post-phases of growth and measured capsule production and MsaB binding to the *cap* promoter. We used binding assays including the electrophoretic mobility shift assay (EMSA) and fluorescent anisotropy in the absence and presence of nutrients to determine if the binding of MsaB is altered by the addition of nutrients. Next, we used Circular dichroism (CD) to determine the secondary structure of MsaB in its native state and in the presence of nutrients. We also used competitive binding assays (EMSA and a fluorescent anisotropy) to explore any interplay between MsaB and other *cap* regulators in presence or absence of nutrients. We found that MsaB binds specifically to the *cap* promoter region to an inverted repeat and that a single nucleotide mutation in the repeat leads to the loss of binding of MsaB. Indeed, we found that nutrients directly affects MsaB binding ability to the *cap* promoter in different growth phases. Under normal nutrient conditions MsaB only binds to the *cap* promoter in late/post-exponential phases. We used a nutrient-depleted medium in the early/mid-phases and found that in low nutrients MsaB now binds to the *cap* promoter. As a result, when MsaB is bound in these conditions capsule is now produced differing from normal conditions when no capsule is produced in this phase of growth. We have also found that MsaB has binding sites for GTP, ATP, and AMP. Further studies will determine the role of these specific nutrients in MsaB binding affinity to its target site. We show that MsaB binds to the *cap* promoter in a nutrient-dependent manner and directly activates the production of capsule. These results suggest that MsaB binding contributes to the nutrient-dependent regulation of capsule production.

Author Disclosure Block:

J.L. Batte: None. **G. Ghag:** None. **V. Rangachari:** None. **M.O. Elasri:** None.

Poster Board Number:

MONDAY-727

Publishing Title:

Expression Analysis of Universal Stress Proteins in *Edwardsiella ictaluri*

Author Block:

A. AKGUL, A. Akgul, Hasan C. Tekedar, Mark L. Lawrence, Attila Karsi; mississippi state Univ., STARKVILLE, MS

Abstract Body:

Edwardsiella ictaluri is a Gram-negative bacterium that causes enteric septicemia of catfish (ESC). The universal stress proteins (USP) are present in archaea, bacteria, plants and fungi, but humans. USPs are expressed differentially to cope with stress during host invasion. We identified 13 USP genes in the *E. ictaluri* genome, but their protective role in *E. ictaluri* under stress is unknown. In this work, we will present the expression analysis of *E. ictaluri* USPs during three different stress conditions (low pH, high H_2O_2, and antibiotic presence). We expect expression analysis will identify essential USPs in *E. ictaluri*, which can be potential targets for live vaccine development.

Author Disclosure Block:

A. Akgul: None. **A. Akgul:** None.

Poster Board Number:

MONDAY-728

Publishing Title:

Differential Expression of Conserved Functions among Genetically Diverse *Escherichia coli* Is Key to Uropathogenic Phenotypes

Author Block:

H. L. Schreiber, IV[1], M. S. Conover[1], M. E. Hibbing[1], W-C. Chou[2], A. Manson-McGuire[2], A. M. Earl[2], J. Livny[2], S. J. Hultgren[1]; [1]Washington Univ Sch of Med., St. Louis, MO, [2]Broad Inst. of MIT and Harvard, Cambridge, MA

Abstract Body:

The ability of *Escherichia coli* to cause urinary tract infections (UTI) is thought to correlate with carriage of putative urovirulence factors (PUFs) and membership within the B2 clade of the *E. coli* phylogenetic tree. However, 25-50% of all uropathogenic *E. coli* (UPEC) isolated from women with UTI are from non-B2 phylogenetic clades (e.g. clades A, B1, and D). Here, we collected 43 B2 and non-B2 *E. coli* strains isolated from a cohort of women during consecutive recurrent UTIs for extensive study of their: i) phylogenetic history and relatedness; ii) shared gene content and carriage of PUFs; iii) transcriptional profiles of core genes shared amongst all of the strains and; iv) fitness in bladder colonization in representative mouse models of UTI. We found that carriage of PUFs correlated to membership in the B2 clade; however, neither specific gene content nor carriage of PUFs was predictive of bladder fitness. Further, some non-B2 isolates of *E. coli* encoding few PUFs had competitive advantages over B2 strains carrying considerably more PUFs in bladder colonization, indicating that carriage of many PUFs was neither necessary nor sufficient for bladder colonization. However, in comparing transcriptional profiles of *E. coli* grown in identical culture conditions, we identified differences in the expression of core genes shared amongst all *E. coli* strains. Importantly, these differences, which were found in genes mediating core bacterial functions such as motility and nutrient utilization, were associated with bladder colonization in both B2 and non-B2 isolates. Thus, our findings show that differential expression of the E. coli core genome, but not gene content and/or membership in the B2 clade of *E. coli*, best distinguish UPEC virulence.

Author Disclosure Block:

H.L. Schreiber: None. **M.S. Conover:** None. **M.E. Hibbing:** None. **W. Chou:** None. **A. Manson-McGuire:** None. **A.M. Earl:** None. **J. Livny:** None. **S.J. Hultgren:** None.

Poster Board Number:

MONDAY-729

Publishing Title:

Elucidation of the Mechanism of Oxidative Stress Regulation in *Francisella tularensis*

Author Block:

Z. Ma[1], V. C. Russo[1], S. M. Rabadi[2], S. V. Catlett[1], C. S. Bakshi[2], M. Malik[1]; [1]Albany Coll. of Pharmacy & Hlth.Sci, Albany, NY, [2]New York Med. Coll., Valhalla, NY

Abstract Body:

Background: *Francisella tularensis* is a highly virulent human pathogen. The ability of *F. tularensis* to cause a fulminate infection is due in part to its ability to survive and replicate in phagocytic cells involved in host's innate immune defenses. In order to establish an intracellular niche, *F. tularensis* has to overcome oxidative stress posed by reactive oxygen and nitrogen species. The genome sequence analysis reveals the presence of OxyR; while both the SoxR/SoxS regulons are absent in *F. tularensis*. The presence of OxyR as the only oxidant stress regulator indicates that regulation of oxidative stress response genes in *Francisella* is unique. However, nothing is known about the wider regulatory role of OxyR during *Francisella* infection. This study investigated the role of OxyR of *F. tularensis* in tularemia pathogenesis and regulation of genes involved in oxidative stress response.**Methods:** We generated a deletion mutant (Δ*oxyR*) and a transcomplemented strain of *oxyR* gene of *Ft* LVS. The *oxyR* mutant was characterized for its sensitivity towards oxidants, capacity to survive in macrophages, and virulence in mice. To investigate the effect of OxyR on gene regulation, we performed iTraq analysis, ChIP analysis and electrophoretic mobility shift assays (EMSA).**Results:** We demonstrate that OxyR of *F. tularensis* is required for resistance to oxidants, survival in macrophages, and virulence in mice. Proteomic analysis revealed differential expression of 123 proteins associated with oxidative stress resistance and cellular functions in the Δ*oxyR* mutant as compared to the wild type *F. tularensis* LVS indicating that OxyR serves as a global regulator of oxidant stress response. Most importantly, OxyR regulates transcription of primary antioxidant enzyme genes *ahpC, katG* and *sodB* by binding directly to their upstream promoter regions.**Conclusions**: These results highlight that OxyR is an important regulator of oxidative stress response and renders *F. tularensis* a pathoadaptive advantage to establish an intracellular niche. An understanding of these unique pathogenic mechanisms is essential for the development of effective therapeutics and prophylactics against this important biothreat agent.

Author Disclosure Block:

Z. Ma: None. **V.C. Russo:** None. **S.M. Rabadi:** None. **S.V. Catlett:** None. **C.S. Bakshi:** None. **M. Malik:** None.

Poster Board Number:

MONDAY-730

Publishing Title:

Contributions of a Two-component Regulatory System and C-Di-Gmp to Differentiation of *Legionella pneumophila*

Author Block:

E. D. Hughes; Univ. of Michigan, Ann Arbor, MI

Abstract Body:

Legionella pneumophila, the causative agent of Legionnaire's disease, is a gram-negative bacterium common in aquatic environments, where it survives and replicates within predatory amoeba and protists, its natural hosts. *Legionella* is capable of differentiating into multiple morphologically distinct cell types, including a highly infectious, metabolically dormant, cyst-like form known as MIFs (Mature Intracellular Forms). The MIF cell type is thought to equip *Legionella* to persist for extended periods outside a host cell in low-nutrient aqueous environments. For example, when compared to *in vitro* broth stationary phase cultures, we determined that MIF cells generated in either HeLa cells or the ciliate *Tetrahymena tropicalis* are more resistant to either antibiotics or detergents.Although MIFs are likely the form of *Legionella* that survives aerosolization to infect humans, little is known about the mechanisms that coordinate MIF cell differentiation. As one approach to investigate MIF development, we are analyzing the locus that includes *lpg0279*, a gene that is more highly expressed in MIF cells than *Legionella* isolated from macrophages. Immediately 3' of *lpg0279* is a putative two-component regulatory system: *lpg0278* is predicted to encode a histidine kinase and *lpg0277* the response regulator—a diguanylate cyclase whose product c-di-GMP regulates lifestyle changes in multiple bacterial species. Deletion mutants generated for each regulatory gene exhibit a prolonged lag phase in broth culture. Compared to WT, the *lpg0278* histidine kinase mutant cells accumulate increased c-di-GMP and exhibit decreased motility and capacity to infect and replicate in primary mouse macrophages. Further examination of the contribution of these genes to MIF cell differentiation will advance our understanding of the environmental persistence of *L. pneumophila* and its subsequent transmission from water aerosols to human lungs.

Author Disclosure Block:

E.D. Hughes: None.

Poster Board Number:

MONDAY-731

Publishing Title:

Rnpp Family Transcription Factors in *Enterococcus faecalis*

Author Block:

L. D. Jordan, N. Schwarting, L. E. Hancock; Univ. of Kansas, Lawrence, KS

Abstract Body:

Infections caused by enterococci are a serious threat to human health, as they represent one of the three most common hospital-acquired pathogens in the United States and around the world. A dichotomy exists between the many benefits of enterococci as a commensal organism and the harmful effects caused to its host in an infective state. Due to its opportunistic nature, enterococci can readily transition from being commensals to pathogens. We recently discovered a peptide transporter, PptAB, involved in the export of small peptide pheromones that induce a mating response in plasmid harboring donor cells. This same peptide transporter was also shown to contribute to biofilm formation and recent evidence suggests that it contributes to virulence in a catheter-associated UTI (CAUTI) model. In Gram-positive bacteria, the RNPP family of transcription factors are known to contribute to a variety of cellular processes, including biofilm formation and pathogenesis and their activity is regulated by binding to small peptides. Here we identify five uncharacterized and predicted RNPP homologs in *E. faecalis* V583 by querying the genome for characteristic elements of the RNPP family. We generated gene deletion mutants for each predicted RNPP homolog and examined these mutants for affects on biofilm development and compared their role in pathogenesis using a mouse model of a CAUTI by using a mixed infection of fluorescently labeled parental and mutant strains. We found that one such homolog displayed an increase in biofilm biomass and resulted in a significant increase in catheter and bladder colonization compared to the parental strain.

Author Disclosure Block:

L.D. Jordan: None. **N. Schwarting:** None. **L.E. Hancock:** None.

Poster Board Number:

MONDAY-732

Publishing Title:

Regulation of Rsma Targets in Mucoid Pseudomonas Aeruginosa

Author Block:

S. D. Stacey, C. Pritchett; East Tennessee State Univ., Johnson City, TN

Abstract Body:

Pseudomonas aeruginosa is an opportunistic pathogen that targets individuals with compromised immune systems like those diagnosed with HIV and cystic fibrosis. Some isolates from cystic fibrosis patients with chronic *P. aeruginosa* infections have a variant phenotype termed mucoid, which is the overproduction of the exopolysaccharide alginate. Alginate synthesis is regulated by the sigma factor AlgU. AlgU is sequestered by the antisigma factor MucA. Cell envelope stress or mutations in the *mucA* gene allow for AlgU hyperactivity resulting in increased alginate biosynthesis. We recently reported that AlgU also regulates the posttranscriptional regulator RsmA, an RNA-binding protein that regulates multiple genes involved in virulence. We propose that an increase in *rsmA* expression leads to increase RsmA activity in mucoid strains. We compared transcriptional and translational fusions of known RsmA targets: *hcnA, tssA1, fha1,* in both PAO1 and *mucA22* to determine RsmA activity. Post-transcriptional activity was increased on the abovementioned RsmA targets in a *mucA22* strain indicating that RsmA had increased activity. We confirmed this result with a leader fusion for *tssA1* and *hcnA.* Increased RsmA activity in *mucA22* strains led us to propose that novel targets of RsmA might exist in mucoid strains. Via iTRAQ labeling in *mucA22* versus *mucA22,* Δ*rsmA* we found known as well as unknown targets of RsmA. Overall, we have shown that in a *mucA* mutant background, RsmA is important in the regulation of genes found in *P. aeruginosa* chronic infections.

Author Disclosure Block:

S.D. Stacey: None. **C. Pritchett:** None.

Poster Board Number:

MONDAY-733

Publishing Title:

Regulation of Flagellar Motility in *Erwinia amylovora* by the Small Rna Arcz

Author Block:

J. K. Schachterle[1], Q. Zeng[2], G. W. Sundin[1]; [1]Michigan State Univ., East Lansing, MI, [2]Connecticut Agricultural Experiment Station, New Haven, CT

Abstract Body:

Background: *Erwinia amylovora*, causative agent of fire blight disease of apple and pear trees, requires flagellar motility for efficient primary infection of flowers. We have shown that the Hfq-dependent sRNA ArcZ positively regulates flagellar motility in *E. amylovora*. However, the mechanism of this regulation is unknown. This study explores the mechanism of ArcZ regulation of flagellar motility and identifies the first known direct target of ArcZ in *E. amylovora*. **Methods:** RNA was isolated from wildtype, *hfq*, and *arcZ* mutant cells, reverse transcribed to cDNA and used in qPCR reactions to determine relative abundances of *fliC*, *motA*, *motB*, *fliA*, *flhD*, and *flhC* mRNA. Translational fusions were developed by cloning of *flhD* 5' UTR in-frame to a GFP reporter. The *arcZ* and *flhD* translational fusion were additionally subjected to site-directed mutagenesis at sites identified using the RNAHybrid online server. Reporter mean fluorescence intensity was measured by flow cytometry.**Results:** Analysis of mutants by qPCR showed that for all genes tested, mRNA levels were significantly lower in *hfq* and *arcZ* mutants relative to wildtype, including the global regulator *flhDC*. RNAHybrid search of ArcZ and *flhD* 5' UTR identified a candidate interaction region. Testing of an *flhD* translational fusion in revealed that translation of FlhD is upregulated 3-fold in *hfq* and *arcZ* mutants. Wildtype *arcZ* complemented the effect, but *arcZ* mutated in the interaction region failed to complement the effect. Introduction of compensatory mutations in the *flhD* translational fusion enabled complementation by the mutated ArcZ.**Conclusions:** These results demonstrate that sRNA ArcZ regulates flagellar motility via regulation of *flhDC*. Mutation and compensatory mutation analysis suggest that the 5' UTR of *flhD* acts as a direct target of ArcZ in *E. amylovora*. However, because mutations of *hfq* and *arcZ* led to opposing effects at the mRNA and translational level, there are likely additional ArcZ targets involved in regulation of flagellar motility.

Author Disclosure Block:

J.K. Schachterle: None. **Q. Zeng:** None. **G.W. Sundin:** None.

Poster Board Number:

MONDAY-734

Publishing Title:

Poly Adenine Tract Length within the Adhesin Encoding Hopz Promoter Influences Transcription in *Helicobacter pylori*

Author Block:

C. R. Acio, A. A. Acio, M. H. Forsyth; Coll. of William & Mary, Williamsburg, VA

Abstract Body:

Helicobacter pylori is a gram-negative bacterium that colonizes the gastric mucosa of the human stomach. *H. pylori* infection is linked to the formation of ulcers, gastritis, and gastric adenocarcinoma. Pathogenesis is dependent, in part, on the bacterium's ability to respond appropriately to the acidic environment of the stomach and evade the immune and inflammatory responses of the host. *H. pylori* utilizes adhesin proteins, such as SabA and HopZ, to adhere to the gastric epithelial cells, allowing increased access to nutrients and preventing clearance as the gastric mucosa is shed. Our previous studies have demonstrated that SabA expression is controlled by multiple mechanisms, including the ArsRS Two Component System and phase variation via slip-strand mispairing. In this study, we investigated the regulatory effects of the poly-adenine tract of the *hopZ* promoter located from -24 to -37, relative to the transcriptional start site, on *hopZ* expression in *H. pylori* strain J99. Through site-directed mutagenesis, we simulated the extensions and truncations of the poly-adenine tract that occur naturally by slip-strand mispairing, followed by the quantification of *hopZ* expression using quantitative real-time PCR (qRT-PCR). Extending or truncating the poly-adenine tract by five adenines significantly increased expression of *hopZ* by approximately threefold. We hypothesize that these changes in the poly-adenine tract modify the DNA topology and influence the binding ability of RNA polymerase and/or other regulatory proteins.

Author Disclosure Block:

C.R. Acio: None. **A.A. Acio:** None. **M.H. Forsyth:** None.

Poster Board Number:

MONDAY-735

Publishing Title:

High-Throughput Identification of Regulatory Pathways of the Locus of Enterocyte Effacement in *Enterohemorrhagic e. coli*

Author Block:

R. Pifer, V. Sperandio; Univ. of Texas Southwestern Med. Ctr., Dallas, TX

Abstract Body:

Enterohemorrhagic *E. coli* (EHEC) O157:H7 is a food borne pathogen of humans that colonizes colonic epithelial cells and causes severe diarrhea. The Locus of Enterocyte Effacement (LEE) of EHEC encodes a type III secretion system that is essential for virulence. The LEE mediates secretion of effector molecules that induce cytoskeletal rearrangements within colonized epithelial cells to form a unique environmental niche. Expression of the LEE is a significant metabolic burden for EHEC and must be carefully regulated. Prior research from us and others have indicated the importance of small molecule metabolites in controlling LEE expression and EHEC virulence; however, uncovering the mechanisms of these signals is often difficult. We have established a high-throughput method using readily available tools to define these LEE regulatory mechanisms. We have developed an ELISA-based approach for evaluating the expression level of EspB, a component of the LEE encoded translocon. Using the *E. coli* K-12 Keio knockout library, we have created a library of LEE-expressing, transcription factor-deficient strains capable of type III secretion and a screen was undertaken to identify genes present in the *E. coli* core genome that regulate the LEE. As a proof of principal of the utility of these tools, we have explored the mechanism of Cysteine as a small molecule regulator of the LEE. We have identified a putative transcription factor, CysR, that is capable of governing type III secretion from K-12. We observe that a *cysR*-deficient strain of EHEC exhibits reduced LEE transcript levels and defective secretion. We observe that this is dependent upon cysteine within the growth media. Using *Citrobacter rodentium* as an EHEC infection model, we have observed that a *cysR*-deficient strain exhibits reduced mortality compared to wild type, suggesting that *cysR* is required for full virulence *in vivo*. The LEE is differentially regulated by a variety of growth conditions and small molecule metabolites, as exemplified in this work by Cysteine. Discovering the mechanism of these processes is often challenging. We describe a framework method that can be used by researchers to quickly determine the requirements of these signaling events.

Author Disclosure Block:

R. Pifer: None. **V. Sperandio:** None.

Poster Board Number:

MONDAY-736

Publishing Title:

Rgfd-dependent Quorum Sensing Affects Fibrinogen Binding, Host Cell Association, and Expression of the *rgf* Operon in Group B *Streptococcus*

Author Block:

R. Parker, D. Knupp, R. Al Safadi, S. Manning; Michigan State Univ., East Lansing, MI

Abstract Body:

Group B *Streptococcus* (GBS) is a leading cause of stillbirth, meningitis, and sepsis in neonates, and capable of causing invasive disease in susceptible adults. While GBS is a normal inhabitant in the microbiome, present in up to 40% of healthy adults, this opportunistic pathogen is able to breach restrictive host barriers and persist in harsh and changing conditions during pathogenesis. Quorum sensing (QS) is a form of cell to cell communication that enables an environmentally-dependent response essential to the virulence of many pathogenic bacteria, though few studies have assessed the importance of this phenomenon in GBS. This study sought to identify a role for QS in the regulation of the regulator of fibrinogen-binding (*rgf*) two-component system and the ability to bind to immobilized fibrinogen and host cells, *in vitro*, through the creation of a deletion mutant lacking the putative autoinducing peptide, RgfD. Expression of the *rgf* operon, assessed using qPCR, was significantly decreased in the mutant throughout exponential growth with the biggest difference (~50-fold) at higher cell densities. Attachment to fibrinogen was found to be 1.6-fold higher in the mutant compared to the wild-type suggesting dynamic regulation of this operon is important to functionality. Association with decidualized endometrial host cells was decreased 1.4-fold in the mutant further identifying this putative QS molecule as important for host colonization in GBS. Taken together, these data identify the putative quorum-sensing peptide, RgfD, as important for GBS pathogenesis and offer a novel target for the development of future therapeutics for the prevention and treatment of GBS disease.

Author Disclosure Block:

R. Parker: None. **D. Knupp:** None. **R. Al Safadi:** None. **S. Manning:** None.

Poster Board Number:

MONDAY-737

Publishing Title:

The Role of sRNAs in Thermoregulation of Gene Expression in Uropathogenic *Escherichia coli*

Author Block:

M. Sutherland, C. A. White-Ziegler; Smith Coll., Northampton, MA

Abstract Body:

In both commensal and uropathogenic *E. coli*, temperature is an important cue used to regulate gene expression and physiology upon entering a human host (37°C). Small regulatory RNAs (sRNAs) offer an additional level of regulation by altering mRNA stability, translation, or processing. The sequences of forty of eighty known sRNAs have been confirmed in the UPEC genome (Luo, Hu, and Zhu, *BMC Genomics* 10, 552 (2009)). My project explores sRNA participation in UPEC CFT073 thermoregulation, with two aims: I. Determine if sRNAs that regulate thermoregulated genes are themselves controlled by temperature and II. Use knock-outs to establish causality between sRNA expression and thermoregulation of genes contributing to virulence, adaptation and immune evasion. Previous transcriptome experiments in our lab demonstrated the thermoregulation of 423 genes in commensal (K-12) *E. coli* and 227 in UPEC.UPEC expression of eleven sRNAs with known thermoregulated targets was confirmed by transcriptome analysis and qRT-PCR; qRT-PCR on RNA from cells grown for 4 hours at 23°C and 37°C has shown six of those to be thermoregulated. Two of the sRNAs identified by this process target thermoregulated outer membrane proteins (OMPs) in a manner that may facilitate adaptation and immune evasion in the human host (37°C environment). The sRNA OmrA is downregulated 2.6-fold at 37°C as compared to 23°C, which could explain the observed seven-fold upregulation of OmrA's target OmpT, a protein known to cleave antimicrobial peptides (Hui, et al. *Microbiology and Immunology* 54 (2010): 452-459). The sRNA CyaR is upregulated 3-fold; this may similarly explain the observed downregulation of OmpX, an immunogenic OMP whose downregulation may decrease motility in low-glucose environments (De Lay and Gottesman. J. Bacteriology 191, 2 (2009): 461-476). Future experiments intend to knock out OmrA and/or CyaR by λ-red mutagenesis and compare the expression patterns of their target genes in wild-type versus mutant cells. This will establish or reject a causal role for these sRNAs in observed temperature-regulated changes in gene expression and physiology. Taken together, our data indicate that several sRNAs are thermoregulated and may be essential or contributory to adaptive thermoregulatory responses in uropathogenic *E. coli*.

Author Disclosure Block:

M. Sutherland: None. **C.A. White-Ziegler:** None.

Poster Board Number:

MONDAY-738

Publishing Title:

Cellobiose-Specific Phosphotransferase System Regulates Virulence Of *e. Coli* Rs218

Author Block:

D. S. S. Wijetunge, M. Dauran, S. Kariyawasam; Pennsylvania State Univ., University Park, PA

Abstract Body:

Neonatal meningitis-associated *E. coli* (NMEC) is one of the major causes of meningitis in newborn babies . The ability to utilize carbohydrates in a variety of host niches appears to be vital to many bacterial pathogens . It has also been demonstrated that these sugar transport systems have a role in regulation of virulence in some pathogenic bacteria . We have recently sequenced the NMEC reference strain *E. coli* RS218 which harbors a genomic island (GI) that encodes a putative cellobiose phosphotransferase system . Blasting of nucleotide sequence of cellobiose operon has revealed a wide distribution of this operon among extra intestinal pathogenic *E. coli*, including NMEC. In this study, we hypothesized that the cellobiose operon is involved in NMEC virulence regulation and is differentially expressed during the course of the infection. To test this hypothesis, we employed a quantitative real-time polymerase chain reaction to compare cellobiose expression of *E. coli* RS218 strain *in-vitro* under laboratory conditions (grown in Luria Bertani broth or human cerebral endothelial cells) and *ex-vivo* (grown in neonatal blood or serum). Our results indicated more than two-fold change in cellobiose gene expression when *E. coli* RS218 was grown in human cerebral endothelial cells, neonatal blood or neonatal serum as compared to LB. Furthermore, the regulatory effects of cellobiose operon on NMEC virulence was evaluated by comparing the expression of major virulence genes of NMEC (*ompA*, *fimH*, *aslA*, *npl*, *ibeA*, *traJ*, and *cnf*) between a mutant of RS218 lacking cellobiose operon and its wild-type strain.

Author Disclosure Block:

D.S.S. Wijetunge: None. **M. Dauran:** None. **S. Kariyawasam:** None.

Poster Board Number:

MONDAY-739

Publishing Title:

Characterization Of A Novel Virulence Regulator In Enterohemorrhagic *escherichia Coli* o157:H7

Author Block:

D. Luzader, M. Kendall; Univ. of Virginia Sch. of Med., Charlottesville, VA

Abstract Body:

Enterohemorrhagic *Escherichia coli* O157:H7 (EHEC) is a deadly food-borne pathogen responsible for outbreaks of bloody diarrhea and hemorrhagic colitis. Ingestion of less than 100 cells is enough to cause disease, making EHEC a serious public health concern. With such a low infectious dose, EHEC must be able to efficiently sense the environment and regulate the expression of virulence genes such as the locus of enterocyte effacement (LEE). The LEE encodes a type three secretion system and effector proteins that influence EHEC pathogenesis through the formation of attaching and effacing (AE) lesions. The regulation of virulence genes EHEC involves the integration of multiple signaling cues. Using a bioinformatics approach, we examined epinephrine and ethanolamine regulatory pathways and identified a putative transcriptional regulator in EHEC which we renamed regulator of EHEC virulence or RevA. Because *revA* is differentially expressed when grown in the presence of either epinephrine or ethanolamine, we hypothesize that RevA plays a role in pathogenesis by the regulation of virulence gene expression in EHEC.To test our hypothesis, we generated a deletion strain of *revA* using lambda-red techniques and then examined the expression of LEE-encoded genes in WT EHEC or the *revA* mutant strain by qRT-PCR. Expression of the LEE was significantly decreased in the *revA* mutant compared to WT. To further assess the influence of RevA on EHEC pathogenesis, we measured the number of AE lesions formed on epithelial cells by fluorescent microscopy through a fluorescein actin staining assay. In agreement with the transcriptional data, the *revA* mutant formed significantly less AE lesions compared to WT EHEC. Preliminary data using electrophoretic mobility shift assays indicate that RevA is a direct regulator of the LEE. These data demonstrate a novel role for RevA in the modulation of EHEC virulence traits. We are currently mapping the RevA regulatory circuits in EHEC and confirming the mechanism through which RevA influences gene expression in EHEC.

Author Disclosure Block:

D. Luzader: None. **M. Kendall:** None.

Poster Board Number:

MONDAY-740

Publishing Title:

The Pneumococcal Tpra Is Essential For Host Derived Sugar Utilisation And Virulence

Author Block:

A. S. Motib, P. Andrew, H. Yesilkaya; Univ. of Leicester, Leicester, United Kingdom

Abstract Body:

Background: *Streptococcus pneumoniae* is a major cause of mortality and morbidity around the world; it causes several serious invasive infections. Virulence factors that lead to increase the pathogenicity of this bacterium are regulated in response to different environmental stimuli. TprA is a transcriptional regulator of *Streptococcus pneumoniae*, which is activated by a peptide, PhrA. However, the role of the TprA/PhrA system in pneumococcal physiology and virulence is not known in detail. Hence, the objective of this study was to further the knowledge on the function of the TprA/PhrA system.**Methods:** Insertion deletion mutants were constructed and their growth was tested in chemically defined medium supplemented with different sugars (glucose, galactose, maltose, and N-acetyl glucosamine) under microaerobic condition. In addition, hyaluronidase and neuraminidase activities were assayed using chromogenic substrates. The expression of *tprA* and *phrA* was determined by *lacZ* reporter assay. Finally, mouse model of pneumococcal pneumonia and systemic infection models were used to determine the virulence of mutant strains compared with the wild type D39.**Results:** We showed by reporter gene assays that the TprA/PhrA system is active in the presence of galactose and mannose. TprA and PhrA mutant strains were significantly attenuated in growth on galactose and mannose compared with the wild type D39 strain. In addition, we also demonstrated that mutation of *tprA* abrogates pneumococcal virulence in a mouse model of pneumococcal pneumonia that develops after intranasal infection, as well as in a septicemia model. Analysis of the TprA mutant indicated that the mutant had decreased production of important virulence factors, including neuraminidase and hyaluronidase compared to the wild type D39 strain.**Conclusions:** Our results showed that TprA is crucial for pneumococcal host-derived sugar metabolism and virulence. We also showed that TprA is essential to produce neuraminidase and hyaluronidase that are important to survive *S. pneumoniae* in host tissue. Further work is underway to determine how TprA contributed to pneumococcal virulence.

Author Disclosure Block:

A.S. Motib: None. **P. Andrew:** None. **H. Yesilkaya:** None.

Poster Board Number:

MONDAY-741

Publishing Title:

SNP Combinations Found *In vibrio cholerae* Clinical Isolates Have an Additive Effect on Virulence Capabilities

Author Block:

B. M. Carignan, K. D. Brumfield, M. S. Son; Plymouth State Univ., Plymouth, NH

Abstract Body:

Background: Cholera is a potentially fatal diarrheal disease caused by the Gram-negative, aquatic bacterium *Vibrio cholerae*. This disease is characterized by vomiting and severe watery diarrhea, and, if left untreated, will result in severe dehydration and ultimately death. Recently, *V. cholerae* clinical isolates demonstrated increased virulence capabilities, causing more severe symptoms with a much faster rate of disease progression than previously observed. **Methods:** We have identified single nucleotide polymorphisms (SNPs) in four key regulatory genes (*hapR, hns, luxO,* and vieA) of a *V. cholerae* clinical isolate, known to affect virulence gene expression. Herein, all possible SNP and SNP combinations were introduced into the prototypical wild-type El Tor strain N16961 using an established allelic exchange protocol, and the effects on production of the master regulator of virulence gene expression, ToxT, and the two main virulence factors, the toxin co-regulated pilus (TCP), and cholera toxin (CT) were determined by Western blot analysis and a colorimetric CT assay, respectively. We hypothesize that various SNP and SNP combinations will affect ToxT levels, and the levels of two main virulence factors. Furthermore, we hypothesize that a minimum combination of SNPs is necessary to recapitulate these increased virulence capabilities observed in *V. cholerae* clinical isolates. **Results:** We observed the varying SNP combinations primarily had additive effects on CT, TCP, and ToxT production relative to the prototypical wild-type strain, while some combinations interestingly had the opposite effect. **Conclusion:** These data strongly suggest that these SNPs in a yet-to-be-determined combination are involved in the increased virulence capabilities observed in *V. cholerae*. However, to what capacity and the molecular basis of these observed increases in virulence capabilities is still unclear, and currently under investigation.

Author Disclosure Block:

B.M. Carignan: None. **K.D. Brumfield:** None. **M.S. Son:** None.

Poster Board Number:

MONDAY-742

Publishing Title:

***Mycobacterium tuberculosis* phoY Proteins Promote Persister Formation by Mediating Phosphate Signaling between Pst/SenX3-RegX3**

Author Block:

S. B. Namugenyi, A. M. Aagesen, A. D. Tischler; Univ. of Minnesota, Minneapolis, MN

Abstract Body:

The *Mycobacterium tuberculosis* (*Mtb*) phosphate-specific transport (Pst) system controls expression of phosphate (P_i)-responsive genes by negatively regulating SenX3-RegX3, a two-component regulatory system, in P_i-rich conditions. Previously we demonstrated that deletion of *pstA1*, which encodes a trans-membrane component of the Pst system, causes *in vivo* attenuation due to constitutive activation of RegX3 and aberrant expression of P_i-responsive genes. In *Escherichia coli* the Pst system inhibits a homologous two-component system through the negative regulator, PhoU. *E. coli* PhoU is also required for formation of persisters, a sub-population of phenotypically antibiotic tolerant bacteria that are genetically identical to drug susceptible bacteria. *Mtb* has two *phoU* homologs, *phoY1* and *phoY2*, which have not been characterized. To determine which PhoY protein participates in signal transduction, we used qRT-PCR to quantify transcript levels of select P_i-responsive genes in *phoY* deletion mutants. The Δ*phoY1* or Δ*phoY2* single deletions did not affect transcript levels, but significant up-regulation was observed in the Δ*phoY1*Δ*phoY2* double mutant. This phenotype was reversed by complementation with either *phoY1* or *phoY2* or deletion of *regX3*, suggesting that both PhoY proteins can negatively regulate SenX3-RegX3 and that they have redundant function. We analyzed the frequency of persister formation in the *phoY* mutants using combinations of antibiotics. Persister frequency was decreased 20- to 40-fold in the Δ*phoY1*Δ*phoY2* mutant compared to wildtype. This decrease in persister frequency was partially *regX3*-dependent. A Δ*pstA1* mutant exhibited a similar decrease in persister frequency that was also *regX3*-dependent. Our data suggest that disrupting P_i signal transduction mediated by the PhoY proteins can dramatically enhance susceptibility of *Mtb* to antibiotics.

Author Disclosure Block:

S.B. Namugenyi: None. **A.M. Aagesen:** None. **A.D. Tischler:** None.

Poster Board Number:

MONDAY-743

Publishing Title:

Phosphotyrosine-mediated Regulation of Enterohemorrhagic *Escherichia coli* Virulence

Author Block:

C. Robertson[1], D. A. Rasko[2], J. B. Kaper[1], **A-M. Hansen**[1]; [1]Univ. of Maryland Sch. of Med., Baltimore, MD, [2]Inst. for Genome Sci., Baltimore, MD

Abstract Body:

Although protein tyrosine phosphorylation is a well-established essential regulatory mechanism in eukaryotes, it is understudied in prokaryotes. We previously identified that the number of tyrosine-phosphorylated proteins in *Escherichia coli* is about 10-fold higher than heretofore appreciated. The newly identified phosphorylated proteins relate to fundamental cell functions and virulence, indicating a central regulatory role of tyrosine phosphorylation. Here, we aim to elucidate the regulatory role of phosphotyrosine-mediated signaling in virulence gene expression of the food-borne pathogen enterohemorrhagic *E. coli* (EHEC) with the focus being on the transcriptional regulator Cra. EHEC infection is characterized by an intestinal attaching and effacing (A/E) histopathology, which is due to the activity of a type III secretion system (T3SS) encoded by the locus of enterocyte effacement (LEE) pathogenicity island. Cra, a glycolytic metabolite-responsive DNA-binding global regulator controlling sugar metabolism, is required for LEE expression under gluconeogenic conditions to ensure successful colonization. Since Cra Y47 identified as being phosphorylated is functionally important and is located in the DNA-binding domain, we hypothesize that phosphorylation of Cra controls the expression of virulence and central carbon metabolism genes by affecting Cra DNA-binding capacity. Comparing the regulatory effect of wild type, non-phosphorylatable and phosphomimetic Cra variants on LEE expression, we demonstrate that phosphorylation of Y47 negatively affects the production and secretion of T3SS proteins under glycolytic conditions where LEE expression is unwarranted. EHEC expressing phosphomimetic Cra versus wild type Cra exhibits decreased A/E lesion formation efficiency, suggesting that Cra phosphorylation negatively influences the EHEC virulence potential. Specifically, we demonstrate that phosphomimetic Cra unlike wild type Cra is incapable of binding to a LEE regulatory region. Our findings indicate that tyrosine phosphorylation of Cra negatively controls LEE expression by interfering with Cra DNA-binding capacity resulting in decreased T3SS activity and A/E lesion formation.

Author Disclosure Block:

C. Robertson: None. **D.A. Rasko:** None. **J.B. Kaper:** None. **A. Hansen:** None.

Poster Board Number:

MONDAY-744

Publishing Title:

Phosphorylated *Pseudomonas aeruginosa* ALGR is Required for Iron Acquisition and Murine Virulence

Author Block:

A. S. Little[1], Y. Okkotsu[1], F. H. Damron[2], M. Barbier[2], A. Oglesby-Sherrouse[3], J. P. Owings[4], J. B. Goldberg[4], W. L. Cody[5], M. J. Schurr[1]; [1]Univ. of Colorado Sch. of Med., Aurora, CO, [2]West Virginia Univ. Sch. of Med., Morgantown, WV, [3]Univ. of Mayland Sch. of Med., Baltimore, MD, [4]Emory Univ. Sch. of Med., Atlanta, GA, [5]Univ. of Dallas, Irving, TX

Abstract Body:

The *P. aeruginosa* AlgZR two-component system controls the expression of several virulence determinants. Three phenotypes (alginate, rhamnolipid and type IV mediated motility) are dependent upon AlgR phosphorylation. A recent ChIP-seq manuscript reported a 154 gene regulon for AlgR. However, this study did not take into account AlgR phosphorylation. While *algR* and *algZ* gene deletions have been assessed in murine infection models, the impact of AlgR phosphorylation on virulence has not been examined. Strains encoding a phospho-mimetic form AlgRD54E and a phospho-defective form AlgRD54N were created to dissect the role of the AlgR phosphorylation state by microarrays and in three different virulence models.Microarrays comparing PAO1*algR*D54E and PAO1*algR*D54N identified differentially regulated genes dealing with iron acquisition. PAO1 *algR*D54N produced considerably less (25%) siderophores than wildtype PAO1. Artificially increased expression of AlgRD54N repressed siderophore production, whereas expression of AlgRD54E had no effect. Mobility shift assays showed that AlgR directly bound to the *prrf, pvdS* and *pvdY* promoters. PAO1*algR*D54N was considerably attenuated in three virulence models. In a *Drosophila melanogaster* oral feeding model, PAO1*algR*54N resulted in 35% mortality compared to near complete mortality of flies infected with PAO1. In an acute murine pneumonia model, PAO1*algR*D54N infected mice had 37.5% mortality, while PAO1 resulted in 100% mortality. PAO1*algR*D54N was also attenuated for growth in a murine wound model.A large group of dysregulated genes were associated with iron, and subsequent assays showed that PAO1*algR*D54N had decreased siderophore production. PAO1*algR*D54N was defective in three different models of virulence, and the dysregulation of iron acquisition genes likely plays a role in the attenuation of virulence with PAO1*algR*D54N, as defects in iron acquisition are known to result in avirulence in several models. However, as the microarray data show a wide range of dysregulated genes, it is unlikely that only one group of related genes resulted in this defect.

Author Disclosure Block:

A.S. Little: None. **Y. Okkotsu:** None. **F.H. Damron:** None. **M. Barbier:** None. **A. Oglesby-Sherrouse:** None. **J.P. Owings:** None. **J.B. Goldberg:** None. **W.L. Cody:** None. **M.J. Schurr:** None.

Poster Board Number:

LB-001

Publishing Title:

Type 6 Secretion System-mediated Killing Generates Clonal Assortment within Microbial Communities

Author Block:

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Abstract Body:

Antagonistic interactions between microbes likely determine the structure and diversity of microbial communities. *Vibrio cholerae* interacts with other microbes in biofilms in aquatic settings and within the digestive tracts of animals including humans, where certain strains can cause fatal cholera. *V. cholerae* utilizes a Type VI Secretion System (T6SS) resembling a phage tail-spike to mediate contact-dependent inter-bacterial antagonism in biofilm communities. The T6SS directly injects toxic effector proteins to lyse neighboring cell that lack cognate immunity proteins. Phenotypic and genomic analyses of a diverse set of >50 isolates we sequenced identified distinct T6SS effector/immunity pairs and several T6SS$^+$ *V. cholerae* isolates that killed one another, i.e. that were mutual killers. By killing competitors but not kin, we reasoned that T6SS-mediated killing could generate structured populations with high clonal assortment. To gain insight into the consequences of T6SS-mediated killing on the dynamics and structure of bacterial populations, we used fluorescence microscopy to visualize competition between two *V. cholerae* mutual killers as they grew from initially well-mixed conditions on solid surfaces. When each isolate was rendered T6SS$^-$ by deletion of an essential apparatus component, a well-mixed population was maintained. Unidirectional killing led to elimination of the T6SS$^-$ mutant by its T6SS$^+$ competitor. In contrast, bidirectional killing generated a structured population with each isolate segregating into distinct clonal patches. These dynamics were recapitulated in an agent-based simulation and partial differential equation models we developed. Imaging of the digestive tract of larval zebrafish by light sheet fluorescence microscopy also revealed that colonization of T6SS$^-$ bacteria was severely impaired in a germ-free host pretreated with T6SS$^+$ but not T6SS$^-$ *V. cholerae*. These results suggest microbes use inter-bacterial antagonism to generate spatial structure, and that T6SSs may have evolved to increase fitness during competition in environmental and host settings. We are currently exploring whether T6SSs establish highly structured populations predisposed to evolving costly behaviors that could not be supported in well-mixed populations.

Author Disclosure Block:

B. K. Hammer, None.

Poster Board Number:

LB-002

Publishing Title:

Rumen Microbial Community Structure of Sheep Fed the Probiotic *Bacillus amyloliquefaciens* H57

Author Block:

B. J. Schofield[1], A. V. Klieve[2], P. Dart[1], O. le[2], N. Lachner[1], Y. Yeoh[1], D. Ouwerkerk[3], D. McNeill[2], P. Hugenholtz[1]; [1]The Univ. of Queensland, St Lucia, Australia, [2]The Univ. of Queensland, Gatton, Australia, [3]Dept. of Agriculture and Fisheries, Dutton Park, Australia

Abstract Body:

The rumen microbiological community in sheep primarily drives the digestion of plant material. Key organisms play specific roles in digestion, ranging from fibre degradation, VFA and microbial protein production, to the regeneration of essential coenzymes such as those involved with methanogenesis. *Bacillus amyloliquefaciens* H57 (H57) is a probiotic isolated specifically for its antimicrobial activities and spore formation. Feeding pregnant Dorper ewes with pellets containing H57 spores at 10^6 g^{-1} led to significantly increased weight gain and nitrogen (protein) retention (Le *et al* 2014). In a project funded by Ridley AgriProducts and the ARC, we examined how H57 might benefit the sheep through effects on the rumen microbial community. Bacterial 16S rRNA genes were sequenced using an Illumina paired-end sequencing platform. There were clear differences in rumen microbial community structure in animals fed H57 compared to the control animals. The dominant organisms in H57 fed animals included a *Prevotella* and *Coprococcus* spp., whereas in the control animals, *Prevotella ruminicola* and a member of the *Succinivibrio* genus dominated. Metagenomic sequencing showed that the dominant *Prevotella* in the H57 fed animals possessed a glycoside hydrolase profile that was dominated by genes for cellulolytic enzymes. By contrast, most known rumen *Prevotella* species are dominated by hemi-cellulolytic gene families that act best on less fibrous materials. The dominance of the cellulolytic *Prevotella* species in the H57 fed animals may have contributed to the increased weight gain observed, as more of the otherwise less digestible cellulosic component of the diet was available for digestion.

Author Disclosure Block:

B. J. Schofield, None..
A. V. Klieve, None..
P. Dart, None..
O. le, None..
N. Lachner, None..
Y. Yeoh, None..
D. Ouwerkerk, None..

D. McNeill, None..
P. Hugenholtz, None.

Poster Board Number:

LB-003

Publishing Title:

Complete Sequences of Plasmids Carrying *bla*$_{IMP-4}$ Carbapenemase Gene from Enterobacteriaceae in Silver Gulls, Australia

Author Block:

M. Dolejska[1], H. Dobiasova[1], M. Medvecky[2]; [1]Univ. of Vet. and Pharmaceutical Sci., Brno, Czech Republic, [2]Vet. Res. Inst., Brno, Czech Republic

Abstract Body:

Background: Carbapenems are among the most important antimicrobials in human medicine. Their clinical efficiency is threatened by carbapenemases disseminated in Gram-negative bacteria worldwide. Recently, we have described high level of colonization by IMP-4-producing Enterobacteriaceae isolates in silver gulls in Five Islands, Australia. The *bla*$_{IMP-4}$ gene was present in the class 1 integron carried by resistance plasmids of various types.
Methods: In this study, a complete nucleotide sequencing of five representative plasmids from Enterobacteriaceae originated from the gulls was performed using MiSeq Illumina system. The sequences were analyzed using bioinformatics tools and compared with *bla*$_{IMP-4}$-carrying plasmids previously reported in clinical isolates in Australia, such as pEI1573.
Results: Multi-drug resistance region (MRR) carrying *bla*$_{IMP-4}$ gene identified in all plasmids was surrounded by IS*26* and showed high similarity to MRR of pEI1573. p1631 was 84 kb IncL/M plasmid with >99 % nucleotide identity with *bla*$_{IMP-4}$-plasmid pEI1573. With respect to pEI1573, the MRR was truncated by 4.5 kb fragment from *Klebsiella pneumoniae* genomes. Plasmid p42.1 contained a typical IncI1 backbone and MRR was bounded by Tn*1721*. p19.1 (185 kb) was related to other multi-drug resistance IncA/C$_2$ plasmids, carrying two variable resistance regions that included *floR-sul2* and pEI1573-like MRR inside Tn*1696*. A 15 kb fragment encoding UV resistance protein, previously identified in pKP13f, was found inside p19.1 backbone. p47.1 (142 kb) had highly mosaic structure formed by conjugative transfer region (tra) of pPry2001 from *Providentia rettgeri*, mercury resistance operon and other segments from genomes of environmental bacteria. Plasmid p77.1 (195 kb) was a mosaic formed by IncHI2 backbone, tra region related to IncX5 plasmids and a novel In*5*-like *bla*$_{IMP-4}$ integron bounded by IS*26*.
Conclusions: We demonstrate high plasticity of resistance plasmids encoding carbapenemase IMP-4 from Enterobacteriaceae in wildlife. This study underscore the spreading potential of large segments carrying resistance determinants, most likely by IS*26* mediated recombination and suggest possible role of environment in evolution of carbapenemase-carrying plasmids.

Author Disclosure Block:

M. Dolejska, None..
H. Dobiasova, None..
M. Medvecky, None.

Poster Board Number:

LB-004

Publishing Title:

***Candida albicans* confers ampicillin resistance to *Streptococcus gordonii* in co-cultures**

Author Block:

A. Maddi, 14214, L. M. Yerke, R. Mancuso, 14214, C. Jennifer, N. Sadhak; State Univ. of new York, Buffalo, NY

Abstract Body:

Background: Oral biofilms are complex and contain various microorganisms including bacteria and fungi. The pioneer oral plaque bacteria, *Streptococcus gordonii* and the most abundant oral pathogenic fungus, *Candida albicans* are known to interact with each other. Past studies have shown that *S. gordonii* binds to hyphae of *C. albicans* in a corncob like structure. However, the exact mechanisms and outcomes of their interactions are not clearly understood. We hypothesized that *C. albicans* and *S. gordonii* confer antimicrobial resistance to one another when grown in co-cultures.
Methods: *C. albicans* and *S. gordonii* were grown in a specially prepared synthetic minimal media [Tryptic Soy Broth Yeast Extract (TSBY) + Yeast Nitrogen Base (YNB)] with and without the antibacterial agent, ampicillin, for 2, 4, 6 and 8 hrs. The cultures were then filtered to eliminate *C. albicans* and the resulting filtrate was then plated on TSBY agar and *S. gordonii* colonies were counted. Similarly *C. albicans* and *S. gordonii* were also grown in co-cultures with caspofungin (antifungal agent) for 2, 4, 6 and 8 hrs. These cultures were plated on YNB agar with ampicillin and the resulting *C. albicans* colonies were counted. Proteomic analysis of secreted protein resulting from the co-cultures was performed using SDS PAGE analysis, silver staining and mass spectrophotometric analysis.
Results: The results from the antibiotic testing showed that *S. gordonii* was able to grow significantly greater ($p<0.05$) at 4 and 6 hrs in the presence of ampicillin (antibacterial) and *C. albicans*, as compared to ampicillin alone. However, when caspofungin (antifungal) was present there was no significant growth of *C. albicans* even when *S. gordonii* was present in the culture. Proteomic and mass spectrometric analysis indicate differentially secreted proteins in the co-cultures as compared to single cultures. **Conclusions:** These data indicate that *C. albicans* protects *S. gordonii* from ampicillin dependent killing and may confer ampicillin resistance. However, *S. gordonii* does not protect *C. albicans* from antifungal killing. The secreted proteins may play a role in the interactions between *C. albicans* and *S. gordonii*.

Author Disclosure Block:

A. Maddi, None..
L. M. Yerke, None..
R. Mancuso, None..

C. Jennifer, None..
N. Sadhak, None.

Poster Board Number:

LB-005

Publishing Title:

Biostimulation And Microbial Community Profiling Reveal Insights On Rdx Transformation In Groundwater Samples Collected From Los Alamos

Author Block:

D. Wang, H. Boukhalfa, B. Newman, O. Marina, D. Ware, P. Reimus, T. Goering; Los Alamos Natl. Lab, Los Alamos, NM

Abstract Body:

Natural attenuation of RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine) is facilitated by a complex interplay of abiotic and biotics activities. In this study, we aim to understand abiotic and microbial signatures of RDX degradation in groundwater. A survey was performed on the bacterial community of nine groundwater samples containing different levels of RDX collected from monitoring wells intercepting shallow and intermediate aquifers located in Los Alamos, New Mexico using the 16S rRNA profiling approach. A total of 827,454 sequence reads were obtained and classified into 997 genera indicating high microbial diversity. Known RDX degrading bacterial genera were found in all samples with Rhodococcus specifically enriched in the groundwater samples containing higher levels of RDX. To identify factors enhancing microbial activity and RDX degradation, groundwater samples from a selected well were stimulated by the addition of acetate or vegetable oil. Results demonstrated that vegetable oil but not acetate promoted RDX degradation in anaerobic conditions. Upon addition of carbon sources, dominant members of Nocardiaceae were outcompeted by bacteria in the family of Comamonadaceae and Pseudomonadaceae, which were underrepresented in groundwater. One of the retrieved sequences related to a previously described RDX-degrading strain (99% sequence identify) was enriched by vegetable oil under anaerobic conditions. The results of this study expand our current understanding of microbial ecology in RDX-contaminated aquifers and emphasize the potential importance of microbes in RDX transformation.

Author Disclosure Block:

D. Wang, None..
H. Boukhalfa, None..
B. Newman, None..
O. Marina, None..
D. Ware, None..
P. Reimus, None..
T. Goering, None.

Poster Board Number:

LB-006

Publishing Title:

Effect of Aerial Fertilization on Microbial Communities Inhabiting Oaks Phyllosphere

Author Block:

a. bani, L. Borruso, P. Panzacchi, M. Ventura, G. Tonon, L. Brusetti; Free Univeristy of Bozen Bolzano, Bolzano, Italy

Abstract Body:

It is widely accepted that anthropic activities have increased nitrogen depositions worldwide. The deposition of atmospheric nitrogen compounds is primarily due to rain and snow harboring nitrate and ammonium. This has an effect on forests plant biodiversity and on the associated biota. Several studies have already investigated the effects of differential nitrogen amendments on soil under plants canopies. On the other hand, there is a general lack of knowledge regarding the effects of aerial nitrogen depositions on the canopy and the associated microbial communities. The goal of the present work is to assess the influence of nitrogen deposition on the microbial communities of the phyllosphere in a temperate forest dominated by sessile oak (*Quercus petraea* Liebl.). The study area is located in Monticolo (South Tyrol, Italy) where a long-term experiment of nitrogen deposition simulation has been established. The experimental design considered six plots: three control plots and three plots treated via aerial fertilization. The fertilizer was a liquid solution containing NH_4NO_3. Twenty kg ha^{-1} y^{-1} of the nitrogen solution were applied monthly from May 2015 until September 2015. Fifteen days after the last fertilization, three biological replicates were collected in each plot. Microbial structure resulted to be differentiated via NMDS into two clusters clearly separating the nitrogen-treated from the non-treated samples.

Author Disclosure Block:

A. bani, None..
L. Borruso, None..
P. Panzacchi, None..
M. Ventura, None..
G. Tonon, None..
L. Brusetti, None.

Poster Board Number:

LB-007

Publishing Title:

A New Pathway for Synthesis of the Biotin Pimelate Moiety in Bacteria

Author Block:

Y. Hu, J. Cronan; Univ. of Illinois at Urbana-Champaign, Urbana, IL

Abstract Body:

Background: Biotin is an essential cofactor for carboxylation, decarboxylation and transcarboxylation reactions in various metabolic pathways. The pathways for the biosynthesis of the precursor pimelate moiety of biotin are diverse, while the enzymes responsible for synthesis of the bicyclic rings are conserved in all biotin-producing bacteria, plants and fungi[(1)]. The most common pathway for synthesis of the biotin pimelate moiety in bacteria is recently demonstrated in *E. coli*. This pathway utilizes a SAM-dependent methyltransferase (BioC) to mask the charged carboxyl group of a malonyl-ACP and to hijack the fatty acid biosynthetic pathway; giving a pimeloyl-ACP methyl ester via two classical fatty acid elongation cycles. A short-chain fatty acid esterase (BioH) then hydrolyzes the methyl group of the pimeloyl-ACP methyl ester, to give pimeloyl-ACP[(2-3)]. Interestingly, several *α-proteobacteria* like the pathogens *Agrobacterium* and *Brucella* have a different means to synthesize the pimelate moiety. **Methods and Results:** We report that these bacteria use a 3-ketoacyl-ACP synthase (condensing enzyme-like) protein called BioZ to synthesize the pimelate moiety. BioZ proteins have around 35% sequence identity with FabH, the fatty acid biosynthesis short chain 3-ketoacyl-ACP synthase[(4-6)]. Expression of various BioZ proteins complement the *E. coli bioC* and *bioH* pimelate moiety deletion mutant strains. Moreover, BioZ proteins contain the same conserved catalytic residues and perform the 3-ketoacyl-ACP synthase reaction, but give products that differ from those of FabH. Modeling of these proteins based on FabH crystal structure templates predicts that the structures are almost identical. The predicted amino acid substitutions that comprise the substrate binding tunnel argue that BioZ has different substrate preferences that differ from those of FabH. **Conclusions:** some *α-proteobacteria* use BioZ to synthesize the pimelate precursor for biotin by performing a FabH-like reaction, but with different substrates.

Author Disclosure Block:

Y. Hu, None..
J. Cronan, None.

Poster Board Number:

LB-008

Publishing Title:

Comparative Genomics of *Devosia* Isolates Involved in the Biotransformation of the Mycotoxin Deoxynivalenol

Author Block:

D. Lepp, Y. I. Hassan, T. Zhou; Agriculture and Agri-Food Canada, Guelph, ON, Canada

Abstract Body:

Deoxynivalenol (DON) is a type B trichothecene mycotoxin that is commonly found in grains infested with fungus of *Fusarium* species. DON contamination is associated with feed refusal and low animal productivity/weight gain, which costs the global economy billions of dollars annually in trade losses. DON is also a documented health hazard for humans, causing both acute and chronic symptoms, and has a maximum tolerable level of less than 2 ppm in human food and 5 ppm in animal feed. Effective methods for controlling DON contamination are therefore urgently needed. We recently reported on the isolation of a bacterial strain (17-2-E-8) that is capable of detoxifying DON, through enzymatic epimerization, with high efficiency under a wide-range of conditions. This strain was subsequently identified as a novel species of *Devosia*, a diverse, poorly characterized genus that includes a number of other strains known for their ability to tolerate or detoxify various toxic chemicals. To identify candidate genes involved in the bio-transformation of DON by strain 17-2-E-8, as well as to characterize the *Devosia* pan-genome, we generated draft genome sequences of eight *Devosia* isolates and performed comparative genomic analyses. An additional 11 publicly available genomes were also included in the analysis, among which two (DBB001 and A16) have been previously reported to bio-transform DON. None of the eight sequenced isolates, aside from 17-2-E-8, were able to epimerize DON, as determined by LC-MS/MS. The total number of predicted genes per genome ranged from 3539[A1] to 5927, which clustered into 9637 orthologous groups and 10854 singletons, as predicted by Orthagogue. The core and pan genome sizes as estimated by PanGP were ~1629 and 20491 genes, respectively. Comparison of 17-2-E-8 with DB001 and A16 identified 307 and 12 conserved genes, respectively, that were not present in any of the non-detoxifying strains, several of which had predicted functions related to the epimerization of DON. Further studies are underway to confirm the function of the identified genes and optimize them for commercial usage, which will both help the feed/food industry and allow for agricultural and industrial applications to mitigate *Fusarium* toxin contamination.

Author Disclosure Block:

D. Lepp, None..
Y. I. Hassan, None..
T. Zhou, None.

Poster Board Number:

LB-009

Publishing Title:

Non-biased Micro RNA Purification from Clinical and Environmental Samples

Author Block:

S. Forman, D. Cabaya; Zymo Res., Irvine, CA

Abstract Body:

Organic extraction, with TRIzol, TRI Reagent or similar, is often a method of choice for pathogen inactivation and sample stabilization and thus plays a significant role in clinical and environmental sample processing prior to nucleic acid analysis. However, the organic extraction methods were shown to introduce significant bias, specifically into the small RNA (miRNA) recovery. We have developed a non-biased method that allows for binding nucleic acids, including miRNAs, directly, i.e., without phase separation or precipitation, following the organic extraction step. We have compared the miRNA profiles obtained through next generation sequencing as well as a hybridization approach with an existing non-biased "double extraction" protocol (Ambion) and confirmed the non-biased purification results. This novel "direct nucleic acid binding" approach is fully automatable and allows the use of organic extraction in high-throughput applications.

Author Disclosure Block:

S. Forman, None..
D. Cabaya, None.

Poster Board Number:

LB-010

Publishing Title:

Multi-Drug Resistant Bacteria in Wound Infections and Tuberculosis Surveillance in a Philippine Tertiary Hospital

Author Block:

J. Velasco[1], M. Valderama[1], K. Nogrado[1], D. Chua Jr.[2], M. Lopez[3], L. Macareo[1], B. Swierczewski[1]; [1]USAMD-AFRIMS, Bangkok, Thailand, [2]V Luna Gen. Hosp., Quezon City, Philippines, [3]AFPMSS, Quezon City, Philippines

Abstract Body:

Background: Antimicrobial resistance is a serious public health threat. Military populations are especially susceptible to wound bacterial infections and diseases which can be spread in close quarters.
Methods and Results: From Aug 2013 - Dec 2015, 1,516 bacterial isolates from patients at a tertiary military hospital underwent drug susceptibility testing using the Microscan Walkaway 40 Plus System. Of 159 *Klebsiella spp.* isolates, 28.3% [95%CI: 21.6-36.1] were imipenem resistant, 28.9% [95% CI: 22.1-36.7] were meropenem resistant and 102 (64.2%) were identified as suspected extended spectrum beta lactamase producers. Of 146 *Escherichia coli* isolates, 2.7% [95% CI: 0.9-7.2] were imipenem resistant, 2.1% [95% CI: 0.6-6.4] were meropenem resistant, 54 (37.0%) were identified as suspected ESBL producers. Of 76 *Acinetobacter spp*. isolates, 30 (39.5%) were non-susceptible to all antibiotics in the negative breakpoint combo 30 or 34 panels. Of 154 *P. aeruginosa* isolates, 30 (19.5%) were non-susceptible to all antibiotics in the NBPC 30 or 34 panels. Of 135 *S. aureus* isolates, 70.4% [95% CI: 61.8-77.8] were identified as methicillin resistant. To further characterize drug susceptibility patterns, analysis was narrowed down to important drug resistance from 454 wound isolates. Testing for carbapenem resistant genes is ongoing. Multidrug resistance tuberculosis testing was performed (Mar 2015-Feb 2016) on 81 sputum samples using the GeneXpert Cepheid PCR with 18/81 (22%) positive for *M. tuberculosis* and rifampicin resistance detected on 4/18 (22%) *M. tuberculosis* positive samples.
Conclusions: This study describes important MDR bacteria in wound infections and provides data on MDRTB in a special population. Strengthening of MDR microbial pathogen surveillance is needed to better characterize circulating drug resistance patterns and to improve early detection and containment strategies.

Author Disclosure Block:

J. Velasco, None..
M. Valderama, None..
K. Nogrado, None..
D. Chua Jr., None..

M. Lopez, None..
L. Macareo, None..
B. Swierczewski, None.

Poster Board Number:

LB-011

Publishing Title:

Reassessment of Penicillin Resistance Breakpoints for *Bacillus anthracis*

Author Block:

D. Sue, J. Bugrysheva, A. J. Carter, C. Lascols, W. A. Bower, S. V. Shadomy, S. K. Pillai, **L. M. Weigel**; CDC, Atlanta, GA

Abstract Body:

Bacillus anthracis, the etiologic agent of anthrax, is designated as a Tier 1 select agent based on the risk assessment of this organism as a biothreat agent. Amoxicillin (AMX) and penicillin (PEN) are recommended for treatment or post-exposure prophylaxis in patients who cannot take the first-line drugs ciprofloxacin or doxycycline. AMX and PEN are recommended as alternatives for children if the organism is documented to be susceptible to PEN. Penicillin is the first choice for treatment of naturally-occurring anthrax in many parts of the world where the disease commonly occurs. *B. anthracis* isolates that are clinically resistant to PEN are rarely reported, although the organism has two β-lactamase genes on the chromosome. We observed numerous strains of *B. anthracis* for which the MIC of PEN crossed the current breakpoint for resistance (≥0.25 µg/ml), but did not approach the MIC of clinically resistant strains (≥256 µg/ml). We performed broth microdilution susceptibility testing following CLSI guidelines for 52 strains of *B. anthracis*, 4 of which had an MIC of PEN ≥256 µg/ml, to determine the frequency of PEN MICs that cross the CLSI breakpoint. MICs were determined on three consecutive test days for PEN, AMX, AMX-clavulanate, ampicillin (AMP), and AMP-sulbactam. For 26 strains (50%), the MIC of PEN was at or just above the current breakpoint (0.12 or 0.25 µg/ml) on one or more days of testing. For 9 strains (19%), the interpretation as PEN-susceptible or -resistant changed on successive test days. The MIC of AMX ranged from ≤0.03 to 0.12 µg/ml without the variability noted for PEN MICs. These data have been submitted to CLSI with a request to reassess the PEN breakpoint and to set a breakpoint for AMX. A new breakpoint of ≥1 µg/ml PEN for resistance will better represent the natural population of PEN-susceptible *B. anthracis and* eliminate inaccurate reports of PEN or AMX resistance.

Author Disclosure Block:

D. Sue, None..
J. Bugrysheva, None..
A. J. Carter, None..
C. Lascols, None..
W. A. Bower, None..
S. V. Shadomy, None..

S. K. Pillai, None..
L. M. Weigel, None.

Poster Board Number:

LB-012

Publishing Title:

Suboptimal Performance of Algorithm Diagnosis of *C. difficile* Infection in Children

Author Block:

E. J. Gomez[1], K. Alby[2], D. P. Robinson[1], S. Montgomery[1], S. S. Roundtree[1], D. L. Blecker-Shelly[1], **K. V. Sullivan**[1]; [1]The Children's Hosp. of Philadelphia, Philadelphia, PA, [2]Hosp. of the Univ. of Pennsylvania, Philadelphia, PA

Abstract Body:

Background: The "algorithm" approach to *C. difficile* infection (CDI) diagnosis is widely used but performance in children has not been rigorously investigated. We compared the performance of algorithmic diagnosis against 4 commercial assays in a tertiary pediatric population. **Methods:** Stool samples submitted for CDI testing in children between 1-18 years of age were tested prospectively at The Children's Hospital of Philadelphia. Duplicate samples within 30 days were excluded. Samples were tested with: an algorithm (C. diff Quik Chek Complete (QCC) reflexed to *illumi*gene *C. difficile*) (ALG); the AmpliVue C. difficile Assay (ACD); the Lyra Direct *C. difficile* Assay (LCD); the BD MAX Cdiff (BDM); and the Xpert *C. difficile* assay (XCD). Sensitivity (SN), specificity (SP), positive predictive value (PPV), and negative predictive value (NPV) were calculated. The gold standard positive was defined as positivity by two tests with different manufacturers. Samples that were positive by one or more tests underwent toxigenic culture. **Results:** 211 non-duplicate samples were analyzed. 41 tested positive by at least one method, 32 by two methods, with a prevalence of 15%. SN, SP, PPV, and NPV were 87.5%, 98.9%, 93.3%, 97.8% for ALG; 21.9%, 98.9%, 77.8%, 87.6% for QCC's toxin A/B component; 96.9%, 98.9%, 93.9%, 99.4% for ACD; 90.6%, 98.9%, 93.5%, 98.3% for LCD; 96.8%, 99.4%, 96.8%, 99.4% for BDM, and 96.9%, 98.9%, 93.9% and 99.4% for XCD. Nine samples were positive by one test only (2 ALG, 2 ACD, 2 LCD, 1 BDM, 2 XCD). 8/9 tested negative by toxigenic culture as did 11/32 (34.3%) of the gold standard positives. **Conclusion:** GDH-based algorithms may detect CDI with suboptimal sensitivity compared to molecular methods in children. QCC's toxin A/B component had false positive results and a sensitivity of 22%. This necessitated reflex testing and delay in reporting in 70% of CDI cases detected with this method. Comparatively, the 4 molecular assays investigated performed with superior (though similar) sensitivity and specificity.

Author Disclosure Block:

E. J. Gomez, None..
K. Alby, None..
D. P. Robinson, None..
S. Montgomery, None..

S. S. Roundtree, None..
D. L. Blecker-Shelly, None..
K. V. Sullivan, None.

Poster Board Number:

LB-013

Publishing Title:

Comparison of TaqMan Array Card and MYCOTB with Conventional Phenotypic Susceptibility Testing in MDR-TB

Author Block:

S. Foongladda[1], S. banu[2], S. Pholwat[3], J. Gratz[3], S. O-Thong[1], N. Nakkerd[1], R. Chinli[1], S. Ferdous[2], S. Rahman[2], A. Rahman[2], S. Ahmed[2], S. Heysell[3], M. Sariko[4], G. Kibiki[4], **E. R. Houpt**[3]; [1]Mahidol Univ., Bangkok, Thailand, [2]Intl. Ctr. for Diarrhoeal Diseases and Res., Bangladesh, Dhaka, Bangladesh, [3]Univ. of Virginia, Charlottesville, VA, [4]Kilimanjaro Clinical Res. Inst., Moshi, Tanzania, United Republic of

Abstract Body:

Background: Phenotypic drug susceptibility testing is endorsed as the standard for second-line drug testing of *Mycobacterium tuberculosis* however it is slow and laborious. **Methods:** We evaluated the accuracy of two faster and easier methodologies that provide results for multiple drugs: a genotypic TaqMan Array Card and the Sensititre MYCOTB plate. Both methods were tested at three central laboratories in Bangladesh, Tanzania, and Thailand with 212 MDR-TB isolates and compared with the laboratories' phenotypic method in use. **Results:** The overall accuracy for ethambutol, streptomycin, amikacin, kanamycin, ofloxacin, and moxifloxacin versus the phenotypic standard was 87% for TAC (range 70-99%) and 88% for the MYCOTB plate (range 76-98%). To adjudicate discordances we re-defined the standard as the consensus of the three methods, against which the TAC and MYCOTB plate yielded 94-95% accuracy while the phenotypic result yielded 93%. There were isolates with genotypic mutations and high MIC that were phenotypically susceptible, and isolates without mutations and low MIC that were phenotypically resistant, questioning the phenotypic standard. **Conclusions:** In our view the TAC, MYCOTB plate, and the conventional phenotypic method perform similarly for second line drugs, however the former methods offer speed, throughput, and quantitative susceptibility information.

Author Disclosure Block:

S. Foongladda, None..
S. banu, None..
S. Pholwat, None..
J. Gratz, None..
S. O-Thong, None..
N. Nakkerd, None..
R. Chinli, None..
S. Ferdous, None..

S. Rahman, None..
A. Rahman, None..
S. Ahmed, None..
S. Heysell, None..
M. Sariko, None..
G. Kibiki, None..
E. R. Houpt, None.

Poster Board Number:

LB-014

Publishing Title:

Coexistence of Antibodies to Severe Acute Respiratory Syndrome Coronavirus and Bat Alphacoronavirus in the same *Rhinolophus* Bats

Author Block:

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Abstract Body:

Background Horseshoe (*Rhinolophus*) bats have been identified as natural reservoirs of severe acute respiratory syndrome coronavirus (SARS CoV) affecting about 8000 people worldwide during 2002 to 2003. In Taiwan, SARS CoV (Betacoronavirus) and bat Alphacoronavirus (*Scotophilus* bat CoV 512) were detected in the feces of Formosan lesser horseshoe bats (*Rhinolophus monoceros*). While genomic analysis has proved coinfection of different CoV in the same bats, no serological evidences were documented due to the difficulties to collect serum from insectivore bats. To understand whether the same *Rhinolophus* bats can be infected with both SARS CoV and bat Alphacoronavirus, recombinant nucleocapsid (N) proteins of both CoVs were used by western blot (WB) analysis to detect antibodies to both CoVs. **Methods** Previous studies showed that full-length N protein with a highly conserved motif among all known CoVs could have cross reactivity, so the carboxyl terminal fragment of N protein (N3) without the motif was used for detecting CoV species-specific antibody. Total 63 serum specimens were collected from 50 female and 13 male *Rhinolophus* bats in Taiwan during 2014 to 2015 for WB analysis with the N3 fragments from SARS CoV Tor2 strain and *Scotophilus* bat CoV 512/CYCU-S1/TW/2013. Recombinant N3 fragments with His-tag were expressed by *Escherichia coli* BL21 and purified by NTA agarose resin. **Results** Out of 63 serum samples, 22 were positive to SARS CoV (35%), 27 were positive to *Scotophilus* bat CoV (43%), and 16 were positive to both CoVs (25%). Females had higher detection rate of CoV-specific antibodies. Only 2 out of the same 63 bats had feces tested positive by RT-PCR targeting RNA-dependent RNA polymerase (RdRp) gene and were tested positive for antibodies to SARS CoV. Serological detection had higher sensitivity than genomic method. **Conclusion** It is the first report describing coexistence of antibodies to SARS CoV and bat Alphacoronavirus in the same *Rhinolophus* bats. The findings suggested that both CoVs not only coexist in the same bat population but also have ability to infect the same individuals. It should be noted for public health due to the potential recombination between human and bat CoVs.

Author Disclosure Block:

Y. Chen, None..
B. Su, None..

H. Chen, None..
H. Cheng, None.

Poster Board Number:

LB-015

Publishing Title:

Diagnosis of *Capnocytophaga canimorsus* Sepsis by a Novel Cell-free DNA Sequencing Assay

Author Block:

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Abstract Body:

Background: Sepsis is an increasingly common condition in hospitalized patients and is associated with a high mortality rate. In up to 40% of cases of sepsis, a causative organism is never identified. There is a need for improved, sensitive diagnostics to inform targeted therapy in patients with sepsis. Given its extreme sensitivity and open-ended nature, next-generation sequencing (NGS) has great potential in filling this need. We report a case of septic shock due to *Capnocytophaga canimorsus* that was diagnosed by a novel plasma-based NGS assay. **Methods:** We developed a minimally invasive NGS-based infectious disease assay that takes advantage of circulating cell-free plasma DNA originating from invasive pathogens. After filtering human sequences, remaining sequences are aligned to a comprehensive pathogen sequence database and abundance is computed for detected pathogens. **Results:** A 60 year-old male with history of asplenia was admitted with septic shock. Initial blood cultures were negative and the patient was treated with multiple antibiotics including coverage for typical and atypical bacterial pathogens, such as rickettsia, and herpesviruses. Due to the uncertainty of the etiology of this illness, a novel NGS-based plasma assay was run which identified *C. canimorsus* within 24 hours. These results were returned in a clinically actionable timeframe resulting in narrowing of treatment for the patient and were subsequently confirmed many days later by 16s sequencing of a positive blood culture bottle. **Conclusions:** We report the first use of an open-ended cell-free DNA NGS assay to identify the causal pathogen in a patient with sepsis in a clinically actionable timeframe. This technology has the potential to improve targeted therapy for patients with sepsis of unclear etiology.

Author Disclosure Block:

A. Strand, None.
D. K. Hong,
Karius, Inc. Role(s): Self, D. Employee.
V. G. Fowler, Jr.,
Pfizer Role(s): Self, C. Consultant.
Novartis Role(s): Self, C. Consultant.
Galderma Role(s): Self, C. Consultant.

Novadigm Role(s): Self, C. Consultant.
Durata Role(s): Self, C. Consultant.
Debiopharm Role(s): Self, C. Consultant.
Genentech Role(s): Self, C. Consultant.
Achaogen Role(s): Self, C. Consultant.
Affinium Role(s): Self, C. Consultant.
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Cerexa Role(s): Self, C. Consultant.
Tetraphase Role(s): Self, C. Consultant.
Trius Role(s): Self, C. Consultant.
MedImmune Role(s): Self, C. Consultant.
Bayer Role(s): Self, C. Consultant.
Theravance Role(s): Self, C. Consultant.
Cubist Role(s): Self, C. Consultant.
Basilea Role(s): Self, C. Consultant.
Affinergy Role(s): Self, C. Consultant.
Merck Role(s): Self, J. Scientific Advisor (Review Panel or Advisory Committee).
Green Cross Role(s): Self, L. Speaker's Bureau.
Cubist Role(s): Self, L. Speaker's Bureau.
Cerexa Role(s): Self, L. Speaker's Bureau.
Durata Role(s): Self, L. Speaker's Bureau.
Theravance Role(s): Self, L. Speaker's Bureau.
B. D. Kraft, None.

Poster Board Number:

LB-016

Publishing Title:

Evaluation of HardyCHROM™ ESBL, a Chromogenic Medium Designed to Screen for Extended-Spectrum-Beta-Lactamase Producing *E. coli, K. pneumoniae, K. oxytoca* and *P. mirabilis* and Recovery of *Enterobacteriaceae* with Reduced Susceptibility to Broad Spectrum Cephalosporins

Author Block:

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Abstract Body:

Background: HardyCHROM™ ESBL is a chromogenic medium designed to screen for ESBL (Extended-Spectrum-Beta-Lactamase) producing *E. coli* (EC), *K. pneumoniae* (KP), *K. oxytoca* (KO) and *P. mirabilis* (PM) from fecal specimens. The medium can also be used for the recovery of *Enterobacteriaceae* with reduced susceptibility to broad spectrum cephalosporins (BSC). In this study, this medium was compared to traditional culture methods utilizing parallel TSB enrichments with 1µg/mL ceftazidime and with 1µg/mL cefotaxime followed by subculture to MacConkey. The results of the reference method were then compared with results from HardyCHROM™ ESBL read at 18 and 24 hours. **Methods:** A total of 1683 fecal specimens were tested on HardyCHROM™ ESBL in parallel with the TSB enrichments. Overall, 242 ESBL-producing EC, KP, KO, and PM recovered from 214 specimens. HardyCHROM™ ESBL was 97.66% sensitive for the screening of ESBL-producing EC, KP, KO, and PM and 83.05% specific after 18 hours of incubation. The reduced specificity was due to the growth of microorganisms resistant to BSC that did not display ESBL phenotype and presented profiles suggestive of AmpC or KPC production. HardyCHROM™ ESBL was 94.69% sensitive and 99.01% specific for the recovery of *Enterobacteriaceae* which were non-susceptible to BSC regardless of ESBL phonotype or not. **Results:** ESBL-producing EC, KP, KO, and PM were recovered from 4.7% of specimens from WI, 5.8% of specimens from NC, and 22.3% of specimens from NY. A total of 572 organisms showed reduced susceptibility to BSC recovered in this study. Of those, 138 also showed reduced susceptibility to carbapenems. These organisms with reduced susceptibility to carbapenems were recovered from 8.7% of specimens from NC, 2.0% of specimens from WI, and 13.3% of specimens from NY. **Conclusions:** Overall, HardyCHROM™ ESBL is reliable for the selective screening of ESBL-producing microorganisms in fecal specimens, as well as for the confirmation of the presence of *Enterobacteriaceae* with reduced susceptibility to BSC.

Author Disclosure Block:

N. Ledeboer, None..
E. Palavecino, None..
R. Myslymi, None..
M. Turner, None..
M. Blevins, None..
M. Faron, None..
J. Connolly, None..
S. Whittier, None.

Poster Board Number:

LB-017

Publishing Title:

Characterization of Multi-drug Resistant *Enterococcus faecalis* Isolated from Research Macaques

Author Block:

M. T. Lieberman[1], S. E. Woods[1], F. Lebreton[2], J. L. Dzink-Fox[1], M. S. Gilmore[2], J. G. Fox[1]; [1]MIT, Cambridge, MA, [2]Harvard Med., Boston, MA

Abstract Body:

Background:
Multi-drug resistant (MDR) *Enterococcus faecalis* is a common and serious cause of nosocomial infections in hospitals. Previous characterization of 14 *E. faecalis* isolates from cephalic recording chambers of research macaques revealed two lineages with marked multi-drug resistance. Lineage A (LA) isolates (n=7) showed differing susceptibilities to gentamicin, with 4/7 isolates displaying high-level gentamicin resistance while Lineage B (LB) isolates (n=7) displayed resistance to neomycin. All isolates displayed marked streptomycin resistance. Resistance to tetracycline, chloramphenicol, erythromycin and trimethoprim-sulfamethoxazole was also noted.
Methods: DNA was extracted from 2 LA and 1 LB isolate and sequenced on a single SMRT cell on a Pacific Biosciences RS2 sequencer. Genomes were assembled and FASTA sequences were analyzed by PubMLST, ResFinder and VirulenceFinder to confirm sequence type (ST) and identify genes of interest.
Results: Two isolates from LA were confirmed to be ST4 and the isolate from LB was confirmed to be ST55. All isolates had a unique antimicrobial resistance gene profile with the *lsa(A)* gene encoding intrinsic resistance to lincosamides and streptogramins A as the only common gene between all 3 isolates. Additional macrolide resistance encoded by *erm(B)* was identified in 2 isolates. Four genes encoding aminoglycoside resistance were identified: *str* and 3 aminoglycoside-modifying enzymes: *aph(3')-III, aac(6')-aph(2")* and *ant(6)-Ia*. Genes encoding tetracycline resistance included mechanisms for efflux pumps (*tetL*) and ribosomal protection (*tetS* and *tetM*). Phenicol resistance was conferred by the *cat* gene and was present in ST4 isolates, but not the ST55 isolate. Trimethoprim resistance encoded by *dfrG* was noted in 1 ST4 isolate. Other virulence factors identified included enterococcal surface protein, aggregation substance, gelatinase, collagen adhesion precursor and endocarditis antigen.
Conclusions: *E. faecalis* isolates from cephalically-implanted macaques display genetic similarities to isolates associated with human nosocomial infections. Macaques represent a unique research model to study human nosocomial infection due to their long-term residence in a healthcare setting and intermittent antimicrobial exposure.

Author Disclosure Block:

M. T. Lieberman, None..
S. E. Woods, None..
F. Lebreton, None..
J. L. Dzink-Fox, None..
M. S. Gilmore, None..
J. G. Fox, None.

Poster Board Number:

LB-018

Publishing Title:

In Vitro Activity Of Ceftazidime-Avibactam (CAZ-AVI) Against CAZ Resistant Isolates Of *Burkholderia pseudomallei* (*Bp*)

Author Block:

K. M. Papp-Wallace[1], S. Chirakul[2], N. Chantratita[3], **H. P. Schweizer**[2], R. A. Bonomo[1]; [1]Cleveland VAMC, CWRU, Cleveland, OH, [2]U of Florida, Gainesville, FL, [3]Mahidol U, Salaya, Thailand

Abstract Body:

Background: *Bp* is an opportunistic and globally emerging pathogen with biothreat potential that is the causative agent of melioidosis. The oxyimino-cephalosporin, CAZ, is used in the treatment of *Bp* infections, but resistance to CAZ (CAZ^R) has been observed. *Bp* possesses class A and class D β-lactamases, PenA and OXA-42-like, which mediate β-lactam resistance. AVI inhibits most class A, C, and some D β-lactamases. Here, we test the ability of AVI to restore susceptibility to CAZ in *Bp*.
Methods: A panel of clinical isolates of *Bp* was tested by broth microdilution MICs using CAZ and CAZ-AVI according to CLSI guidelines; AVI was maintained at 4 µg/mL. All experiments with virulent *Bp* clinical isolates were performed at BSL-3 in Select Agent certified laboratory facilities and employing compliant standard operating procedures. Select agent excluded *Bp* laboratory strains with defined mutations causing CAZ^R were also tested.
Results: MIC testing reveals that AVI restores CAZ activity in several virulent CAZ resistant clinical isolates as well in laboratory generated CAZ^R strains (Table). Strain 1026b, the prototype Thai clinical isolate, is CAZ susceptible, and is the parent to the attenuated derivative, Bp82. Strain 699d is CAZ^R due to deletion of penicillin binding protein 3 (Δ*pbp3*); AVI does not enhance CAZ activity in this strain. The four other strains are CAZ^R due to mutations in the bla_{PenA} gene or its promoter (P↑↑). The CAZ^R mechanism of strain 5041a is unknown, however AVI decreases the CAZ MIC suggesting it is β-lactamase-mediated. Using a panel of select agent excluded Bp82 mutants expressing various CAZ^R PenA variants were found to be susceptible to the CAZ-AVI combination.
Conclusions: With the exception of the clinical isolate of *Bp* Δ*pbp3*, the results suggest that CAZ-AVI is an effective combination against CAZ^R strains of *Bp*. The CAZ-AVI combination offers an opportunity for the treatment of this difficult-to-treat pathogen with biothreat potential.

MICs in µg/mL for CAZ and CAZ-AVI against clinical and laboratory strains of Bp

Strain	CAZ	CAZ-AVI	Resistance Mechanism
Clinical:1026b	2	1	None
699d	128	64	Δ*pbp3*

5041a	32	1	unknown
Bp1651	256	2	P↑↑ *penA*$_{S72F\&D240G}$
490f	>256	2	*penA*$_{A172T}$
577ci	>256	2	P↑↑ *penA*$_{C69Y\&S72F}$
979bii	>256	8	P↑↑ *penA*$_{P167S}$
Laboratory:Bp82	2	1	None
Bp82	16	1	P↑↑ *penA*
Bp82	64	1	*penA*$_{C69Y}$
Bp82	16	1	*penA*$_{P167S}$
Bp82	8-16	1	*penA*$_{D240G}$

Author Disclosure Block:

K. M. Papp-Wallace,
AstraZeneca Role(s): Self, H. Research Contractor.
Actavis Role(s): Self, H. Research Contractor.
Merck Role(s): Self, H. Research Contractor.
Wockhardt Role(s): Self, H. Research Contractor.
S. Chirakul, None..
N. Chantratita, None.
H. P. Schweizer,
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Merck Role(s): Self, H. Research Contractor.
Wockhardt Role(s): Self, H. Research Contractor.
GSK Role(s): Self, H. Research Contractor.

Poster Board Number:

LB-019

Publishing Title:

AmpR Effectors in *Pseudomonas aeruginosa* Regulating β-lactam Resistance

Author Block:

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Abstract Body:

Pseudomonas aeruginosa is an opportunistic pathogen infamous for causing a wide range of infections in immunocompromised, burn wound and cystic fibrosis patients among others. Extensive resistance to antibiotics confounds its treatment. Resistance to β-lactams that is often used to treat *Pseudomonas* infection is conferred by expression of hydrolytic β-lactamases. The predominant β-lactamase AmpC in *P. aeruginosa* is regulated by AmpR, a LysR-type transcriptional regulator. Activation of AmpR in Enterobacteriaceae is shown to be mediated by signaling molecules generated during cell wall recycling known as muropeptides. The identity of this muropeptide in *P. aeruginosa* is yet to be elucidated. The goal of this study was to identify *P. aeruginosa* AmpR ligand. The objectives were to identify and quantify *P. aeruginosa* muropeptides in the absence and presence of induction using cefoxitin, a strong β-lactamase inducer through liquid chromatography/mass spectrometry (LC/MS). To address this, *ampG* and *ampP* deletion mutants in PAO1 encoding permeases involved in transport of muropeptides into the cytoplasm and periplasm, respectively were constructed. Whole cell LC/MS analyses of PAO1 and the mutant strains led to the identification of 20 muropeptides as compared to the parent PAO1. The total muropeptides decreased in the cell wall recycling mutants. In addition there was also a decrease in the levels of total muropeptides upon induction. The most abundant muropeptide in all the samples were *N*-acetyl glucosamine anhydro-*N*-acetylmuramic acid tetrapeptide. The muropeptide that was detected only upon induction *N*-acetylglucosamine anhydro *N*-acetylmuramic acid (NAG-anhNAM) pentapeptide (L-Ala-γ-D-Glu-m-DAP-D-Ala-D-Ala) was. This finding suggests that NAG-anhNAM pentapeptide may be the ligand for AmpR leading to *ampC* expression. Identifying the AmpR ligand would enable us to synthesize its analogs and disrupt AmpR function impairing *P. aeruginosa* virulence.

Author Disclosure Block:

S. Dhar, None..
M. Lee, None..
D. Hesek, None..
H. Kumari, None..
S. Mobashery, None..
K. Mathee, None.

Poster Board Number:

LB-020

Publishing Title:

Innovative Use of the Tetracyclines to Activate Bacterial Suicide Genes and Combat Antibiotic Resistance Mechanisms

Author Block:

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Abstract Body:

The tetracyclines are a broad spectrum group of antibiotics whose antibacterial efficacy has been greatly reduced due to the development of resistance. They have been used for decades as antimicrobials (antibacterial, antiviral and antiprotozooan) and anti-inflammatory. The established 16S rRNA binding mechanism for the antibacterial action does not explain their wide range of activity. We explored possible target sites that could explain their mechanism of action and wide range of activity. This would help improve effective use, provide opportunities to discover new therapeutic usefulness of the drugs and offer insights on how to combat drug resistance. Our recent studies using biophysical and molecular techniques indicate that the tetracyclines bind dsRNA, inhibit processing and function of cellular RNAs under certain conditions. The *hok*/*sok* locus is a well established toxin/antitoxin plasmid stability element, which has been associated with multi-drug (ampicillin, chloramphenicol, kanamycin, streptomycin and sulphonamides) resistance plasmids. It is one of the most frequently reported plasmid addiction systems in ESBL-encoding plasmids, and ensures plasmid maintenance by post-segregational killing of plasmid-free daughter cells via RN:RNA interactions. The *hok*/*sok* locus also occurs in the chromosomes of enterobacteria, and is more abundant in pathogenic strains. We investigated the effect of the *hok*/*sok* locus on bacterial pathogenicity and stress response using *E. coli* growing in various stressful conditions. Our studies showed that the *hok*/*sok* locus enhances host cell survival in conditions such as high temperature, antibiotic stress and low cell concentrations. Interestingly, we also observed that although the *hok*/*sok* locus increases bacterial tolerance to β-lactam antibiotics, it increases the susceptibility of these bacteria to doxycycline by inducing the self-killing plasmid maintenance system due to the interference of the tetracyclines with the RNA:RNA processing pathway. Since the *hok*/*sok* locus ensures the transmission of plasmids containing multi-drug resistance genes, these results may represent a drug target that will open up opportunities for the innovative use of tetracyclines in the global war to contain the rise of antimicrobial resistance.

Author Disclosure Block:

C. chukwudi, None..
L. good, None.

Poster Board Number:

LB-021

Publishing Title:

A Quinolone-type Compound is Showing Surprising Activity as an Efflux Pump Inhibitor on the RND-type Efflux Pump, AdeABC in *Acinetobacter baumannii*

Author Block:

Y. Zhu[1], J. L. Auer[1], S. Jamshidi[1], J. M. Sutton[2], K. M. Rahman[1]; [1]King's Coll. London, london, United Kingdom, [2]Publ. Hlth.England, Salisbury, United Kingdom

Abstract Body:

Introduction: Multidrug-resistant (MDR) clinical pathogens overexpress a series of efflux pumps, which effectively exclude or reduce the intracellular concentration of many antibiotics, making the pathogens significantly more resistant. These efflux pumps are the topic of considerable interest as targets for novel adjunct therapies. We have designed and synthesized a series of quinolone based compounds that have shown notable activity as efflux pump inhibitors (EPI) compared to PAβN which is widely regarded as a potent EPI. **Methods**: The quinolone type EPI compound IZ-75-52 and its analogues were synthesized using solution phase chemistry. All synthesized compounds were fully characterized using NMR and Mass spectroscopic techniques. The activity of the synthesized compounds were tested for their ability to inhibit the AdeABC efflux pump of *A. baumannii* using different types of fluorescence accumulation tests including Hoechst assays. A novel assay, based on the enzymatic-activation of a fluorescent probe by a bacterial enzyme, was also used to evaluate the efflux inhibition. GOLD molecular docking and MD simulations with AMBER were carried to understand the molecular level interaction between IZ-75-52 and the AdeB efflux pump. **Results**: IZ-75-52 showed strong inhibitory activity against AdeABC efflux pump of *A.baumannii* in fluorescent accumulation tests. In Hoechst assay, 25µg/mL of IZ-75-52 showed similar levels of inhibition activity to PaβN and this was exceeded at 50µg/mL. Evaluation of IZ-75-52 using the enzyme-activated fluorescent probe-based efflux assay demonstrated similar levels of efflux inhibition to that seen with PAβN. Computational analysis showed IZ-75-52 binds to the multisite binding pocket of AdeB and the Phe-loop located between the proximal and distal binding pocket plays a key role in the IZ-75-52 mediated inhibition. **Conclusion:** The level of EPI activity observed by IZ-75-52 is surprising due to its relatively low molecular weight (MW 349.3). The scaffold is amenable to medicinal chemistry modifications and the novel assay format together with information obtained from advanced *in silico* techniques will be used to design new EPIs based on IZ-75-52 with a view to develop a pre-clinical candidate.

Author Disclosure Block:

Y. Zhu, None..
J. L. Auer, None..

S. Jamshidi, None..
J. M. Sutton, None..
K. M. Rahman, None.

Poster Board Number:

LB-022

Publishing Title:

Mesenchymal Stem Cells Improves Survival in a Mouse Model of Severe Pneumococcal Pneumonia

Author Block:

C. A. Hinojosa[1], L. Reyes[1], D. A. Hunt[2], A. Rodriguez[3], R. Johnson[2], N. Soni[1], A. Anzueto[1], J. Peters[1], M. Moyer[2], C. J. Orihuela[1], X-D. Chen[1], M. Restrepo[4]; [1]Univ. of Texas Hlth.Sci. Ctr. San Antonio (UTHSCSA), San Antonio, TX, [2]Incell Corp. LLC, San Antonio, TX, [3]Hosp. Joan XXIII, Tarragona, Spain, [4]South Texas Veterans Hlth.Care System (STVHCS), San Antonio, TX

Abstract Body:

Background/Purpose: Community acquired pneumonia (CAP) is the leading cause of morbidity, and mortality worldwide. *Streptococcus pneumoniae* is the principal etiologic agent causing CAP. There is a need to identify novel therapies that can further improve the morbidity and mortality in patients with CAP. Novel therapies with Mesenchymal Stem Cells (MSCs) may potentially advance the science by modulating the often excessive host-immune response and enhancing tissue repair. This study assessed the survival effect of two different MSCs, one derived from the adipose tissue (Ad-MSCs) compared to the one derived from the bone marrow (BM-MSCs) in a murine pneumococcal pneumonia model.
Methods: Mice were infected intranasally with 10^7 CFU of *S. pneumoniae.* After 24 hours, mice were randomly assigned to three groups: 1) pre-labeled Ad-MSCs (10^6, n=7); 2) pre-labeled BM-MSCs (10^6, n=6); and 3) control (n=5-8). The primary outcome was survival assessed from the time of inoculation. Secondary outcomes include bacterial burden and time to develop bacteremia, concentration of the cells in the lung parenchyma.
Results: Bacterial burden and pathological analysis uncovered no significant differences between groups. Pre-labeled MSCs were visualized in the lungs. At 60 hours post-inoculation the Ad-MSCs had a survival rate of 43% compared to 0% in the control group (p=0.08). At 120 hours the BM-MS had a survival rate of 80% vs. 0% in the control group (p=0.03).
Conclusions: We observed a beneficial overall survival effect of intratracheal administration of MSCs whether derived from the adipose or the bone marrow tissue in a murine model of pneumococcal pneumonia. Further translational studies should assess the mechanisms, safety and time of administration of MSCs in invasive pneumococcal disease.

Author Disclosure Block:

C. A. Hinojosa, None..
L. Reyes, None..
D. A. Hunt, None..
A. Rodriguez, None..

R. Johnson, None..
N. Soni, None..
A. Anzueto, None..
J. Peters, None..
M. Moyer, None..
C. J. Orihuela, None..
X. Chen, None..
M. Restrepo, None.

Poster Board Number:

LB-023

Publishing Title:

Lfnp24 As A Therapeutic Vaccine Candidate: A Phase 1 Randomized, Double Blind, Placebo Controlled Safety And Immunogenicity Study In Hiv-1 Infected Chinese Subjects

Author Block:

Y. Lu[1], T. Zhang[2], W. Xia[2], B. Su[2], R. Wang[2], Y. Ji[2], X. Lu[2], B. Lu[1], Y. Liu[1], S. Yao[1], S. Li[1], H. Peng[3], Y. Ou[3], N. Touzjian[4], N. Kushner[4], A. Strong[4], H. Wu[2]; [1]Haikou VTI Biological Inst., Haikou, China, [2]Beijing You'an Hosp., Beijing, China, [3]Co-CRO Med. Dev. Co., Ltd, Beijing, China, [4]Vaccine Technologies Inc, Wellesley, MA

Abstract Body:

Background: Detoxified anthrax lethal factor fused to HIV-1 p24 of the B/C subtype (LFn-p24B/C) as a therapeutic vaccine can complement the antiretroviral therapy in boosting the host immune system. We report data from the randomized, double-blind, placebo controlled, phase 1 clinical trial of LFn-p24B/C in China**.**
Methods: HIV-1 infected patients with $CD4^+$ T cell counts >350 and viral loads <50 under stable ART were recruited. 32 eligible subjects were randomized into two groups (vaccine:placebo, 3:1): 16 received 300ug of vaccine or placebo and 16 received 450ug of vaccine or placebo im at 0, 4 and 12 weeks. Safety and immunogenicity (ELISA, $CD4^+$ counts, IFN-γ ELISPOT and ICS, as measured by IFN-γ expression) were evaluated up to 48 weeks post first immunization.
Results: Most adverse events were mild or moderate, and no SAEs were reported. All subjects maintained undetectable plasma viral levels throughout the trial. Higher anti-p24 titers were observed in 21/24 vaccinees compared to placebos. $CD4^+$T cell counts increased significantly in vaccinees with durable responses up to 36 weeks following first immunization (p=0.003,p=0.009 and p=0.034 at 2,14 and 36 weeks respectively). No significant change was observed in placebo (p=0.98,p=0.54 and p=0.84 respectively at the same time points). Dose-related gag specific T cell response by IFN-γ ELISPOT assay was observed after three immunizations of LFn-p24B/C (81.8%,450ug and 50%, 300 ug, 25% placebo) Parallel ICS assay demonstrated higher gag-specific $CD8^+$ and $CD4^+$ responses in vaccinees compared to placebo (29.2% for both, p=0.009 and p=0.048 respectively). Vaccinees had decreased expression of HLA-DR on $CD4^+$T cells (-10% p=0.0005) and CD38 on $CD8^+$T cells (-11.5%, p=0.0016) and increase CD38 expression on $CD4^+$T cells (+7.37%, p=0.004). No such observations were found in placebos.
Conclusions: LFn-p24 immunization in virologically suppressed, HIV-1 infected subjects led to increased CD4+T cells, induction of HIV-1 specific response and modulation of immune activation.

Author Disclosure Block:

Y. Lu,
Vaccine Technologies Inc; Matrivax Inc Role(s): Self, A. Board Member.
Vaccine Technologies Inc Role(s): Self, D. Employee.
Vaccine Technologies Inc; Matrivax Inc Role(s): Self, K. Shareholder (excluding diversitied mutual funds).
T. Zhang, None..
W. Xia, None..
B. Su, None..
R. Wang, None..
Y. Ji, None..
X. Lu, None.
B. Lu,
Haikou VTI Biological Institute, Ltd Role(s): Self, D. Employee.
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S. Li,
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Vaccine Technologies Inc Role(s): Self, D. Employee.
A. Strong,
Vaccine Technologies Inc Role(s): Self, D. Employee.
H. Wu, None.

Poster Board Number:

LB-024

Publishing Title:

ETX2514, a Novel, Rationally Designed Inhibitor of Class A, C and D β-lactamases, for the Treatment of Gram-negative Infections

Author Block:

A. B. Shapiro[1], S. Guler[1], N. Carter[1], M. McLeod[1], B. De Jonge[2], R. McLaughlin[2], H. Huynh[2], N. Gao[2], T. Durand-Reville[1], A. Miller[1], J. Mueller[1], R. Tommasi[1]; [1]Entasis Therapeutics, Waltham, MA, [2]AstraZeneca, Waltham, MA

Abstract Body:

Introduction: A rationally designed series of novel diazabicyclooctenone β-lactamase inhibitors led to the identification of ETX2514 which has Class A, C and broad Class D activity. We tested ETX2514 against eight representative β-lactamases and high-molecular mass PBPs. We evaluated its ability to restore activity to β-lactam (BL) partners against multidrug resistant (MDR) Gram-negative pathogens, with a focus on *A. baumannii* since β-lactamase expression, especially Class D, is a prevalent resistance mechanism in this organism. **Methods:** Rate constants for reaction of ETX2514 with β-lactamases were measured with nitrocefin as substrate. Rate constants for ETX2514 reaction with purified PBPs were measured with a fluorescence polarization BOCILLIN FL competition assay. The *in vitro* antibacterial activity of ETX2514 alone, or of eight BL partners in combination with 4 mg/L of ETX2514 against recent MDR clinical isolates of *Escherichia coli* (n = 202), *Klebsiella pneumoniae* (n = 198), *Pseudomonas aeruginosa* (n = 209) and *A. baumannii* (n = 195) was measured by broth MIC following CLSI guidelines. BL content for select strains was determined by whole genome sequencing. **Results:** ETX2514 reacted with β-lactamases with k_{inact}/K_i of ~10^6 - 10^7 $M^{-1}s^{-1}$ for class A (CTX-M-15 and TEM-1), class C (AmpC and P99) and OXA-48 from class D; and ~10^4 $M^{-1}s^{-1}$ for OXA-10 and OXA-24/40 from class D. ETX2514 reacted with *P. aeruginosa* PBP 1a, 2, and 3 with k_{inact}/K_i of 12, 24, and 57 $M^{-1}s^{-1}$, respectively; with *A. baumannii* PBP 1a, 2, and 3 with k_{inact}/K_i of 180, 1800, and 3 $M^{-1}s^{-1}$, respectively; and with *E. coli* PBP1a, 2, and 3 with k_{inact}/K_i of 120, 17000, and 2, respectively. The MIC_{90} of any BL combined with ETX2514 was $\leq$ 0.12 mg/L against both *K. pneumoniae* and *E. coli.* Imipenem was the most effective BL partner for ETX2514 against *P. aeruginosa* (MIC_{90} of 2 mg/L) while sulbactam was the most potent partner against *A. baumannii* (MIC_{90} = 4 mg/L). **Conclusions:** ETX2514 potently inhibits Class A, C, and a broad spectrum of Class D β-lactamases. Intrinsic antibacterial activity is likely due to PBP2 inhibition. *In vitro* susceptibility testing suggests sulbactam/ETX2514 could be an effective treatment for infections caused by MDR *A. baumannii*.

Author Disclosure Block:

A. B. Shapiro, None..
S. Guler, None..
N. Carter, None..
M. McLeod, None..
B. De Jonge, None..
R. McLaughlin, None..
H. Huynh, None..
N. Gao, None..
T. Durand-Reville, None..
A. Miller, None..
J. Mueller, None..
R. Tommasi, None.

Poster Board Number:

LB-025

Publishing Title:

Weakening the Enemy: Novel Whole Cell Reporter Technology for Natural Product Screening

Author Block:

P. Premnath[1], M. Reck[1], B. Kunze[1], R. Jansen[1], S. Bhuyan[1], K. Wittstein[1], J. Niggemann[1], J. Herrmann[2], R. Müller[2], M. Stadler[1], I. Wagner-Döbler[1]; [1]Helmholtz Ctr. for Infection Res., Braunschweig, Germany, [2]Helmholtz Inst. for Pharmaceutical research, Saarland, Germany

Abstract Body:

The escalating problem of drug resistance calls for the development of novel screens targeting less studied pathogenic mechanisms in a microbe. We have designed a novel whole cell fluorescent screening system, incorporating three virulence mechanisms of *Streptococcus mutans* to find natural products from Myxobacteria and fungi specifically weakening the bug, rather than just killing it. Each screen employs a reporter strain where, a *gfp+* fluorophore preceded by the promoter of the target gene, is integrated into the native locus of the bacterial genome via single homologous recombination. Three primary screens individually consisting of promoters of (1) lytic transglycosylase, (2) a self-acting competence mediating murein hydrolase and (3) mutacin VI, a bacteriocin, have been constructed and validated in *S. mutans.* Screening of 310 myxobacterial and fungal compounds from different classes were performed. Of those, promising were selected which include seven compounds acting on the mutacin VI and tuscolid on murein hydrolase, all of them showing more than 50% effect and eighteen compounds stimulating the transglycosylase beyond 75%. Our study revealed that tuscolid and myxopyronin A which showed an activity on the murein hydrolase, simultaneously triggered the transglycosylase promoter indicating a dual target activity or that they acted on a common conserved mechanism. Sorangicin A, an RNA polymerase inhibitor from the myxobacterium *Sorangium cellulosum* and the glykenins 17 and 19, from the fungus *Dacrymyces stillatus*, stimulated strong induction (approx. 2-fold) of the transglycosylase promoter. A diterpene compound B5-1 erinacine, from the edible medicinal mushroom *Hericium erinaceus*, strongly suppressed mutacin VI induction (50% reduction). The system, thus, provides a suitable platform to discover drugs capable of modulating the bacteria's own mechanism to reduce it to a less pathogenic phenotype.

Author Disclosure Block:

P. Premnath, None..
M. Reck, None..
B. Kunze, None..
R. Jansen, None..

S. Bhuyan, None..
K. Wittstein, None..
J. Niggemann, None..
J. Herrmann, None..
R. Müller, None..
M. Stadler, None..
I. Wagner-Döbler, None.

Poster Board Number:

LB-026

Publishing Title:

Rifampicin Resistant Leprosy in Southern India; analysis of efflux pump gene expressions and molecular modelling of rifampicin-RpoB interactions in <i>Mycobacterium leprae<i>

Author Block:

S. Vedithi, M. Das, L. Rajan; Schieffelin Inst. of Hlth.Res. and Leprosy Ctr., Vellore, India

Abstract Body:

Background: Mis-sense mutations within *rpoB* gene of *Mycobacterium leprae* were identified to be a possible reason for rifampicin resistance in leprosy. We investigated mutations within *rpoB* gene of *M. leprae* in relapsed leprosy cases from 4 tertiary care leprosy centres in south India. Further the differences in mRNA expression levels of 8 efflux pump genes in rifampicin resistant and sensitive strains were studied. In-silico modelling of rifampicin interaction with native and mutant RpoB protein models was also performed.
Methods: DNA and RNA were extracted from skin biopsies of 35 relapse/MDT non-respondent leprosy cases and PCR was performed to amplify 276bp rifampicin resistance determining region of *rpoB* gene of *M. leprae*. DNA Sequencing was performed to detect mutations. Homology models of mutant and native RpoB proteins were developed using iTasser Suite and docking experiments using Molecular Docking Server (AutoDock 4). mRNA expression levels of efflux pump genes (ML0556, ML1349, ML1388, ML1562, EMRB Efflux Pump and ML2350-2352) were analyzed in rifampicin resistant and sensitive strains.
Results: Mutations were detected in 5 out of the 35 relapse leprosy cases which included codon positions "Asp441Tyr" in two cases, "Leu436Ser", "Leu436Pro" and "His476Arg" in one case each. Molecular docking analysis revealed that native RpoB interacts with Rifampicin with two hydrogen bonds with estimated change in free energy of binding of -7.71 kcal/mol. This energy decreased in mutants with Asp441Tyr = -5.68 kcal/mol, Leu436Ser = -6.70 kcal/mol, Leu436Pro = -4.78 kcal/mol and His476Arg = -3.34 kcal/mol. Asp441Tyr mutation indicated complete loss in hydrogen bonding with rifampicin and rest of the mutations indicated only a single hydrogen bond. The estimated inhibition constant “Ki” has also increased in all mutants when compared to native RpoB. mRNA expression levels of ML1349-drrB family and ML1388-drug efflux membrane protein coding genes increased by 14.55 and 7.93 fold respectively in rifampicin resistant strains (normalized with 16SrRNA).
Conclusion: Structural variations induced by point mutations in RpoB protein can be a possible reason for loss in interaction with rifampicin and efflux pump gene expression is associated with rifampicin resistance in leprosy.

Author Disclosure Block:

S. Vedithi, None..
M. Das, None..
L. Rajan, None.

Poster Board Number:

LB-027

Publishing Title:

Translational Mouse Model of *Clostridium difficile* Infection

Author Block:

T. J. De Wolfe, M. N. Duster, J. L. Steele, B. J. Darien, N. Safdar; Univ. of Wisconsin - Madison, Madison, WI

Abstract Body:

Background: Current animal models to study *Clostridium difficile* infections (CDI) are beneficial to understand pathogenesis, however they contain several limitations when considering the effect of therapeutic intervention. The modifications made in our mouse model allow better approximation of clinical CDI for patients in healthcare settings and will allow researchers to study new treatment options, efficacy of supportive care, mechanisms of action, and CDI recurrence. **Methods:** Male 5-7 week old C57BL/6 wildtype mice obtained from Jackson Laboratories were housed with autoclaved food, bedding, and water. After 1 week of acclimation in the vivarium, mice were randomized and administered an antibiotic pre-treatment (0.25mg/ml cefoperazone) for 5 days in their drinking water. At the onset of antibiotic pre-treatment, mice were administered single strains (10^8 CFU/day) of *Lactobacillus casei* probiotics until the study endpoint. Two days after the last antibiotic pre-treatment, mice were gavaged with 10^2 *C. difficile* VPI 10463 spores and monitored for 48 hours. **Results:** Only 60% of mice survived the *C. difficile* challenge without probiotic administration while 80% of mice survived if administered *L. casei* M36 or *L. casei* BL23. Mice that were administered *L. casei* M36 and *L. casei* BL23 throughout the experimental timeline had significantly reduced weight loss and health scores compared to the *C. difficile* control. **Conclusions:** Modification of the antibiotic pre-treatment from a reputable mouse model has allowed us to establish reproducible CDI with moderate lethality. This lethality better approximates clinical CDI in humans. As a proof of concept, we have demonstrated probiotic efficacy for CDI prophylaxis. Moving forward this model will allow researchers to study alternative treatment options for CDI including mechanisms.

Author Disclosure Block:

T. J. De Wolfe, None..
M. N. Duster, None..
J. L. Steele, None..
B. J. Darien, None..
N. Safdar, None.

Poster Board Number:

LB-028

Publishing Title:

Dissecting the Microbiome in a Spatial Context

Author Block:

K. Duncan, S. Vaishnava; Brown Univ., Providence, RI

Abstract Body:

A dysregulated gut microbiome has been shown to contribute to gastrointestinal pathologies such as ulcerative colitis and Crohn's disease, but the mechanism is poorly understood. Traditional methods for studying the gut microbiome hint as to which organisms are present, but lack spatial context of the local environment. The microbiome contains a consortium of bacteria coming into close contact with the host (Fig A). Only by understanding the gut flora in its ecological framework can we begin to look into the host-microbe and microbe-microbe interactions that exist in this ecosystem. There is increasing evidence supporting the need for looking at the gut microbiome in a spatial context, but current methods have limited access to answering such questions.

We have developed a robust method that allows us to isolate distinct transverse regions to elucidate the biogeography of the microbiome. Specific fixation and sectioning techniques are employed that maintain the structural integrity of the gut flora, and by coupling laser-capture microdissection and 16s rRNA sequencing, the community composition of each layer is revealed.

By comparing the bacterial composition of 50um deep "layers" from the epithelium into the luminal space we are able to show that the specified regions do in fact vary in community composition (Fig B). A PCoA plot comparing consecutive 50um layers for small intestine and colon (Fig C) shows the layers from the same tissue cluster, but also that there is variation in each of the layers, showing that they are distinct.

Our approach of spatial microdissection of microbes *in situ* will be applied towards exploring the immune mechanisms that maintain biogeography of intestinal microbial communities. We anticipate that this will provide insight into the metabolic networks that govern the assembly of complex bacterial communities during health and disease.

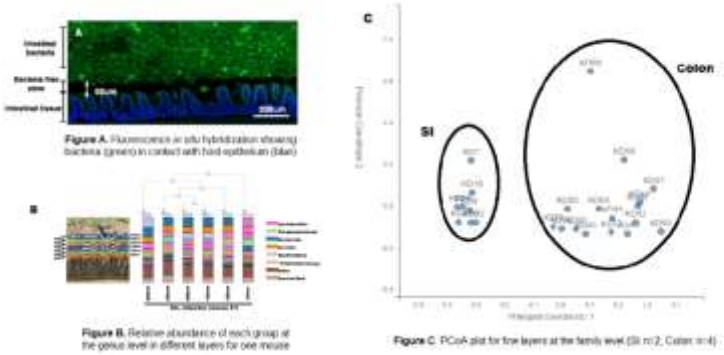

Figure A. Fluorescence in situ hybridization showing bacteria (green) in contact with host epithelium (blue)

Figure B. Relative abundance of each group at the genus level in different layers for one mouse

Figure C. PCoA plot for fow layers at the family level (SI: n=2, Colon: n=4)

Author Disclosure Block:

K. Duncan, None..
S. Vaishnava, None.

Poster Board Number:

LB-029

Publishing Title:

Investigation of Signaling Mechanisms that Control *Vibrio cholerae* Quorum Sensing Receptor VpsS

Author Block:

S. A. Jung, W-L. Ng; Tufts Univ., Boston, MA

Abstract Body:

Bacteria use quorum sensing (QS) for cell-cell communication to carry out group behaviors. This intercellular signaling process relies on cell density-dependent production and detection of chemical signals called autoinducers (AIs). *Vibrio cholerae*, the causative agent of cholera, uses four histidine kinases—CqsS, LuxQ, VpsS, and CqsR—to control virulence factor production through QS. At low cell density (LCD), these four signaling receptors function in parallel to activate the key regulator LuxO, while at high cell density (HCD), upon signal detection, the activity of these receptors is turned off, which leads to subsequent deactivation of LuxO and repression of virulence factor production. The CqsS and LuxQ systems have been well-studied: CAI-1 regulates the kinase activity of the CqsS system, while AI-2 controls LuxQ activity. However, little is known about the signals and their biosynthetic pathways that control receptors VpsS and CqsR. We have created transposon mutant libraries in a QS triple receptor mutant expressing only VpsS. Using a dual reporter system, we have identified a set of mutants with altered QS responses. Many of these transposon mutants carry insertions in genes responsible for generating key metabolites in the central metabolic pathway. Thus, our preliminary data suggest that certain intracellular signals could affect VpsS activity and could be key for modulating the QS response in *V. cholerae*.

Author Disclosure Block:

S. A. Jung, None..
W. Ng, None.

Poster Board Number:

LB-030

Publishing Title:

Characterization of a Murine Model of ZIKV Infection

Author Block:

A. Muruato[1], S. Rossi[1], S. Weaver[1], R. Tesh[2]; [1]The Univ. of Texas Med. Branch, Galveston, TX, [2]UTMB, Galveston, TX

Abstract Body:

Background: Zika virus (ZIKV) is a rapidly emerging mosquito-borne flavivirus that is currently causing an explosive outbreak across Central and South America. ZIKV infection results in a disease known as Zika fever, which is typically characterized by a mild febrile illness, rash, and conjunctivitis in adults and may be linked a variety of severe neurological and congenital illnesses. Currently, no vaccine or antiviral exists to combat Zika disease. Here we describe a murine model to test the safety and efficacy of therapeutics.
Methods: To establish a murine model of ZIKV infection, we used groups of immunocompromised mice lacking either the interferon alpha/beta receptor (A129) or IFN alpha/beta/gamma receptors (AG129). Mice ranging in age from 3-11 weeks were infected with an intraperitoneal (i.p.) dose of 10^5 PFU of ZIKV (low passage contemporary Cambodian isolate) and monitored daily for signs of illness (ruffling, decreased mobility, etc) and weight loss. Blood was collected daily to evaluate viremia. Furthermore, mice were necropsied as various times post infection to quantify the viral load in key organs.
Results: Immunocompromised mice are susceptible to ZIKV infection. All A129 mice exhibited signs and symptoms of disease such as ruffled fur and hunched posture. ZIKV was detected in the brain of 3-week-old A129 mice by 3 days post infection (dpi) and caused observable signs of neurologic disease by 6 dpi. Virus was detected in all organs tested with the highest viral titers observed in the brain, spleen, and testes. All 3iweek-old mice became moribund and succumbed to illness by 7dpi. Meanwhile, 5- and 11-week-old A129 mice exhibited signs of illness, viremia, and weight loss, but did not naturally succumb to infection. This age-dependent mortality is not observed in AG129 mice. Three-week-old AG129 mice have a similar disease progression and reach similar titers in the blood and organs as same-aged A129 mice. However, the neurologic disease observed is more pronounced. A similar outcome was observed in 9-week-old AG129 mice, suggesting that mortality is not age-dependent in AG129 mice.
Conclusions: This is one of the first characterizations of a murine model for ZIKV infection and therefore further characterization of this model could provide valuable insight into disease pathogenesis and serve as an effective platform to test the efficacy of antivirals and vaccines in vivo for ZIKV.

Author Disclosure Block:

A. Muruato, None..
S. Rossi, None..
S. Weaver, None..
R. Tesh, None.

Poster Board Number:

LB-031

Publishing Title:

The Structural Basis of Asymmetry in DNA Binding and Cleavage as Exhibited by the I-SmaMI LAGLIDADG Meganuclease

Author Block:

B. W. Shen[1], A. Lambert[1], B. C. Walker[2], B. L. Stoddard[1], **B. K. Kaiser**[2]; [1]Fred Hutchinson Cancer Res. Ctr., Seattle, WA, [2]Seattle U, Seattle, WA

Abstract Body:

• LAGLIDADG homing endonucleases ('meganucleases') are highly specific DNA cleaving enzymes that are considered selfish elements because they facilitate the spread of the gene that encodes them. Like other enzymes that act on DNA targets, meganucleases often display binding affinities and cleavage activities that are dominated by one protein domain.
• To decipher the underlying mechanism of asymmetric DNA recognition and catalysis, we identified and characterized a new meganuclease (I-SmaMI) that belongs to a superfamily of homologous enzymes that recognize divergent DNA sequences. I-SmaMI is a “monomeric” meganuclease that contains two LAGLIDADG domains on a single polypeptide.
• We solved a series of crystal structures of the enzyme-DNA complex representing a progression of sequential reaction states, and compared the structural rearrangements within each LAGLIDADG domain against their relative contribution to binding affinity. These results demonstrate that the N-terminal LAGLIDADG domain contributes the majority of DNA binding affinity, and that full binding involves significant conformational changes that position the C-terminal LAGLIDADG domain. We also tested how equivalent point mutations in each of the two actives sites affected DNA cleavage. These mutations generated partial nickase enzymes, with a point mutation in the N-terminal domain having a more significant effect on cleavage activity.
• Together, these results demonstrate the structural basis for 'dominance' by one protein domain over the other in a pseudodimeric DNA cleaving enzyme.

Author Disclosure Block:

B. W. Shen, None..
A. Lambert, None..
B. C. Walker, None..
B. L. Stoddard, None..
B. K. Kaiser, None.

Poster Board Number:

LB-033

Publishing Title:

Phenotypic Analysis Of New Genes Appears To Be Responsible For Acid And Cold Resistance In *Bacillus Cereus* ATTC 14597

Author Block:

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Abstract Body:

Background: *Bacillus cereus* is a toxin producing bacterium responsible for food-borne toxi-infections. To monitor the ability of the food-borne opportunistic pathogen *Bacillus cereus* to survive during minimal processing of food products conditions were established which allowed the cells to adapt to heat and acid stresses.
Methods: To determine the relationship between the response to cold and acidity condition, we tried to see if certain genes involved in the adaptation to cold may occur in the acid stress response in B. cereus ATCC14579. The effect of acidity on the mutants that were identified in our laboratory as sensitive to cold: 109H2 (ydbR mutant RNA helicase ATPdépendante), 111D1 (RZC3747) 108C12 (porB mutant pyruvate synthase), 134G8 (mutS mutant / porB) and 110H1 (yqeC mutant, 6-phospho gluconate dehydrogenase) was studied. Growth in microculture at different pHe 7.0; 5.0; 4.8; 4.7; 4,6 and 4, 55 were conducted on the wild type and mutant sensitive to cold
Results: Results show that the cold 109H2 sensitive mutant is not affected by acidity and it is growing faster than the wild type, the other mutants are sensitive to acidity.
Conclusions: Our results Suggest that the mutated genes play an important role in the growth of B. cereus in acidic medium.

Author Disclosure Block:

K. Senouci-rezkallah, None..
M. P. jobin, None..
P. Schmitt, None.

Poster Board Number:

LB-034

Publishing Title:

Outer Membrane Protein LptD/OstA (PA0595) Has a Novel Role in the Regulation of Alginate Synthesis in *Pseudomonas aeruginosa*

Author Block:

S. Pandey[1], C. Delgado[2], L. FLorez[1], H. Kumari[1], K. Mathee[1]; [1]Florida Intl. Univ., Miami, FL, [2]New York Univ., New York, NY

Abstract Body:

Pseudomonas aeruginosa, a Gram-negative bacterium, is a leading opportunistic pathogen in chronic cystic fibrosis (CF) and other lung diseases such as chronic obstructive pulmonary disease (COPD). The majority of *P. aeruginosa* isolates from the lungs of chronic CF patients are mucoid due to overproduction of an exopolysaccharide known as alginate. Alginate helps *P. aeruginosa* in immune invasion, antibiotic resistance, scavenging free oxygen radicals, and retention of water and nutrients. Sigma factor AlgT/U is the master regulator of alginate production in *P. aeruginosa*. An anti-sigma factor, MucA, sequesters AlgT/U and downregulates alginate production under normal conditions. It is well established that mutations in *mucA* lead to alginate overproduction. All the known proteins involved in alginate regulatory process reside in periplasm, innermembrane, or cytoplasm. To date, no outermembrane component involved in the regulation of alginate production has been identified. We hypothesized that novel components can be identified using nonmucoid variants of the constitutively alginate-overproducing strain PDO300 (PAO*mucA22*). Our analyses of a nonmucoid variant of PDO300, *sap27*, led to the identification of a cosmid, pMO012217, harboring 18 open reading frames (ORFs), to rescue the mucoid phenotype. Transposon mutagenesis of this cosmid revealed the insertion to be in a three-gene operon consisting of *lptD-surA-pdxA*. The *lptD* gene encodes an outer-membrane protein (OMP) involved in lipopolysaccharide transport. SurA is the cognate chaperone protein of LptD while PdxA is responsible for vitamin B_6 synthesis. We showed that expression of *lptD* alone restores the mucoid phenotype. Thus, we propose a novel role of LptD in alginate regulation in *P. aeruginosa* and this is the first report of an OMP involved in the process.

Author Disclosure Block:

S. Pandey, None..
C. Delgado, None..
L. FLorez, None..
H. Kumari, None..
K. Mathee, None.

Poster Board Number:

LB-035

Publishing Title:

A Classroom Research Module to Assess Antibiotic-Resistant Microbes in the Environment

Author Block:

C. A. Bascom-Slack; Tufts Univ. Sch. of Med., Boston, MA

Abstract Body:

Providing discovery-based research opportunities to students at an early stage and establishing partnerships between different institution types are recommended strategies for retaining students in the sciences. Implementing authentic research in the classroom, however, can be challenging. The PARE (**P**revalence of **A**ntibiotic-**R**esistance in the **E**nvironment) program aims to improve the quality of undergraduate education in a transformative way by initiating a large-scale classroom project that is low cost and short in duration. The hypotheses of this study were that participating students would 1) report gains in items related to the process of science 2) increase in number each year 3) engage in partnerships with high school students and 4) contribute reliable environmental resistance data.

Currently, there are no systematic surveillance methods for reporting levels of antibiotic-resistant microbes at environmental sites, yet environmental exposure to antibiotics is high in certain areas. In the PARE program, students use systematic methods to assess and report prevalence of tetracycline-resistant (tet^R) microbes in soil samples. In the Undergraduate Research Student Self-Assessment (URSSA) survey, a majority of PARE students reported gains in confidence and attitudes relating to the process of science and that the experience prepared them for more advanced work. The project was disseminated to 18 instructors in the first year and to over 50 instructors across the nation this year; the number of students participating doubled to 1,000. 87% percent of students report feeling part of a scientific community, indicating that partnerships were created. Over 700 data points reporting the prevalence of tet^R microbes at various soil collection sites around the country have been reported by students. Ongoing formative assessment of the program using a student skills survey and monitoring of raw data entries has helped to identify factors contributing to data entry errors.

The PARE project has been transformative in providing an authentic research experience to hundreds of students who report gains in self-efficacy and attitudes about science. It has introduced instructors to classroom-accessible authentic research. Assessment measures are informing ongoing changes to protocols and instructional materials to improve reliability of student-entered data.

Author Disclosure Block:

C. A. Bascom-Slack, None.

Poster Board Number:

LB-036

Publishing Title:

Tick-borne *Rickettsia* Pathogens in Southern Kazakhstan

Author Block:

T. Nurmakhanov[1], Y. Sansyzbayev[2], O. Yeskhodzhayev[1], A. Vilkova[1], A. Berdibekov[3], A. Matzhanova[4], R. Sailaubek[5], H. K. St. John[6], C. M. Farris[6], A. L. Richards[6]; [1]Kazakh Scientific Ctr. for Quarantine and Zoonotic Diseases, Almaty, Kazakhstan, Almaty, Kazakhstan, [2]Kazakh Scientific Ctr. for Quarantine and Zoonotic Diseases, Almaty, Kazakhstan, Amaty, Kazakhstan, [3]Taldykorgan Anti-plague Station, Taldykorgan, Kazakhstan, [4]Kyzylorda Anti-plague Station, Kyzylorda, Kazakhstan, [5]Shymkent Anti-plague Station, Shymkent, Kazakhstan, [6]Naval Med. Res. Ctr., Silver Spring, MD

Abstract Body:

Background: In Kazakhstan the distribution and prevalence of rickettsial pathogens is not well known. Thus, the focus of this study was to detect and identify rickettsiae in ticks from 3 southern Kazakhstan oblasts. **Methods:** Ticks collected from vegetation and farm animals (n=2,358) were identified by morphological methods and placed into pools of 10-20 individuals of the same species, homogenized and the DNA extracted. The nucleic acid preparations were assessed utilizing *Rickettsia* genus- and species-specific qPCR assays. **Results:** Ticks included: *Hyalomma asiaticum* (n=768/44 pools), *Hyalomma scupense* (n=660/44 pools), *Hyalomma anatolicum* (n=15/1 pool), *Haemaphysalis sulcata* (n=165/11 pools), *Haemaphysalis punctata* (n=30/2 pools), *Haemaphysalis* spp. (n=60/4 pools), *Dermacentor niveus* (n=30/2 pools), *Dermacentor* spp. (n=615/41 pools), and *Argas* spp. (n=15/1 pool). Of the 150 pooled tick samples rickettsiae were detected (n=27; 18%) and the agents identified as *R. slovaca* (n=6; 4%), *R. raoultii* (n=5; 3/3%), *R. aeschlimannii* (n=2; 1.3%), and *Rickettsia* spp. (n=14; 9.3%). **Conclusions:** The human pathogens *R. slovaca, R. raoultii,* and *R. aeschlimannii* were detected in tick pools from southern Kazakhstan.

Author Disclosure Block:

T. Nurmakhanov, None..
Y. Sansyzbayev, None..
O. Yeskhodzhayev, None..
A. Vilkova, None..
A. Berdibekov, None..
A. Matzhanova, None..
R. Sailaubek, None..
H. K. St. John, None..

C. M. Farris, None..
A. L. Richards, None.

Poster Board Number:

LB-038

Publishing Title:

Selective Signaling Response Inhibition Of A *Pseudomonas aeruginosa* Mucoid Variant Caused By Alginate Overproduction

Author Block:

J. Yang[1], M. Toyofuku[1], K. Tateda[2], N. Nomura[1]; [1]Univ. of Tsukuba, Tsukuba, Japan, [2]Toho Univ., Tokyo, Japan

Abstract Body:

Most microbes live in the environment in communities, called biofilms, rather than as solitary planktonic cells. Bacteria communicate with each other within their communities by releasing signaling molecules and by receiving them with specific signaling receptors. Bacteria use cell-cell communication to control the group behavior such as biofilm formation, virulence factors and respiration in their communities by regulating gene expression. In cell-cell communication, it is important that the signal reach the target cell and is received by its cognate receptor. Simultaneously, bacteria in biofilms produce an extracellular matrix (ECM) that consists of extracellular proteins and polysaccharides. The effect of the ECM on the signaling response of cell-cell communication is still not fully understood. The ubiquitous bacterium *Pseudomonas aeruginosa* produces various signaling molecules to communicate with one another. *P. aeruginosa* produce two acy-homoserine lactones (AHL), 3-oxo-C12-HSL and C4-HSL, and a quinolone signal, called *Pseudomonas* quinolone signal (PQS). In this study, we use a mucoid variant of *P. aeruginosa*, which overproduces the extracellular polysaccharide, alginate, to investigate the effect of ECM production on signaling response. We use a signaling molecule synthesis gene promoter fused to a plasmid to detect gene expression that is regulated by signaling molecules themselves. We find that the mucoid mutation causes a defect of the response to quinolone signal while the response to AHLs is partially inhibited. Next, we examine the effect of alginate production on the signaling response by deleting the alginate production gene of mucoid variant. Surprisingly, the deletion restores the PQS response but has no effect on the response to AHLs. These data indicate that alginate production interferes with only the quinolone signal response but has no effect upon AHL. We also confirm this phenomenon by measuring pyocyanin production, which is regulated by PQS. We show that the mucoid variant has low pyocyanin production while deletion of alginate restores production. Furthermore, the mucoid variant does not respond to the PQS from WT when cultured together. Our study implies that ECM production alters the signaling responses, which may result in varied behaviors within a group of bacteria.

Author Disclosure Block:

J. Yang, None..
M. Toyofuku, None..
K. Tateda, None..
N. Nomura, None.

Poster Board Number:

LB-039

Publishing Title:

Application of Biochemical Antifouling Coating to Reduce Biocorrosion of Minnesota's Maritime Transportation Infrastructure

Author Block:

S. Huang; Univ. of Minnesota, Duluth, Duluth, MN

Abstract Body:

Background: Biocorrosion of steel structures in the Duluth-Superior Harbor (DSH) is a severe problem for Minnesota's maritime transportation industry. It is clear from previous research that corroding steel surfaces and tubercles in the DSH are covered by complex microbial biofilms that contain bacteria of the types responsible for corrosion of steel in other environments. Thus, it may be possible to reduce the rate of the corrosion by impeding microbial attachment and growth or modifying microbial communities that develop on the steel surfaces. **Methods:** We have conducted lab experiments testing anti-biofouling coatings by exposing steel coupons in harbor water environment. Steel coupons were surface coated with crosslinked silica coatings containing different non-toxic antifouling biochemical including surfactin, capsaicin, magnesium peroxide, gramicidin. After 2 months of exposure, coupons were retrieved and analyzed. Biocorrosion was evaluated by quantities and coverage of corrosion tubercle, and also by SEM imaging of the coupon surface. Microbial communities within tubercles that develop on the corroding coupons were sampled and DNA extracted. The extracted DNA was used to describe changes in the overall composition of bacterial communities with 16S rDNA-amplicon V4 region miseq 2x300 Illumina sequencing. **Results:** Corrosion tubercle analysis show that Surfactin and MgO_2 treated coupons have significant reduction in number (31%, 36%) and percent coverage (37%, 20%) of corrosion tubercles on steel coupons. NMDS Beta diversity analysis of 15M+ sequences data suggests the treatments have significantly changed the composition of bacteria communities. Burkholderiales (13%) and Rhizobiales (8%) are the dominated orders of bacteria found in all of the coupon samples. **Conclusions:** Result suggests the enzyme and biochemical treatment can change the composition of microbial communities and reduce the formation of corrosion tubercles on steel surface. Approaches to applying these antifouling enzymes and biochemical to steel structures will be further evaluated to provide reliable, cost-effective methods to treat steel structures in the DSH.

Author Disclosure Block:

S. Huang, None.

Poster Board Number:

LB-040

Publishing Title:

Degradation of Polyester Polyurethane by An Indigenously Developed Bacterial Consortium Isolated from Soil

Author Block:

A. A. Shah, M. Gulzar, Z. Shah, F. Hasan; Quaid-i-Azam Univ., Islamabad, Pakistan

Abstract Body:

Background: In the current study, a consortium of two polyester polyurethane degrading bacterial strains *Bacillus subtilis* MZA-75 and *Pseudomonas aeruginosa* MZA-85 was developed and its degradation rate was compared with that of individual strains.
Methods: The degradation was analyzed through measurement of weight loss and CO_2 evolved (Sturm test), as a result of breakdown of PU. The surface morphology of PU film was checked through scanning electron microscopy (SEM). Extracellular esterase activity was estimated using para-Nitrophenyl acetate (*p*NPA) as chromogenic substrate. The degradation products were detected through gas chromatography-mass spectrometry (GC-MS).
Results: More than 50% of weight loss was observed in PU film within 20 days of incubation. When plastic films pieces were subjected to consortium in Sturm test as a sole carbon source; the quantity of carbon dioxide released was much higher in comparison to control as well as to the individual strains. SEM micrographs showed surface changes in the treated PU film in the form of cracks originating from the point of bacterial attachment. A sharp increase in amount of extracellular esterase was observed in the presence of polyester PU with consortium as compared to pure culture. GC-MS results indicated that besides adipic acid and 1,4-butanediol, an additional peak for methylene bis di-isocyanate (MDI) was also detected in cell free supernatant of consortium samples that was not detected in pure cultures.
Conclusions: Hence, it is concluded that the consortium might be applied in polyurethane waste management and as a tool in the process of biochemical monomerization for purified monomers recycling.

Author Disclosure Block:

A. A. Shah, None..
M. Gulzar, None..
Z. Shah, None..
F. Hasan, None.

Poster Board Number:

LB-041

Publishing Title:

Microbe Today: *Bordetella pseudohinzii* sp. nov. Infects C57BL/6 Mice

Author Block:

Y. V. Ivanov[1], B. Linz[1], K. B. Register[2], J. D. Newman[3], D. L. Taylor[1], K. R. Boschert[4], S. Le Guyon[5], E. F. Wilson[1], L. M. Brinkac[6], R. Sanka[6], S. C. Greco[4], P. M. Klender[4], L. Losada[6], E. T. Harvill[1]; [1]Pennsylvania State Univ., University Park, PA, [2]United States Dept. of Agriculture, Ames, IA, [3]Lycoming Coll., Williamsport, PA, [4]Washington Univ., St. Louis, MO, [5]Nanyang Technological Univ., Singapore, Singapore, [6]J. Craig Venter Inst., Rockville, MD

Abstract Body:

Background. Clinical studies rely heavily on mouse models of infection, making precise identification and control of contaminating pathogens in animal facilities an essential task. Over the past decade, there have been several reports documenting the isolation of *Bordetella* spp. from specific-pathogen-free C57BL/6 mice housed in various facilities in the United States, Europe and Asia.
Methods. To accurately speciate isolates of *Bordetella* from mice, we first sequenced the genomes of two from the United States and one from Germany and compared them with genome sequences of other *Bordetella*. We reconstructed whole-genome-based and 16S-rRNA-gene-based phylogenies for all *Bordetella* spp. sequenced to date. We then determined estimated DNA-DNA Hybridization (eDDH) values and Average Nucleotide Identity (ANI) scores. Metabolic profiles and antimicrobial sensitivities have been assayed with Biolog PM1 and PM2 tests, Biolog GENIII, API20 NE system, and Epsilometer tests. The ability of each isolate to colonize the lungs of mice was assessed using a previously described murine model of infection.
Results. Each of the two reconstructed phylogenies placed the three murine isolates into a distinct monophyletic cluster, most closely related to *B. hinzii*. Furthermore, distinctly different eDDH, ANI, gene content, metabolic profiles, and host specificity all provide compelling evidence for delineation of murine isolates from the *B. hinzii* group. Therefore, we designate these murine isolates as *B. pseudohinzii* spp. nov.
Conclusions. Our study provides means to precisely identify *B. pseudohinzii* sp. nov. and to assess its prevalence among laboratory-raised mice either based on the presence of *B. pseudohinzii*-specific genes such as the type II-C CRISPR-Cas genes, which are absent in all other bordetellae, or by a unique pattern of mutations observed in their 16S-rRNA sequence. Using the latter approach, we revealed that all *Bordetella* isolates, cultured from mice across Japan, most likely represent *B. pseudohinzii* sp. nov.

Author Disclosure Block:

Y. V. Ivanov, None..
B. Linz, None..
K. B. Register, None..
J. D. Newman, None..
D. L. Taylor, None..
K. R. Boschert, None..
S. Le Guyon, None..
E. F. Wilson, None..
L. M. Brinkac, None..
R. Sanka, None..
S. C. Greco, None..
P. M. Klender, None..
L. Losada, None..
E. T. Harvill, None.

Poster Board Number:

LB-042

Publishing Title:

Revealing Conserved DNA Motifs in the 3' End of the Type IIA CRISPR Leader Region

Author Block:

P. Klein, F. Z. Najar, M. Van Orden, **R. Rajan**; Univ. of Oklahoma, Norman, OK

Abstract Body:

The Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated (Cas) proteins consist of RNA-protein complexes that provide bacteria and archaea sequence-specific immunity against bacteriophages and conjugative plasmids. The bacteria or archaea become immune to phage or plasmid infections by inserting short pieces of the intruder DNA site-specifically into the leader-repeat (repetitive units of CRISPR RNA gene) junction, in a process called adaptation. The hypothesis for the present research is that sequence/structural motifs present in the leader-repeat junction recruits type-specific Cas proteins for successful adaptation. As part of our ongoing investigation on adaptation, we analyzed type IIA CRISPR loci belonging to 55 *Streptococcal* and 74 other bacterial genera. Our dataset consisted of 150 nucleotides of the CRISPR leader, the first repeat, and the protein sequences of Cas1, Cas2, Cas9, and Csn2. After performing multiple sequence alignments using MUSCLE, phylogenetic trees were generated in UGENE. Our results showed an over-representation of two distinct 3' leader ends in all the 129 loci analyzed, i) 5'-ATTTGAG-3' and ii) 5'- CYACGAG-3'. Interestingly *Streptococcus thermophilus* DGCC7710, a model bacterium used for adaptation studies, possesses two type IIA CRISPR loci of which locus 1 (Sth-CR1) has the ATTTGAG sequence motif and locus 3 (Sth-CR3) has the CYACGAG motif. A phylogenetic analysis of the repeat and the Cas proteins revealed segregation reflecting the 3' leader end conservation and pointed to the coevolution of the leader region and the CRISPR components. Based on previous studies (1) which experimentally demonstrated that the 3' end of the leader is essential for adaptation in Sth-CR1 locus and due to certain bacteria only carrying Sth-CR3 locus, we hypothesize that the two DNA motifs are essential for type IIA adaptation. We currently are investigating the importance of the two DNA motifs and its co-relation to locus-specific Cas proteins by *in vitro* assays. Similar studies can be used to identify conserved leader sequences in other CRISPR systems.

Author Disclosure Block:

P. Klein, None..
F. Z. Najar, None..
M. Van Orden, None..
R. Rajan, None.

Poster Board Number:

LB-043

Publishing Title:

Rapid Detection of Polymyxin-resistant *Enterobacteriaceae* from Blood Cultures

Author Block:

A. Jayol[1], V. Dubois[1], L. Poirel[2], **P. Nordmann**[2]; [1]Univ. of Bordeaux, Bordeaux, France, [2]Univ. of Fribourg, Fribourg, Switzerland

Abstract Body:

Enterobacterial strains resistant to polymyxins are increasingly reported worldwide. MIC testing by broth microdilution (reference technique) for determining colistin susceptibility remains time-consuming (24 to 48 h) and many other techniques such as disk diffusion are not reliable. Therefore, the Rapid Polymyxin NP (Nordmann/Poirel) test was developed for a rapid identification of colistin-resistant *Enterobacteriaceae*. This test is based on the detection of the glucose metabolization related to bacterial growth in liquid medium in presence of a defined concentration of colistin (3.75 mg/L) (or polymyxin B) indicating colistin resistance. Formation of acid metabolites consecutive to the glucose metabolization was evidenced by a color change (orange to yellow) of a pH indicator (red phenol). The Rapid Polymyxin NP test has been evaluated to detect colistin-resistant *Enterobacteriaceae* directly from spiked blood cultures and from clinical positive blood cultures. It was evaluated with 80 blood culture sets positive with *Enterobacteriaceae* isolates belonging to various genera (*Escherichia, Klebsiella, Enterobacter, Citrobacter, Hafnia, Proteus, Providencia, Serratia, Morganella*). The test showed excellent performances to discriminate colistin-resistant and -susceptible enterobacterial isolates and results were rapidly obtained within 2-4 hours. The method was easy-to-perform, requiring neither subculture nor centrifugation of the blood culture (only a ten-fold dilution). This rapid test is well adapted for an early identification of polymyxin-resistant *Enterobacteriaceae* directly from blood cultures. It may be useful for the management of septic patients, especially in countries where carbapenemase producers are highly prevalent and where polymyxins are last resort antibiotics.

Author Disclosure Block:

A. Jayol, None..
V. Dubois, None..
L. Poirel, None..
P. Nordmann, None.

Poster Board Number:

LB-044

Publishing Title:

***H. pylori* PCR Test can Predict Presence of Clarithromycin Resistance-associated Mutations from Stool with High Correlation to Patient Eradication**

Author Block:

E. Beckman[1], G. Fiorini[2], I. Saracino[2], C. Clark[1], V. Slepnev[1], D. Patel[1], C. Gomez[1], R. Ponaka[1], S. Elagin[1], B. Vaira[2]; [1]Meridian BioSci., Cincinnati, OH, [2]Univ. of Bologna, Bologna, Italy

Abstract Body:

Background: *Helicobacter pylori* is associated with gastric illness including gastric cancer and peptic ulcers. *H. pylori* can be detected using samples obtained from both invasive and non-invasive methods. The prevalence of clarithromycin resistance among *H. pylori* infected population is increasing and there is a need to identify the resistance preferably with non-invasive methods. This PCR method can be used to detect *H. pylori* in stool as well as predict presence of mutations associated with resistance to clarithromycin.**Methods:** A total of 244 frozen stool samples predominantly positive according to the gold standard (culture and/or histology plus rapid urease test and ^{13}C-urea breath test) were tested. Genomic DNA was extracted from the stool using QIAamp FAST Stool DNA Kit (QIAGEN). A qPCR Exogenous Control (Bioline, UK) was added to samples prior to the extraction. Real-time PCR amplification was performed using *H. pylori* Analyte Specific Reagents (ASR) primers and probes (Meridian Bioscience, Cincinnati) and exogenous control specific primers and probes. The Real-time PCR reaction was prepared with SensiPLUS master mix (Bioline) and ran on RotorGene (QIAGEN). Presence of resistance-associated mutations to clarithromycin was determined by signal difference between two test fluorophores or DNA melt analysis. PCR results were compared to final outcome of the patient after combination therapy including clarithromycin. **Results:** The sensitivity of the assessement above mentioned was 90% according to the gold standard. Out of the 176 true positives tested, a total of 61 samples or 34.7% showed point mutations associated with clarithromycin resistance based on the PCR analysis. The resistance samples contain mutations that includes A2143G, A2412G and A2142C which were confirmed by DNA bidirectional sequencing. Eradication results from clarithromycin therapy showed 86% correlation to the PCR results from stool samples.
Conclusions: The Real-time PCR method is capable of detecting *H. pylori* in stool and detecting the point mutations associated with clarithromycin resistance. This assay may present a useful option for patient management in areas with high prevalence of resistance to clarithromycin.

Author Disclosure Block:

E. Beckman, None..
G. Fiorini, None..
I. Saracino, None..
C. Clark, None..
V. Slepnev, None..
D. Patel, None..
C. Gomez, None..
R. Ponaka, None..
S. Elagin, None.
B. Vaira,
Meridian Bioscience Role(s): Self, C. Consultant.

Poster Board Number:

LB-045

Publishing Title:

Colistin Resistance of Gram-negative Clinical Bacterial Isolates

Author Block:

Q. Luo, W. Yu, Y. Xiao, L. Li; The First Affiliated Hosp., Coll. of Med., Zhejiang Univ., Hangzhou, Zhejiang, China, Hangzhou, China

Abstract Body:

Recently, researchers have discovered that bacteria worldwide are sharing *mcr-1*-a plasmid mediated gene that confers resistance to colistin, a 'last resort' antibiotic. The plasmid-mediated colistin resistant gene *mcr-1* has mainly been identified in bacterial species from livestock animals. However, it was also detected in humans from patients and asymptomatic people.
We used agar dilution method to measure MIC of 2866 non-duplicate Gram-negative clinical bacterial isolates collected from a tertiary hospital during 2014-2015. We screened out 40 *Escherichia coli* (3.15%; 40/1270), 8 *Klebsiella pneumoniae* (0.87%; 8/915), 4 *Acinetobacter baumannii* (0.67%; 4/600), 0 *Pseudomonas aeruginosa* (0/81) colistin resistant strains (MIC>2 μg/mL). Of note, *A. baumannii* and *P. aeruginosa* has much lower colistin resistant ration than *E. coli*.
PCR and DNA sequencing were employed to screen for the presence of *mcr-1* in the colistin resistant strains. Results indicated that *mcr-1* was only present in *E. coli*.
MLST according to warwick database indicated that these colistin resistant *E. coli* strains belonged to multiple sequence type are dispersedly distributed. S1-PFGE and Southern hydridization showed that *mcr-1* were located on plasmids of different sizes. These plasmids were successfully transferred to J53. In the clinical isolate *E. coli*, colistin resistant levels conferred by *mcr-1* are relatively lower than non-*mcr-1* colistin resistant strains. The mechanism of this phenotype is still unknown. We have discovered that lipid A structure was different between *mcr-1* containing and non-*mcr-1* colistin resistant strains through MALDI-TOF measurement.
Colistin resistance of gram-negative clinical bacterial isolates is definitely worth further study, including epidemiologic study and resistance mechanism research.

Author Disclosure Block:

Q. Luo, None..
W. Yu, None..
Y. Xiao, None..
L. Li, None.

Poster Board Number:

LB-046

Publishing Title:

Targeted Amplicon Sequencing versus Standard Culture Methods for Detection of Cystic Fibrosis Pathogens

Author Block:

R. M. Davidson, E. Epperson, K. C. Malcolm, J. A. Nick, M. Strong; Natl. Jewish Hlth., Denver, CO

Abstract Body:

Individuals with cystic fibrosis (CF) often acquire chronic, polymicrobial lung infections due to excessive mucus production and defective airway clearance of bacteria. Diagnostic tests capable of detecting a range of pathogenic bacteria are essential for determining effective treatment regimens. Rapid and accurate detection is especially important for nontuberculous mycobacteria (NTM), which are emerging CF pathogens that currently take days to weeks to identify using laboratory culture techniques. Microbiome profiling using targeted amplicon sequencing is a promising approach for clinical diagnostics given the rapid turn around time and potential to detect most bacterial genera in a human sample. The method uses PCR to amplify target DNA directly from complex samples followed by high throughput sequencing. Our goal is to compare amplicon sequencing to gold standard culture methods for three CF pathogens: *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Mycobacterium* species (NTM). We hypothesize that molecular methods will recapitulate culture results. First, we performed 16s rRNA amplicon sequencing on sputa samples from 32 adult CF patients and evaluated the presence of 16s reads compared to positive microbiologic culture results. We observed the highest sensitivity and specificity for *P. aeruginosa* (sens:spec; 0.94: 1.0), lower accuracy for *S. aureus*; (0.85: 0.89) and the least precision for *Mycobacterium* species (0.60: 0.64). To improve upon the low predictive power of the 16s target for NTM, we sequenced a gene target designed to specifically detect mycobacterial pathogens, and significantly improved our NTM detection accuracy. Our results suggest that the choice of sequencing target is an important consideration for detection of desired pathogens, and rapid molecular methods may be feasible tools for diagnostic laboratories.

Author Disclosure Block:

R. M. Davidson, None..
E. Epperson, None..
K. C. Malcolm, None..
J. A. Nick, None..
M. Strong, None.

Poster Board Number:

LB-048

Publishing Title:

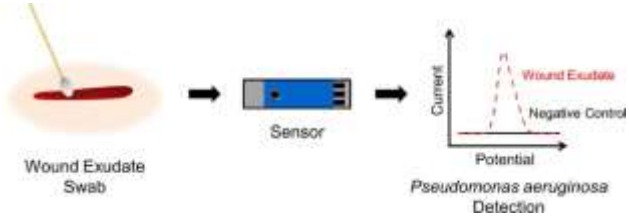
Rapid Detection of *Pseudomonas aeruginosa* in Wound Exudate from Patients with Chronic Wounds

Author Block:

H. J. Sismaet[1], A. Banerjee[2], S. McNish[2], Y. Choi[3], M. Torralba[3], S. Lucas[3], A. Chan[3], V. K. Shanmugam[2], E. D. Goluch[1]; [1]Northeastern Univ., Boston, MA, [2]George Washington Univ., Washington, DC, [3]J. Craig Venter Inst., Rockville, MD

Abstract Body:

A significant limitation to antibiotic stewardship and improved patient care is the delay between the obtainment of a biological sample and its bacterial identification from culture results. In the field of wound care in clinical practice, the use of plate cultures inoculated from swab samples is the standard practice for positive identification, taking anywhere from 24 to 48 hours before a positive result is produced. In the clinical setting, *Pseudomonas aeruginosa* is one of the leading causes for bacterial infection and is an important bacterium to study given its impact on people with chronic wounds and compromised immune systems. Because *Pseudomonas aeruginosa* infections are currently identified using plate cultures, a rapid detection for this bacterium would allow a doctor to switch from broad-spectrum antibiotics to more direct targeted therapy, lowering antibiotic resistance and improving patient care outcomes. Here we report the use of an inexpensive, disposable electrochemical sensor to detect pyocyanin, a unique, redox-active quorum sensing molecule released by *Pseudomonas aeruginosa*, in wound exudate from patients with chronic wounds. This electrochemical detection strategy eliminates sample preparation and takes less than a minute to complete analysis. The electrochemical results were compared against 16S rRNA profiling where blind identification yielded 9 correct matches, 2 false negatives, and 3 false positives giving a sensitivity of 71% and specificity of 57% for detection of *Pseudomonas aeruginosa*. The results from this study will help in improving the sensor for point-of-care diagnostics.



Author Disclosure Block:

H. J. Sismaet, None..
A. Banerjee, None..
S. McNish, None..
Y. Choi, None..
M. Torralba, None..
S. Lucas, None..
A. Chan, None..
V. K. Shanmugam, None.
E. D. Goluch,
Dr. Goluch has a financial interest in QSM Diagnostics, Inc., which commercializes infection diagnostic technology. Role(s): Self, N. Other.

Poster Board Number:

LB-049

Publishing Title:

Detection of *Clostridium difficile* Binary Toxin Genes, *cdtA* and *cdtB,* in Buenos Aires, Argentina

Author Block:

N. Rios Osorio[1], D. Cejas[1], R. Quirós[2], M. A. Berger[3], R. Sadorin[2], **G. Gutkind**[1], L. Fernández Canigia[3], M. Radice[1]; [1]Univ. de Buenos Aires, Buenos Aires, Argentina, [2]Hosp. Austral, Buenos Aires, Argentina, [3]Hosp. Alemán, Buenos Aires, Argentina

Abstract Body:

Background: Hypervirulent *Clostridium difficile* strains are characterized by the presence of two large toxins, toxins A (TcdA) and B (TcdB), and the binary toxin (CDT), encoded by *cdtA* and *cdtB*. During the last decades, *C. difficile* BI/NAP1/027 strain has been reported in North America and Europe. This binary toxin producing strain displayed high-level fluoroquinolone resistance and has been related to severe nosocomial outbreaks. Epidemiological studies on *C. difficile* are scarce in our country. The aim of this study was to investigate the presence of binary clostridial toxins in Buenos Aires **Methods:** 29 *C. difficile* isolates were recovered from diarrheal stool samples of hospitalized patients between March and November 2015 at 2 hospitals in Buenos Aires. All these samples rendered positive Glutamate Dehydrogenase (GDH) test. Both GDH and A/B toxins were investigated by Quick Check Complete enzyme immunoassay (EIA). Regardless of the toxin results, all samples were shocked in 95% alcohol for 30 min and appropriately inoculated for *C. difficile* isolation. Identification was confirmed by MALDI-TOF. Susceptibility to fluoroquinolones (levofloxacin (LEV), moxifloxacin (MXF) and gatifloxacin (GAT)), vancomycin (VAN) and metronidazole (MTZ) was determined according to CLSI. A multiplex PCR was carried out in order to detect *tcdA, tcdB, cdtA* and *cdtB,* according to *Persson et* al 2008, using genomic DNA obtained from *C. difficile* cultures as template. All amplicons were sequenced **Results:** 12/29 isolates were positive for toxin A/B by EIA. Both *tcdA* and *tcdB* were detected in 16 isolates. Many discrepancies were observed between phenotypic and genotypic approaches. No *tcdA*-/*tcdB*+ isolates were observed. Coding genes for CDT were identified in 4 *tcdA*+/*tcdB*+ isolates, 2 of them characterized as toxin- by EIA. MIC90 (μg/ml) of LEV, MXF, GAT, VAN and MTZ were 4, 16, 16, 0.5, 0.5 respectively **Conclusions:** To the best of our knowledge, binary toxin has been reported only once in our country in stool samples using GeneXpert. CDT positive isolates were susceptible to the fluoroquinolones studied, suggesting that these isolates would correspond to other clone different from BI/NAP1/027

Author Disclosure Block:

N. Rios Osorio, None..
D. Cejas, None..
R. Quirós, None..
M. A. Berger, None..
R. Sadorin, None..
G. Gutkind, None..
L. Fernández Canigia, None..
M. Radice, None.

Poster Board Number:

LB-050

Publishing Title:

Transferable Resistance Gene *optrA* in *Enterococcus faecalis* Isolated from Swine in Brazil

Author Block:

L. M. Almeida[1], F. Lebreton[2], P. Bispo[2], J. T. Saavedra[2], J. Wurster[2], C. Pires[1], M. R. Fernandes[1], L. T. Cerdeira[1], N. Lincopan[1], A. M. Moreno[1], E. M. Mamizuka[1], M. S. Gilmore[2]; [1]Univ. of São Paulo, São Paulo, Brazil, [2]Harvard Med. Sch., Boston, MA

Abstract Body:

Background: The ABC transporter gene *optrA* is a transferable phenicol/oxazolidinone resistance gene identified very recently in staphylococci and enterococci isolates of human and animal origins in China and Italy. Here we investigate *Enterococcus faecalis* strains, isolated from swine in Brazil and carrying *optrA* on a previously uncharacterized genetic element. **Methods:** A total of 15 *E. faecalis* strains isolated from porcine fecal swabs exhibited high chloramphenicol and linezolid MIC levels. All strains were screened by PCR for the presence of the *optrA* gene. The multilocus sequence types (ST) were determined according to the MLST database. Finally, whole genome sequencing (Illumina Hiseq), de novo assemblies (CLC Genomics Workbench 8.0.3) and annotations (Rapid Annotation Server - RAST) were performed to investigate the genetic environment of *optrA*. **Results:** A conserved *optrA*-carrying DNA segment of 4439 bp was identified in all 15 *E. faecalis* strains, which grouped into genetically distant STs (ST29, ST330, ST590, ST591, ST592). Upstream of *optrA*, genes coding for a hypothetical protein and an AraC family transcriptional regulator were detected. Downstream of *optrA*, a gene coding for a protein of unknown function was found to display 100% DNA identity with the *optrA* genetic element previously reported in *E. faecalis* and *Staphylococcus sciuri*. Further downstream, a partially disrupted ISSep1-like transposase and a protein coding for DNA repair RadC were present. While in most of the *E. faecalis* strains, the *optrA*-carrying segment was inserted downstream of a complete copy of the site-specific transposable element Tn554, in one of them the 4439 bp *optrA* region inserted inside the *tnpC* gene of Tn554. **Conclusions:** The flanking regions of the *optrA*-carrying segment in the epidemiologically unrelated *E. faecalis* strains of this study differed from the elements described in other strains and species so far. The analysis of these segments suggests that the transposon Tn554 might be involved in the integration and transfer of *optrA*. Finally, our data suggest that *optrA* acquisition result from both vertical inheritance and horizontal transfer, possibly transposon mediated, in enterococci.

Author Disclosure Block:

L. M. Almeida, None..
F. Lebreton, None..

P. Bispo, None..
J. T. Saavedra, None..
J. Wurster, None..
C. Pires, None..
M. R. Fernandes, None..
L. T. Cerdeira, None..
N. Lincopan, None..
A. M. Moreno, None..
E. M. Mamizuka, None..
M. S. Gilmore, None.

Poster Board Number:

LB-051

Publishing Title:

Combinations of Daptomycin Plus Gentamicin Exert Early Synergistic Killing of Streptococcus mitis In Vitro

Author Block:

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Abstract Body:

Background. Among viridans group streptococci, *S. mitis* has emerged as the most common cause of endocarditis and the toxic strep syndrome in immunocompromised hosts. *S. mitis* isolates are frequently β-lactam-resistant, rendering daptomycin (DAP) as a potential option for treatment of invasive infections with these strains. Of note, many clinically-derived *S. mitis strains* **rapidly develop high-level DAP-resistance (DAP-R) both *in vitro* and *in vivo* (≤ 48 hr exposure to DAP; AAC 2013)**. The objective of this study was to investigate the ability of DAP in combination with 'old' gentamicin (GEN) or 'new' antibiotics [tedizolid (TED), linezolid (LNZ) and ceftaroline (CPT)] to kill a prototypical *S. mitis* endocarditis isolate (SF100). We focused on early 'cidal effects of such combinations in time-kill assays.

Methods.

MICs. Determined both by Etest and broth microdilution assay (CLSI recommendations for *S. mitis*).

***In vitro* time-kill curves.** Time-kill curves (0, 2, 4, and 6 hr) were performed at an initial inoculum of 10^5 CFU/ml in the presence of a range of sub-inhibitory-to-inhibitory concentrations of all study antibiotics.

Results.

Although most combinations yielded enhanced early killing, only DAP + GEN combinations demonstrated significant early synergistic killing vs DAP or GEN alone (see **Table below**; data given as $\log_{10}$ CFU/ml ± SD; means of two separate runs)**.**

Time (hr)	**Control**	**DAP 1X**	**GEN 1X**	**DAP 1X + GEN 1X**
0	5.0±0.2	5.0±0.1	4.9±0.3	4.9±0.3
2	5.4±0.2	5.1±0.0	4.9±0.1	3.3±1.0
4	6.3±0.0	4.1±0.1	5.0±0.2	1.6±0.2*°¨

6	7.8±0.4	4.0±0.4	4.9±1.0	1.9±0.1*
DAP MIC =0.75µg/ml; GEN MIC=6 µg/ml ; *p-value <0.05 vs Control; p-value <0.05 vs DAP 1X; ¨ p-value <0.05 vs GEN 1X				

Conclusions.
Combinations of DAP + GEN are a promising therapeutic option for the treatment of invasive *S. mitis* syndromes. Current studies in our lab are examining additional DAP combination regimens (e.g. DAP + rifampin, trimethoprim-sulfa, imipenem or ampicillin) for ability to not only evoke early synergistic killing, but also to prevent the emergence of DAP-R upon longer *in vitro* exposures to DAP.

Author Disclosure Block:

B. Zapata, None..
D. N. Alvarez, None..
T. T. Tran, None..
C. Garcia-de-la-Maria, None..
J. M. Miro, None.
C. A. Arias,
Forest Role(s): Investigator, Speaker's Bureau. Theravance Role(s): Consultant, Grant Investigator, Speaker's Bureau. Pfizer Role(s): Speaker's Bureau. AstraZeneca Role(s): Speaker's Bureau. Cubist Ro Role(s): Self, N. Other.
M. J. Rybak, None..
P. Sullam, None.
A. S. Bayer,
Research grants from: NIH-NIAID; ContraFect Corp; Theravance Pharmaceuticals Scientific Advisory Board: ContraFect Corp. Role(s): Self, N. Other.
N. N. Mishra,
MercK Pharmaceuticals Role(s): Self, E. Grant Investigator.

Poster Board Number:

LB-052

Publishing Title:

The Prp8 Intein as a Target for Inhibition of Pathogenic Fungi

Author Block:

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Abstract Body:

Abstract: Invasive fungal infections (IFIs) remain a major public health challenge. Globally, over 300 million people are affected by IFIs, with estimated deaths of over 1 million people every year. IFIs are largely caused by *Candida, Cryptococcus, and Aspergillus* species. Among the IFI pathogens, *Cryptococcus neoformans (Cne), C. gattii* (Cga), and *Aspergillus fumigatus (Afu)* cause significant human disease, and are difficult to treat. Currently, IFI treatment is usually achieved with combination therapy for a lengthy period of time. Despite the availability of anti-fungal drugs, mortality rates associated with these infections often exceed 50%. In addition, drug resistance is a significant problem. The *Prp8* intein is one of the most widespread eukaryotic inteins, occurring in important pathogenic fungi including *Cryptococcus* and *Aspergillus* species. Because the processed Prp8 carries out essential and non-redundant cellular functions in pathogenic fungi, a Prp8 intein inhibitor could be a mechanistically novel anti-fungal agent. Here, we developed high throughput screening assays to monitor Prp8 splicing. Using the assays, we demonstrated that cisplatin, a broad spectrum cancer drug, not only inhibited the Prp8 splicing *in vitro*, but also inhibited the growth of *Cne*, *Cga*, and *Afu*. In contrast, *Candida* species that do not harbor the Prp8 intein were only poorly inhibited by cisplatin, suggesting that cisplatin inhibition is through specific targeting of the Prp8 inteins. Furthermore, the inhibitions were found as fungicidal. We also investigated the binding of cisplatin to Prp8, using mass spectrometry and mutagenesis approaches. Overall these results indicated that the Prp8 intein is a novel anti-fungal target.

Author Disclosure Block:

Z. Li, None..
J. Zhang, None..
S. Chaturvedi, None..
C. Green, None..
M. Belfort, None..
H. Li, None.

Poster Board Number:

LB-053

Publishing Title:

PK-PD Driver of Efficacy for CF-301, a Novel Anti-Staphylococcal Lysin: Implications for Human Target Dose

Author Block:

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Abstract Body:

Lysins are a new class of antimicrobials consisting of bacteriophage-derived cell-wall hydrolases. CF-301, the first lysin to enter US clinical trials, has concluded a First-in-Man Phase 1 trial (0.04-0.4 mg/kg in healthy volunteers) and is being developed for the treatment of *S. aureus* bacteremia. CF-301 exhibits rapid *S. aureus*-specific bacteriolysis, potent anti-biofilm activity, low propensity for resistance and pronounced synergy with antibiotics. Here, the PK-PD of CF-301 against *S. aureus* was characterized using the traditional murine thigh-infection (MTI) model.

Mice infected with $1x10^6$ CFU *S. aureus* were administered CF-301 divided into 1, 2, 3, or 4 doses over 24 h. Change in $\log_{10}$ CFU at 24 hours post infection was used as the dependent variable in a regression analysis to examine the predictive value of AUC:MIC ratio, Cmax:MIC ratio, %T>MIC, or a combination of these indices, using 20 different models ranging from linear to nonlinear, with model fit to the data assessed by comparing the residual standard errors. Efficacy of CF-301 (1 to 60 mg/kg in 4 divided doses) was evaluated against 10 clinical *S. aureus* isolates (MIC range: 4-64 ug/mL) and a subset in the presence of sub-therapeutic 0.5-5 mg/kg Daptomycin (DAP), and various models were fit to the data.

While all indices and index combinations displayed an association with CF-301 efficacy, the AUC:MIC index alone had the best predictive value. AUC:MIC ratios of $\geq$1.5 (single-agent) and $\geq$0.5 (DAP combo) are associated with maximal bacteriolytic activity (3 $\log_{10}$ CFU reduction from controls). Based on the CF-301 MIC_{90} value in human serum of 1 µg/mL, the AUC:MIC ratio target values, and estimated AUCs from a Phase 1 study in humans (in press), it is projected that CF-301 doses between 0.1-0.2 (single agent) and 0.03-0.1 (DAP combo) mg/kg will be efficacious in humans.

Data demonstrate that the AUC:MIC ratio is the PK-PD index most predictive of CF-301 efficacy against *S. aureus.* AUC:MIC ratio targets of approximately 1.5 and 0.5 are expected to be achieved in humans at CF-301 doses in the ranges of 0.1-0.2 (single agent) and 0.03-0.1 mg/kg (DAP combo). Additional studies incorporating population PK analyses and simulation will further refine the targets and evaluate optimal dosing regimens for CF-301.

Author Disclosure Block:

J. A. Rotolo, None..
R. A. Ramirez, None..
R. Schuch, None..
M. Machacek, None..
T. Khariton, None..
P. Ghahramani, None..
M. Wittekind, None.

Poster Board Number:

LB-054

Publishing Title:

Small Molecule Library Screening for *Pseudomonas aeruginosa* AmpR Inhibitors

Author Block:

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Abstract Body:

Failure of current antibiotic therapy to treat *Pseudomonas aeruginosa* infections is predominantly responsible for many life threatening acute and chronic human infections. This study aims to identify novel therapeutic agents against *P. aeruginosa* that can target its virulence and antibiotic resistance at the same time. For this, a high throughput screening approach is undertaken to identify inhibitors of a global regulator, AmpR, which is critical for its antibiotic resistance and acute virulence. Previous studies have shown that besides positively regulating ß-lactam resistance through AmpC ß-lactamase, AmpR regulon includes an extensive list of genes involved in non-ß-lactam resistance, quorum sensing associated virulence, T6SS protein, biofilm formation, regulation of cycling di-GMP, protein phosphorylation, and physiological processes. The objective of this study is to screen for non-bactericidal compound(s) that interfere with the AmpR function, with two specific aims: 1) Screening a chemical library for AmpR inhibitors, and 2) Verification of AmpR inhibitors. With a P_{ampC}-luxCDABE reporter system introduced in *P. aeruginosa* PAO1, a unique scaffold based combinatorial library was screened to identify potential AmpR inhibitors by screening millions of compounds using just 79 samples. Nine positive hits, showing more than 20% reduction in luciferase activity, were identified from the primary screening. The compounds identified in the primary screening contained one of the two core structures: pyrrolidine-based or guanidine-based molecules. Further, these hits will be used for counter selection using other AmpR regulated reporters, such as P_{phzA} and P_{mexE} to confirm specificity of AmpR inhibition. Positional scanning based secondary screening will be used to identify individual target compounds. The best hits from Aim 1 will be tested for human cell-cytotoxicity and their ability to affect *P. aeruginosa* virulence and antibiotic resistance using various *in-vitro* and *in-vivo* assays. The compounds identified in this study will hold promise to be developed as novel drugs that would prevent acute to chronic progression as well as greatly improve current antibiotic treatments against *P. aeruginosa* and other pathogens harboring AmpR homologues.

Author Disclosure Block:

H. Kumari, None..
M. Giulianotti, None..
S. Dhar, None..

C. Bernard, None..
K. Morales, None..
K. Mathee, None.

Poster Board Number:

LB-055

Publishing Title:

Gram-negative Sepsis During Alemtuzumab Conditioning for Hematopoietic Stem Cell Transplantation (HSCT) in Chronic Granulomatous Disease (CGD)

Author Block:

M. PARTA[1], D. Hilligoss[2], M. Marquesen[2], C. Kelly[2], N. Kwatemaa[2], H. Malech[2], E. M. Kang[2]; [1]Leidos BioMed. Res., Inc., Bethesda, MD, [2]Natl. Inst. of Allergy and Infectious Diseases/NIH, Bethesda, MD

Abstract Body:

Neutropenia with an absolute neutrophil count (ANC) <500/mm^3 is the dominant risk for bacterial infection in the early phase of HSCT. Four patients with CGD undergoing HSCT had bacteremia prior to neutropenia with bacteria that often cause disease in CGD without neutropenia.Four male patients, age 3-24 years, underwent HSCT for X-linked CGD. Three donors were matched/unrelated, one matched-related. All received alemtuzumab, day -8 to -4, dose-adjusted busulfan days -3 and -2. Total body irradiation of 300 cGy was used for recipients from unrelated donors.All patients had fever with alemtuzumab. Fever after completion of alemtuzumab was recurrent (3 cases) or persistent. *K. pneumoniae* (2 cases), *Serratia* sp. and *Burkholderia cepacia* complex bacteremias occurred on days -4 to 4. Average absolute lymphocyte count (ALC) and ANC on the days of bacteremia were 37/mm^3 and 2.89/mm^3. The interval from bacteremia to neutropenia was 5.5 days (range 1-10). Prolonged *Burkholderia* bacteremia and hypotension required intensive care unit support for 3 days. Engraftment occurred at an average of 23 days (range 16-39) in all.In conclusion, 4 patients with X-linked CGD undergoing HSCT had Gram-negative bacteremias during immunosuppressive conditioning prior to neutropenia. These events have led us to institute antibacterial prophylaxis at the start of conditioning. Funded by the NCI Contract No. HHSN261200800001E.

Author Disclosure Block:

M. Parta, None..
D. Hilligoss, None..
M. Marquesen, None..
C. Kelly, None..
N. Kwatemaa, None..
H. Malech, None..
E. M. Kang, None.

Poster Board Number:

LB-056

Publishing Title:

Non-hydroxamic Acid Lpxc Inhibitors, Their In Vitro And In Vivo Antimicrobial Activities

Author Block:

m. teng, D. Puerta, K. taganov; Forge Therapeutics, San Diego, CA

Abstract Body:

Background: Gram-negative infections continue to present threats to public health. With increasing resistance to the existing antibiotics, antibiotics with new mechanism of actions or through new targets are needed. LpxC is a zinc-dependent UDP-3-*O*-(*R*-3-hydroxymyristol)-*N*-acetylglucosamine deacetylase. It is responsible for the biosynthesis of lipid A which provides an anchor for lipopolysaccharide of the outer membrane of Gram-negative bacteria. LpxC inhibitors inhibit the biosynthesis of lipid A, disable the outer membrane assembly, and render the antibacterial activity. Though LpxC has attracted attention from drug hunters, no inhibitor had advanced to clinic. Nearly all known potent LpxC inhibitors contain hydroxamates as war-heads to coordinate the Zn(II) metal ion in the enzyme. Researches have suggested that part of the failures of these compounds are due to the liability of hydroxamate group, including high clearance, known toxicities (Ames, micronucleus) and poor target selectivity. We set out to discover non-hydroxamate LpxC inhibitors using fragment based design strategy starting out from the Zn-binding fragments. **Methods:** Through a screening of our metal binding fragment library against *E. coli* LpxC at 200 μM, we identified a handful of fragments. These were modeled into the LpxC. Those that provided the right trajectory into the hydrophobic tunnel were selected for further structural elaborations.
Results: Compounds were tested against *E. coli*, *P. aeruginosa* , *K. pneumoniae* and selected biodefense microbes. The most potent non-hydroxamate compounds possess MIC of 0.25 ug/mL against wild type *E. coli*. and 4 ug/mL against pump knock-out *P. aeruginosa* and showed no activity towards *S. aureus*. These compounds displayed potent IC_{50} against both Kpn and Pae enzymes. Cross screen against MMPs, HDACs, lysine demethylases, and carbonic anhydrase showed these inhibitors are highly selective for LpxC. A few compounds were evaluated in the murine thigh model against wild type E. coli infection and showed efficacy.
Conclusions: We have identified potent and selective non-hydroxamate inhibitors. These compounds showed good potency against broad panel GN bacteria with superb target selectivity. In vivo efficacy has been demonstrated in murine thigh infection model. Further effort will be focused on improving Pae activity, overcoming efflux pump and membrane permeability.

Author Disclosure Block:

M. teng, None..
D. Puerta, None..
K. taganov, None.

Poster Board Number:

LB-057

Publishing Title:

Safety and Pharmacokinetics of Multiple Doses of CD101 Injection: Results from a Phase 1, Dose-escalation Study

Author Block:

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Abstract Body:

CD101 Injection is a novel echinocandin being developed as a high-exposure, once-weekly agent for the treatment and prevention of invasive fungal infections. CD101 has potent in vitro and in vivo activity against a broad range of *Candida* and *Aspergillus* spp. CD101 Injection was evaluated in a randomized, double-blind, placebo-controlled, dose-escalation study to establish the safety, tolerability, and pharmacokinetics (PK) of multiple intravenous doses. In 3 sequential cohorts of 8 healthy subjects (n=6, active; n=2, placebo), CD101 Injection was infused over 1 h, once weekly (100 mg × 2 doses [Cohort 1], 200 mg × 2 doses [Cohort 2], 400 mg × 3 doses [Cohort 3]), with dose escalation by predefined safety criteria. Extensive plasma and urine sampling over 28 (Cohorts 1 and 2) or 35 days (Cohort 3) was performed for PK analysis. Safety and tolerability was assessed by adverse events (AEs), vital signs, physical exams, electrocardiograms (ECGs), and hematology and clinical chemistry laboratories up to 21 days after dosing. Subjects (N=24, randomized and completed all assessments) were primarily White (88%), Hispanic or Latino (75%); males and females were equally represented. There were no serious or severe AEs. The majority of AEs were mild; all AEs completely resolved by study completion. There was a higher incidence of AEs and mild, transient infusion reactions in Cohort 3. There were no clinically significant hematology or clinical chemistry laboratory abnormalities at any dose. Additionally, there were no safety issues related to ECGs, vital signs, or physical exam findings. CD101 plasma exposures were dose-proportional. The average C_{max} ranged from 6.49 (Cohort 1, Day 8) to 30.5 µg/mL (Cohort 3, Day 15); corresponding area under the curve ($AUC_{0\text{-}168h}$) values were 390 to 1840 µg·h/mL. Accumulation was minor (30% to 55%, $AUC_{0\text{-}168h}$ ratio of last/first dose). Apparent clearance of CD101 was low (<0.28 L/hour), and its half-life was long ($t_{1/2}$ >80 h). Excretion in urine was minimal (<1%). CD101 Injection was safe and well tolerated at multiple doses up to 400 mg once weekly for 3 weeks, exhibited long plasma $t_{1/2}$, and maintained plasma exposures that support once-weekly dosing. The overall safety, tolerability, and PK profile of CD101 support continued development as a once-weekly therapy for invasive fungal infections.

Author Disclosure Block:

T. Sandison,
Cidara Therapeutics, Inc. Role(s): Self, D. Employee.
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Celerion Role(s): Self, D. Employee.
Cidara Therapeutics, Inc. Role(s): Self, H. Research Contractor.
V. Ong,
Cidara Therapeutics, Inc. Role(s): Self, D. Employee.
J. Lee,
Cidara Therapeutics, Inc. Role(s): Self, D. Employee.
Cidara Therapeutics, Inc. Role(s): Self, K. Shareholder (excluding diversitied mutual funds).
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Cidara Therapeutics, Inc. Role(s): Self, D. Employee.
Cidara Therapeutics, Inc. Role(s): Self, K. Shareholder (excluding diversitied mutual funds).

Poster Board Number:

LB-058

Publishing Title:

Multiple Active Site Substitutions in the Native Class D β-lactamase of *Acinetobacter baumannii*, OXA-51/66, Result in Increased Hydrolytic Activity Toward Carbapenems

Author Block:

E. C. Schroder[1], Z. Klamer[1], A. Saral[1], K. A. Sugg[1], C. M. June[1], R. A. Bonomo[2], A. Szarecka[1], D. A. Leonard[1]; [1]Grand Valley State Univ., Allendale, MI, [2]Cleveland VA, Cleveland, OH

Abstract Body:

Background Class D β-lactamases confer carbapenem resistance in *Ab*. Until recently, the chromosomally-encoded carbapenemase of *Ab* (OXA-51/66) was considered of little threat because of its inherent low affinity and turnover rate for carbapenems. Notably, several hundred clinical variants of OXA-51/66 have been reported, many with substitutions of active site residues. Herein, we investigated the kinetic properties of OXA-66 and clinical variants toward β-lactam antibiotics.
Methods OXA-66 and five clinical variants of OXA-66 were studied. Kinetic constants for imipenem, doripenem (Dori) and a variety of penicillins, cephalosporins and monobactam substrates were determined for all 5 variants. To explain the kinetic results, both OXA-66 and OXA-66 P130Q (OXA-109) were simulated for 200 nsec using CHARMM Molecular Dynamics (MD) package and force-field.
Results The presence of a P130Q mutation in OXA-66 results in a 15-40 fold increase in carbapenem hydrolysis efficiency (e.g. Dori k_{cat}/K_M 0.010 → 0.360 μM^{-1} s^{-1}), with modest or no gain in activity toward a variety of penicillins and cephalosporins. Compared to OXA-66, an OXA-66 triple-substitution clinical variant (I129V/W222L/P226S) displayed 20-70 fold increases against penicillin and carbapenems (Dori k_{cat}/K_M 0.010 → 0.710 μM^{-1} s^{-1}). Interestingly, while OXA-66 does not hydrolyze cephalosporins or monobactams, the triple-variant also showed increased hydrolytic activity toward cefotaxime, ceftriaxone, ceftazidime, cefepime and aztreonam (Azt $k_{cat} < 0.05$ → 0.340 s^{-1}). MD simulations show that in OXA-66, P130 inhibits side-chain rotation of I129 leading to steric prevention of Dori binding. A Gln substitution at position 130 greatly enhances I129 mobility, lessening this steric clash.
Conclusions Analysis of several clinical variants of the OXA-66 carbapenemase of *Ab* show that active site substitutions are emerging that lead to increased hydrolytic activity toward carbapenems and many other classes of β-lactam antibiotics. This evolution of substrate specificity represents a very worrisome development for the use of β-lactams against this troublesome pathogen.

Author Disclosure Block:

E. C. Schroder, None..
Z. Klamer, None..
A. Saral, None..
K. A. Sugg, None..
C. M. June, None.
R. A. Bonomo,
Astra-Zeneca Role(s): Self, I. Research Relationship.
Merck Role(s): Self, I. Research Relationship.
Wockhardt Role(s): Self, I. Research Relationship.
CheckPoints Role(s): Self, I. Research Relationship.
Actavis Role(s): Self, I. Research Relationship.
NIH Role(s): Self, I. Research Relationship.
VA Role(s): Self, I. Research Relationship.
A. Szarecka, None..
D. A. Leonard, None.

Poster Board Number:

LB-059

Publishing Title:

Alterations in Membrane Fluidity Appears to Influence Drug Resistance in Opportunistic Pathogen, *Candida albicans*

Author Block:

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Abstract Body:

Cell membrane serves as a permeability barrier for the entry of biomolecules. Physical properties of membrane strongly influence their biological functions. This study is an effort to characterize and correlate the membrane fluidity with observed fluconazole resistance in a pair of isogenic clinical isolates (F1-sensitive and F5-resistant) of *Candida albicans* isolated from AIDS patient with recurrent oropharyngeal candidiasis (OPC). Microviscosity is the reciprocal of fluidity and gives measure of fractional resistance to rotational and translational motion of the molecule. The fluorescent probe, 1,6-Diphenyl-1,3,5-hexatriene (DPH) has been used to determine the fluidity and microviscosity. The resistant isolate F5 was found to attain a more rigid membrane than the sensitive isolate F1. Increased membrane rigidity may be responsible for reduced drug entry by passive diffusion in the resistant isolate. Classical MDR is mostly attributed to the overexpression of the drug efflux pump proteins. High expression levels were previously reported in the resistant isolate F5 for *MDR1* gene, encoding MFS superfamily transporter and *ERG11* gene, encoding the azole target sterol 14α-demethylase. In view of the multitude of factors responsible for MDR, it was pertinent to characterize the changes in structure and function of cell membrane between resistant and sensitive clinical isolates for complete elucidation of the MDR mechanism and to design better therapeutic strategies for MDR reversal. Changes in membrane fluidity, a parameter of physical state of the membrane may also affect MDR by influencing entry or efflux of drugs. Observed drug resistance also appeared to be influenced by host factors, prior drug treatment and efflux pump overexpression. With the change in growth phase of cells, there was an observed change in membrane fluidity and the *Candida* cells appeared to adapt to environmental changes through modulation of their membrane properties and lipid composition leading to altered membrane fluidity.

Author Disclosure Block:

T. Prasad, None..
V. S. Radhakrishnan, None..
A. Kumar, None..
R. Singh, None..

M. Kumar, None..
N. N. Mishra, None.

Poster Board Number:

LB-060

Publishing Title:

Microbiome Antagonism of *Pseudomonas aeruginosa* Gastrointestinal Colonization

Author Block:

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Abstract Body:

P. aeruginosa is found as a prominent member of the human gut microbiome, but the oral dose required to establish measurable colonization in a healthy subject is high. We hypothesized that there is a role for resident host microbiota in preventing gastrointestinal (GI) colonization and dissemination by *P. aeruginosa*. Germ-free and conventional BL-6 mice treated with antibiotics were challenged with *P. aeruginosa*. Twenty-four hours post-gavage, intestines were harvested and the CFU of surviving *P. aeruginosa* measured. Germ-free mice mono-colonized with *Bacteroides fragilis,* a dominant member of the human GI microbiome, were challenged similarly with *P. aeruginosa*. We found that a significant reduction in the presence of culturable microbiota was a prerequisite for mouse GI tract colonization. Mice colonized by *B. fragilis* showed more resistance to dissemination by *P. aeruginosa* compared to germ-free mice. We identified a transposon insertion mutant of *P. aeruginosa* that colonizes the intestines of both conventional antibiotic treated mice as well as *B. fragilis* mono-colonized mice at levels approximately 1,000-fold higher than that observed for the wild-type parental strain. The transposon insertion carried by this mutant causes a 300-fold increase in transcription of a *P. aeruginosa* efflux pump. Inactivation of the same efflux pump also significantly reduced intestinal colonization relative to the parental strain. The human GI microbiota act as protectors in the GI tract by preventing the dissemination of pathogenic bacteria throughly largely unknown mechanisms. Utilizing *P. aeruginosa* (pathogen) and *B. fragilis* (host commensal) as model organisms, we showed that *B. fragilis* alone restricts dissemination of *P. aeruginosa*. The level of expression of a *P. aeruginosa* efflux pump system correlated with the level of intestinal colonization. This result suggests that the host commensal microbiome restricts pathogen colonization and dissemination through the production of antagonistic small molecules that are transported by the efflux systems of pathogens. Together with mounting evidence that the host microbiome can modulate immune and metabolic homeostasis, commensal microorganisms may protect against invading bacterial pathogens by producing antibacterial compounds that are active *in vivo*.

Author Disclosure Block:

K. E. R. Bachta, None..
W. Zheng, None..

J. E. Hudak, None..
S. Oh, None..
D. L. Kasper, None..
J. J. Mekalanos, None.

Poster Board Number:

LB-061

Publishing Title:

Quorum Sensing Regulation of the CRISPR-Cas Adaptive Immune System in Pseudomonas aeruginosa

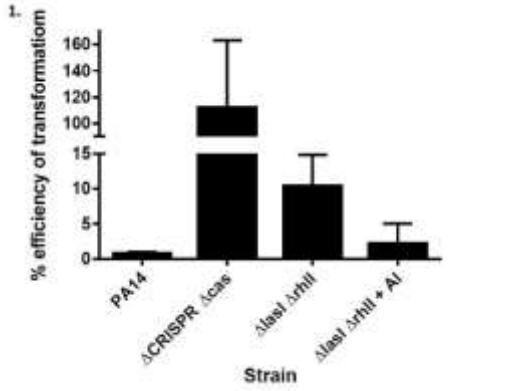
Author Block:

N. M. Hoeyland-Kroghsbo[1], J. E. Paczkowski[1], S. Mukherjee[1], E. R. Westra[2], J. Bondy-Denomy[3], B. L. Bassler[4]; [1]Princeton Univ., Princeton, NJ, [2]Univ. of Exeter, Penryn, United Kingdom, [3]The Univ. of California, San Francisco, San Francisco, CA, [4]Howard Hughes Med. Inst., Bethesda, MD

Abstract Body:

In a process called quorum sensing (QS), bacteria communicate, monitor population density, and control collective behaviors including virulence. QS relies on the production, release, and detection of diffusible signaling molecules called autoinducers (AI). Bacteria at high cell density maximally engage in QS. These cells are particularly vulnerable to bacteriophage infections. QS control of antiphage activities could enable bacteria to activate phage defense mechanisms specifically when they are at the highest risk of infection. CRISPR-Cas systems provide bacteria and archaea with acquired and heritable sequence-specific immunity against their parasites, such as phages and plasmids. CRISPR transcripts guide Cas proteins to cleave complementary parasite genomes, which provides immunity. There is little understanding of the regulation of these immune systems. In *Pseudomonas aeruginosa* PA14, we used qRT-PCR to measure the effect of QS on *cas* gene expression. We assayed the effect of QS on the activity of the PA14 CRISPR-Cas system to successfully eliminate a CRISPR-targeted plasmid containing a protospacer sequence (Fig. 1). We find that PA14 uses QS through the Las and Rhl systems to increase the relative transcript levels of the *cas* genes, which in turn leads to a potent increase in CRISPR-Cas activity. QS control of the CRISPR-Cas adaptive immune system has a profound influence on the success of CRISPR-mediated defense. Our discovery may prove particularly important for the development of QS-inhibitors and phage therapies as alternatives to traditional antibiotics.

1.



Author Disclosure Block:

N. M. Hoeyland-Kroghsbo, None..
J. E. Paczkowski, None..
S. Mukherjee, None..
E. R. Westra, None..
J. Bondy-Denomy, None..
B. L. Bassler, None.

Poster Board Number:

LB-062

Publishing Title:

Antibiotic Resistance is Induced by a Bacterial Starvation Signal in Vibrio cholerae

Author Block:

H. Kim, Y. Oh, S. Yoon; Yonsei Univ. Coll. of Med., Seoul, Korea, Republic of

Abstract Body:

When bacteria encounters variety of growth-inhibitory stresses, the stringent response (SR) is activated by accumulation of (p)ppGpp, a small nucleotide regulator known as a stress alarmone. Accumulation of (p)ppGpp results in a profound reprogramming of global gene expression. The genes *relA* and *relV* are involved in the production of (p)ppGpp, whereas the *spoT* gene encodes an enzyme that hydrolyzes it in *Vibrio cholerae*. Here, we show that the bacterial capability to produce (p)ppGpp is deeply involved in regulating antibiotic resistance in *V. cholerae*. N16961, a 7th pandemic *V. cholerae* strain, became resistant to tetracycline, when (p)ppGpp accumulation was induced. N16961 and *ΔrelAΔspoT*, a (p)ppGpp-overproducing mutant strain, were more resistant to the treatment of lethal concentration of tetracycline, when compared with (p)ppGpp^0 (i.e.*ΔrelAΔrelVΔspoT* mutants) and *ΔdksA* mutants, which cannot synthesize nor utilize (p)ppGpp for transcription, respectively. Similar effects were also induced, in response to the treatment with other antibiotics, such as erythromycin and chloramphenicol. No remarkable differences, however, were observed when bacterial strains were treated with aminoglycosides. Furthermore, the *ΔrelAΔspoT* mutant, which mounted resistance, lost typical curved-rod shape morphology and looked smaller in size based on our scanning electron microscope image analysis. N16961, when harvested at stationary phase, also exhibited similar cell shape change, while (p)ppGpp^0 and *ΔdksA* mutants maintained their normal cell shape. We also found that N16961 gained smaller cell shape phenotype, when (p)ppGpp accumulation was induced by serine hydroxamate at exponential phase. Together, our results demonstrate that the alteration of bacterial cell phenotype by accumulation of intracellular (p)ppGpp is associated with antibiotic resistance in *V. cholerae*. Future research will clearly reveal a previously unexplored role of the bacterial starvation signal in regulating bacterial resistance phenotype.

Author Disclosure Block:

H. Kim, None..
Y. Oh, None..
S. Yoon, None.

Poster Board Number:

LB-063

Publishing Title:

Understanding Intragenic Transcription and its Regulation

Author Block:

L. E. Lamberte, B15 2TT, S. S. Singh, B15 2TT, D. C. Grainger; Univ. of Birmingham, Birmingham, United Kingdom

Abstract Body:

Horizontally acquired DNA, which is AT-rich in nature, is known to be toxic to bacterial cells. Furthermore, the nucleoid-associated protein H-NS counteracts this toxicity. Despite its central role in shaping the evolution of bacterial genomes, molecular explanations on the mechanisms behind the toxicity of AT-rich DNA and regulation of AT-rich DNA by H-NS are lacking. It was previously thought that AT-rich genes are subject to canonical regulation by H-NS (i.e. H-NS silences activity coming from the genuine promoter). However, this work demonstrates the phenomenon of "pseudo-regulation". Using an array of biochemical, genetic, and bioinformatic approaches, we show that H-NS silences activity coming from internal promoters within the coding sequence of an AT-rich gene, rather than the genuine promoter. In addition, we also show that the toxicity of this gene is due to the presence of these internal promoters. This phenomenon is likely widespread, and is demonstrated by this study in a number of AT-rich genes. In addition, the transcription terminator Rho provides an additional mechanism for regulation by terminating intragenic transcripts coming from the H-NS-targeted AT-rich genes. We predict that all AT-rich genes are likely to be enriched for internal promoters. This study therefore provides a molecular explanation for the toxicity of AT-rich DNA that may extend to all domains of life.

Author Disclosure Block:

L. E. Lamberte, None..
S. S. Singh, None..
D. C. Grainger, None.

Poster Board Number:

LB-064

Publishing Title:

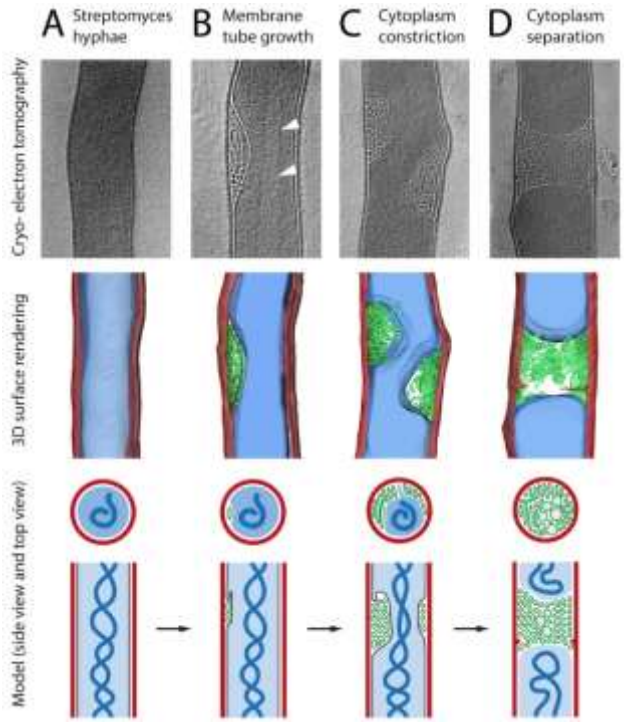
Cross-membranes Orchestrate Compartmentalization and Morphogenesis in Streptomyces

Author Block:

G. P. van Wezel[1], K. Celler[1], J. Willemse[1], R. Koning[2], A. J. Koster[2]; [1]Leiden Univ., Leiden, Netherlands, [2]Leiden Univ. Med. Ctr., Leiden, Netherlands

Abstract Body:

Bacteria have complex social behaviour and organization, some living as coherent, multicellular entities. The filamentous streptomycetes grow as a multicellular syncytial mycelium with physiologically distinct hyphal compartments separated by cross-walls. The viability of mutants devoid of cell division, which can be propagated from fragments, suggests a different form of compartmentalization. We have discovered that complex membrane structures, visualized by cryo-correlative light microscopy and electron tomography, fulfil this role. Membranes form small assemblies between the cell wall and cytoplasmic membrane, or, as evidenced by FRAP, large protein-impermeable cross-membrane structures, which compartmentalize the multinucleoid mycelium. All areas containing cross-membrane structures are nucleoid-restricted zones, suggesting that the membrane assemblies also act to protect nucleoids from cell-wall restructuring events. Our work reveals an entirely novel mechanism of controlling compartmentalization and chromosome segregation in multicellular bacteria. A model for the role of cross-membranes in compartmentalization and multicellular growth will be presented.



Author Disclosure Block:

G. P. van Wezel, None..
K. Celler, None..
J. Willemse, None..
R. Koning, None..
A. J. Koster, None.

Poster Board Number:

LB-065

Publishing Title:

Understanding Copper Uptake in Gram Negative Bacteria

Author Block:

A. González Sánchez, C. A. Ramirez Cubillas, A. Dávalos, A. García-de-los-Santos; Ctr. for Genomics Sci., UNAM, Cuernavaca, Morelos, Mexico

Abstract Body:

Copper (Cu^{2+}) is an essential micronutrient for all forms of life. Oxidation states (Cu^{+}/Cu^{2+}) this metal is ideal cofactor of enzymes catalyzing redox reactions in vital biological process as respiration and others. Uptake of Cu^{2+} is the unknowing part of a fine regulated Cu-trafficking network mediated by protein-protein interactions that deliver Cu^{2+} to target proteins and efflux excess of metal to avoid toxicity. To date, inner and outer membrane proteins (OMP) involved with import of copper has not been characterized in Gram negative bacteria.
In this study we report the identification and partial characterization of a putative OMP involved with Cu^{2+} uptake in *Rhizobium etli* CFN42, a facultative symbiotic diazotroph that must ensure appropriate Cu^{2+} supply for living either free in the soil or as intracellular symbiont of leguminous plants. The putative Cu^{2+}-uptake OMP described in this study was identified in an isogenic mutant, more tolerant to Cu^{2+} than the parental strain, which spontaneously lost 200 kb of plasmid p42e. This Cu^{2+}-resistance phenotype suggested that a putative Cu^{2+}-uptake protein may be encoded in such region. Copper tolerant mutants with smaller deletions of plasmid p42e shared the absence of different putative transporters including an OMP annotated as RopAe. Disruption of *ropAe* gene was the only mutation that increased copper tolerance to *R. etli.* Bioinformatic predictions, genetic experiments and RT-qPCR analyses supported the hypothesis that *ropAe* gene maintain a basal transcription level that facilitates copper uptake. Under copper limitations, the transcription of *ropAe* increases and the putative OMP worked as a high-affinity copper β-barrel channel that permits the copper uptake across outer membrane.

Author Disclosure Block:

A. González Sánchez, None..
C. A. Ramirez Cubillas, None..
A. Dávalos, None..
A. García-de-los-Santos, None.

Poster Board Number:

LB-066

Publishing Title:

Increased Expression of Genes Involved in Uptake and Degradation of Murein Tripeptide under Nitrogen Starvation in Escherichia coli

Author Block:

U. Choi[1], Y-J. Seok[2], C-R. Lee[1]; [1]Myongji Univ., Yongin, Korea, Republic of, [2]Seoul Natl. Univ., Seoul, Korea, Republic of

Abstract Body:

Background: Peptidoglycan (also known as murein) is an important envelope component of bacteria, and its turnover usually takes place at low levels during normal growth and at considerable levels under stress conditions. Degraded amino sugars and murein tripeptide are used for re-synthesis of peptidoglycan or as self-generated nutrients or energy sources for cell growth. PgrR (regulator of peptide glycan recycling; formerly YcjZ) was recently identified as a repressor of several genes participating in uptake and degradation of murein tripeptide. **Methods:** To identify genes regulated by PgrR, the genome-wide transcription profile in the *pgrR* mutant was carried out by RNA sequencing. These transcriptome data were confirmed by quantitative RT-PCR (qRT-PCR) analysis. To determine whether transcriptions of identified genes are directly regulated by PgrR, an electrophoretic mobility shift assay (EMSA) using DNA fragments upstream of the identified genes as a probe was also performed. Eenvironmental conditions that induce the expression of PgrR-regulated genes were determined using several *lacZ* fusion constructs, such as *ycjY-lacZ*, *mpaA-lacZ*, and *mppA-lacZ* fusion constructs. We also examined the phenotype of the *pgrR* mutant strain. **Results:** The *ycjG* gene involved in murein tripeptide degradation as a new direct target of PgrR. The expression of these PgrR-regulated genes was repressed in the presence of a good nitrogen source, but their expression increased under poor nitrogen conditions because of a nitrogen starvation-induced decrease in the PgrR protein level. Under nitrogen starvation, the *pgrR* mutant cells exhibited faster growth than wild-type cells, implying that derepression of genes under the control of PgrR may help cells overcome nitrogen limitation. **Conclusions:** Nitrogen starvation induces PgrR-mediated derepression of genes involved in uptake and degradation of murein tripeptide, and this may stimulate the utilization of murein tripeptide as a nitrogen source.

Author Disclosure Block:

U. Choi, None..
Y. Seok, None..
C. Lee, None.

Poster Board Number:

LB-067

Publishing Title:

Structure-based Engineering of Type II Acyl Carrier Protein Interactions

Author Block:

J. C. Milligan, D. R. Jackson, A. J. Schaub, S. Tsai; Univ. of California, Irvine, Irvine, CA

Abstract Body:

Background: The *E. coli* type II fatty acid synthase (FAS) has long been a target for biofuel production given its ability to efficiently produce hydrocarbon chains. Though the individual enzymes in the pathway have been structurally and functionally characterized, uncovering the key protein-protein interactions, particularly between the acyl carrier protein (ACP) and its partner enzymes, has remained elusive due to the transient nature of the interactions. Without this structural information, engineering efforts and combinatorial biosynthesis have proved unsuccessful.
Methods: Here, we present the application of a mechanism-based crosslinker to trap an ACP-ketosynthase (KS) complex and solve its crystal structure.
Results: The structure of this ACP-KS complex dimer reveals the key interactions between the ACP (AcpP) and one of the KS enzymes in the pathway (FabB). Structural analysis suggests that allosteric mechanisms exist to facilitate enzyme turnover by forcing the dissociation of one AcpP upon docking of a second AcpP to the opposite FabB monomer. This hypothesis was tested using molecular dynamics simulations which predict that a network of interactions exists from one AcpP across the FabB dimer to the second AcpP, forcing its dissociation. Using this structural and dynamics data, several surface mutants of AcpP and FabB were generated and overexpressed in *E. coli* BL21 cells. As hypothesized, the lipid profile of the cultures was altered due to the disruption of this key protein-protein complex that is known to affect chain length and saturation.
Conclusions: This work represents the first crystal structure of an ACP-KS complex as well as the first successful attempt to alter the product profile of this metabolic pathway by specifically targeting protein-protein interactions.

Author Disclosure Block:

J. C. Milligan, None..
D. R. Jackson, None..
A. J. Schaub, None..
S. Tsai, None.

Poster Board Number:

LB-068

Publishing Title:

Acquisition of Non-transferable Plasmid and its Deleterious Effect upon UV Exposure

Author Block:

M. shahnaij; icddr,b, Dhaka, Bangladesh

Abstract Body:

Background: Serendipitily an *E. coli* strain (ID SK-13) which can acquire non-transferable plasmid via conjugation in considerably high rate has been isolated from stool sample. Non-conjugable plasmids of Shigella flexneri 2a, 2b, 3a, 3b and X variant were transferred to this recipient *E. coli* strain SK-13 (Lac+, Ara+ and F-). UV light susceptibility test were performed on exconjugant and their cured counterpart to analyze the effect of UV light on non-transferable plasmid.
Methods: Host range was determined using different serotypes of *Shigella* spp.(n=50), *E. coli* (n=20) and *Salmonella* (n=20). Broth mating technique was performed to prepare ex-conjugants, acridine orange facilitated curing of transferable plasmid (20-100Mda) and confirmed by plasmid profile analysis. Exconjugants and its cured strains having different plasmid profile were subjected to varying doses of UV light to elucidate the effect of acquired non-transferable on survival after UV irradiation.
Result: Donor *S. flexneri* 2a, 2b, 3a, 3b and X variant transferred non-transferable plasmid along with conjugative plasmid to recipient *E. coli* strain SK-13. On the other hand, other tested donor strains, different serotype of *Shigella* spp. except above mentioned 5 sub-serotypes of *S. flexneri*, *E. coli* and *Salmonella* spp., did not mobilize non-transferable plasmid but rather transfer only conjugative plasmid. Plasmid transfer rate of conjugative plasmid in SK-13 was 5×10^{-4} (median) and in which 5-30% ex-conjugants was harbor non-transferable plasmid/s in ascending order of molecular weight and in cumulative manner. Comparison of UV light susceptibility test on different plasmid pattern of ex-conjugant and their respective cured counterparts indicated that upon UV exposure, acquisition of non-transferable plasmid/s might be increased DNA breakage, thus reduced number of UV induced bacteria were survived by DNA repair system. In summary, this study explained the natural phenomenon, why mobilization of non-transferable plasmid is rare and low.

Author Disclosure Block:

M. shahnaij, None.

Poster Board Number:

LB-069

Publishing Title:

Survey of Antibiotic Resistance Genes in Soil Near Two Antarctic Research Stations in Terra Nova Bay

Author Block:

F. Wang[1], **R. D. Stedtfeld**[2], O-S. Kim[3], S. Hashsham[2], J. M. Tiedje[2], W. Sul[4]; [1]Inst. of Soil Sci., Chinese Academy of Sci., Nanjing, China, [2]Michigan State Univ., E. Lansing, MI, [3]Korea Polar Res. Inst., Incheon, Korea, Republic of, [4]Chung-Ang Univ., Anseong, Korea, Republic of

Abstract Body:

Soil is one of the most important environmental carriers of antibiotic resistance genes, which are increasingly perceived as environmental contaminants. Methods to assess the risks associated with the acquisition or transfer of resistance mechanisms are still underdeveloped. Assessment of this risk requires quantification of background levels of antibiotic resistance genes in different matrices. Towards this goal, samples were collected from soils near the 30-year old Gondwana Research Station and before and during newly developed Jangbogo Research Station from the Terra Nova Bay in Antarctica. A qPCR array with 384 primer sets targeting antibiotic resistance genes and mobile genetic elements was used to detect and quantify these genes. A total of 74 antibiotic resistance genes and mobile genetic elements encompassing eight major antibiotic resistance gene categories were detected. Seven antibiotic resistance genes were present in most samples, potentially indicating natural existence in Antarctica soil. In addition, samples significantly grouped based on spatial proximity to research stations.These results suggest that certain types of antibiotic resistance genes may occur in pristine environmental soils regardless of anthropogenic activity.

Author Disclosure Block:

F. Wang, None..
R. D. Stedtfeld, None..
O. Kim, None..
S. Hashsham, None..
J. M. Tiedje, None..
W. Sul, None.

Poster Board Number:

LB-070

Publishing Title:

CSA-131 and CSA-138, Cationic Steroid Antibiotics, Inhibit Pseudomonas aeruginosa Biofilm Formation

Author Block:

C. Bozkurt Güzel[1], M. Tuysuz[1], G. Inci[1], P. B. Savage[2]; [1]Istanbul Univ., Istanbul, Turkey, [2]Brigham Young Univ., PROVO, UT

Abstract Body:

Background: *Pseudomonas aeruginosa* can cause hard to treat life-threatening infections due to its high resistance to antibiotics and to the ability to form antibiotic tolerant biofilms. The biofilm-forming capability of *P. aeruginosa* is a crucial virulence attribute that is responsible for its increased resistance to different classes of antibiotics and disinfectants. As chronic infections are difficult to treat, attempts have been made to discover new therapeutics targeting novel sites. The ceragenins, designed to mimic the activities of antimicrobial peptides, represent a new promising group of agent that revealed a potent *anti-P. aeruginosa* action.
Methods: A total of 25 *P. aeruginosa* isolates and control strain were used. Firstly, biofilm formation and determination of MIC values of CSA 13, CSA 44, CSA 131, CSA 138, ciprofloxacin and colistin were evaluated with crystal violet staining method and microbroth dilution technique, respectively. Among these strains four good biofilm producing strains and the control strain were chosen for biofilm studies. Sessile MICs and inhibition of cells adhesion and biofilm formations were evaluated.
Results: Susceptibility testing demonstrated that the MIC_{50} (µg/ml) values of CSA 13, CSA 44, CSA 131, CSA 138, ciprofloxacin and colistin were 8, 8, 8, 16, 1 and 2, respectively. The SMICs were found greater than MICs. Inhibition of adhesion rates depended on time. CSA 13, CSA 44 and CSA 131 were found more efficient after 4 hour incubation although CSA 138, ciprofloxacin and colistin after 1 hour incubation. The most efficient agent for inhibition of adhesion was found colistin (up to %45). Although inhibition of biofilm formation rates depended at concentration, the highest inhibition rates were shown at MICs for all agents, as expected. CSA 131, CSA 138 and colistin were found most efficient agents for inhibition of biofilm formation (up to %90).
Conclusion:
Overall, our study highlighted the potential of CSA-131 and CSA-138 to be used as effective alternatives to the conventional antibiotics against biofilms of *P. aeruginosa*. Future studies should be performed to correlate safety, efficacy and pharmacokinetic parameters of these molecules.

Author Disclosure Block:

C. Bozkurt Güzel, None..
M. Tuysuz, None..
G. Inci, None..
P. B. Savage, None.

Poster Board Number:

LB-071

Publishing Title:

High-yield production of 3-Hydroxybutyric Acid in the acetogen C. ljungdahlii

Author Block:

B. M. Woolston, **D. H. Currie**, G. N. Stephanopoulos; Massachusetts Inst. of Technology, Cambridge, MA

Abstract Body:

Clostridium ljungdahlii has recently emerged as an attractive candidate for the bioconversion of synthesis gas (CO, CO_2, H_2) to a variety of fuels and chemicals through the Wood-Ljungdahl (WL) pathway. Here we examine another use of the WL pathway; namely the augmentation of fermentation yields on heterotrophic substrates by using reducing equivalents produced in glycolysis to fix the CO_2 released, thus resulting in complete carbon conversion. As a proof of concept, we focused on 3-hydroxybutyric acid (3HB), a chiral compound that serves as feedstock in a variety of applications. First, we varied promoters, codon usage, and ribosome binding site spacing to optimize 3HB production, achieving titers of 1 g/L. Second, we implemented a CRISPRi approach to reduce flux to unwanted byproducts. Finally, we quantified the activity of the WL pathway during fermentation using isotopic tracers. The results demonstrate the ability to significantly enhance fermentation yields above those that can be achieved in typical engineering hosts such as *E. coli* or *S. cerevisiae*.

Author Disclosure Block:

B. M. Woolston, None..
D. H. Currie, None..
G. N. Stephanopoulos, None.

Poster Board Number:

LB-072

Publishing Title:

High Throughput Chemical Screening for Inhibitors of Rumen Methanogens

Author Block:

v. carbone[1], k. lunn[1], s. Muetzel[1], m. tavendale[1], s. ganesh[1], Y-L. Zhang[1], C. Sang[1], D. dey[1], R. atua[1], M. Weimar[2], j. cheung[2], p. J. edwards[3], w. B. whitman[4], M. Morrison[5], c. McSweeney[6], y. Kobayashi[7], B. denny[8], L. R. Schofield[1], G. Cook[2], R. S. Ronimus[1]; [1]AgRes. Ltd, Palmerston North, New Zealand, [2]Univ. of Otago, Dunedin, New Zealand, [3]Massey Univ., Palmerston North, New Zealand, [4]Univ. of Georgia, Athens, GA, [5]The Univ. of Queensland, Woolloongabba, QLD, Australia, [6]CSIRO, brisbane, Australia, [7]Hokkaido Univ., Hokkaido Prefecture, Japan, [8]The Univ. of Auckland, auckland, New Zealand

Abstract Body:

Methane emissions from ruminants are widely recognized as one of the significant factors contributing to global climate change. Methane stemming from the fermentation process in the rumen is produced by methanogenic archaea that are typically only a small proportion of the total microbial community (1-4%). One strategy for mitigating these emissions is to develop inhibitory compounds that specifically target rumen methanogens. To accelerate inhibitor discovery, high-throughput screening techniques were developed that simultaneously target the specialized biochemical pathways of methanogens (including methanogenesis, cofactor biosynthesis and archaeal lipid synthesis). Large chemical compound libraries (representing a variety of well-known chemotypes) and natural product libraries (representing unique and diverse chemistry) were sourced from commercial distributors. These were screened for their effects against methanogens using a newly-developed cell-based assay measuring the optical density of pure cultures of methanogens in 96-well plates and a new high-throughput rumen fluid-based assay where methane production is monitored. Several novel methanogen-specific compounds with potential for mitigating methane emissions have been discovered using these methods.

Author Disclosure Block:

V. carbone, None..
K. lunn, None..
S. Muetzel, None..
M. tavendale, None..
S. ganesh, None..
Y. Zhang, None..
C. Sang, None..
D. dey, None..

R. atua, None..
M. Weimar, None..
J. cheung, None..
P. J. edwards, None..
W. B. whitman, None..
M. Morrison, None..
C. McSweeney, None..
Y. Kobayashi, None..
B. denny, None..
L. R. Schofield, None..
G. Cook, None..
R. S. Ronimus, None.

Poster Board Number:

LB-073

Publishing Title:

Co-Detection and Association of O Antigen-specific Genes with Major Virulence Genes of Shiga Toxigenic *Escherichia Coli* Using Digital PCR Technology

Author Block:

X. Liu[1], L. Noll[1], X. Shi[1], A. O'Guin[2], J. Mitchell[3], B. Dalke[3], T. Nagaraja[1], G. Anderson[1], **J. Bai**[1]; [1]Kansas State Univ., Manhattan, KS, [2]Fluidigm Corp., South San Francisco, CA, [3]Thermo Fisher Scientific, Cambridge, MA

Abstract Body:

Shiga toxin-producing *E. coli* (STEC) are major foodborne pathogens and seven major serogroups, O157, O26, O45, O103, O111, O121, and O145 that carry *stx*1 and/or *stx*2 and *eae* genes have been declared as adulterants in ground beef by the USDA-FSIS. The USDA-FSIS recommended culture-based method takes at least a week, and it does not fit high throughput settings. A number of PCR detection assays have been developed; none is capable of associating virulence genes with the STEC O-groups. Some multi-channel digital PCR systems disseminate a PCR reaction into hundreds or thousands of tiny reactions. Each reaction chamber can only hold a single to a couple of templates, providing the opportunity to differentiate if 2 or 3 genes are from a single genome or multiple genomes. Using Thermo Fisher QuantStudio 3D system (20K chambers/chip), we demonstrated, with both pure culture and culture-spiked cattle feces, that the gene association rates of *rfb*E-O157 and *stx*2 from the same genome was significantly higher than that from two separate genomes. The Fluidigm Biomark 37K chip (48 samples/chip, 770 chambers/sample) allowed us to associate each of the seven major O-groups with one of the *stx* genes. Gene association rates for strains that carry two genes were 67.3 to 90.5, with an average of 77.3 for culture and 74.6 to 88.0 with an average of 81.1 for culture-spiked fecal samples. The rates for the two genes carried by separate genomes were 8.7 to 28.8, with an average of 21.4 for culture, and 8.3 to 30.9, with an average of 13.9 for culture-spiked fecal samples. In addition, we were able to associate three genes, *rfb*E-O157, *stx*2 and *eae* from the same *E. coli* genome. We identified three O103 strains that carried *eae*, and three O45 strains that carried *stx*1 from cattle feces, and the whole process took two days. These results were confirmed by the traditional IMS-based isolation, followed by PCR confirmation. To our knowledge, this is the first report of the application of digital PCR system to detect and to associate major virulence genes with O-groups of STEC.

Author Disclosure Block:

X. Liu, None..
L. Noll, None..
X. Shi, None..

A. O'Guin, None..
J. Mitchell, None..
B. Dalke, None..
T. Nagaraja, None..
G. Anderson, None..
J. Bai, None.

Poster Board Number:

LB-074

Publishing Title:

Photoarsenotrophy in Action

Author Block:

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Abstract Body:

Anoxygenic photosynthetic arsenite oxidation "photoarsenotrophy" is a recently identified arsenic metabolism involving the inter-conversion of arsenite to arsenate and coupling to cellular energy production. Because arsenate is less toxic than arsenite, these microbes mediate the attenuation of arsenic toxicity in the environment. Little is known about the impact of photoarsenotrophic bacteria on arsenic biogeochemical cycling. Photoarsenotropy was first identified in *Ectothiorhodospira sp.* Str. PHS-1, cultured from a hot spring on Paoha Island Mono Lake, California. Big Soda Lake (Nevada), a hypersaline alkaline arsenic rich lake (similar to Mono Lake) is also known to have seasonal blooms of similar purple sulfur bacteria (PSB) (e.g. *Ectothiorhodospira* and *Chromatium*). The activities of photosynthetic arsenite oxidizing bacteria were investigated using anaerobic microcosm experiments with Big Soda Lake water collected within the PSB layer. High performance liquid chromatography-inductive coupled plasma-mass spectroscopy was used to detect and quantify arsenite and arsenate. Arsenite oxidation was observed initially in light incubated microcosms containing 50 µM arsenite. Cyclic arsenite oxidation/arsenate reduction was observed when shifting microcosms between light and dark conditions. However arsenate reduction occurred only in the dark. Neither reduction nor oxidation of arsenic was observed in autoclaved water samples, indicating that oxidation and reduction reactions were biologically mediated. The anaerobic arsenite oxidase gene, *arxA,* was detected in light-incubated samples, which was most similar to those within *Ectothiorhodospira* spp. The *arrA* gene for arsenate respiratory reductase was also detected in microcosms incubated in the dark, indicating that *arxA* and *arrA* might be mediating arsenic redox reactions. Big Soda Lake microcosm results support the potential occurrence of light dependent arsenite oxidation and coupling to arsenate reduction in the dark. These results suggest that light-dependent arsenic redox cycling could occur in other arsenic-impacted aquatic systems irrigated with arsenic rich groundwater, such as rice paddies in Bangladesh.

Author Disclosure Block:

J. Hernandez, None..
B. Stoneburner, None..
M. Rosen, None..

L. Miller, None..
R. Ronald Oremland, None..
C. Saltikov, None.

Poster Board Number:

LB-075

Publishing Title:

Parallel Uniform Sequencing of Microbial Aggregates by Barcode Droplet MDA

Author Block:

Y. K. LIght[1], M. Rhee[2], R. J. Meagher[1], A. P. Arkin[3], A. K. Singh[1]; [1]Sandia Natl. Lab., Livermore, CA, [2]Illumina Inc., San Diego, CA, [3]Lawrence Berkeley Natl. Lab., Berkeley, CA

Abstract Body:

Microbial aggregates naturally occurring in an environmental niche are representative of direct microbial interactions, thus understanding this scale of interactions allows us to predict relationships between species and environmental traits with high resolution. To be able to study microbial aggregates at the micron scale, genomic amplification prior to sequencing is required, owing to limited amount of genomic content from microbial aggregates. Multiple displacement amplification (MDA) is robust but it has shown amplification bias that results in uneven sequence coverage. Here, we have developed a platform for unbiased genomic amplification and high-throughput sequencing library preparation to process large number of microbial aggregates in parallel utilizing barcode MDA emulsion droplets. Initially, we used *Ecoli* K-12 MG1655 to perform droplet MDA and compare the evenness of sequencing coverage to that of conventional tube MDA. *E.coli* genomic DNA was denatured and fragmented after heat lysis of cells and each fragmented DNA was partitioned in an emulsion droplet containing MDA reaction mixture and hexamer primer. MDA DNA was pulled together and sequenced. The coverage of genomic DNA was determined by mapping contigs to the reference sequence after *de novo* assembly. For microbial aggregate study, hexamer primer with specific barcode was assigned to each aggregate to handle many aggregates in parallel and whole genome assemblies of members of each microbial aggregate and network analysis of microbial aggregates were performed from the multi-flexed sequence reads. For a sample containing 0.1 pg/µL of *E. coli* DNA (equivalent of ~3/1000 of an *E. coli* genome per droplet), droplet MDA achieved a 65-fold increase in coverage in de novo assembly compared to conventional tube MDA. Indexing of each microbial aggregate together with unbiased amplification utilizing droplet MDA enables us to sequence microbial aggregates with high-throughput and to provide improved sequence coverage of aggregate members. The promise, accordingly, is the ability to link phylogenetics to functional genetics of the members of the community.

Author Disclosure Block:

Y. K. LIght, None..
M. Rhee, None..
R. J. Meagher, None..

A. P. Arkin, None..
A. K. Singh, None.

Poster Board Number:

LB-076

Publishing Title:

Determination of Vancomycin Susceptibility for *Staphylococcus aureus* by Flow Cytometry

Author Block:

A. P. Silva, A. Silva-Dias, R. Teixeira-Santos, **S. Costa-de-Oliveira**, A. G. Rodrigues, C. Pina-Vaz; Faculty of Med., Univ. of Porto, Porto, Portugal

Abstract Body:

Background: Vancomycin MIC determination is recommended whenever considering its use in case of a severe *S. aureus* infection. Moreover, whenever therapeutic failure is suspected, testing for heteroresistant vancomycin intermediate *S. aureus* (hVISA) is also recommended. The methods available for detection of susceptibility to vancomycin are based on MIC determination by broth microdilution, by E-test or automated systems. The main disadvantage of such procedures, is that they require a long incubation period, needing a time to result (TTR) of at least 24h since they are based on growth. Flow cytometry (FC) is an accurate and fast approach for the analysis of cell architecture and its functional parameters. Herein, we propose a FC based approach to determine vancomycin MIC and hVISA strains. **Methods:** Thirty-five *S. aureus* isolates with vancomycin MICs ranging between 0.125 to 2 µg/mL; the Mu50 strain, a vancomycin intermediate *S. aureus* (VISA) strain, with MIC 8 µg/mL, and the Mu3 (hVISA) strain, with MIC 2 µg/mL, both kindly provided by Rafael Canton (Hospital Ramon y Cajal, Madrid), were studied. The vancomycin MIC values were determined by broth microdilution method. *S. aureus* cells were exposed to serial concentrations of vancomycin (from 0.03 to 16 µg/mL) during 1 h/37ºC and stained with a fluorescence probe for assessing membrane depolarization. Non-treated cells were used as control. Afterwards, cells were analyzed in a flow cytometer (BD Accuri™ C6 Cytometer) and a dedicated software. The percentage of depolarized cells exposed to each vancomycin concentration was compared to control cells. Regarding hVISA detection, the Mu3 strain was incubated with 4 µg/mL of vancomycin for 2 h/37ºC and the MIC value re-determined by FC. **Results:** The percentage of depolarized cells significantly increased comparing to control at MIC value plus/minus one dilution. For lower concentrations no significant changes were observed. The test revealed an excellent correlation with the microdilution method. Using FC test we were able to detect the heteroresistant strain; the MIC value increased after pre-incubation with vancomycin. **Conclusions:** We propose a new method for vancomycin MIC determination. The method is fast (TTR of 2h) and easy to perform; its high sensitivity can also be useful for detection of hVISA.

Author Disclosure Block:

A. P. Silva, None..
A. Silva-Dias, None..

R. Teixeira-Santos, None..
S. Costa-de-Oliveira, None..
A. G. Rodrigues, None..
C. Pina-Vaz, None.

Poster Board Number:

LB-077

Publishing Title:

Improved Efficiency with Kiestra Total Laboratory Automation

Author Block:

D. R. Shibib, I. K. Dusich, R. B. Thomson, Jr.; NorthShore Univ. Hlth.System, Evanston, IL

Abstract Body:

Background:
Total laboratory automation (TLA) in Microbiology includes automated specimen processing, incubation, plate imaging, reading of high resolution plate images, and plate discard when results are finaled. We were first in the USA to install the Kiestra TLA System (BD, Sparks, MD) in December, 2014. As of February, 2016, all bacteriology specimen types have been validated and added to the TLA system. To assess the relative efficiencies of TLA compared to manual methods, all bacteriology tasks common to both systems were compared for similar lengths of time pre- and post-TLA installation.
Methods:
The number of bacteriology tests performed, hours worked, and FTEs used for a two week period pre-TLA (2014) and a corresponding two week period post-TLA (2016) were determined by reviewing laboratory records. Time and personnel required for all tasks, including specimen processing and inoculation, incubation, plate reading, identification of all isolates, antimicrobial testing of all pathogens, results reporting and discard of culture plates were counted. Time periods with full laboratory staffing were selected. A comparison of the data was performed.
Results:
During a two week period before TLA, when manual methods were used, 4,534 bacteriology tests were performed with 2,062.5 hours worked, amounting to 25.78 FTEs. During a two week period after TLA installation and validation of all bacteriology specimens, 5,032 bacteriology tests were performed with 1,828.25 hours worked, amounting to 22.85 FTEs. There was an 11.0% increase in bacteriology tests performed (498 tests) and an 11.4% reduction in FTEs required (2.93 FTEs) after the introduction of TLA. Manual methods resulted in 176 tests performed/FTE, while Kiestra TLA resulted in 220 tests/FTE, a 25% increase.
Conclusion:
Data from our first year of use indicate that Kiestra TLA, compared to manual methods, reduces the number of FTEs required to perform all bacteriology tasks resulting in a 25% increase in the number of tests performed/FTE. Ongoing development of automated MALDI-TOF target spotting and antimicrobial testing may further improve efficiency.

Author Disclosure Block:

D. R. Shibib, None..
I. K. Dusich, None..
R. B. Thomson, None.

Poster Board Number:

LB-078

Publishing Title:

Performance of Ten Phenotypic Assays for the Detection of Carbapenemase-producing *Enterobacteriaceae*

Author Block:

P. D. Tamma, B. N. A. Opene, **P. J. Simner**; Johns Hopkins Hosp., Baltimore, MD

Abstract Body:

Background: The objective of this study was to compare the accuracy of 10 phenotypic assays for the detection of carbapenemase-producing Enterobacteriaceae (CPE).
Methods: A collection of 184 isolates including 112 isolates producing a variety of carbapenemases (CP) and 72 non-CP-producing (ESBL or AmpC producers +/- porin mutations) isolates were included in this study. All tests were set up on the same day from a common blood culture plate containing a pure culture of the isolate. The testers were blinded to the expected results. The sensitivity and specificity of each test was determined, with the molecular genotype serving as the gold standard.
Results: Table 1: Performance of ten phenotypic assays for the detection of CPE.

	RAPIDEC® CARBA NP[a]	**Neo-Rapid Carba Screen ®[b]**	**Rapid CARB Blue Screen ®[b]**	**Manual Carba NP CLSI**	**Manual Blue Carba**	**Modified Carba NP**	**Boronic Acid Synergy Test**	**Metallo-β-lactamase IP/IPI Etest[b]**	**Modified Hodge Test**	**Carbapenem Inactivation Method**
	% Sensitivity (95% CI)									
bla$_{KPC}$ (n=42)	90 (76-97)	83 (68-92)	79 (63-89)	90 (76-97)	95 (83-99)	86 (71-94)	69 (53-82)	--	97 (90-99)	90 (76-97)
bla$_{NDM}$ (n=36)	89 (73-96)	92 (76-98)	97 (84-99)	92 (76-98)	97 (84-99)	94 (80-99)	--	47 (31-64)	92 (82-97)	97 (87-99)
bla$_{VIM}$ (n=13)	100 (72-100)	100 (72-100)	100 (72-100)	100 (72-100)	92 (62-99)	100 (72-100)	--	38 (15-68)	98 (88-99)	100 (72-100)

bla$_{IMP}$ (n=6)	100 (52-100)	100 (52-100)	100 (52-100)	100 (52-100)	100 (52-100)	100 (52-100)	--	0 (0-48)	100 (90-100)	83 (36-99)
bla$_{OXA}$ (n=15)	60 (33-83)	53 (27-78)	9 (4-19)	73 (45-91)	73 (45-91)	73 (45-91)	--	--	100 (91-100)	53 (27-78)
	% Specificity (95% CI)									
bla$_{KPC}$ (n=42)	95 (85-99)	97 (87-99)	98 (90-99)	98 (90-99)	92 (81-97)	98 (90-99)	90 (79-96)	--	82 (59-94)	97 (87-99)
bla$_{NDM}$ (n=36)	95 (85-99)	97 (87-99)	98 (90-99)	98 (90-99)	92 (81-97)	98 (90-99)	--	100 (92-100)	82 (59-94)	97 (87-99)
bla$_{VIM}$ (n=13)	95 (85-99)	97 (87-99)	98 (90-99)	98 (90-99)	92 (81-97)	98 (90-99)	--	100 (92-100)	82 (59-94)	97 (87-99)
bla$_{IMP}$ (n=6)	95 (85-99)	97 (87-99)	98 (90-99)	98 (90-99)	92 (81-97)	98 (90-99)	--	100 (92-100)	82 (59-94)	97 (87-99)
bla$_{OXA}$ (n=15)	95 (85-99)	97 (87-99)	91 (81-96)	98 (90-99)	91 (81-97)	98 (90-99)	--	--	82 (59-74)	97 (87-99)

[a]Availability in US pending clinical trial completion & FDA clearance; [b]RUO product in US
Conclusions: Overall, the accuracy to detect CPE varied across the phenotypic tests. The local epidemiology of CP genotypes should be considered when selecting a phenotypic assay for clinical use.

Author Disclosure Block:

P. D. Tamma, None..
B. N. A. Opene, None..
P. J. Simner, None.

Poster Board Number:

LB-079

Publishing Title:

Predictors for Mortality in MERS (Middle East Respiratory Syndrome)-Infected Patients: Emphasis on Viral Load, Antibody Response and Clinical Manifestations

Author Block:

S. Park[1], K-H. Hong[2], J-Y. Lee[3], B-N. Kim[4], H. Pai[5], S-H. Park[6], E-C. Shin[6], J-P. Choi[2], S-H. Kim[1]; [1]Asan Med. Ctr., Seoul, Korea, Republic of, [2]Seoul Med. Ctr., Seoul, Korea, Republic of, [3]Dankook Univ. Hosp., Cheonan, Korea, Republic of, [4]Sanggye-Paik Hosp., Seoul, Korea, Republic of, [5]Han Yang Univ. Hosp., Seoul, Korea, Republic of, [6]KAIST, Daejeon, Korea, Republic of

Abstract Body:

Background: The combined interpretation of clinical manifestations with viral load and immune response help us understand the pathogenesis of viral infection. We thus analyzed the clinical manifestations with detailed viral shedding and antibody kinetics in MERS patients. **Methods:** We enrolled all patients admitted into 3 MERS-designated hospitals and obtained sputum and sera over the course of disease. The samples were tested by reverse-transcriptase polymerase chain reaction (RT-PCR) and the whole-virus indirect immunofluorescence antibody (IFA) test. **Results:** Of 30 patients with MERS, 6 (20%) died. The median time (IQR) to death from the symptom onset was 23 days (13-28). The median times (IQR) to the negative conversion of RT-PCR for MERS-CoV and the positive conversion of IFA titer (≥ 1:640) for MERS-CoV from the symptom onset were 19 days (15-21) and 18 days (17-23), respectively. Risk factors for mortality are shown in Table 1. Old age, low albumin, altered mentality, and high pneumonia severity index (PSI) score at admission were identified as risk factors for mortality. High viral loads in respiratory samples on 2 weeks (median Ct value 30.2 [survivor] vs. 27.1 [non-survivor], *P*=0.02) and low antibody titer (< 1:640) on 3 weeks (33% [3/9, survivor] vs. 100% [4/4, non-survivor], *P*=0.03) from the symptom onset during the course of disease were found as risk factors for mortality. **Conclusions:** These data suggest that patients with old age as well as those who neither control viral control nor mount adequate immune responses are associated with mortality in MERS-infected patients.

Table 1. Univariate analysis for predictors of mortality in MERS patients.

Value	**Total (n=30)**	**Survivor (n=24)**	**Death (n=6)**	***P***
At admission				
Age, mean (SD)	49 (13)	46 (13)	59 (8)	0.03
Albumin (IQR)	3.8 (3.5 - 4.4)	3.8 (3.5 - 4.4)	3.2 (2.5 - 3.7)	0.02

Mental change (%)	2 (7)	0	2 (33)	0.003
Pneumonia severity index, median (IQR)	49 (37 - 75)	46 (36 - 63)	119 (47 - 169)	0.02
During the course of disease				
The presence of MERS viremia (%)	15/23 (65)	13/19 (68)	2/4 (50)	0.48
MERS viral loads of (RT-PCR Ct value) of respiratory samples at 2 weeks, median (range)	36.8 (26.8 - 38.3)	37.9 (28.0 - 38.3)	27.1 (26.8 - 28.1)	0.02
MERS viral loads of (RT-PCR Ct value) respiratory samples at 3 weeks, median (range)	36.8 (27.1 - 39.0)	38.7 (36.8 - 39.0)	30.2 (27.1 - 33.2)	0.20
Low antibody titer (< 1:640) at 3 weeks (%)	7/13 (54)	3/9 (33)	4/4 (100)	0.03

Author Disclosure Block:

S. Park, None..
K. Hong, None..
J. Lee, None..
B. Kim, None..
H. Pai, None..
S. Park, None..
E. Shin, None..
J. Choi, None..
S. Kim, None.

Poster Board Number:

LB-080

Publishing Title:

Emergence of KPC-producing Urease-negative *Klebsiella pneumonia* in Corrientes, Argentina

Author Block:

J. Di Conza[1], M. E. Badaracco[2], Y. Calza[2], P. Chavez[2], R. Goyechea[2], M. Almuzara[1], C. Vay[1], M. Bangher[2], L. Soler[2], **G. Gutkind**[1], L. Peña[2]; [1]UBA, CABA, Argentina, [2]ICC, Corrientes, Argentina

Abstract Body:

Background: KPC-producing *K. pneumoniae* (KPC-Kpn) are severe nosocomial pathogens. To our knowledge, Corrientes has been free of KPC-Kpn outbreaks. This province limits and is a key for regional transit with Paraguay and Brazil. Dissemination of the hiperendemic ST 258 clone as well as ST11 and ST23 have been already reported. Our aim was to describe the molecular and clinical epidemiology of an atypical urease-negative KPC-Kpn clone. **Methods:** We conducted a prospective descriptive analysis of all patients with KPN-KPC infection (August 2015 to January 2016) in Corrientes, Argentina. Identification included conventional, semiautomated panels and MALDI-TOF. Antimicrobial susceptibility was performed by disk diffusion according to CLSI. Screening for carbapenemases (KPC) was performed by using APB disks, a modified Hodge and blue-Carba tests. bla_{KPC} was confirmed by PCR and sequencing. The clonal relatedness was investigated by ERIC-PCR and MLST. **Results:** A patient derived in August 2015 from a limiting province rendered a KPC-producing isolate (characterized as *Klebsiella* (*Raultella*) *terrigena* by API 20E (98%)) from an urine colonization. As the resistance profile made was unlikely, it was submitted for MALDI-TOF confirmation, which placed it as *K. pneumoniae*; urease production was negative. Since then, other 12 microorganisms with the same profile were recovered in two hospitals, 4 from rectal swabs, and 8 from clinically relevant samples (3 UTIs, 1 from an hepatic abscess, 2 from respiratory syndrome, a pancreatic abscess and a mediastinitis; the last 4 died). All isolates presented the same biotype with an antibiotic multiresistance profile, being only fully susceptible to amikacin. Further, all of them displayed identical ERIC-PCR electropherotype belonging to a single clonal type ST340. **Conclusions:** Our findings report a KPC-producing clone with an atypical urease reaction associated with nosocomial outbreaks. It also constitutes the first description of ST340 in Argentina alerting for the emergence of a non-ST258 clone, especially considering that urease-negative BLEE-producing *K. pneumoniae* have already been reported in Brazil.

Author Disclosure Block:

J. Di Conza, None..
M. E. Badaracco, None..

Y. Calza, None..
P. Chavez, None..
R. Goyechea, None..
M. Almuzara, None..
C. Vay, None..
M. Bangher, None..
L. Soler, None..
G. Gutkind, None..
L. Peña, None.

Poster Board Number:

LB-081

Publishing Title:

Impact of Implementing Direct Stool Pathogen Detection on Test Volume and Results Delivery in a Patient-Centered Microbiology Laboratory Serving a Pediatric Population

Author Block:

S. Holt[1], M. V. Powers-Fletcher[2], A. Phillips[1], R. Grand-Pre[1], M. Dickey[1], A. Pavia[2], C. Stockmann[2], A. Blaschke[2], J. Daly[1]; [1]Primary Children's Hosp., Salt Lake City, UT, [2]Univ. of Utah, Salt Lake City, UT

Abstract Body:

Diagnosis of infectious gastroenteritis (IGE) is complicated by the variety of potential causative pathogens with similar disease presentations. Laboratory detection of these pathogens often requires a combination of individual tests, including culture and molecular assays, which can delay diagnosis. Multiplex PCR panels, which test for multiple pathogens at once, have the potential to reduce turnaround time (TAT) and improve patient care. The purpose of this study was to determine the impact of implementation of the FilmArray (FA) GI panel (BioFire Diagnostics, Salt Lake City, UT) on ordering practices and results delivery in a pediatric patient-centered laboratory.

The FA GI panel assay, which contains 22 bacterial, parasitic, and viral targets, was available for clinical use at Primary Children's Hospital (PCH) during a 5 month study period (October 2015 - February 2016). All other stool tests for IGE remained orderable and included stool culture, viral antigen and molecular tests, and microscopy for ova/parasites. FA GI testing was performed on-demand, as soon as specimens were received by the laboratory and an instrument was available. Ordering practices, laboratory test volumes, and TAT were analyzed.

From the beginning to the end of the study period, the volume of FA GI tests ordered increased by 87.5%, while the volume of other stool testing decreased by half. The most striking declines were in adenovirus and rotavirus testing, followed by stool culture, with a 97.4%, 78.5% and 51.2% decrease, respectively. FA GI results were available within an average of 3.0 hrs from the time of specimen receipt, which was faster than the average time of 33 hrs ($p < 0.05$ using Student's t-test) required to obtain results for all of the same targets using traditional methods.

The results of this study suggest that physicians are willing to utilize direct molecular detection assays to assist with the diagnosis of IGE in a pediatric patient population. Implementation of this assay correlated with a reduction in traditional stool tests ordered and decreased results TAT; future studies to determine the impact on clinical outcomes and healthcare costs are required.

Author Disclosure Block:

S. Holt, None..
M. V. Powers-Fletcher, None..

A. Phillips, None..
R. Grand-Pre, None..
M. Dickey, None..
A. Pavia, None..
C. Stockmann, None..
A. Blaschke, None..
J. Daly, None.

Poster Board Number:

LB-082

Publishing Title:

Metagenomic Evaluation of Sink Trap Biofilms as a Nosocomial Reservoir for Carbapenemase Producing Gammaproteobacteria

Author Block:

P. Subramanian[1], N. A. Hasan[1], C. Grim[2], A. Mathers[3], R. Colwell[1]; [1]CosmosID, Inc., Rockville, MD, [2]CFSAN U.S. FDA, Laurel, MD, [3]Univ of Virginia Sch of Med, Charlottesville, VA

Abstract Body:

Background: Carbapenemase producing bacteria are an increasing threat to hospitalized patients and a full understanding of nosocomial transmission has yet to be realized. The biofilm in sink traps has long been recognized as a biologically active community but little is known about how to evaluate the presence of highly resistant organisms or the risk they could pose to patients. We adapted a novel genomic approach to evaluate a sink trap as a hospital reservoir for carbapenemase organisms. **Method:** 15 *Klebsiella pneumoniae* carbapenemase (bla_{KPC}) *Aeromonas* sp. and Enterobacteriaceae clinical isolates collected between 2/2012-5/2013 paired with 9 sink trap biofilm samples from the same hospital (3/2014) underwent whole genome shotgun sequencing using an Illumina MiSeq and metagenomic analysis using CosmosID's MetaGenID software. To determine if patient isolates were present in the biofilm, their genomes were added to the MetaGenID bacterial database. The biofilm metagenomes were examined using this supplemented database. Both biofilm samples and patient isolates were also profiled using the MetaGenID antimicrobial resistance and virulence gene database. Genes with partial match to a biofilm sample at >50% coverage and > 80% isolate coverage were aligned, using BLAST, to a characterized bla_{KPC} plasmid. **Result:** 7 patient isolates were identified as present in the sink biofilms indicating that the sink trap biofilm can act as a sustained reservoir over time. Of 303 antibiotic resistance and virulence associated genes found in the biofilm samples and patient isolates, the majority (n=190) were plasmid associated. The remaining genes not on the plasmid, with the exception of a single virulence factor, were found only in the biofilms. All genes present and common to multiple biofilm samples and patient isolates (including those isolates not identified in the biofilms) were plasmid associated. **Conclusion:** We have applied a novel approach to understand a nosocomial reservoir of drug resistance which can sustain plasmid based genes of resistance shared between environmental and patient isolates. Based on these results, we conclude that biofilms can create a niche for sustaining clinically relevant multidrug resistance organisms (MDROs) in a hospital.

Author Disclosure Block:

P. Subramanian,
CosmosID, Inc. Role(s): Self, D. Employee.
N. A. Hasan,
CosmosID, Inc. Role(s): Self, D. Employee.
C. Grim, None..
A. Mathers, None.
R. Colwell,
CosmosID, Inc. Role(s): Self, A. Board Member.
CosmosID, Inc. Role(s): Self, D. Employee.

Poster Board Number:

LB-083

Publishing Title:

Creation GARD (Genotype-linked Antibiotic Resistance Database)

Author Block:

J. W. Kim, 95060[1], P. Chan[2], T. M. Lowe[2], M. Barlow[3], M. Camps[1]; [1]Univ. of California, Santa Cruz, Santa Cruz, CA, [2]Maverix Biomics, Inc, San Mateo, CA, [3]Univ. of California, Merced, Merced, CA

Abstract Body:

We are establishing a cloud-based data-sharing and sequence analysis platform called GARD (Genome-Linked Antibiotic Resistance Database), which is decentralized by design and has data-sharing protocols that are compatible to the exchange standards for broad sharing of clinical human genomic data.
We are populating this platform by sequencing strains of extraintestinal pathogenic *E. coli* exhibiting unique profiles of extended-spectrum β-lactam resistance (ESBL), as measured using MIC assays for a panel of nine β-lactam antibiotics. These isolates were obtained through a collaboration with Mercy Hospital in Merced, California.
Here, we present the results of sequencing 22 of these strains using MiSeq technology. Based on MLST typing and phylogenetic analysis across all protein-coding SNPs, our samples are largely comprised of ST131 (13 of 22 strains), with the remaining 9 strains distributed across 7 sequence types.
We detected four classes of β-lactamase genes: plasmid-borne TEM (10), CTX-M (16) and OXA (13), and chromosomal AmpC. Nine of the TEM genes and all the OXA and AmpC genes lacked the mutations associated with ESBL activity. Genes encoding four CTX-M variants were found: CTX-M-14, 15, 65 and 27. The presence of CTX-M genes showed a high correlation with resistance to 3rd generation (and to a lesser degree 4th generation) cephalosporins, particularly ceftriaxone ($r = 0.99$ with 22 samples, $p = 0.0$). Our clinical data also suggests that TEM-1 can be sufficient for conferring resistance against cefazolin (2nd generation), consistent with prior experimental reports.
Overall, our initial results suggest that β-lactamase sequences can be used to classify ESBL phenotypes more accurately than previously reported, although additional mechanisms of resistance still appear to be at play. As it develops further, GARD should serve as a knowledge base for monitoring the spread of resistance to ESBLs and other classes of antibiotics, for identifying mobile genetic elements facilitating its spread, and for diagnosis of antibiotic resistance.

Author Disclosure Block:

J. W. Kim, None.
P. Chan,
Maverix Biomics, Inc Role(s): Self, D. Employee.
T. M. Lowe,
Maverix Biomics, Inc Role(s): Self, A. Board Member.
M. Barlow, None..
M. Camps, None.

Poster Board Number:

LB-084

Publishing Title:

Genotyping and Phenotypic Analysis of Hybrid Strains of *Escherichia coli* (EAEC/UPEC) Isolated From Urinary Tract Infections

Author Block:

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Abstract Body:

Background: The diversity of behaviors showed by *E. coli* strains is supported by a high genetic plasticity that allows the transfer and maintenance of a diversity of virulence genes among *E. coli* strains. The aim of this study was caried out the genotyping and the phylogenetic analysis of *E. coli* strains isolated from urinary tract infections treated in a hospital of Brasília-DF, Brazil. **Methods:** Strains were tested for the presence of 6 virulence genes of UPEC (*focA*, *pap*, *vat*, *fyuA*, *chuA* and *yfcV*), 2 of MNEC (*cnf* and *sfa*), 2 of EAEC besides *csgA* (curli fimbriae) and *ag43*. Amplification and sequencing of the genes *dinB* (DNA polymerase) and *icdA* (isocitrate dehydrogenase) were employing in the phylogenetic analysis. *dinB-icdA* concatenated sequences (966 base-long sequences) were used in bootstrap analysis (with 1000 replications) in order to infer phylogenetic relationship among strains. **Results:** The most frequently detected genes were *chuA* (78% - 62/78), *csgA* (76% - 61/78) and *fyuA* (69% - 54/78). EAEC genes (*p*CVD432 and *agg*R) were detected in 3,8% (3/78) of UPEC strains. Forty different genotypes were detected among tested strains. Hybrid genotypes (EAEC/UPEC) were identified in 3 strains as follows: *fyuA+ chuA+ pap+ csgA+ ag43+ pCVD+ aggR+* in EAEC strain 1.1; *fyuA+ vat+ focA+ pap+ csgA+ ag43+ pCVD+ aggR+* in EAEC strain 1.2; and *fyuA+ chuA+ pap+ csgA+ pCVD+ aggR+* in EAEC strain 1.3. Twenty one strains with the most common genotypes and the hybrid strains EAEC/UPEC were selected for the phylogenetic analysis. In phylogenetic tree, EAEC/UPEC strains 1.1 and 1.3 compose a large cluster along with 7 UPEC strains. Branching of this cluster was supported by a bootstrap value of 98. In this cluster, 6 of out 7 strains displayed the combination *fyuA+ chuA+ pap+ csgA+* as shared gene set. **Conclusions:** Our data showed that heteropathogenic EAEC/UPEC strains can cause extraintestinal infections. Additionally, the data raise the possibility that typical genes of EAEC strains can be transferred to and stably maintained by UPEC strains, what can lead to the emergency of heteropathogenic categories.

Author Disclosure Block:

F. B. M. Lara, None..
D. R. Nery, None..
P. M. D. Oliveira, None..
M. L. Araujo, None..

L. B. Ferreira, None..
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Poster Board Number:

LB-085

Publishing Title:

Intradermal Accumulation of Doxycycline Could Explain its Anti-Inflammatory Action on Rosacea

Author Block:

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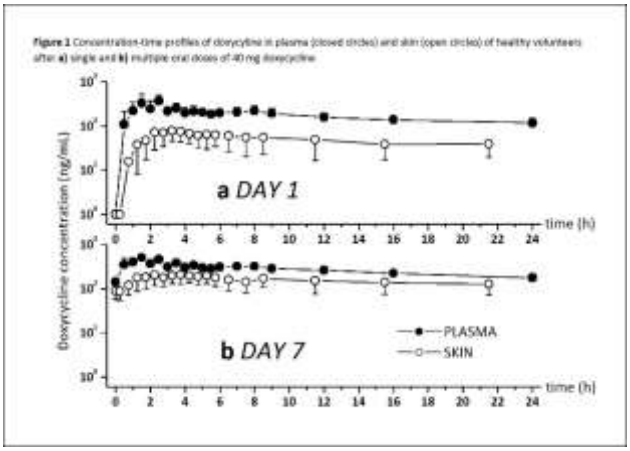
Abstract Body:

Background: Doxycycline (DOX) is approved for the treatment of rosacea. The drug's therapeutic efficacy is believed to be based on its anti-inflammatory properties rather than on a direct antibacterial effect. However, DOX pharmacokinetics (PK) in human skin, i.e. the target site for this indication, has to date not been described. This study was designed to measure single (SD) and repeated dose (RD) PK of DOX in plasma and skin of healthy volunteers (HV) during oral therapy with DOX. **Methods**: 6 HV received 40 mg DOX delayed release capsules orally once daily for 7 days. After dosing on days 1 and 7, DOX levels were assessed in plasma and skin of HV. Skin measurements were done with microdialysis. **Results:** Concentration-time profiles of DOX in plasma and skin of HV are shown in figure 1. Mean ± SD C_{max} and $AUC_{0\text{-}24h}$ as well as median (min-max) t_{max} of total DOX in plasma were 409.5 ± 145.9 µg/L, 4051.2 ± 985.3 µg·h/L and 1.5 (1.5-2.5) h after SD and 529.8 ± 80 µg/L, 6432.6 ± 853.6 µg·h/L and 1.5 (1.5-2.5) h after RD, respectively. Mean ± SD C_{max} and $AUC_{0\text{-}24h}$ as well as median (min-max) t_{max} of unbound drug in skin of HV were 90.7 ± 35.2 µg/L, 1036 ± 519.7 µg·h/L and 2.8 (2.3-6.5) h after SD and 255.4 ± 82.6 µg/L, 3232.2 ± 1130.6 µg·h/L and 3.8 (1.3-8.5) h after RD, respectively. Assuming 90% plasma protein binding, skin to plasma ratios of C_{max} and $AUC_{0\text{-}24h}$ of unbound DOX were 2.4 and 2.1 on day 1. They increased to 4.1 and 4.8, respectively, after 7 days of therapy. Interestingly, post-dose C_{min} to pre-dose C_{min} ratios of > 1 for both compartments indicate incomplete achievement of steady-state. **Conclusions:** Pronounced accumulation in the interstitium of human skin may help explaining the activity of DOX against inflammatory skin diseases such as rosacea. This accumulation might be enhanced at steady-state and in intracellular compartments.



Author Disclosure Block:

P. Matzneller, None..
E. Lackner, None..
M. Zeitlinger, None.

Poster Board Number:

LB-086

Publishing Title:

Robust Potentiation of Known Antimycotic Agents with Molecular Replacement Therapy for the Topical Treatment of Tinea Infections

Author Block:

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Abstract Body:

Dermatophytoses, also known as tinea, are fungal infections of the skin rampant among the human population. *Trichophyton* spp. are dermatophyte fungi that invade and grow in the keratin of skin, hair and nails and are responsible for causing these infections. Depending on the site of infection, various topical anti-mycotics show efficacious therapeutic benefits in the treatment of tinea while many fail to achieve the desired therapeutic outcome. In recent years there have also been reports of drug resistance in dermatophytes that further aggravate the clinical scenario. Potentiation of known agents and achieving their effective concentrations in skin thus remains an area of active research for the development of anti-tinea products. In this study we report that our proprietary Molecular Replacement Therapy (MRT) platform potentiates the *in vitro* antifungal activity of different classes of antimycotics including azoles and allylamines against *Trichophyton* spp. as demonstrated through time kill assays. Our data suggest a synergistic action of the MRT agent with various antifungals against *T. rubrum*. We further show that clinical isolates of *Trichophyton* spp. resistant to fluconazole and terbinafine are efficiently inhibited by topical formulation(s) of certain azoles or terbinafine with MRT. This platform technology not only imparts enhanced *in vitro* fungal killing but also causes enhanced skin penetration and allows higher deposition of the active anti-mycotic agent in rat skin as observed in an *in vivo* dermal PK study. Therefore a two-pronged effect comprising an immediate impact of the formulation on fungus residing in the upper layers of the skin followed by a long term sustained effect of higher retained amount of actives on fungus residing in deeper layers of the skin becomes evident.

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Poster Board Number:

LB-087

Publishing Title:

Superior Efficacy and Improved Renal and Bone Safety After Switching from a Tenofovir Disoproxil Fumarate (TDF) Regimen to a Tenofovir Alafenamide (TAF) Based Regimen Through 96 Weeks (W96) of Treatment

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Abstract Body:

Background: TAF is a tenofovir (TFV) prodrug with a 90% reduction in free TFV plasma levels compared to TDF. Week 48 (W48) data from this large switch study demonstrated a significantly higher rate of virologic suppression and statistically significant improvements in renal function and bone density. Data from W96 comparing those maintaining their prior TDF-based regimen to those switching to elvitegravir 150 mg, cobicistat 150 mg, emtricitabine 200 mg, & TAF 10 mg (E/C/F/TAF) are presented.
Methods: GS-US-292-0109 is an ongoing, active controlled, open-label international study in which virologically suppressed subjects on one of three TDF-based regimens (E/C/F/TDF, EFV/FTC/TDF, or boosted ATV + FTC/TDF) were randomized 2:1 to switch to E/C/F/TAF or to maintain their prior regimen. Efficacy and safety data through W96 following the switch are summarized.
Results: 910/959 (95%) of subjects randomized to E/C/F/TAF and 434/477 (91%) of those who continued on a TDF-based regimens completed W96 in this study. Virologic success rates (HIV-1 RNA less than 50 copies/mL): E/C/F/TAF 93%; TDF-based regimen 89% (p=0.017; difference 3.7%; 95% CI 0.4-7.0%). AE-related discontinuations occurred in 0.9% of those on E/C/F/TAF and in 2.5% of those who maintained their TDF-based regimen. Key safety data at W48 and W96 are shown in the table below. **Conclusions:** Switching to E/C/F/TAF from a TDF-based regimen resulted in superior virologic control and in significant improvements in both renal function and in bone density through W96. For those switching to E/C/F/TAF, these differences persisted from W48 to W96.

Safety data, W48 & W96

		E/C/F/TAF		TDF-based regimen		p value
		W48	W96	W48	W96	
% with Adverse Event	Grade 3	7.6	9.2	10.5	10.9	--

	Grade 4	1.1	1.4	0.8	1.3	--
Total cholesterol, median change from baseline	Ratio to HDL	+0.2	+0.3	+0.1	+0.1	<0.001
Bone mineral density, mean % change from baseline	Hip	+1.9	+2.4	-0.1	-0.5	<0.001
	Spine	+1.9	+2.1	-0.2	-0.1	<0.001
Urine protein, median % change from baseline	Ratio to urine creatinine (mg/g)	-20.9	-25.5	+9.6	+9.0	<0.001
Urine albumin, median % change from baseline		-17.9	-13.7	+8.5	+11.1	<0.001

Author Disclosure Block:

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Poster Board Number:

LB-088

Publishing Title:

Efficacy of a Human Monoclonal Antibody with Biofilm Disrupting Activity in a Rat Infective Endocarditis Model due to Methicillin-Resistant *Staphylococcus aureus* (MRSA)

Author Block:

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Abstract Body:

Background: Many serious bacterial infections are associated with biofilm formation. TRL1068 is a high affinity native human antibody against a biofilm epitope conserved across both Gram-positive and Gram-negative bacterial species. **Methods:** TRL1068 was evaluated in a catheter-induced aortic valve IE rat model (Xiong et al., 2005, *AAC)*. Rats were infected with a clinical isolate (MRSA strain 300-169; associated with persistent bacteremia). Animals were treated with 6 days of vancomycin (n=8; VAN: 120 mg/kg, SC, bid) alone or in combination with TRL1068 (n=10) or isotype control antibody (n=8); each at 15 mg/kg, iv, QD on days 1 and 4. MRSA burden was quantified at day 7 sacrifice (mean $\log_{10}$CFU/g tissue ± SD) (cardiac vegetations, intracardiac catheter, kidney, spleen and liver). **Results:** MRSA densities in vegetations showed ≥ 1.75 log reduction in the TRL1068 + VAN arm vs. isotype control + VAN arm ($P<0.001$). Significant reductions in CFUs was also observed for intracardiac catheters, kidneys, spleens and livers ($P<0.05$) for the VAN + TRL1068 arm vs both VAN + isotype and isotype alone arms. A trend towards mortality reduction in the VAN + TRL1068 arm was also observed ($P=0.09$). **Conclusions:** The reduced bacterial burden and mortality following combination therapy with TRL1068 + VAN supports further development of TRL1068 for the treatment of endovascular and other serious infections associated with biofilm formation.

Author Disclosure Block:

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Poster Board Number:

LB-089

Publishing Title:

Cytotoxic Properties of 50kDa Protein Isolated from *Streptomyces A13* Against A549 Human Lung Cancer Cells and AA8 Healthy Hamster Cells

Author Block:

M. L. Delos Santos[1], H. Banocnoc[1], M. Badong[1], J. Nacional[1], T. Zulaybar[2]; [1]Dept. of Ed., Makati City, Philippines, [2]Inst. of Molecular Biology & Biotechnology UP Los Banos, Laguna, Philippines

Abstract Body:

Background: *Streptomyces* are known to produce anticancer compounds that are effective inhibitors of some cancer cells. As such, we would like to determine if 50 kDa protein from *Streptomyces* A13 have cytotoxic properties against A549 lung cancer cells and AA8 Healthy Hamster Cells. **Methods:** A13 were grown on Croatian Vegetative Medium. Cells were separated by centrifugation at 3,000 rpm. Extraction of the proteins from the cells was performed through ammonium-sulfate precipitation. Protein content was measured by the Bradford Method with bovine serum albumin as the reference protein. The precipitated proteins were analysized for size by denaturing PAGE using standard procedures. The ammonium sulfate-precipitated broth (B1) sample and cell extract were used for the MTT assay. Identification of A13 was done using the 16s rRNA full gene sequence. **Results:**The protein concentration was highest with the B1 (446.323ug/mL) followed by the spent medium (215.700 ug/mL), cell extract (96.158ug/mL) and least in the PEI pellet (65.166ug/mL).This high protein concentration in B1 as compared to the cell extract may be explained by their extrusion from the cell. A13 cell extract and B1 were found cytotoxic against the A549 lung cancer cells having mean IC_{50} values of 4.37013 µg/ml and 4.54037 µg/ml respectively while the positive control had a mean IC_{50} value of 2.27627 µg/ml. It was also found that the crude extract of A13 was less cytotoxic against AA8 healthy hamster cells having an IC_{50} value of 3.6794 µg/ml compared to the IC50 value of the positive control which was 1.8370. BLAST result of the 16s rRNA for A13 yielded 99% homology to *Streptomyces costaricanus, Streptomyces padanus* and other 15 Stre*ptomyces sp*. isolates. **Conclusions:** We conclude that the 50kDa protein from *Streptomyces* A13 is a potential source of chemotherapeutic agent against A549 and its less toxic compared to the positive control.

Author Disclosure Block:

M. L. Delos Santos, None..
H. Banocnoc, None..
M. Badong, None..
J. Nacional, None..
T. Zulaybar, None.

Poster Board Number:

LB-090

Publishing Title:

Novel Compound from *Variovorax paradaxux S110* Kills *Mycobacterium tuberculosis*

Author Block:

M. Espinoza-Moraga, H. I. Boshoff, C. E. Barry, III; NIH, Bethesda, MD

Abstract Body:

Bacterial natural products are a varied and rich source of undiscovered anti-tuberculosis (TB) drugs with unique scaffolds and/or novel mechanisms of action. Due to the threats of anti-TB drug resistance keep growing, we are in urgent need of new drugs to treat the disease. Sphagnum peat bogs are acidic and hypoxic environments, with similar conditions to those encountered in tuberculous granulomas. Moreover, these habitats are also major natural reservoirs of mycobacterial and competing species. These factors support sphagnum peat bogs as ideal sources of novel anti-mycobacterial compounds. Here we reported a novel compound from a bog bacteria capable to inhibit selectively *M. tuberculosis.*

Sphagnum bog samples were collected across Northeastern US. Bog bacteria producing inhibitory activity against *M. tuberculosis* (MIC ≤50 μg/ml) were isolated and genotyped by their 16S rRNA gene sequence. PacBio was used to sequence the whole bacterial genome, and biosynthetic gene clusters were identified using automated bioinformatics software PRISM and anti-SMASH 3.0. Active metabolites were purified from bacterial biomass samples using gel filtration chromatography and further characterized by LC-MS and NMR. Subsequent transcriptional profiling of *M. tuberculosis* treated with the most active metabolite yielded signatures indicating novel mechanisms of action.

Bog bacteria, including *Pedobacter*, *Pantoea*, *Pseudomonas*, *Chryseobacterium*, *Streptomyces*, and *Variovorax* were found to produce secondary metabolites against *M. tuberculosis.* One positive hit, identified as *Variovorax paradaxus strain S110*, was selected for whole genome sequencing based on scarce prior reports of associated natural products and exclusive and potent activity of the active agent against *M. tuberculosis* (MIC <0.39 μg/ml; other species MICs >200 μg/ml). Three novel non-ribosomal peptide (NRP) or hybrid NRP/polyketide (PK) biosynthetic gene clusters were identified. Based on the *in silico* predict structure, bioactivity-guided fractionation and spectrometric tools, a novel peptide was identified.

Our findings indicate that a novel compound with unique scaffolds and/or novel mechanism of action can be fully isolated and characterized from *V. paradaxus S110*. This new compound has the potential to form a basis for new anti-TB classes addressing bacterial targets that are currently underexploited.

Author Disclosure Block:

M. Espinoza-Moraga, None..
H. I. Boshoff, None..
C. E. Barry, None.

Poster Board Number:

LB-091

Publishing Title:

Treatment of Adenovirus Infection in Solid Organ Transplant with Brincidofovir

Author Block:

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Abstract Body:

Background: Disseminated adenovirus (AdV) infection is increasingly described in solid organ transplant (SOT) recipients, particularly in pediatric patients, in whom the infection can be rapidly fatal following diagnosis. The investigational antiviral, Brincidofovir (BCV), has been administered through an expanded access trial (Study CMX001-350) and an open-label trial of serious AdV infection (Study CMX001-304) to patients who are immunosuppressed following SOT. Herein we describe the characteristics and outcomes of 26 SOT recipients who received BCV for serious AdV infection. **Methods:** Pediatric and adult patients who had undergone solid organ transplantation and who were diagnosed with serious AdV infection were treated with BCV 100 mg twice weekly (BIW) or, for children <50 kg, 2 mg/kg BIW. Descriptive statistics are presented for categorical variables, and medians (range) for continuous variables. **Results:** 26 pediatric and adult patients were treated with BCV. 73% of patients were pediatric (n=19; <18y), with a median age 8y (range 8m to 58y) and received BCV for a median of 8.5 wks. The most prevalent transplant type was liver (n=12; 11 pediatric subjects), although in adults lung (n=3) and kidney or kidney/pancreas (n=3) were predominant. 54% (n=14) of AdV infections were disseminated disease. 69% (n=18) subjects presented with median 3.7 $\log_{10}$ c/mL, baseline viremia (range <LLOQ to >ULOQ). Of these, 89% (n=16) were not quantifiable (<190 c/mL) at some point on treatment and 83% (n=15) were not quantifiable at end of treatment. 19% (n=5) of subjects died while in the study, with a median follow-up to 22 wks (range 1 week to 39 weeks). **Conclusions:** In this limited cohort of predominantly pediatric patients who had undergone SOT, a significant, and sustained adenoviral suppression was noted that might be attributed to treatment with BCV. BCV continues in development as the first potential treatment for serious AdV infection in immunocompromised patients. Additional study of BCV in treatment of AdV infections in SOT recipients is needed to address the clinical improvement associated with viral suppression of AdV viremia.

Author Disclosure Block:

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Poster Board Number:

LB-092

Publishing Title:

Daptomycin Resistance Associated with Loss of Function Mutation in Staphylococcus aureus

Author Block:

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Abstract Body:

Antibiotic resistance is increasing among *Staphylococcus aureus,* and often spreads throughout the bacterial population rapidly. Recently introduced drugs, such as daptomycin, represent the last line treatment options. However, resistance to daptomycin is also beginning to emerge. The mechanisms of daptomycin resistance (DAP-R) in *S. aureus* are diverse and complex, and remain to be fully elucidated. To identify genetic changes that can confer DAP-R in *S. aureus,* we used a highly saturating library of transposon insertions in the genome of *S. aureus* HG003 to screen for resistant mutants with enhanced survival in presence of daptomycin. Sixteen colonies were selected for further characterization. In this collection, transposon insertions were observed in two genes and they were further identified by arbitrarily primed PCR and sequence analysis. One gene encodes a hypothetical lipoprotein (*362*) and the other an alkaline shock protein (Asp23). Targeted deletions constructed by homologous recombination were generated for each of the two genes, followed by phenotypic analysis. Mutants *Δ362* and *Δasp23* exhibited a slightly higher MIC (2 μg/ml) to daptomycin than wild type. Mutant 362 also showed a slightly higher MIC (2 μg/ml) to vancomycin. Importantly, when the MBC was examined, a much higher tolerance for vancomycin was noted for both mutants (32 μg/ml for *Δ362*; 64 μg/ml for *Δasp23* and 2 μg/ml for wild type strain). By killing curve assay the mutant strains showed reduced bacterial mortality in presence of high antibiotic concentration (5X MIC) compared to the wild type strain. Neither mutation was associated with a fitness cost. Both mutants were able to grow in the presence of 1ug/mL daptomycin. These findings identified novel genes that contribute to the tolerance of *S. aureus* to daptomycin and vancomycin. Reduced susceptibility associated with loss of function mutations that has not previously been observed.

Author Disclosure Block:

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Poster Board Number:

LB-093

Publishing Title:

Development and Application of Microfluidic Devices in Time Course Studies of Microbial Symbiosis in Individual, Living *Steinernema* Nematodes

Author Block:

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Abstract Body:

Background: Animal-microbe symbioses are ubiquitous phenomena with great importance in medical and agricultural sciences. *Steinernema* nematodes and *Xenorhabdus* bacteria have been established as a successful model system to investigate microbial pathogenesis and mutualism, the two extremes of symbiotic relationships. The bacterium *Xenorhabdus nematophila* forms a mutualistic relationship with *Steinernema carpocapsae* nematodes, colonizing a specialized intestinal pocket of the nematode host in a species-specific manner, and together they infect a wide range of insects. During their lifecycle, certain networks of bacterial genes are regulated to confer either pathogenic or mutualistic states depending on the environment and host. The *X. nematophila* global transcription regulator, leucine-responsive regulatory protein (Lrp), is crucial for controlling gene expression necessary for both pathogenesis and mutualism. However, current understanding of Lrp-regulated bacteria-nematode interactions is based solely on population level, single time point studies, since investigating *in vivo* temporal dynamics of these interactions is impeded by the difficulty of isolating and maintaining individual living nematodes in which bacteria can be observed over time. **Methods:** To overcome this challenge, we developed a microfluidic device that enables us to isolate and microscopically observe individual, living *Steinernema* nematodes. We characterized both wild type and *lrp* null mutant *X. nematophila* bacterial populations within these nematodes over 5 days. **Results:** Our data are consistent with population level studies of Lrp-dependent bacteria-nematode symbiosis. We also find that compared to wild type, nematodes co-cultured with *lrp* mutant carry a more loosely packed symbiont population and display higher mortality. **Conclusions:** We present our current progress in this research, which provides new perspectives into animal-bacterial symbioses with, to the best of our knowledge, the first direct time course study of individual living animal hosts and their bacterial symbionts.

Author Disclosure Block:

M. D. Stilwell, None..
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Poster Board Number:

LB-094

Publishing Title:

Iron Sensing via a Catalytically Divergent Rhomboid Protease

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Abstract Body:

Environmental sensing in prokaryotes can be accomplished by the release of regulatory proteins from the membrane following two sequential proteolytic events. In *Mycobacterium tuberculosis* (Mtb), final release of anti-sigma factors is mediated by Rip1, a zinc metalloprotease similar to other described site-2 proteases. However, orthologs of known periplasmic site-1 proteases that sense environmental signals and initiate the cascade in Mtb were shown to be inactive. We report here, a previously un-annotated and highly divergent member of the rhomboid family of intramembrane proteases, which we term, catalytically divergent 'cdRhom', performs this activity. Despite lacking a canonical rhomboid active site, Rv3193c (RomA) uses distinct catalytic residues to perform the site 1 cleavage of the anti-sigma factor (RsmA), priming it for processing by Rip1. A physical interaction between the PDZ domain of Rip1 and a proline-rich region in extracytoplasmic domain of RomA facilitate the signaling cascade. Structural analysis of the extracytoplasmic domain revealed a conserved iron coordination site, indicating a likely environmental trigger for this system. Indeed, we found that RsmA processing was altered by iron concentration, and RomA-deficient bacteria were unable to replicate in low iron conditions due to altered levels of iron homeostatic genes. The substrate topology, active site, and sensory domains of cdRhom impart an environmental sensing function that is unprecedented for this family of proteins.

Author Disclosure Block:

S. Nambi, None.

Poster Board Number:

LB-095

Publishing Title:

The ArlRS-MgrA Regulatory Cascade Modulates Clumping and Virulence in Staphylococcus aureus

Author Block:

H. A. Crosby, P. M. Schlievert, A. R. Horswill; Univ. of Iowa, Iowa City, IA

Abstract Body:

Staphylococcus aureus causes a wide variety of superficial and invasive infections, including septicemia, osteomyelitis, and endocarditis. Its pathogenicity is dependent on expression of virulence factors such as toxins, secreted enzymes, and adhesins. Several *S. aureus* adhesins bind to fibrinogen, an abundant component of human plasma. When *S. aureus* interacts with fibrinogen or whole plasma it forms macroscopic clumps, a process termed clumping. This behavior is thought to aid in immune evasion, and it can also promote the formation of vegetations on heart valves, leading to endocarditis. Our laboratory recently demonstrated that clumping of *S. aureus* is regulated by the two-component system ArlRS. Little is known about ArlRS, although multiple studies have shown that *arlRS* mutants are less virulent. An *arlRS* mutant fails to form clumps in the presence of human plasma or fibrinogen, and the mutant is attenuated in a rabbit model of infective endocarditis. How ArlRS affects clumping and virulence was not well understood, though. We demonstrate here that ArlRS activates expression of the global regulator MgrA. Like *arlRS* mutants, *mgrA* mutants are also unable to clump, and are attenuated in infective endocarditis. RNA-seq analysis of an *mgrA* mutant showed that MgrA represses expression of up to eight putative and known adhesins, including Ebh, a giant ~1.1 MDa membrane-anchored surface protein, and SraP, a large cell wall attached protein. EMSA analysis, combined with transcriptional reporters and qRT-PCR, confirmed that MgrA directly represses *ebh* and *sraP*. We found that clumping could be restored to an *mgrA* mutant by deleting the genes for Ebh and SraP, suggesting that overexpression of these large surface proteins blocks clumping through steric hindrance. Deletion of *ebh* and *sraP* also partially restores virulence to an *mgrA* mutant. Taken together, these results demonstrate that ArlRS and MgrA constitute a regulatory cascade that controls expression of many genes, including the genes for eight large surface proteins. Regulation of these surface proteins has broad effects on the behavior of *S. aureus* in the presence of host proteins and inside a host.

Author Disclosure Block:

H. A. Crosby, None..
P. M. Schlievert, None..
A. R. Horswill, None.

Poster Board Number:

LB-096

Publishing Title:

Virulence Factor Regulators Control CRISPR-Cas Gene Expression in *Pseudomonas aeruginosa*

Author Block:

B. Castro, **J. Bondy-Denomy**; Univ. of California, San Francisco, San Francisco, CA

Abstract Body:

CRISPR-Cas systems are bacterial immune systems that present a formidable barrier to bacteriophage (phage) infection. Although many aspects of CRISPR-Cas function and mechanism have been well characterized *in vitro* and *in vivo*, the regulatory cues that control these processes are poorly understood in most microbes. This is the case in *Pseudomonas aeruginosa*, an organism that has become a widely used model system for studying all aspects of CRISPR-Cas function. Here, we describe a genetic screen to identify regulators of CRISPR-Cas expression and efforts to map the regulatory components for CRISPR-Cas operons in this organism. We have detected *kinB* as a sensor kinase/phosphatase that is required for optimal CRISPR-Cas expression. Through *in vivo* mutant analysis, we show that the important activity of KinB is not its kinase function but its ability to dephosphorylate its cognate response regulator, AlgB. In the absence of KinB, phosphorylated AlgB activates previously identified global virulence regulators AlgR and AlgU, which we show leads to CRISPR-Cas repression. Why do virulence regulators in *P. aeruginosa* control CRISPR-Cas expression? We speculate that this could be optimized to reflect phage infection risk, and/or that this CRISPR-Cas system may possess alternative gene regulatory functions. Further, through crRNA engineering, we have shown that the Type I CRISPR system in *P. aeruginosa* is able to repress and activate gene expression through base pairing in the absence of cleavage. Therefore, this work provides novel insight into the regulatory inputs for CRISPR-Cas function and also suggests regulatory outputs for CRISPR-Cas-dependent gene regulation through base pairing. Finally, the ability to design crRNAs rationally provides a facile and exciting new technology for gene regulation manipulation in this and other microbes.

Author Disclosure Block:

B. Castro, None..
J. Bondy-Denomy, None.

Poster Board Number:

LB-097

Publishing Title:

A Novel Type of Mobile DNA: How the *qacC* Gene Has Recently Spread Between Rolling Circle Plasmids of *Staphylococcus*

Author Block:

T. M. Wassenaar[1], H. Ingmar[2], D. W. Ussery[3]; [1]MMGC, Zotzenheim, Germany, [2]Univ. of Copenhagen, Frederiksberg C, Denmark, [3]Oak Ridge Natl. Lab., Oak Ridge, TN

Abstract Body:

Background: Resistance of *Staphylococcus* species to quaternary amonium compounds, frequently used as desinfectants and biocides, is due to presence of various Qac genes. Various Qac genes belong to the Small Multidrug Resistant protein family; these are mostly, though not exclusively, found on rolling-circle replicating (RC-) plasmids. Four classes of Qac genes are found in *Staphylococcus* species: QacC, QacG, QacJ and QacH. Within their class these genes are conserved, but QacC is extremely conserved, while present in variable plasmid backgrounds. The lower degree of sequence identity of these plasmids compared to the strict nucleotide conservation of their QacC gene suggests that this gene is a recent addition to these plasmids. **Methods:** By sequence comparison, we searched for an explanation how this gene was mobilized in absence of insertion sequences or other mobile elements. Publically available sequences of *qacC*, their flanking sequences and the replication gene that is invariably present in RC-plasmids were compared, to reconstruct the evolutionary history of these plasmids and the recent spread of QacC. **Results:** A new model of gene mobility is proposed that explains how *qacC* can be transfered to recipient RC-plasmids without assistance of other genes, by means of its location in between the double strand replication origin DSO and the single-strand replication origin SSO. **Conclusions:** The proposed mobilization model explains all sequence conservation observations. It represents a novel mechanism of gene mobilization in RC-plasmids, by which other genes, such as *lnuA* (conferring lincomycin resistance) is likely to be transferred as well.

Author Disclosure Block:

T. M. Wassenaar, None..
H. Ingmar, None..
D. W. Ussery, None.

Poster Board Number:

LB-098

Publishing Title:

Expression of Active Human Akt Protein Kinase in *Saccharomyces cerevisiae* Overruns PtdIns4,5P_2 Signaling Pathways

Author Block:

I. Rodriguez-Escudero, T. Fernandez-Acero, M. Molina, **V. J. Cid**; Univ. Complutense de Madrid, Madrid, Spain

Abstract Body:

The Akt protein kinase acts in mammalian cells as the core transducer of phosphatidylinositol-3,4,5-*tris*phosphate (PtdIns3,4,5P_3)-dependent signaling, thus controlling cell growth, proliferation and survival. The yeast *S. cerevisiae*, an eukaryotic model organism, lacks this phosphoinositide. However, co-expression of Akt with its upstream activator, mammalian class I phosphatidylinositol 3-kinase (PI3K), allows the heterologous reconstitution of this signaling module. Simultaneous expression of PI3K and Akt inhibited yeast growth, an effect that relied on the activity of both kinases. Both fluorescence and transmission electron microscopy revealed large plasma membrane (PM) intracellular invaginations in PI3K/Akt-expressing yeast cells. Actin patches supported the development of such altered membranes, which were also associated to the peripheric endoplasmic reticulum. Occasionally, invaginated PM promoted abnormal intracellular deposits of cell wall material, showing concomitant markers of activation of the cell wall integrity (CWI) signaling pathway, such as local recruitment of the Pkc1 protein kinase and general activation of the downstream MAPK pathway. Moreover, phosphatidylserine (PS) and, paradoxically, the PI3K substrate, phosphatidylinositol-4,5-*bis*phosphate (PtdIns4,5P_2) were enriched in such invaginations. Overproduction of the PtdIns4,5P_2-effector Slm1 largely mimicked the effects of PI3K-Akt. Furthermore, we found that Slm1 was hyperphosphorylated in PI3K-Akt expressing cells. We hypothesize that mammalian Akt is able to take over substrates of endogenous yeast AGC kinases, leading to hyperactivation of PtdIns4,5P_2-dependent pathways. These observations underscore that phosphorylation by PtdIns4,5P_2-dependent AGC kinases regulates PtdIns4,5P_2, and that such mechanisms are key actors in maintaining the homeostasis of the yeast PM.

Author Disclosure Block:

I. Rodriguez-Escudero, None..
T. Fernandez-Acero, None..
M. Molina, None..
V. J. Cid, None.

Poster Board Number:

LB-099

Publishing Title:

The Heterogeneity Of Primary And Alternative Ribosome Proteins During Drug Tolerance In Mycobacterium

Author Block:

y. li, 12208, a. ojha; Wadsworth Ctr., New York State Dept. of Hlth., Albany, NY

Abstract Body:

Background
Ribosomes, made of 23S, 16S, 5S RNA and 54 ribosomal proteins in Mycobacterium , are molecular machine for protein biosynthesis. .Generally, most prokaryotic genomes have only one gene for each ribosomal protein, but several species have additional copy of one or several ribosomal proteins. Cys-rich (CXXC) zinc binding motif is contained in the primary ribosomal proteins (C^+) but not in alternative ribosomal proteins (C^-). And there are five (L28, S14, L33, L28, L31) or four (L28, S14, L33, L28) alternative ribosomal proteins (C^-) encoded by genomes in Mycobacterium smegmatis and Mycobacterium tuberculosis respectively. C^- ribosome proteins are strongly induced in the low-zinc condition in Mycobacterium. The role of these alternative ribosomal proteins in ribosomal functionality, however, remains unclear.
Methods
In this study, we are focusing on the role of C^- ribosomal proteins in Mycobacterium by the Fluorescence microscope observations, RT-PCR, Survive experiment, iTRAQ analysis, in vitro translation assay and other methods.
Results
In this study, we showed that C- ribosomal proteins are required for the zinc homeostasis in Mycobacterium. Furthermore, we discovered that C^- ribosome is more resistant to aminoglycoside (Kanamycin, Streptomycin, Tetracycline and Hygromycin) than the C^+ ribosome, using Mycobacterium as the working model. Moreover, we can increase the sensitivity Mycobacterium smegmatis to Kanamycin and Streptomycin by repressing the expression of C^- ribosome protein through adding the zinc. Similar results are also found in Mycobacterium tuberculosis. And the proteomics iTRAQ analysis shows that zinc could regulate a switch C^+ and C^- ribosomal proteins and result in the changes of non-ribosomal proteins in Mycobacterium smegmatis. It may indicate the differences in the structure and functionality between C^- and C^+ ribosome. Furthermore, we found that the translation rate of C^- ribosome is slower than the C^+ ribosome but more resistant to Kanamycin inhibition in vitro translation assay.
Conclusions
Overall, our results suggest that C^- ribosomal proteins of Mycobacterium play an important role in the drug tolerance and the heterogeneity of primary and alternative ribosomal proteins.

Author Disclosure Block:

Y. li, None..
A. ojha, None.

Poster Board Number:

LB-100

Publishing Title:

MIC Distribution of MGIT 960 Susceptible and Resistant Strains of *Mycobacterium tuberculosis* and Correlation with Established Critical Concentrations

Author Block:

I. Martin, K. Dionne, K. Carroll, N. Parrish; Johns Hopkins Med. Inst., Baltimore, MD

Abstract Body:

Antimicrobial susceptibility testing (AST) of *M. tuberculosis* (MTB) has been based on established critical concentrations (CCs) of drugs rather than minimum inhibitory concentrations (MICs). A microbroth dilution method (TREK MYCOTB plate) allows determination of MICs for 12 drugs. We used the MYCOTB plate to determine the MIC distribution of MTB strains identified as susceptible or resistant to isoniazid (INH), rifampin (RIF), and ethambutol (EMB) in the Mycobacterial Growth Indicator Tube (MGIT)-960 system. A total of 193 unique clinical and control strains of MTB were subjected to AST using both the MGIT and the MYCOTB plate. Susceptibility/resistance in the MGIT was dependent upon growth relative to a static CC for the following drugs: INH (0.1 µg/ml), RIF (1µg/ml) and EMB (5µg/ml). For the MYCOTB plate, MICs were determined for the same drugs after 14 days of incubation. MICs were compared to established CC or the next nearest concentration represented in the MYCOTB plate. For INH, RIF, and EMB, the MYCOTB concentrations used for susceptible/resistant breakpoints were 0.06µg/ml, 1µg/ml and 4µg/ml, respectively (≤ concentration = susceptible; > concentration = resistant). Categorical agreement was calculated for all strain/drug combinations. Categorical agreement for all strains/antibiotics considered together was 98% between the 2 methods. This included a total of 424 susceptibility data points (n: RIF=137, EMB=145, INH=142). MIC distributions relative to established CC varied between antibiotics. For RIF, 100% (109/109) of MGIT susceptible strains had MICs < the CC of 1µg/ml; 100% of resistant isolates had MICs > 1µg/ml. For INH, 98% (97/99) of MGIT susceptible strains had MICs ≤0.06 µg/ml, whereas 100% of MGIT resistant strains (43/43) had MICs > 0.06 µg/ml. For EMB, 100% of MGIT susceptible strains (128/128) had MICs <4 µg/ml. However, only 53% (9/17) MGIT resistant strains had MICs >4; 8 strains identified as EMB resistant in the MGIT had MICs of 4µg/ml. Perfect categorical agreement and dichotomous MIC distribution was observed between methods for RIF, whereas for EMB and INH results were less concordant with MICs within one 2-fold dilution of the MGIT-based CC. These results demonstrate that categorical agreement decreases when the MGIT-based CC is not included in the MYCOTB plate.

Author Disclosure Block:

I. Martin, None..
K. Dionne, None..

K. Carroll, None..
N. Parrish, None.

Poster Board Number:

LB-101

Publishing Title:

Detection Of Cres In Us Clinical Laboratories

Author Block:

J. Johnson[1], V. Albrecht[2], M. Karlsson[2], G. Robinson[1], V. Stevens[2], B. Werner[2], A. Schuetz[3], D. Wolk[4], E. Marlowe[5], E. Babady[6], M. Miller[7], K. Culbreath[8], S. Butler-Wu[9], D. Diekema[10], J. Rasheed[2], B. Limbago[2]; [1]Univ. of Maryland Sch. of Med., Baltimore, MD, [2]Ctr. of Disease Control and Prevention, Atlanta, GA, [3]WCM/NY Pres, X, NY, [4]Geisinger Hlth.System, X, PA, [5]Permanente MG, Baltimore, CA, [6]MSK,, Baltimore, NY, [7]UNC, X, NC, [8]TriCore, X, NJ, [9]UWMC, X, WA, [10]UI, Baltimore, MD

Abstract Body:

Carbapenem-resistant *Enterobacteriaceae* (CRE) are an emerging threat to patients and are often resistant to all primary therapies. One aim of this study was to determine how well US laboratories identify CRE using standard methods. Targeted clinical isolates of CRE and carbapenem-susceptible *Enterobacteriaceae* (CSE) were collected from 9 US clinical laboratories between Oct. 2013-Sept. 2015 and sent to the CDC. MICs were determined using reference broth microdilution and confirmed CRE were tested by PCR for KPC, NDM, OXA-48, and other carbapenemases. 392 CRE isolates were evaluated. Of these, 339 (86.5%) were confirmed as CRE, the majority were *Klebsiella pneumoniae* (73%), *Escherichia coli* (7.4%), *Enterobacter cloacae* (8.8%) and other *Enterobacter* species (7.1%). The most common CRE not confirmed by reference testing were *E. coli* (29%), *K. pneumoniae* (27%), *E. aerogenes* and *E. cloacae* (17% each). Among 752 CSE evaluated, 12 were determined to be CRE, including 3 *E. cloacae*, 3 *E. coli*, 3 *K. pneumoniae*, and a *Proteus mirabilis*, *P. vulgaris*, and *Serratia marcescens.* The majority of confirmed CRE (n=351) were carbapenemase producers (CP), including KPC (75%), OXA-48 (1.1%) or NDM (0.6%). Non-CP CRE were resistant to ertapenem (99%), and 52% were also resistant to at least one other carbapenem; one was resistant only to doripenem. The most common non-CP CRE included *K. pneumoniae* (34%), *E. coli* and *E. cloacae* (16% each), and *E. aerogenes* (12%). Although most CP tested as CRE, 7 KPC isolates were not resistant to any of the carbapenems; these included 4 *K. pneumoniae*, and an *E. coli*, *E. cloacae*, and *E. aerogenes*. In conclusion, primary testing at clinical laboratories over-called carbapenem resistance in 14% of suspected CRE compared to reference methods. Most CP were phenotypically resistant to at least one carbapenem, but 2.5% were not resistant to any carbapenem. Discrepancies occurred among multiple species of *Enterobacteriaceae* and among both CP and non-CP CRE.

Author Disclosure Block:

J. Johnson, None..
V. Albrecht, None..
M. Karlsson, None..
G. Robinson, None..
V. Stevens, None..
B. Werner, None..
A. Schuetz, None..
D. Wolk, None..
E. Marlowe, None..
E. Babady, None..
M. Miller, None..
K. Culbreath, None..
S. Butler-Wu, None..
D. Diekema, None..
J. Rasheed, None..
B. Limbago, None.

Poster Board Number:

LB-102

Publishing Title:

Emergence of Colistin Resistant *Klebsiella pneumoniae* in an Oncology Center in Eastern India

Author Block:

G. Goel, M. Chandy, S. Banerjee, S. Gupta, S. Mullick, K. Sengupta, S. Bhattacharya; Tata Med. Ctr., Kolkata, India

Abstract Body:

Colistin is one of the last antibiotic resorts in the armamentarium in treatment of multi—drug resistant Gram negative bacilli (MDR-GNB) like carbapenem resistant enterobacteriaceae (CRE), which comprise up-to 30% of all the GNB isolated from blood cultures in developing nations. This has caused increasing dependence on colistin and thereby emerging colistin resistance in GNB is becoming a real challenge in successful treatment. There are hardly any new antibiotics in pipeline for GNB. There is dearth of epidemiologic data about colistin resistance. In this observational study we analyzed the epidemiology and outcome of patients with colistin resistant *Klebsiella*. Data was collected retrospectively from February 2014 to September 2015. A total of 12,957 cultures were processed including 6290 blood cultures. Predominant MDR-GNB were *Escherichia coli* (482), *Klebsiella pneumoniae* (343), *Pseudomonas aeruginosa* (56), *Enterobacter cloacae* (13), *Proteus* spp. (6) and *Citrobacter* spp. (4). Extended spectrum beta lactamase producers were 479 while 396 were CRE. Fifteen patients had colistin resistant *Klebsiella pneumoniae* (CRK) isolated from blood (3), respiratory samples (4), urine (3), pus (2) and 1 each from tissue, bile and stool surveillance culture. All CRK isolates were pan-resistant except fosfomycin (all 15 sensitive-S), tigecycline (8-S; 6 intermediate-I); doxycycline (1-S; 2-I); amikacin (1-S; 1-I); co-trimoxazole (3-S) and chloramphenicol (3-S). The patients were 10 males and 5 females (age range 12 to 80 years; median 44). Underlying malignancies were hematological (9), gastro-intestinal tract (3) and urinary bladder (3). Three patients have had bone marrow transplantation and 7 had neutropenia. Prior to isolation of CRK, 11 patients had infections with carbapenem resistant *Klebsiella pneumoniae* (73%), while 3 had it in stool surveillance cultures. Previous use of colistin was noted in 53% (8 patients). All cause mortality was 47% (7). The study highlights CRK is associated with high mortality. Colistin resistance can arise denovo due to selection pressure or horizontal spread as a breach in infection control practices. There is a need to optimize colistin use and dosing adjustments in deranged renal profile. Further molecular research is required to study predilection of *Klebsiella pneumoniae* for colistin resistance.

Author Disclosure Block:

G. Goel, None..
M. Chandy, None..
S. Banerjee, None..
S. Gupta, None..
S. Mullick, None..
K. Sengupta, None..
S. Bhattacharya, None.

Poster Board Number:

LB-103

Publishing Title:

Evaluation of a New Rapid Multiplexed Molecular System for the Direct Detection of Bacterial Pathogens in Blood

Author Block:

T. Alavie[1], P. Lo[2], A. Khine[1], F. Mohammadzadeh[3], T. Mazzulli[2]; [1]Qvella Corp., Richmond Hill, ON, Canada, [2]Mount Sinai Hosp., Toronto, NT, Canada, [3]Qvella Corp., Richmond Hill, ON

Abstract Body:

Background: A key element to improve the treatment of sepsis is rapid identification of bloodstream pathogens and susceptibility testing. The objective of this study was to compare pathogen detection directly from blood samples using Qvella's FAST ID™ prototype to conventional blood culture results for samples collected from patients with suspected sepsis in the emergency department (ED).
Methods: An additional "test" blood culture bottle was collected from patients ≥18 years presenting to an academic hospital ED with suspected sepsis from Feb-Nov 2015. 800 microliters of blood from this test bottle was used for the FAST ID method prior to incubation, with the reminder of the sample subjected to routine incubation up to 6 days. Any positive results detected from the test bottle (either FAST ID or culture) were cross-referenced with routine bacterial identification by MALDI-TOF from the same patient.
Results: Among 589 test bottle samples from patients with suspected sepsis, 28 samples were positive using both FAST ID and standard culture techniques, 1 culture-negative sample was positive using FAST ID while 1 culture-positive sample was negative using FAST ID. This yielded a sensitivity for bloodstream infection detection of 96.6% and specificity of 99.8% using the FAST ID when compared to standard microbial detection. Pathogen identification results from FAST ID were compared with the identification obtained through routine microbiology according to the hospital's standard clinical workflow. For 26 out of 28 positive samples (92.5%), FAST ID results were in agreement with the identification results obtained employing the routine microbiology workflow.
Conclusions: This study demonstrated the automated, rapid and direct detection and identification of a panel of bacterial pathogens in clinical blood samples. Rapid identification of microorganisms in blood samples should allow clinicians to expedite and tailor initial antimicrobial therapy for patients with suspected sepsis, resulting in improved clinical outcomes and a reduction in the use of unnecessary antibiotics. Additional clinical studies are needed to determine the clinical impact of new technologies capable of detecting microorganisms directly in blood samples collected from patients with suspected sepsis.

Author Disclosure Block:

T. Alavie, None..
P. Lo, None..
A. Khine, None..
F. Mohammadzadeh, None..
T. Mazzulli, None.

Poster Board Number:

LB-104

Publishing Title:

Inflammatory and Nutrition Biomarkers as Predictors of Growth in Environmental Enteric Dysfunction

Author Block:

S. Syed[1], K. P. Manji[2], C. McDonald[1], R. Kisenge[2], S. Aboud[2], E. Liu[1], W. Fawzi[3], C. P. Duggan[1]; [1]Boston Children's Hosp., Boston, MA, [2]Muhimbili Univ. of Hlth.& Allied Sci., Dar es Salaam, Tanzania, United Republic of, [3]Harvard Sch. of Publ. Hlth., Boston, MA

Abstract Body:

Background: Environmental enteric dysfunction (EED) is an acquired subclinical infectious condition of the small intestine among residents of low-income countries. Insulin-like growth factor 1 (IGF-1) is the circulating mediator of growth hormone activity. Adequate concentrations of IGF-1 during infancy are crucial for promoting linear growth. The suppression of IGF-1 and IGF-BP-3 (principal binding protein of circulating IGF-1) may be a primary mechanism through which EED affects growth. We aimed to assess the association between IGF-1 & IGF-BP3 with child growth among young Tanzanian children at risk of EED.**Methods:** Blood samples were obtained from 590 children at age 6 wks & 6 mths. Measurement of serum IGF-1 and IGF-BP3 was by ELISA. Growth was measured monthly. Cox proportional hazards models were constructed to estimate hazard ratios (HRs) and corresponding 95%CIs of stunting (HAZ<-2), wasting (WHZ<-2), & underweight (WAZ<-2) across quartiles of IGF-1 and IGF-BP3.**Results:** In multivariate analyses, children with IGF-1 concentrations in the highest quartile had a 51% (HR 0.49, 95% CI: 0.3, 0.8; p for trend=0.05) decreased risk of stunting and a 57% (HR 0.43, 95% CI: 0.23, 0.78; p for trend=0.048) lower risk of underweight than children with concentrations in the lowest quartile after adjusting for covariates. Compared to infants with IGF-BP3 levels in the lowest quartile, those with values in the highest quartile had a 51% (HR 0.49, 95% CI: 0.27, 0.91; p for trend=0.049) lower risk of underweight compared to those in the lowest quartile. Neither biomarker was related to the risk of wasting.**Conclusions:** Serum levels of IGF-1 and IGF-BP3 in early infancy are associated with stunting and underweight. The relationship between these growth markers and their biological significance with regards to EED require further study.

Author Disclosure Block:

S. Syed, None..
K. P. Manji, None..
C. McDonald, None..
R. Kisenge, None..
S. Aboud, None..

E. Liu, None..
W. Fawzi, None..
C. P. Duggan, None.

Poster Board Number:

LB-105

Publishing Title:

Killing *Vibrio cholerae* by a Chemical Modulator for Glucose Metabolism

Author Block:

Y. Oh, H. Kim, S. Yoon; Yonsei Univ. Coll. of Med., Seoul, Korea, Republic of

Abstract Body:

Vibrio cholerae, a Gram-negative bacterium, causes pandemic cholera. An oral rehydration solution (ORS), which contains a large amount of glucose, has been used as the primary treatment for cholera. Meanwhile, it has also been speculated that continuous administration of ORS may create glucose-enriched microenvironments in favor of *V. cholerae* growth. Previous studies revealed that the capability of *V. cholerae* 7th pandemic strain N16961 to produce acetoin is essential to maintain the survival fitness under glucose-rich environment. Production of acetoin, a neutral fermentation end-product, allows *V. cholerae* metabolizing glucose without pH drop, which is mediated by organic acid production. This notion also suggests that inhibition of acetoin fermentation may end up with killing *V. cholerae* by acidic pH stress under glucose-rich conditions. Here, we developed a simple high-throughput screen to identify **i**nducers of **m**etabolism-mediated **ac**idification (termed as iMAC). Out of 8,364 compounds, we identified a chemical, 5-(4-chloro-2-nitrobenzoyl)-6-hydroxy-1,3-dimethylpyrimidine-2,4(1H,3H)-dione (iMAC17191) that killed glucose-metabolizing N16961 by pH drop. When N16961 was grown with extra glucose in the presence of 50 μM iMAC17191, acetoin production was completely suppressed and concomitant accumulation of lactate and acetate was observed. Beta-galactosidase activity assay using a single-copy P_{VC1589}::*lacZ* reporter fusion demonstrated that iMAC17191 likely inhibited acetoin production at the transcriptional level. Together, our results suggest that iMAC17191, acting as a metabolic switch, has a therapeutic potential to be developed as a novel antibacterial agent against cholera.

Author Disclosure Block:

Y. Oh, None..
H. Kim, None..
S. Yoon, None.

Poster Board Number:

LB-106

Publishing Title:

HCV Antigen Testing Instead of HCV Antibody Testing to Screen for HCV Infections

Author Block:

M. M. Konstantinovski, H. J. Gerritsen, J. J. C. de Vries; Leiden Univ. Med. Ctr., Leiden, Netherlands

Abstract Body:

Background: Anti-HCV serology is routinely used for screening for active and past HCV infections in the Netherlands. HCV core antigen (Ag) testing is not delayed by a window phase between infection and immune response as is the case with anti-HCV, also HCV Ag testing is inexpensive compared to PCR. This study investigated the diagnostic characteristics of HCV Ag testing in the setting of an academic center.
Methods: 4 types of clinical serum and plasma samples were selected and HCV Ag testing was performed conform the manufacturers manual. HCV RNA load was used as the golden standard. Group 1(N=23): Active HCV infection with a RNA load of <100 000 IU/mL ;Group 2 (N=18): Active HCV infection with a RNA load of >100 000 IU/mL; Group 3 (N=17): Cleared HCV infection with undetectable RNA load; Group 4 (N=2): Acute infections with positive PCR but negative HCV ab serologyOverall 11 samples were from HIV positive patients, 14 samples had an unknown HIV status and the rest of the samples was HIV negative.
Results: All 18/18 samples with high HCV RNA load tested positive for HCV Ag. Whereas 9/23 samples with a RNA load <100 000 IU/mL tested negative for HCV Ag. The positive HCV Ag samples from group 2 had a viral load of 7900-79000IU/mL and the negative samples of 54-25400 IU/mL, 4 negatives belonged to HIV positive patients. All 17 cleared HCV infection samples tested negative. The two acute infections tested positive for HCV Ag with a viral load at the time of diagnosis of 79000 IU/mL and > 1,000,000 IU/mL Another seroconversion was identified. This patient had a negative HCV Ag test along with a positive HCV Ab and a low viral load of 158 IU/mL.
Conclusions: From other studies it is known that the HCV Ag test performs less within the lower range of viral load, that has been confirmed in this study. We identified 2 patients who would have benefited from the HCV Ag test with a more timely diagnosis of HCV.
Discussion: We demonstrated that the HCV Ag test should be used in addition to HCV Ab testing due to possible missed cases if there is a low viral load, either in the acute phase or later on. HCV Ag testing may be a more appropriate choice if a recent infection is suspected.Our medical center decided to implement the HCV Ag testing as per march 2016 as a routine part of the periodical screening of HIV patients in addition to the HCV Ab test, being the first laboratory in Europe implementing this test.

Author Disclosure Block:

M. M. Konstantinovski, None..
H. J. Gerritsen, None..
J. J. C. de Vries, None.

Poster Board Number:

LB-107

Publishing Title:

Cobas HPV Assay; Suboptimal Reproducibility and Effect of Re-testing to Detect False Negative Results

Author Block:

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Abstract Body:

The FDA-cleared, Cobas human papillomavirus (HPV) assay (Roche, Pleasenton, CA) detects 14 high risk (HR) HPVs and distinguishes HPV 16 and 18 from the other 12 HR viruses. It is a qualitative, multiplex, real time (RT) PCR. Cobas HPV results are reported as positive or negative for HPV 16, 18 or for the other 12 HR HPVs. Samples that have a crossing point (Cp) value less than the manufacturer's assigned cut-off value, are reported positive. Those with no Cp or a Cp greater than the cut-off value are reported negative. Upon retesting of clinical samples we found that 4 - 6% of results were not reproducible. The discrepancies occur in samples with Cp values near the cut off value. Minor variations in Cp in RT PCR assays are well known, but such small changes are usually inconsequential. However, near the cut-off value, these variations may change test result from negative to positive and vice versa. With the new proposed screening algorithm that uses HPV with genotype as the primary screening tool for cervical cancer, false negative HPV results can have significant impact on patient care. Following the discovery of suboptimal reproducibility of the Roche HPV assay, and to avoid false negative results, we started to test all HPV samples in duplicate. After testing 2200 clinical samples, we observed a 4% discrepancy between duplicate results. All discrepant results belonged to the samples with Cp values close to the cut-off value of the assay. Running all HPV samples in duplicate imposed additional costs to our lab. We discussed the issue with Roche and requested access to Cp value of those samples reported as negative. Our goal was to limit repeat testing to negative samples with Cp values near the cut off. Our request was temporarily approved by Roche for research and investigational purposes, but they feel that, since the test is not FDA approved in this format, it constitutes an off-label use. Physicians should be alerted to the possibility of false-negative results, so they can make appropriate clinical decisions about patient follow up.

Author Disclosure Block:

H. Salimnia,
GenMark, NanoSphere, Accelerate, GenePoc, Luminex Role(s): Self, I. Research Relationship.

P. Lephart,
luminex, Nansphere, accelerate, GenePoc Role(s): Self, I. Research Relationship.
C. Rajan, None..
D. Prebelich, None..
E. Paulson, None..
J. Howell, None..
S. Hallock, None..
K. Fabis, None..
M. Fairfax, None.

Poster Board Number:

LB-108

Publishing Title:

Prevalence of *Trichomonas vaginales* in ThinPrep Specimens from Asymptomatic Patients of Various Age Groups to Improve Sexually Transmitted Disease Diagnosis and Management

Author Block:

M. Martinez, P. Rodriguez, C. Lanteri, J. Perez, L. Hamilton; San Antonio Military Med. Ctr., San Antonio, TX

Abstract Body:

Background: The Center for Disease Control states that the prevalence of *Trichomonas vaginales* infections increases with age. However, *Trichomonas vaginales* testing is not routinely in asymptomic patient populations, suggesting that the prevalence is underdiagnosed. The purpose of our study was two-fold: 1) to confirm CDC's age stratification observations and 2) determine the prevalence of *T. vaginales* in ThinPrep samples in asymptotic patients.
Methods: PANTHER nucleic acid amplication testing using Aptima *T. vaginales* primer and probe kit was used to detect *T. vaginales* in 1000 ThinPrep clinical specimens during an eight week month period. Results were tallied by age groups and prevalence determined.
Results: Of the 1000 samples tested, 400 samples were collected from patients >30, 300 from ages 25-30, and 300 from those less than 25-years of age. Overall, 2.8% of samples tested positive for *T. vaginales*. The highest prevalence was in those >30 years with a 3.75% (37/1000) positivity rate. Patients between the ages of 25-30 had a 2.3% (7/300) positive rate while those samples less than 25 years were positive in 2% (6/300) of the samples.
Conclusions: Our results confirm that the prevalence of *T. vaginales* is greater in older woman than younger cohorts. Furthermore, samples that otherwise would not have been tested for *Trichomonas vaginales* were found to be positive, suggesting that routine screening of asymptotic patients is warranted.

Author Disclosure Block:

M. Martinez, None..
P. Rodriguez, None..
C. Lanteri, None..
J. Perez, None..
L. Hamilton, None.

Poster Board Number:

LB-109

Publishing Title:

Decreasing Overall Turnaround Times for Targeted and Whole Genome Sequencing

Author Block:

C. Davidson[1], M. Landes[2], R. Qi[1], C. Parikh[1], D. Mandelman[2], H. Latif[2], W. Zhang[2], A. Harris[2], N. Hasan[3], S. Ghosh[1]; [1]Thermo Fisher Scientific, South San Francisco, CA, [2]Thermo Fisher Scientific, Carlsbad, CA, [3]CosmosID, Inc, Rockville, MD

Abstract Body:

Background: Currently next generation sequencing (NGS) is handicapped by slow and complex workflow processes. Reducing overall turnaround time is critical for the wide adoption of targeted and whole genome sequencing (WGS) for microbiological applications. Here we describe improvements to the four steps of the NGS workflow: i) library preparation; ii) template preparation, iii) sequencing; iv) and data analysis. Together, these improvements dramatically decrease the overall sequencing turnaround times. **Material/Methods:** The new rapid approach was applied to two different library preparation protocols: i) a highly-multiplexed PCR approach consisting of 1200 amplicons targeting the 16S rRNA gene as well as species and antimicrobial resistance determinants; ii) and a WGS approach using a MuA transposon-based library preparation method. To assess the accuracy of detection with the optimized workflow, nucleic acid from six bacterial cultures (*A. baumannii, E. cloacae, E. faecium, K. pneumoniae, P. aeruginosa,* and *S. aureus)* were extracted as input for targeted and WGS sequencing. Both targeted and WGS libraries were clonally amplified using an isothermal amplification approach that saves 3 hours over the standard methods. Sequencing times were improved by reducing flow times and the total number of flows. The implementation of On-Instrument Analysis (OIA) enabled near real-time base calling reducing the total primary analysis time. **Results:** Targeted and WGS libraries were generated, sequenced, and analyzed in 6.5 hours with sequencing and analysis taking 50 minutes compared to 2.5 hours and 1 hour for standard sequencing and analysis, respectively. Analysis of sequencing accuracy revealed a raw read accuracy >99.5%, comparable to data from the standard workflow. The read length distribution for the targeted libraries was similar to standard workflow with 100% specificity for species identification and antimicrobial resistance determinants for targeted and WGS libraries indicating rapid sequencing without compromising detection accuracy. **Conclusions:** For the targeted and WGS assays described, the total turnaround time could be completed in a standard workday.

Author Disclosure Block:

C. Davidson,
Thermo Fisher Scientific Role(s): Self, D. Employee.
M. Landes,

Thermo Fisher Scientific Role(s): Self, D. Employee.
R. Qi,
Thermo Fisher Scientific Role(s): Self, D. Employee.
C. Parikh,
Thermo Fisher Scientific Role(s): Self, D. Employee.
D. Mandelman,
Thermo Fisher Scientific Role(s): Self, D. Employee.
H. Latif,
Thermo Fisher Scientific Role(s): Self, D. Employee.
W. Zhang,
Thermo Fisher Scientific Role(s): Self, D. Employee.
A. Harris,
Thermo Fisher Scientific Role(s): Self, D. Employee.
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CosmosID Role(s): Self, D. Employee.
S. Ghosh,
Thermo Fisher Scientific Role(s): Self, D. Employee.

Poster Board Number:

LB-110

Publishing Title:

First Report of *E. coli* Isolates Harboring the *mcr-1* Gene in Brazil

Author Block:

S. A. M. Lentz[1], D. Lima-Morales[2], V. M. L. Cuppertino[1], A. S. Motta[1], A. L. Barth[2], **A. F. Martins**[1]; [1]Univ. Federal do Rio Grande do Sul - UFRGS, Porto Alegre, Brazil, [2]Hosp. de Clínicas de Porto Alegre, Porto Alegre, Brazil

Abstract Body:

Background: The recent identification of the plasmid-mediated transferable colistin resistance *mcr-1* gene is of great concern to public health as polymyxins are considered the last resource for treatment of infections caused by carbapenemase producing Enterobacteriaceae. The *mcr-1* gene, was first described in China, and was then reported in Europe, Asia, Canada and Africa. In view of the concerning spread of antibiotic resistance and the prevalence of *mcr-1* gene among ESBL producers, we screened ceftazidime resistant isolates collected in a poultry slaughterhouse. **Methods:** A total of 295 isolates obtained from poultry chicken rectal swab in 2015 in southern Brazil, were screened by PCR for the presence of the *mcr-1* gene. We were able to find the *mcr-1* gene in 3 out of the 295 isolates (1,0%). **Results:** The 3 amplicon fragments were sequenced using the CLR5-F (5′-CGGTCAGTCCGTTTGTTC-3′) and CLR5-R (5′-CTTGGTCGGTCTGTAGGG-3′) primers and showed a 100% identity with the *mcr-1* sequence (GeneBank Accession Number KP347127). According to 16S RNA analysis, all three isolates were identified as *Escherichia coli.* The minimum inhibitory concentration (MIC) of polymyxin, performed by broth microdilution, was 1, 2 and 8 mg/L. The isolates were susceptible to piperacillin-tazobactam, amikacin, cefepime, meropenem and gentamicin and non-susceptible to cefuroxime, amoxicilin-clavulanate, ampicillin, ampicillin-sulbactam, sulfametoxazol/trimetoprim, ciprofloxacin and ceftazidime by the disc diffusion method. Further studies are being carried out in order to evaluate the presence and type of plasmids and to establish the MLST of the *E. coli mcr-1* positive isolates. **Conclusions:** To the best of our knowledge, this is the first report of *mcr-1* in Brazil and, probably in Latin America, highlighting the worldwide spread of this novel resistance determinant.

Author Disclosure Block:

S. A. M. Lentz, None..
D. Lima-Morales, None..
V. M. L. Cuppertino, None..
A. S. Motta, None..
A. L. Barth, None..
A. F. Martins, None.

Poster Board Number:

LB-111

Publishing Title:

Moxifloxacin Improves Treatment Outcomes in Patients with Ofloxacin-resistant Multidrug-resistant Tuberculosis

Author Block:

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Abstract Body:

Background: It is unclear whether the use of moxifloxacin (MFX), a fourth-generation synthetic fluoroquinolone, results in better outcomes in patients with ofloxacin (OFX)-resistant multidrug-resistant tuberculosis (MDR-TB). **Methods:** During the period April 2006 to December 2013, a total of 2511 patients with culture-confirmed tuberculosis (TB) were treated at a TB referral hospital in southern Taiwan. Of them, 325 (12.9%) had MDR-TB, and of those patients, 81 (24.9%) had OFX-resistant MDR-TB and were included in the study. **Results:** Among the 81 patients with OFX-resistant MDR-TB, 50 (61.7%) were successfully treated and 31 (38.3%) had unfavorable outcomes, including treatment failure (n=25; 30.9%), loss to follow-up (n=2; 2.5%) and death (n=4; 4.9%). Patients with MFX-susceptible and low-level MFX-resistant isolates who were treated with MFX had a significantly higher rate of treatment success (77.3% vs. 43.2%; odds ratio [OR]=4.46; 95% confidence interval [CI]=1.710-11.646; *P*=0.002) than without MFX. Multivariate logistic regression analysis showed that treatment with MFX (OR=3.46; 95% CI=1.18-10.15; *P*=0.024) and second-line injectable agents during the intensive phase (OR=3.29; 95% CI=1.11-9.74; *P*=0.031) were independent predictors of treatment success. Mutation at codon 94 in the *gyrA* gene was the most frequent mutation (68.0%) associated with high-level MFX resistance. Multivariate Cox proportional hazards regression analysis showed that treatment with MFX was an independent factor associated with early culture conversion (hazard ratio=2.02, 95% CI=1.14-3.56; *P*=0.016). **Conclusions:** Our results show that a significant proportion of OFX-resistant MDR-TB isolates were susceptible or had low-level resistance to MFX, indicating that patients with OFX-resistant MDR-TB benefit from treatment with MFX.

Author Disclosure Block:

J. Chien, None..
C. Yu, None..
P. Hsueh, None.

Poster Board Number:

LB-112

Publishing Title:

Pyrvinium Inhibits the Growth and the Biofilm Formation of *Candida albicans* by Affecting Yeast Casein Kinase 2

Author Block:

M. Macias, L. Yeo, J. Garcia, C. Truong, H. Park; California State Univ. Los Angeles, Los Angeles, CA

Abstract Body:

Background: As an opportunistic pathogen, *Candida albicans* cause serious infection in immunocompromised individuals. The pathogenicity of *C. albicans* can be attributed to its ability to form hyphae and biofilm on host cells. Our previous study discovered that yeast casein kinase 2 (*YCK2*), a member of Casein Kinase I (CK1) family of eukaryotic systems, negatively regulates hyphal transition and biofilm formation (Jung et al). Recently, an anthelmintic drug, pyrvinium pamoate, has been reemphasized as a potential anti-cancer drug as it targets Casein Kinase I (CKI), which governs cell cycle (Deng et al). Thus we hypothesized that pyrvinium also affects *YCK2* that shares a significant homology to CK1, thus may inhibit hyphal transition and biofilm formation in *C. albicans*.
Methods: *C. albicans* wild type and *yck2* mutant strains were tested for morphology, growth, and biofilm formation in various concentrations of pyrvinium. To determine the molecular mechanisms by which pyrvinium affects on *C. albicans* hyphal transition and biofilm formation, transcriptional analysis on *YCK2* and its homologs were conducted with pyrvinium compared to no drug control.
Results: The morphology of cells grown in pyrvinium, demonstrated signs of cell apoptosis for 2.5 μg/mL, with the most cell death in the 5 μg/mL. Growth curve analysis showed significant growth inhibition by pyrvinium as a dose dependent manner. The biofilm formation was also inhibited by pyrvinium as a dose dependent manner. It was also noted that the *yck2* mutant strain was less affected by pyrvinium compared to the wild type strain in biofilm inducing condition, still remains 30-40 % more biofilm than the wild type strain. Transcriptional analysis demonstrated the mRNA expression of all three CK1 homologs was not changed by pyrvinium, suggesting the inhibitory effect of pyrvinium to *C. albicans* growth and biofilm formation is likely by affecting *YCK2* at post translational level.
Conclusions: Pyrvinium has inhibitory effect on both growth and biofilm formation of *C. albicans.* In particular, the drug seems to function via manipulating the YCK2 signaling cascade. As pyrvinium is a FDA approved and safe to treat patients, we will further look into the possibility of utilizing pyrvinium as a new antifungal agent.

Author Disclosure Block:

M. Macias, None..
L. Yeo, None..
J. Garcia, None..
C. Truong, None..
H. Park, None.

Poster Board Number:

LB-113

Publishing Title:

Computational Predictions of Substrate Specificity in Nonribosomal Peptide Synthetases through Comparative Adenylation Domain Trees

Author Block:

M. G. Chevrette[1], C. R. Currie[1], M. H. Medema[2]; [1]Univ. of Wisconsin-Madision, Madison, WI, [2]Wageningen Univ., Wageningen, Netherlands

Abstract Body:

Microbial nonribosomal peptide synthetases (NRPSs) are an important source of complex natural molecules of high therapeutic and biotechnological value. These modular protein-systems are found in a variety of microbes and produce structurally and functionally diverse peptide metabolites used in medicine as antibiotics, anticancers, and more. The order and identity of peptides incorporated by an NRPS are dictated by its assembly-line module and domain structure. Various methods have been developed to computationally predict NRPS adenylation domain (NRPS-AD) substrate specificity directly from genomes. The ability to deduce this information from sequence data has revolutionized natural product drug discovery by greatly increasing throughput, prioritizing novelty, and lowering the rate of costly rediscoveries. However, it is unclear how available algorithms compare in terms of accuracy. Here, we gathered 314 NRPS-AD sequences from recently experimentally characterized NRPSs from literature, and used these as a test set for systematic benchmarking. Algorithms based on active site motifs (using pattern matching or support-vector machines) performed better than whole-domain-based algorithms (using profile hidden Markov models; pHMMs), at 56% and 42% accuracy respectively. All algorithms were unable to reliably predict highly similar (>80% protein identity; PID) NRPS-ADs that had shifted or expanded substrate specificity due to recent evolutionary events. To address this, we describe a comparative genomics algorithm (Predictions through Comparative A-domain Trees; prediCAT) which constructs NRPS-AD trees and predicts query specificity by monophyly. PrediCAT increased precision to 65% accuracy within the test set. Incorporating the entire assembled dataset into a jackknife benchmarking scheme resulted in the correct substrate predictions for 86% of NRPS-ADs by prediCAT compared to 63% by pHMMs. At >80% PID, prediCAT was 86% accurate versus 72% in pHMMs. Queries at low similarity (<60% PID) to the training data had accuracies of 81% and 40% for prediCAT and pHMMs respectively. Our findings suggest that comparative genomics can be used as a powerful tool to predict NRPS scaffolds to assist investigations of chemical diversity and drug discovery efforts.

Author Disclosure Block:

M. G. Chevrette, None..
C. R. Currie, None..
M. H. Medema, None.

Poster Board Number:

LB-114

Publishing Title:

Systemic Delivery of Multiple siRNA Treatment for Prevention of Neuro-inflammation and Viral Encephalitis, Induced by Japanese Encephalitis Virus in BALB/c mice.

Author Block:

T. N. Dhole, D. Singh, A. Zia, P. Kumar, S. Saxena, R. Kapoor; Sanjay Gandhi Post Graduate Inst. of Med. Sci., Lucknow, UP, India

Abstract Body:

Background: Japanese Encephalitis Virus (JEV) is a member of the family Flaviviridae. JEV is transmitted by Culex mosquitoes between wild and domestic birds and pigs. Therapy for JE is supportive and no clearly effective antiviral agents exist. In recent years RNAi has been explored to inhibit viral diseases. However, the development of siRNA-based antiviral strategies hampered by a number of challenges related to the in vivo delivery. Rabies virus glycoprotein (RVG) is able to cross the blood brain barrier. Complexing or encapsulating siRNA with liposomes has been shown to protects RNAi from degradation and improve delivery through the vasculature.**Methods:** siRNA against structural proteins of JEV, designed in-silico, synthesized commercially. Cationic liposome prepared and physically characterized by Zeta sizing and electron microscopy. Mice were challenged with 3×10^5 PFU of GP-78. siRNA were given at 0 hour, 24 hour, day 3, and day 5. BALB/c mice sacrificed at 3d.p.i., 5d.p.i., 7 d.p.i., and 9 d.p.i. Virus was estimated in brain by plaques and rRT-PCR. CBA was done for cytokine levels. Brain histopathology and IHC was done for the JEV, MMPs (2, 7 and 9) and TIMP (1 and 3) for virus and CNS inflammations. Mice were observed for mortality for 30 days.
Results: Viral replication was inhibited in N2A by 100 pmole of siRNA. Decreased mean log pfu viral load has been observed (3.46 on 3dpi, 3.47 on 5dpi, 2.37 on 7dpi and 2.59 on 9dpi) as compared to infection control mice. Over expression of MMPs and TIMPs has been suppressed followed by reduced neuronal degradation. Mice were survived up to 30 days whereas controls died up to 9th day. Virus was cleared from brain suggested by histopathology and IHC examinations. **Conclusions:** Study could be used as effective clinical strategy in epidemic outbreak for JEV positive patients even after vaccination.

Author Disclosure Block:

T. N. Dhole, None..
D. Singh, None..
A. Zia, None..
P. Kumar, None..
S. Saxena, None..
R. Kapoor, None.

Poster Board Number:

LB-115

Publishing Title:

Extended Protection by DAV131 against Antibiotic-Induced *Clostridium difficile* Infection in Hamsters

Author Block:

N. Saint-Lu[1], S. Sayah-Jeanne[1], F. Sablier-Gallis[1], M. Pulse[2], C. Burdet[3], T. T. Nguyen[3], W. J. Weiss[2], F. Mentré[3], E. Chachaty[4], **A. Andremont**[3], J. de Gunzburg[1]; [1]Da Volterra, Paris, France, [2]Univ. of North Texas Hlth.Sci. Ctr., Fort Worth, TX, [3]Univ. Paris Diderot and INSERM, UMR 1137, IAME, Paris, France, [4]Inst. Gustave Roussy, Villejuif, France

Abstract Body:

Background: We previously showed that DAV131, an oral adsorbent-based product, could protect hamsters against *C. difficile* infection (CDI) induced by moxifloxacin. Besides fluoroquinolones, cephalosporins and clindamycin are major risk factors for the development of CDI. We investigated if DAV131 could also protect hamsters against CDI after treatment with these antibiotics.
Methods: Male Syrian hamsters were administered 90 mg/kg ceftriaxone subcutaneously oad for 5 days (D1 to D5), and infected orally at D3 with 10^4 *C. difficile* spores from a non-epidemic toxigenic strain. Alternatively, hamsters were infected orally at D1 with 10^4 *C. difficile* spores as above, and administered 10 mg/kg clindamycin subcutaneously at D2. Groups of 10 animals, in either experimental settings, were orally administered 100, 300, 600 or 900 mg/kg DAV131 twice per day, from D1 to D8. Survival was monitored up to D12, and feces were collected to determine free clindamycin concentrations by bioassay.
Results: When administered at 900 and 600 mg/kg doses, DAV131 protected 100 % of hamsters from mortality induced by clindamycin, whereas 30% and no protection were achieved with 300 and 100 mg/kg doses respectively. This was associated with a significant and dose-dependent reduction of clindamycin concentrations in feces. A similar dose-dependent protection from ceftriaxone-induced CDI was also achieved.
Conclusions: Oral DAV131 exhibited a dose-dependent protection of hamsters against ceftriaxone-, and clindamycin-induced lethal CDI, as it did with moxifloxacin. This study clearly shows that DAV131 constitutes the first preventive strategy that can potentially protect against CDI when applied concomitantly with diverse causative antibiotic treatments. The development of this promising strategy for the prevention of CDI in humans (named DAV132) is ongoing.

Author Disclosure Block:

N. Saint-Lu,
Da Volterra Role(s): Self, D. Employee.
S. Sayah-Jeanne,

Da Volterra Role(s): Self, D. Employee.
F. Sablier-Gallis,
Da Volterra Role(s): Self, D. Employee.
M. Pulse,
Da Volterra Role(s): Self, H. Research Contractor.
C. Burdet,
Da Volterra Role(s): Self, H. Research Contractor.
T. T. Nguyen,
Da Volterra Role(s): Self, H. Research Contractor.
W. J. Weiss,
Da Volterra Role(s): Self, H. Research Contractor.
F. Mentré,
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E. Chachaty,
Da Volterra Role(s): Self, C. Consultant.
A. Andremont,
Da Volterra Role(s): Self, C. Consultant.
J. de Gunzburg,
Da Volterra Role(s): Self, A. Board Member.
Da Volterra Role(s): Self, C. Consultant.
Da Volterra Role(s): Self, K. Shareholder (excluding diversitied mutual funds).

Poster Board Number:

LB-116

Publishing Title:

Ridinilazole Preserves Major Components of the Intestinal Microbiota During Treatment of *Clostridium difficile* Infection

Author Block:

J. Chang[1], A. Kane[1], L. McDermott[1], R. J. Vickers[2], D. R. Snydman[1], C. M. Thorpe[1]; [1]Tufts Med. Ctr., Boston, MA, [2]Summit Therapeutics, Abingdon, United Kingdom

Abstract Body:

Background: *Clostridium difficile* infection (CDI) and the subsequent high recurrence rates have been shown to be associated with perturbation of the native microbiota following the use of broad-spectrum antibiotics such as vancomycin to treat CDI. There is a need for *C. difficile* treatment that may have minimal impact on the normal protective microbiota. **Methods:** Stool samples were obtained from 82 patients enrolled in a phase II randomized, blinded clinical trial comparing vancomycin and ridinilazole. Real-time quantitative PCR was used to assess changes in 5 different bacterial groups (*Bacteroides, Prevotella, Enterbactericeae, C. coccoides* and *C. leptum*) as well as all bacteria present using universal 16S ribosomal DNA primers. Plasmid-based standard curves were utilized to interpolate copy numbers per sample. Patient samples were obtained at study entry, days 5 and 10 of treatment, day 25 and day 40 post-entry, as well as at the time of any recurrence. **Results:** Treatment with vancomycin significantly decreased ($P < 0.001$) levels of the groups *Bacteroides, Prevotella, C. coccoides* and *C. leptum* during treatment days 5 and 10, with an average drop of 3 logs for each of these groups. Vancomycin also resulted in significant decrease of all bacteria as detected by the 16S primers. Treatment with ridinilazole resulted in no significant decrease in these specific microbiota groups and total bacteria. Neither vancomycin nor ridinilazole resulted in any significant change in *Enterobacteriaceae* levels. **Conclusions:** These findings indicate that ridinilazole is superior to vancomycin in sparing these major components of the native microbiome during treatment of CDI, which may be responsible for the marked reduction in disease recurrence rates following ridinilazole therapy.

Author Disclosure Block:

J. Chang, None..
A. Kane, None..
L. McDermott, None.
R. J. Vickers,
Summit Therapeutics Role(s): Self, D. Employee.
D. R. Snydman, None..
C. M. Thorpe, None.

Poster Board Number:

LB-117

Publishing Title:

In Vitro* and *In Vivo* Efficacy of the Novel β-lactamase Inhibitor ETX2514 Combined with Sulbactam Against Multidrug Resistant *Acinetobacter baumannii

Author Block:

J. O'Donnell[1], J. Newman[2], A. Tanudra[1], A. Chen[1], B. De Jonge[2], H. Shankaran[2], J. Mueller[1], R. Tommasi[1]; [1]Entasis Therapeutics, Waltham, MA, [2]AstraZeneca, Waltham, MA

Abstract Body:

Background: The novel diazabicyclooctenone β-lactamase inhibitor ETX2514 is active against Class A, C and D serine β-lactamases and effectively restores the antibacterial activity of sulbactam against multidrug resistant (MDR) *A. baumannii*. To further evaluate the therapeutic potential of this combination, its activity against a large collection of clinical isolates and its PK/PD drivers *in vitro* and *in vivo* were assessed. **Methods:** The *in vitro* antibacterial activity of sulbactam with 4 mg/L of ETX2514 was determined at IHMA against a globally diverse collection of *A. baumannii* from 2011-2014 (n = 1223), by broth MIC using CLSI guidelines. The *in vitro* activity of ETX2514 / sulbactam was characterized using an *in vitro* hollow-fiber model. Dose range and dose fraction experiments varying concentration (C_{max}, AUC) and frequency of administration of ETX2514 and sulbactam (q3h, q6h, q12h, and q24h) were used to determine PK/PD endpoints against five clinical *A. baumannii* strains. The same strains were evaluated *in vivo* by bacterial burden reduction in a tissue-based abscess in neutropenic thigh and lung infection models to validate exposure targets established *in vitro*. **Results:** The MIC_{90} of sulbactam/ETX2514 was 4 mg/L across all years tested with no carbapenem cross-resistance observed. Initial results of *in vitro* and *in vivo* investigations with sulbactam alone vs. a sulbactam-sensitive *A. baumannii* isolate (MIC = 2 mg/L) suggested Time > MIC as the PK/PD driver with 21 to 38% Time > MIC associated with a 1-log kill *in vivo*. Against MDR strains (with sulbactam MICs > 8 mg/L), ETX2514 restored MIC potency to < 2 mg/L and demonstrated a PK/PD driver of Time > critical threshold (C_T) in the hollow-fiber model system. E_{max} modelling of *in vivo* data from lung and thigh models suggested ETX2514 effectively restores sulbactam activity, and > 1-log kill is realized with Time > MIC for the combination ranging from 26 to 58% of the dosing interval. **Conclusions:** The combination of ETX2514 and sulbactam was highly effective against clinical *A. baumannii* strains *in vitro* and *in vivo*. These results support further evaluation of this combination to treat MDR *A. baumannii* infections.

Author Disclosure Block:

J. O'Donnell, None..
J. Newman, None..
A. Tanudra, None..

A. Chen, None..
B. De Jonge, None..
H. Shankaran, None..
J. Mueller, None..
R. Tommasi, None.

Poster Board Number:

LB-118

Publishing Title:

A Phase 1 Safety and Tolerability Study of Radavirsen (AVI-7100), an Anti-sense Oligonucleotide Targeting Influenza A M1/M2 Translation

Author Block:

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Abstract Body:

Background: Therapeutic agents with novel mechanisms of action are needed for treating influenza A viruses. Radavirsen, formerly AVI-7100, is an antisense oligomer (specifically a phosphorodiamidate morpholino oligomer with positive charge (PMOplus®)) that has been shown to inhibit the translation of M1 and M2 protein of influenza A and has demonstrated preclinical efficacy in mouse and ferret models. **Methods:** A Phase 1 safety, tolerability and pharmacokinetic study of Radavirsen was performed in healthy adult volunteers in the Clinical Trials Unit at the National Institutes of Allergy and Infectious Diseases, NIH. The study was divided into 2 parts. The first was a single-ascending dose study of 5 cohorts, 40 subjects total, randomized 6:2 to receive single intravenous doses of Radavirsen ranging from 0.5 mg/kg to 8 mg/kg or control, and followed for 28 days. The second part was a multiple dose study of 16 subjects randomized 12:4 to receive 8 mg/kg/day or control x 5 days and followed for 32 days. **Results:** A total of 66 subjects signed informed consent, and 56 subjects were dosed. All doses of Radavirsen were well tolerated. The most common AE in the single dose cohorts were headaches, hyperglycemia, hypomagnesemia, and hypoglycemia. Headaches, hypomagnesemia, and hypoglycemia were similar between the subjects receiving Radavirsen and controls. Transient grade 1 hyperglycemia occurred more frequently in subjects receiving Radavirsen, and was more common with the higher doses. There were no apparent dose related changes in other laboratory parameters or EKG measurements. Following a single dose, Radavirsen conforms to a linear and dose-proportional two-compartmental pharmacokinetic model, with a terminal half life of approximately 2.7-3.0 hours. The multi-dose cohort had an increase in reversible proteinuria in the AVI treated cohort. PK analysis of this cohort reveled an effective half life of 2-3 hours, though there was a third compartment with an extended half-life without drug accumulation. **Conclusions:** With the data available to date, Radavirsen appears to be well tolerated. Full safety and pharmacokinetic data from the single and multi-dose cohorts will be presented.

Author Disclosure Block:

J. Beigel, None..
J. Voell, None..
P. Munoz, None.
P. Iversen,
Sarepta Therapeutics Role(s): Self, D. Employee.
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Sarepta Therapeutics Role(s): Self, D. Employee.
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Sarepta Therapeutics Role(s): Self, D. Employee.
R. T. Davey, None.

Poster Board Number:

LB-119

Publishing Title:

Identification of Genes Mediating Antibiotic Sensitivity in *Mycobacterium tuberculosis* Using Tn-seq

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Abstract Body:

The treatment of tuberculosis (TB) is challenging - the standard HRZE (isoniazid, rifampicin, pyrazinamide and ethambutol) regimen for drug-sensitive TB needs at least 6 months, whereas more complicated cases of multidrug-resistant (MDR) and extensively-drug-resistant (XDR) TB require the additional use of second-line drugs for an extended duration of 18 months and beyond[1]. These lengthy treatment regimens result in toxic side-effects for many patients and often lead to patient non-compliance, which contributes to treatment failure and the development of drug-resistant TB. Consequently, there is a pressing need for the development of a shorter therapeutic regimen, as well as a better understanding of the factors limiting the effectiveness of current drugs.

We screened a saturated *Mycobacterium tuberculosis* (Mtb) transposon mutant library for mutants that exhibit altered fitness under partially-inhibitory concentrations of rifampicin, ethambutol, isoniazid, vancomycin and meropenem. By analyzing the change in frequency of the various transposon mutants using the TRANSIT[2] transposon insertion sequencing (Tn-seq) analysis platform, we were able to identify and rank genes mediating antibiotic sensitivity in Mtb. We verified the data by drug sensitivity assays with individual mutants and with information from existing literature. Multiple cell envelope mutants were predicted to be strongly sensitive to the drugs tested, whereas inactivation of individual efflux pumps did not appear to contribute majorly to drug sensitivity.

Author Disclosure Block:

W. Xu, None..
M. A. DeJesus, None..
M. G. Wright, None..
T. R. Ioerger, None..
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Poster Board Number:

LB-120

Publishing Title:

Protein acetylation is involved in *Salmonella enterica* serovar Typhimurium virulence

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Abstract Body:

Salmonella causes a range of diseases in different hosts, including enterocolitis and systemic infection. Lysine acetylation regulates many eukaryotic cellular processes, but its function in bacteria is largely unexplored. The acetyltransferase Pat and nicotinamide adenine dinucleotide (NAD^+)-dependent deacetylase CobB are involved in the reversible protein acetylation in *Salmonella* Typhimurium. Here, we used cell and animal models to evaluate the virulence of *pat* and *cobB* deletion mutants in *S.* Typhimurium, and found that *pat* is critical for bacterial intestinal colonization and systemic infection. Next, to understand the underlying mechanism, genome-wide transcriptome was analyzed. RNA-seq data showed the expression of *Salmonella* pathogenicity islands 1 (SPI-1) is partially dependent on *pat*. In addition, we found that HilD, a key transcriptional regulator of SPI-1, is a substrate of Pat. The acetylation of HilD by Pat maintained HilD stability and was essential for the transcriptional activation of HilA. Taken together, these results suggest that protein acetylation system regulates SPI-1 expression by controlling HilD in a post-translational manner to mediate *S.* Typhimurium virulence.

Author Disclosure Block:

Y. Sang, None..
J. Ren, None..
Y. Yao, None.

Poster Board Number:

LB-121

Publishing Title:

Alpha-ketoglutatate Mediated Regulation of *Pseudomonas aeruginosa* Pathogenesis by MifS-MifR Two-Component System

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Abstract Body:

Pseudomonas aeruginosa is a Gram-negative opportunistic pathogen that causes incapacitating infections in immunocompromised patients. Successful infection and disease progression extensively depends on any pathogen's ability to effectively utilize available nutrients that are essential for its growth and survival. *P. aeruginosa* preferentially utilizes tricarboxylic acid (TCA) cycle intermediates as carbon, nitrogen and energy sources. In *P. aeruginosa*, two-component system (TCS) signaling proteins have played an integral part in regulating the uptake of TCA cycle intermediates, specifically, C_4-dicarboxylates such as succinate, fumarate and malate. Recently, MifS and MifR TCS proteins were identified to regulate utilization of a key TCA cycle intermediate, the C_5-dicarboxylate α-ketoglutarate (α-KG). Importantly, in the recent years, α-KG has gained significant importance as a biological signaling molecule in bacteria, animals and plants. To date, very little is known of the physiology of *P. aeruginosa* when provided with α-KG as the sole carbon source and their role in virulence. We postulate that MifSR TCS is required to regulate *P. aeruginosa* pathogenesis in the presence of α-KG. Multiple virulence phenotypes were tested in the presence and absence of α-KG. Loss of *mifSR* had no effect on *P. aeruginosa* virulence in the presence of other TCA cycle intermediates, except α-KG. However, in comparison to the wild-type PAO1 strain, *mifSR* deletion strains exhibited differential regulation of pigment production, motility, phenazine production and cell cytotoxicity in the presence of α-KG. Thus, MifSR TCS regulates the expression of *P. aeruginosa*'s key virulence determinants in α-KG-dependent manner. Simultaneous regulation of multiple mechanisms involved in *P. aeruginosa* pathogenesis suggests a complex mechanism of MifSR action. Thus, understanding the physiological cues and regulation would provide a better stratagem to fight the often indomitable infections.

Author Disclosure Block:

G. D. Tatke, None..
S. Mustafi, None..
M. A. Barbieri, None..
K. Mathee, None.